CULTURAL AND MOLECULAR CHARACTERIZATION OF *Fusarium* ISOLATES CAUSING WILT DISEASE OF SOLANACEOUS VEGETABLES

RAKIBUL HASAN NITOL

DEPARTMENT OF PLANT PATHOLOGY
SHER-E-BANGLA AGRICULTURAL UNIVERSITY
DHAKA-1207

JUNE, 2020

CULTURAL AND MOLECULAR CHARACTERIZATION OF *Fusarium* ISOLATES CAUSING WILT DISEASE OF SOLANACEOUS VEGETABLES

BY

RAKIBUL HASAN NITOL
REGISTRATION NO. 12-04756

A Thesis
Submitted to the Faculty of Agriculture
Sher-e-Bangla Agricultural University, Dhaka
in partial fulfilment of the requirements
for the degree of

MASTER OF SCIENCE
IN
PLANT PATHOLOGY
SEMESTER: JANUARY-JUNE, 2020

Approved by:

Dr. Md. Rafiqul Islam **Professor** **Department of Plant Pathology** **Sher-e-Bangla Agricultural University** **Supervisor**	**Abu Noman Faruq Ahmmed** **Professor** **Department of Plant Pathology** **Sher-e-Bangla Agricultural University** **Co-Supervisor**

Prof. Dr. Fatema Begum
Chairman
Examination Committee
Department of Plant Pathology
Sher-e-Bangla Agricultural University

Department of Plant Pathology
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207

PABX: +88029144270-9
Fax: +88029112649
Web site: www.sau.edu.bd

CERTIFICATE

This is to certify that the thesis entitled, **"CULTURAL AND MOLECULAR CHARACTERIZATION OF *Fusarium* ISOLATES CAUSING WILT DISEASE OF SOLANACEOUS VEGETABLES"** *submitted to the Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirement for the degree of* **MASTER OF SCIENCE IN PLANT PATHOLOGY** *embodies the results of a piece of bona fide research work carried out by* **RAKIBUL HASAN NITOL** *bearing Registration No.* **12-04756** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma, elsewhere in the country or abroad.*

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Dated: 15 September, 2020

Place: Dhaka, Bangladesh

(Dr. Md. Rafiqul Islam)
Professor
Department of Plant Pathology
Supervisor

Dedicated

To

My Beloved Parents & Teachers

List of Abbreviations of Technical Symbols and Terms

Full Word	**Abbreviation/ Symbol**
Agricultural	Agril.
Agriculture	Agric.
Analysis of Variance	ANOVA
And	&
And Others	*et al.*
Bangladesh Agricultural Research Institute	BARI
Basic Local Alignment Searching Tools	BLAST
Centimeter	cm
Coefficient of Variation	CV
Colony Forming Unit	CFU
Complete Randomized Design	CRD
Cubic Foot	Cft
Days After Inoculation	DAI
Days After Transplanting	DAT
Degree Centigrade	℃
Deionized Distilled Water	ddH_2O
Deoxyribonucleic Acid	DNA
Expectation Value	E-Value
Forma specialis	f. sp.
Gram	g
Honestly Significant Difference	HSD
Internal Transcribed Spacer	ITS
Journal	*J.*
Least Significant Difference	LSD
Litre	L
Metric Ton	MT
Microliter	µl

(Cont'd)

Micrometer	µm
Millimeter	mm
Molecular Evolutionary Genetic Analysis	MEGA
Namely	viz.
National Center for Biotechnology Information	NCBI
Negative Logarithm of Hydrogen Ion Conc.	pH
Neighbor-Joining Tree	NJ Tree
Nucleotide	nt
Percentage	%
Percentage of Disease Incidence	% DI
Polymerase Chain Reaction	PCR
Potato Dextrose Agar	PDA
Randomized Complete Block Design	RCBD
Rotation Per Minute	rpm
Sher-e-Bangla Agricultural University	SAU
Sodium Hypochlorite	NaClO
That is	i.e.
Ton	T

ACKNOWLEDGEMENT

Firstly, I am expressing my deep sense of gratitude to the ***"Almighty Allah" (SWT),*** *the most gracious and benevolent who has made me able to perform this research work and to submit the thesis successfully for the degree of Master of Science (M.S.) in Plant Pathology, Sher-e-Bangla Agricultural university, Dhaka, Bangladesh. My deepest respect and love form the core of my heart is for* ***"Prophet Muhammad" (SM)*** *who is forever a torch of knowledge and guidance for humanity.*

It is my pleasure to express my earnest reverence, appraisal and enormous indebtedness to my respected research supervisor, ***Dr. Md. Rafiqul Islam****, Professor, Department of Plant Pathology, Sher-e- Bangla Agricultural University, Dhaka, Bangladesh, for his sincere guidance, constructive suggestions, encouragement and amiable behavior during the whole period of study. I was able to gather a lot of pleasant experience and enjoyed an independent working environment under his supervision.*

I would like to express my deep respect and gratitude to my research co-supervisor, ***Abu Noman Faruq Ahmmed,*** *Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka Bangladesh for his guidance, suggestions and co-operation throughout the whole research period.*

My love, respect and gratefulness also goes to ***Dr. Abu Bakar Siddique****, alumni of Sher-e-Bangla Agricultural University and Postdoc researcher, Dept. of Plant virology, UMBR, Biology Center, Branišovská ,1160/31, 370 05 České Budějovice, Czech Republic. I like to express my immense gratefulness to him for his heartiest cooperation for molecular identification of the pathogen.*

*I am also thankful to "****Ministry of Science & Technology****" for funding the research as a fellow of "National Science and Technology (NST) Fellowship" program in the year 2018 that helped me a lot to conduct the research smoothly.*

I am also extremely grateful to ***Prof. Dr. Khadija Akhter****, former Chairman as well as to* ***Prof. Dr. Fatema Begum,*** *Chairman, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka. Both of them were very supportive and helpful to provide research facilities as a chairman of the department.*

My cordial respect goes to ***Professor Dr. M. Salahuddin M. Chowdhury, Professor Dr. Nazneen Sultana, Professor Dr. F. M. Aminuzzaman, Professor Dr. Md. Belal Hossain, Professor Dr. Nazmunnahar Tonu****, Associate Professor* ***Shukti Rani Chowdhury,*** *Assistant professor* ***Md. Ziaur Rahman Bhuiyan, Hosna Ara Chowdhury Nisha, Sayed Mohammad Mohsin*** *and all other respected teachers, Department of Plant Pathology, Sher-e-Bangla*

Agricultural University, Dhaka for their valuable teaching, academic advices, and countless encouragement.

I want to acknowledge ***Dr. Md. Anwarul Islam****, Librarian, Sher-e-Bangla Agricultural University, Dhaka for providing various information, journal, Books related to research.*

Special thanks goes to my fellow mates and friends ***Sanzida Mokarroma, Asif Noor****,* ***Md. Sajeduzzzaman*** *and* ***Md. Tanvir ahmed****. They all supported me a lot during the period of experiment and without their co-operation the whole journey would have been very difficult.*

I am also thankful to my senior fellow ***Nahid Akter****, Lecturer, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for her sister like attitude and helpful mind.*

I would like to express my thankfulness to ***Moksedur Rahman****, Maintenance Engineer, Central laboratory Sher-e-Bangla Agricultural University, Dhaka for his cordial help during the experimental period.*

I also want to acknowledge the cooperartion of our lab attendant ***Rehana Akhter, Pritilata Sarder*** *and* ***Khorshed Al-Alam*** *and all other staffs of Department of Plant Pathology as well as all staffs of Sher-e-Bangla Agricultural University, Dhaka, for their cordial support all time.*

Finally, I recall indebtedness to my beloved parents and the family members for their boundless affection and well wishes.

The Author

CONTENTS

CHAPTER	TITLE	PAGE NO.
	LIST OF ABBREVIATIONS OF TECHNICAL SYMBOLS AND TERMS	**i-ii**
	ACKNOWLEDGEMENT	**iii-iv**
	CONTENTS	**v-xi**
	LIST OF TABLES	**vii**
	LIST OF FIGURES	**ix**
	LIST OF PLATES	**x**
	LIST OF APPENDICES	**xi**
	ABSTRACT	**xii**
I	**INTRODUCTION**	**1-5**
II	**REVIEW OF LITERATURE**	**6-22**
	2.1.Economic importance and epidemiology of Fusarium wilt	6
	2.1.1.Economic importance of Fusarium wilt	6-7
	2.1.2.Epidemiology of Fusarium wilt	7-8
	2.2.Symptomatology of Fusarium wilt	8-9
	2.3.Morphological features of *Fusarium* spp.	10
	2.4.Geographical distribution and host range of *Fusarium* spp.	10-11
	2.5.Dispersal of the pathogen (*Fusarium* spp.)	11-12
	2.6.Infection and disease progression process of *Fusarium* spp.	12-14
	2.7.Effect of temperature on growth of *Fusarium* spp	14
	2.8.Effect of pH on growth of *Fusarium* spp.	14-15
	2.9.Effect of culture media on growth of *Fusarium* spp.	15
	2.10.Morphological, physiological and cultural variability of *Fusarium* spp.	16-18
	2.11.Molecular identification & characterization of *Fusarium* spp.	19-21
	2.12.Pathogenic variation of *Fusarium* spp.	21-22
III	**MATERIALS AND METHODS**	**23-37**
	3.1.The experimental site	23
	3.2.Duration of the experiment	23
	3.3.Design of the experiment	23
	3.4.Collection of diseased sample	24
	3.5.Lab Experiment	25-33
	3.5.1.Equipment and Chemicals used	25

CHAPTER	TITLE	PAGE NO.
	3.5.2.Preparation of Potato Dextrose Agar (PDA) media and culture plates	25-26
	3.5.3.Isolation and identification of the pathogen	26-27
	3.5.4.Purification and preservation of the pathogen	28
	3.5.5.Nomenclature of the Isolates	28-29
	3.6.Cultural and morphological variability of *Fusarium* spp. isolates	29
	3.6.1.Cultural variability among isolates of *Fusarium* spp	29
	3.6.2.Measurement of mycelial (radial) growth of the isolates	30
	3.6.3.Determination of time (days) required for sporulation of the isolates	30
	3.6.4.Counting of sporulation rate (conidia/ml suspension) of the isolates	30
	3.7.Molecular identification of *Fusarium* spp. isolates	31
	3.7.1.Extraction of genomic DNA, amplification and sequencing	31
	3.7.2.Analyzing the sequence for molecular identification and phylogenetic tree construction	32-33
	3.7.2.1.Performing similarity search in “GenBank”	32
	3.7.2.2.Construction of “Phylogenetic Tree”	32
	3.8. Pot Experiment (Pathogenicity test)	34-37
	3.8.1.Preparation of soil mixture	34
	3.8.2.Soli sterilization	34
	3.8.3.Preparation of inoculum and inoculation in the soil	34
	3.8.4.Collection and transplantation of seedlings	36
	3.8.5.Confirmation of the disease	36
	3.8.6.Assessment of wilt incidence	36-37
	3.9.Statistical analysis	37
IV	**RESULTS AND DISCUSSION**	**38-81**
	4.1.Symptomological study of Fusarium wilt	38
	4.2.Identification of *Fusarium* spp.	39
	4.3.Cultural and morphological variability among isolates of *Fusarium* spp.	40-52
	4.3.1.Colony characteristics of *Fusarium* spp. isolates	40-44
	4.3.2.Radial mycelial growth of isolates of *Fusarium* spp**.**	45-48

CHAPTER	TITLE	PAGE NO.
	4.3.3.Sporulation rate and sporulation time of the isolates of *Fusarium* spp.	49-50
	4.3.3.1.Rate of sporulation	49
	4.3.3.2.Time required for sporulation	50
	4.3.4.Correlation and Regression study between mycelial growth and mycelial growth and sporulation rate	50-51
	4.3.5.Correlation and Regression study between mycelial growth and mycelial growth and sporulation time	51-52
	4.4.Molecular identification & characterization	53-78
	4.4.1.Molecular identification & phylogenetic tree construction of isolate-DSAUT	53-56
	4.4.2.Molecular identification &phylogenetic tree construction of isolate-GBARIB	56-59
	4.4.3.Molecular identification & phylogenetic tree construction of isolate-MMsB	59-62
	4.4.4.Molecular identification & phylogenetic tree construction of isolate-MMsC	62-64
	4.4.5.Molecular identification & phylogenetic tree construction of isolate-BGT	65-67
	4.4.6.Molecular identification & phylogenetic tree construction of isolate-BGP	68-70
	4.4.7.Molecular identification & phylogenetic tree construction of isolate-JJhB	70-73
	4.4.8.Molecular identification & phylogenetic tree construction of isolate-MeGP	73-75
	4.4.9.Molecular identification & phylogenetic tree construction of isolate-PBC	76-78
	4.5.Pathogenic variability of *Fusarium* spp. isolates	79-81
V	**SUMMARY AND CONCLUSION**	82-84
VI	**REFERENCES**	85-98
VII	**APPENDICES**	99-104

LIST OF TABLES

Sl. No.	Title	Page No.
01.	List of Isolates of *Fusarium* spp. based on locations and host plants	29
02.	Primer pair used in the PCR amplification	31
03.	Colony characteristics of *Fusarium* spp. isolates grown on PDA media	41
04.	Radial mycelial growth of *Fusarium* spp. isolates grown on PDA Media	47
05.	Sporulation rate and time required for sporulation of *Fusarium* spp. isolates	50
06.	Percentage of nucleotide identities of DSAUT isolate with selected *Fusarium* fungi reported worldwide	55
07.	Percentage of nucleotide identities of GBARIB isolate with selected *Fusarium* fungi reported worldwide	58
08.	Percentage of nucleotide identities of MMsB isolate with selected *Fusarium* fungi reported worldwide	61
09.	Percentage of nucleotide identities of MMsC isolate with selected *Fusarium* fungi reported worldwide	63
10.	Percentage of nucleotide identities of BGT isolate with selected *Fusarium* fungi reported worldwide	63
11.	Percentage of nucleotide identities of BGP isolate with selected *Fusarium* fungi reported worldwide	69
12.	Percentage of nucleotide identities of JJhB isolate with selected *Fusarium* fungi reported worldwide	72
13	Percentage of nucleotide identities of MeGP isolate with selected *Fusarium* fungi reported worldwide	74
14.	Percentage of nucleotide identities of PBC isolate with selected *Fusarium* fungi reported worldwide	77
15.	Wilt incidence and virulence level of *Fusarium* spp. isolates at 30, 45 and 60 DAT	80

LIST OF FIGURE

Sl. No.	Title	Page No.
01.	Macro and Micro conidia of *Fusarium* sp. under compound microscope (40X)	39
02.	Grouping of *Fusarium* spp. isolates based on radial mycelial growth/day	48
03.	Relationship between average mycelial growth/day and No. of spore/ ml suspension	51
04.	Relationship between average mycelial growth/day and sporulation time (day)	52
05.	Neighbor joining (NJ) Phylogenetic tree of DSAUT isolate in relation to other *Fusarium* isolates generated with 1000 bootstrap replicates	56
06.	Neighbor joining (NJ) Phylogenetic tree of GBARIB isolate in relation to other *Fusarium* isolates generated with 1000 bootstrap replicates	59
07.	Neighbor joining (NJ) Phylogenetic tree of MMsB isolate in relation to other *Fusarium* isolates generated with 1000 bootstrap replicates	62
08.	Neighbor joining (NJ) Phylogenetic tree of MMsC isolates in relation to other *Fusarium* isolates generated with 1000 bootstrap replicates	64
09.	Neighbor joining (NJ) Phylogenetic tree of BGT isolate in relation to other *Fusarium* isolates generated with 1000 bootstrap replicate	67
10.	Neighbor joining (NJ) Phylogenetic tree of BGP isolate in relation to other *Fusarium* isolates generated with 1000 bootstrap replicates	70
11.	Neighbor joining (NJ) Phylogenetic tree of JJhB isolate in relation to other *Fusarium* isolates generated with 1000 bootstrap replicates	73
12.	Neighbor joining (NJ) Phylogenetic tree of MeGP isolate in relation to other *Fusarium* isolates generated with 1000 bootstrap replicates	75
13	Neighbor joining (NJ) Phylogenetic tree of PBC isolates in relation to other *Fusarium* isolates generated with 1000 bootstrap replicates	78

LIST OF PLATES

Sl. No.	Title	Page No.
01.	Collection of diseased samples from infected fields	24
02.	Isolation and identification of *Fusarium* spp.	27
03.	Hyphal tip culture of *Fusarium* spp. inside of laminar air flow cabinet	28
04.	Performing of sequence analysis for molecular identification and phylogenetic tree construction	33
05.	Soil Preparation for pathogenicity test	35
06.	Seedling transplantation in inoculated pot for pathogenicity test	36
07.	Fungal wilt symptoms in solanaceous vegetables	38
08.	Colony characteristics *Fusarium* spp. isolates grown on PDA Media	43-44
09.	Pathogenicity test for *Fusarium* spp. isolates	79

LIST OF APPENDICES

Sl. No.	Title	Page No.
01.	Map showing the sample collection regions under the study	99
02.	Chloroform based protocol of fungal cDNA Extraction	100
03.	PCR amplification protocol	101
04.	DNA sequence (FASTA format) for BLAST	102-103
05.	Field visit for sample collection	104
06.	View of pot preparation for pathogenicity test	104

CULTURAL AND MOLECULAR CHARACTERIZATION OF *Fusarium* ISOLATES CAUSING WILT DISEASE OF SOLANACEOUS VEGETABLES

ABSTRACT

Fusarium spp. represent one of the noxious groups of plant pathogen causing wilt disease of many economically important crops. Due to presence of remarkable variability among its isolates, management options against this pathogen are quite difficult. Therefore, the present experiment was conducted to study the variability of different isolates of *Fusarium* spp. collected from major vegetable growing regions of Bangladesh. Those isolates varied morphologically with cottony white, cottony white with brownish center, cottony pinkish white, pinkish white, whitish purple, purplish and creamy colored colony where the colony shape was regular, regular with concentric ring to irregular. Margin of the colony were entire, wavy, curled, filiform and undulated with crateriform, fluffy, flat and convex colony texture. Mycelial growth rate of the isolates was between 2.00−10.32 mm day^{-1} where, the isolates DSAUB and JhKT exhibited the highest and the lowest growth, respectively. The isolates were also clustered into three groups based on colony diameter viz. fast growing, moderate growing and slow growing fungi. The variation of conidia production was between $2.66\times10^6-12.33\times10^6$ ml^{-1} suspension with sporulation time 3.33−7.33 days. Molecular identification of *Fusarium* spp. isolates was preformed through amplification of fungal DNA at ITS region, sequencing the DNA molecules and analyzing the sequence in "NCBI-BLAST" database and then six isolates viz. DSAUT, MMsB, MMsC, BGT, BGP and JJHB were identified as *Fusarium oxysporum* and another three isolates i.e. GBARIB, MeGP and PBC were identified as *F. fujikuroi*, *F. solani* and *F. commune*, respectively. Phylogenetic trees of the queried isolates in relation to globally reported *Fusarium* isolates revealed that there was a strong ancestral relationship among the native isolates and the respective globally reported isolates of *Fusarium* spp. Virulence level of the isolates also varied significantly. The isolates were grouped as highly virulent (HV), virulent (V), moderately virulent (MV) and avirulent (AV) pathogen based on disease incidence. The overall results demonstrated the existence of cultural, morphological, pathogenic and molecular variation of *Fusarium* spp. isolates in native environment.

CHAPTER I

INTRODUCTION

CHAPTER I
INTRODUCTION

Vegetables are the cheapest and richest sources of calories, natural vitamins, minerals & dietary fibre, antioxidant, phytochemical compounds etc. They are mostly soft and succulent, annual or perennial horticultural crops, with certain sections (roots, stalks, flowers, fruits, leaves, stem etc.) that can be consumed wholly or partially, cooked or raw form (Pennington and Fisher, 2009). Consumption of vegetables in adequate amount can be protective against some chronic diseases such as diabetes, cancer, obesity, metabolic syndrome, cardiovascular diseases as well as improve risk factors related to these diseases (Septembre-Malaterreb *et al.,* 2018).

Solanaceae family is one of the most important crop families whereas, solanaceous vegetables are top ranked consumed vegetables, among different vegetables grown in the world. Solanaceae, a family of mainly tropical flowering plants, is also termed as nightshade or potato family. Members of this family are found throughout the world but are mostly abundant and widely distributed in the tropical regions of Latin America. However, very few members are also found in temperate regions (Rubatzky and Yamaguch, 1997). This family contains total 102 genera and nearly 2,500 species, many of them have considerable economic importance as food and drug plants (Petruzzello, 2015).

Potato (*Solanum tuberosum*); eggplant (*Solanum melongena*); tomato *(Solanum lycopersicum*); peppers (various *Capsicum* species); tobacco (*Nicotiana tabacum* and *N. rustica*); the poisonous jimsonweed (*Datura stramonium*) and nightshades (*S. nigrum, S. dulcamara,* and others) etc. are the most known crops among this family. Solanaceous crops have also its significance in nutritional aspects as consumption of solanaceous vegetables in regular diet has undeniable positive effects on health since phytochemicals present of these vegetables can protect the human body from several types of chronic diseases. Dietary fibre presents in tomato, peppers, brinjal and potato may also contribute to the overall

health benefits by lowering cholesterol, managing blood glucose concentrations, and by transporting a significant amount of minerals and phytochemicals linked to the fibre matrix through the human gut (Kumari *et al.,* 2017).

Agriculture is the main strength of our economy which contributes about 13.20 % of our total GDP (BER, 2019). More than 156 types of vegetables are cultivated over the country by nearly 16.2 million farmers and in recent years, Bangladesh ranks third among the countries which posted highest growth in vegetable production (Imam, 2018). In 2017-18, total vegetable production of Bangladesh was 4335305 MT and the contribution of solanaceous crops in production was 1015145 MT (except potato) which was approximately 23% of total produced vegetable and total potato production was 9744000 MT (BBS, 2018).

Inspite of a huge production of agricultural crops, our farmers are facing many problems during farming practices. A large amount of crops is lost every year due to the occurrence of severe pest and disease outbreak, where fungal pathogens play a vital role in developing plant diseases. Presently, about 80% of plant diseases are associated with fungi. However, solanaceous crops are known to suffer from 12 major diseases and most of them are fungal (Khan, 1991).

Among different reported fungi, the genus *Fusarium* represents one of the most important and troublesome groups of fungi belongs to the class Hyphomycetes and family Tuberculariaceae under the subdivision Deuteromycotina causing devastating plant diseases like wilt and root/stem rot in numerous economically important crops around the world (Charoenporn *et al.,* 2010). The members of this genus are distributed across the globe where they are responsible for huge economic losses of both standing crops and post-harvest products (Booth,1971).

Fungal wilt caused by pathogenic strains of several species of *Fusarium* including *F. oxysporum, F. solani, F. eumartii, F. avenaceum, F. sulphureum* and *F. tabacinum* has been treated as a destructive disease that causes upto 100% yield loss (Gunua, 2010). However, the most commonly known destructive

species is *Fusarium oxysporum* that kills a wide range of solanaceous crops comprising of tomatoes, potatoes, pepper, eggplants etc. Other plants like palm oil, legumes, strawberries, sugarcane, lettuce, watermelon and bananas are also infected by this pathogen (Joshi, 2018).

A survey represented that at least 80% of all cultivated plants in the world are associated with at least one disease caused by a *Fusarium* species (Lesile and Summerell, 2006). Between the year 2008-2009, a severe outbreak of Fusarium wilt in tomato field was occurred in South Africa and in the consequences approximately 12 million metric ton yield was lost (Joshi, 2018). Another occurrences of wilt, vessel necrosis and death of plants of tomato, aubergine (eggplant), potato and pepper were reported in Serbia caused by *Fusarium oxysporum* during the early 80s, where the maximum intensity of infection was recorded nearly 80% and yield loss was more or less than 85% (Lević *et al.*, 2009). Those above facts have made *Fusarium* spp. one of the most devastating plant pathogens around the world.

Fusarium wilt occurs mainly in two forms also called syndromes. The first form of the disease manifestation is the 'acute wilt' a case where the plants leaves dry out very quickly and die while attached to the original position. The second form of the syndrome is known as 'chronic wilt' where the plants live with the fungi for a longer period which can even proceed to years. In chronic wilt syndrome, the plants remain stunted but do not die immediately (Flood, 2006). Slightly vein clearing on the outer parts of younger leaflets is the first visible symptoms of Fusarium wilt. Subsequently, the older leaves show epinasty caused by drooping of the petioles and in case of severe infection, plants become wilted and die suddenly (Agrios, 2005).

The fungus infects the plant through the root system, after penetration in the roots, they colonize in the root cells and then starts to spread to other parts of the plants through the vascular bundles with the help of upstream flow of water also called the transpiration stream pull of water in the vascular tissues specifically

in the xylem. The fungus grows and produces micro and macro conidia while spreading in the vascular tissues. The mycelia grown in the vascular tissue leads to blockage of the vessels resulting to water stress, development of wilt symptoms and finally death of the infected plants (Bishop and Cooper, 1983). As this fungus attacks through root of the plant, internal discoloration of the vascular bundles is the main diagnostic feature for Fusarium wilt (Zitter, 1998).

Fusarium spp. is known as a dynamic fungus because of high variability in respect to their cultural, morphological, physiological, molecular as well as pathogenic characteristics (Moss and Smith, 1984). More than 100 strains (formae speciales: ff. spp.) have been identified having a wide host range from herbaceous plants to trees (Gordon, 2017).

Though *Fusarium* spp. is a soil borne fungi, it can be spread and survive through infected plant parts like seed, bulb, corm etc. (Flood, 2006). As a soil inhabitant, this fungus is able to survive in the soil for an extensive period, may be upto 30 years forming resting spores known as chlamydospore (Haware *et al.*,1992). That is why it is difficult to control the disease through crop rotation as well as other cultural practices. Variability among different isolates and changing of pathogenic races are another reasons of difficulties to manage this pathogen. Resistance shown against chemicals and bio-agents by the pathogen might be due to the variability and development of new races that needs to be addressed for the proper management of the disease.

Above facts suggest that, development of resistant varieties might be the most effective and pragmatic solution against *Fusarium* spp. Though several number of resistant cultivars are reported around the world, their performance varies with locations and changing of pathogenic races (Singh and Reddy, 1991). So, urgent attention needs to be given on the variability studies of *Fusarium* spp. that could contribute in developing resistance cultivars and proper management strategies of the disease.

The present study was undertaken to characterize the different isolates of *Fusarium* spp. present in the major vegetable growing regions of Bangladesh.

Objectives of the Study

1. To isolate and identify different isolates of *Fusarium* spp. causing wilt of solanaceous vegetables;
2. To characterize the different isolates of *Fusarium* spp. causing wilt of solanaceous vegetables; and
3. To ascertain the variability of different *Fusarium* isolates causing wilt of solanaceous vegetables.

CHAPTER II

REVIEW OF LITERATURE

CHAPTER II
REVIEW OF LITERATURE

Fungal wilt of solanaceous crops caused by *Fusarium* spp. is a serious problem for vegetable cultivation and occurs almost all vegetable growing regions in Bangladesh. It is a very virulent pathogen which can cause upto 100% yield loss. Due to wide range of variability, it is difficult to control. Whatever, the works and researches regarding, epidemiology, economic importance, symptomatology morphological features as well as cultural, morphological, molecular and pathogenic variability of *Fusarium* spp. isolates have been reviewed and presented in this chapter.

2.1. Economic importance and epidemiology of Fusarium wilt

2.1.1. Economic importance of Fusarium wilt

Joshi (2018) described that *Fusarium* is a destructive plant pathogen and has been reported to cause huge field crop losses. For instance, in South Africa alone between 2008 and 2009, tomato yield loss as a result of Fusarium wilt was estimated to be above 12 million metric tons.

Lević *et al.* (2009) described that severe outbreak of wilt, vessel necrosis and death of plants of tomato, aubergine (eggplant), potato and pepper were reported during the decade of 1980 in Serbia caused by *Fusarium oxysporum*. The maximum intensity of infection was recorded nearly 30%, but in some cases it ranged from 80 -100% causing a huge amount of yield loss.

Chaudhary *et al.* (2009) described that Fusarium wilt epidemics depends on crop stages (seedling, adult and flowering), environment and crop variety and it is a part of disease complex under field condition. They mainly worked with the prevalence of wilt-root rot complex of lentil caused by several pathogens in India where, the main pathogens were *Fusarium oxysporum* f.sp. *lentis* (62%),

Rhizoctonia bataticola (25.2%) and *Sclerotium rolfsii* (9.8%). According to the report, wilt incidence can reach 50 -78% under natural condition and 100% yield loss may occur if the crop is affected at the seedling stage.

Ploetz (2005) described that "Gros Michel" (triploid cultivar of banana) was the first exported banana cultivar in the history of global trade but it was highly susceptible to panama disease (wilt of banana) caused by race 1 of *Fusarium oxysporum*. During the first half of 20th century, losses caused by panama disease were estimated at USD 2.3 billion in Panama only. Due to the impact of this incidence, the export of "Gros Michel" cultivar was replaced with "Cavendish" which was resistant to race 1 and race 2 of *Fusarium oxysporum.*

2.1.2. Epidemiology of Fusarium wilt

Pattison *et al.* (2014) reported that the growth and survival of the pathogen in the root zone were favored by dry soil condition under which the fungus was able to extract sufficient water for its growth and reproduction. After infection, disease development was depended on sufficient water being available for pathogen growth and dispersion in the xylem fluid. An internal water deficit caused by dry condition or waterlogging promotes symptom expression.

Peng *et al.* (1999) noted that the growth of Fusarium wilt pathogens was usually greatest at 28°C. The growth of the pathogens was inhibited above 35°C and below 17°C temperature. On the other hand, disease incidence increased as temperature increased from 24°C to 34°C.

Epp (1987) studied the epidemiology of Fusarium wilt of Cavendish type banana viz. 'Umalag' for 4 years in Philippines. He observed that symptoms developed after rain events and there was a positive correlation between heavy rains and disease incidence. He also reported that 95% of the symptomatic plants were matured or were about to mature.

Smith *et al.* (1981) and Colyer (2007) noted that in some plants, nematodes predispose the host to be infected by *Fusarium* spp. The interaction between the root-knot nematode *Meloidogyne incognita* and strains of *Fusarium oxysporum* f. sp. *vasinfectum* causing Fusarium wilt of cotton in the USA was found to be the most widely recognized disease complex in the world.

Rishbeth (1957) found that fungal wilt incidence was greatest when condition was most favorable for plant growth. There was a slow but steady appearance of diseased plants during dry condition, but the incidence of wilted plants increased four-fold following two months of heavy rainfall.

Stover (1956) described that some soil factors play a vital role in disease development. The disease is more serious in light sandy soil than in heavy clay soil. This is probably due to the effect of the sandy soil on the water relations of the host plant. *Fusarium* species are strongly aerobic and are favored by soil water contents of less than field capacity.

2.2. Symptomatology of Fusarium wilt

Miller *et al.* (2011) stated that slow and stunted growth of the plant followed by yellowing and wilting of the leaves and reddish discoloration of the xylem vessels are the general symptoms of wilt disease caused by *Fusarium* spp.

Flood (2006) found that Fusarium wilt occurs in two forms called syndromes. The first form of the disease manifestation is the “acute wilt” where the plants leaves dry out very quickly and die while attached to the original position. The second form of the syndrome is known as “chronic wilt” where the plants live with the fungi for a longer period which can even proceed to years. In chronic wilt syndrome, the plants remain stunted but do not die immediately.

According to, Agrios (2005) slightly vein clearing on the outer parts of younger leaflets is the first visible symptoms of Fusarium wilt. Subsequently, the older leaves show epinasty caused by drooping of the petioles. At the seedling stage, plants usually become wilted and die soon after the appearance of the first

symptoms. In case of favorable environmental condition, severe infection may occur in older plants and become wilted and die suddenly. However, vein clearing and leaf epinasty followed by stunting of the plants, yellowing of the lower leaves, occasional formation of adventitious roots, wilting of leaves and young stems, defoliation, marginal necrosis of the remaining leaves and finally death of the plant are the most common symptoms in older plants.

Most commonly symptoms appear on only one side of the stem and progress upward until leaf necrosis and death of the stem. In cross sections near the base of the infected plant stem, a brown ring is evident in the area of the vascular bundles and upward extension of the discoloration is also possible depending upon the severity. Occasionally, fruit infection can also be occurred followed by dropping off without becoming spotted.

Hutmacher *et al.* (2003) and Elliot (2009) observed that at the seedling stage or in young plants, cotyledons and leaves are wilted and dropped off which lead to bare stems. Early detection of Fusarium wilt is difficult because early symptoms may resemble some types of other seedling diseases and are easily confused with those of crown or root rot, stem cankers, pest injury, drought, nutrient deficiency, bacterial and verticillium wilt.

According to, Zitter (1998) internal discoloration of the vascular bundles is the main symptom that is used as a diagnostic feature of Fusarium wilt. Production of gummy red ooze from the lesion is another common symptom of Fusarium wilt but according to various reviews on the symptoms this should not be mistaken with ooze as a result of bacterial wilt. In some rare occasions Fusarium wilt has been associated with sudden collapse of some plants.

2.3. Morphological features of *Fusarium* spp.

Booth (1971) noted that *Fusarium* spp. is a filamentous fungus of the class Hyphomycetes and family Tuberculariaceae under the subdivision Deuteromycotina.

Barnett and Hunter (1972) described that extensive and whitish cottony mycelium is the first identifying cultural characteristics of *Fusarium* spp. Sometimes pink, purple or yellow mycelium are also found. Conidiophores of genus *Fusarium* are slender, simple, short and irregularly branched and conidia born on conidiophore. Conidia produced by *Fusarium* spp. are hyaline and principally of two kinds: macroconidia and microconidia. Macroconidia are hyaline, two to several celled, fusiform- to sickle shaped, slightly curved or bent at the pointed ends. Microconidia are 1 to 2 celled, hyaline, pyriform, fusiform to ovoid, straight or curved.

2.4. Geographical distribution and host range of *Fusarium* spp.

Gordon (2017) stated that *Fusarium oxysporum* is a fatal soil borne fungi prevalent in almost all agricultural fields around the world. More than 100 strains (formae speciales) of this fungi have been identified having a wide host range from herbaceous plant to trees.

Mosert *et al.* (2017) reported that *Fusarium oxysporum* f. sp. *Cubense* in banana fields of various countries including, Asia, Australia, Philippines, Zimbabwe, South Africa, Nigeria, Pakistan, China, Costarica, Kenya, and Uganda.

Miller *et al.* (2011) noted that *Fusarium oxysporum* has a wide host range. Tomato and other solanaceous crops, sweet potato, legumes, cucurbits and banana are the most susceptible host plants. Besides that, it also infects other herbaceous plants as well as cotton, ornamentals and palms.

Wong (2003) stated that the distribution of this fungi is as wide as the distribution of its host plants. For example, three physiological races of *Fusarium oxysporum* f. sp. *lycopersici* have been reported worldwide so far. Among them, Race 1 is the most widely distributed and has been reported from most geographical areas while race 2 has been specifically reported in Ohio, Florida, Brazil, Netherlands, Britain, Mexico, Australia, Morocco, Israel, Iraq, India and Bangladesh. Race 3 has been reported in Brazil, Australia, California and Florida.

Francis and Burgess (1975) stated that it is a soil-inhabiting fungus which is distributed worldwide. Some species of *Fusarium* are restricted to the tropics, some are predominant in the temperate zones, while others are found in desert, alpine, and arctic areas, where harsh climatic conditions prevail.

Armstrong and Armstrong (1948) first observed that vascular wilt of *Fusarium* spp. was able to parasitize the roots of plants without pathogenesis. Grass and weed populations may serve as a reservoir of inoculum that influences the occurrence of disease in plantations. They are also possibly involved in the contamination of irrigation sources and rivers with the pathogen. However, alternative hosts are usually regarded as a mechanism of long term survival of the pathogen.

Armstrong and Armstrong, (1981) stated that though *Fusarium oxysporum* has a wide range, individual pathogenic strains within the species have a limited host range and strains with similar or identical host ranges are assigned as same formae speciales (f. sp.).

2.5. Dispersal of the pathogen (*Fusarium* spp.)

According to, Flood (2006) though *Fusarium oxysporum* is a soil borne fungal pathogen, it can be spread through infected plant parts like seeds, bulb, corm etc.

Wong (2003) described that, farm cultivation tools and machinery contributes to high percentage of pathogen locomotion from one field to another especially

when the implements are used in an infected field and simultaneously in virgin soil without cleaning.

Su *et al.* (1986) described that, flood as well as irrigation water also play a vital role in the spread of this fungus. When plantations have been irrigated from contaminated dams or rivers and when flood waters from infested land have inundated alluvial river valleys, propagules of the pathogen and infested organic residues move a considerable distance and contaminate new lands.

Pathogen can spread through either passive or active methods. Stover (1962) reported that, the only means of active spread (spreading of an existing infection) of the pathogen through the soil is from plant to plant by root proximity. After the death of an infected plant the roots are decayed and the fungus present in the roots of the infected plant release spores into the soil. The short-lived secondary and tertiary roots are more likely to be infected than the major roots, and they release a small but constant supply of inoculum into the soil while the plant is still growing.

2.6. Infection and disease progression process of *Fusarium* spp.

According to, Beckman (1987), the life cycle of *Fusarium* spp. can be divided into dormant, parasitic and saprophytic stages. The dormant stage comprises inhibition and germination of resting structures in soil. The parasitic stage comprises penetration of roots, colonization of the root cortex and endodermis, movement to the xylem; colonization of the xylem of stems and leaves, symptom expression and finally death of the host. The saprophytic stage is the formation of resting structures in the dead host.

Huisman (1982) stated that in the dormant phase, mycelia, chlamydospores, macroconidia and microconidia (propagules) present in infested soil are inhibited from germinating because of mycostasis (stopping the growth of fungi).

Steinkellner *et al.* (2008) reported that when resting structures come into contact with root exudates released in the rhizosphere by a host plant (e.g. tomato), inhibition of resting spores from germination can be reversed by a non-host plant (e.g. grass) and it is non host specific.

Inoue *et al.* (2002); Wanjiru *et al.* (2002) and Mandeel (2007) reported that *Fusarium* spp. enter into the parasitic phase when any of the propagules or germ tube of spore penetrates the host through cracks formed by emerging lateral roots, wounds at the root cap, root hairs or branch roots.

Walter *et al.* (2009) observed that the fungi also produce cell wall degrading enzymes such as cutinase, pectinase, polygalacturonase etc. to enhance penetration process.

Morrell and Bloom (1981) reported that there is an association with Fusarium wilt and nematode colonization. Nematodes provide a potential entry point (wound) for the fungus, that's why severity of fungal wilt is higher in nematodes infested field. Interaction between fungi, nematodes and bacteria also create wilt complex.

Bishop and Cooper (1983) reported that after penetration in the roots, they colonize in the root cells and then starts to spreads to other parts of the plants through the vascular bundles with the help of upstream flow of water also called the transpiration stream pull of water in the vascular tissues specifically the xylem. The fungus produces macro and micro conidia while spreading in the vascular tissues. The mycelia grown in the vascular tissue leads to blockage of the vessels thus resulting to water stress, development of wilt symptoms and finally death of the infected plants.

Ortiz *et al.* (2014) stated that, resistant plants try to counter the spread of the fungi by producing defense mechanism and antifungal compounds such as tyloses, gels and gums to inhibit the growth and spread of the fungi. Early productions of the antifungal compounds and defense mechanism before

reaching the fungi in the stem vessels may enable the plant to survive. Development of disease symptoms is very rapid in susceptible plants which leads to collapse of the whole plant.

2.7. Effect of temperature on growth of *Fusarium* spp.

Khilare and Ahmed (2012) reported that 30°C temperature is the most suitable for the growth of *F. oxysporum* and growth drastically reduced below 15°C and above 35°C.

Gangadhara *et al.* (2010) studied the temperature effect on growth of *Fusarium. oxysporum* f. sp. *vanillae* isolates where 25°C was identified as most suitable temperature for maximum growth of this pathogen. Drastically reduction of growth was observed above 40°C and below 15°C temperature.

Mina and Dubey (2010) studied the relationship between the environmental factors and wilt disease development in chickpea. They reported that at 28°C (*in-vitro*) temperature, maximum colony diameter (85 mm) was observed in case of *Fusarium* spp.

Chi and Hanson (1964) noted that optimum temperature for the growth and development of *Fusarium solani* is 28°C but it can grow in a wide temperature ranging from 10-35°C. However, reduction of growth of this fungus starts below 15°C and above 30°C. The growth stops at 40°C and 5°C temperature.

2.8. Effect of pH on growth of *Fusarium* spp.

Farooq *et al.* (2005) reported that pH level can play a vital role in the growth of *Fusarium* spp. This report revealed that neutral pH (pH 7) was preferable for maximum growth (88.33 mm) and development of the fungus. Both increasing pH and decreasing pH from neutral pH were not favorable for the fungal growth.

Kistler (1997) and Hayes (1978) stated that pH ranged from 6.5 to 7.0 is the most favorable for growth of *F. oxysporum* f. sp. *ciceri*. and the growth was maximum at pH level 7.

Jamaria (1972) stated that that *F. oxysporum* f. sp. *niveum* (wilt pathogen of watermelon) has ability to grow in a wide pH range which is varied from 3.2 to 8.3. Although most preferable pH for the growth of this fungal isolates is between 5.5-6.5.

2.9. Effect of culture media on growth of *Fusarium* spp.

Teixeira *et al.* (2016) worked with four isolates of *Fusarium oxysporum* from wilted Passion fruit plants in Triângulo Mineiro, Brazil. They noted that Malt extract media was the best for mycelial growth and sporulation capacity of sleeted isolates. Both macroconidia and microconidia was observed and color of the isolates varied from white to pink to violet.

Khilare and Ahmed (2012) studied the performance of six culture media on mycelial growth of *Fusarium oxysporum*. Among those six tested media, PDA as well as Czapek's dox agar media were reported as the best for growth of this fungus.

Farooq *et al.* (2005) trailed eight culture media among them Czapek's dox agar and chickpea seed-meal agar media performed well for the growth of *Fusarium oxysporum* f. sp. *ciceri.*

Dikkar and Deshmukh (2003) found that Richard agar medium followed by PDA and Czapeck's agar medium was the best for mycelial growth and development of *Fusarium* spp.

2.10. Morphological, physiological and cultural variability of *Fusarium* spp.

Carmona *et al.* (2020) investigated variability of 120 *Fusarium oxysporum* isolates associated with wilt of tomato in Colombia. Their macroscopic analysis of the colony showed a variation in color between white, pale-to-dark violet and magenta and microscopic characteristics showed the formation of a slightly curved macroconidia over the sporodochia with 3-4 septation. The microconidia were oval, curved, or kidney shaped, without or with septation. They also reported that micro conidia were the most abundant structure in the culture media and all isolates also formed intercalary or terminal chlamydospores on hyphae.

Sahu *et al.* (2017) studied variability among eight isolates of *Fusarium oxysporum* f. sp. *udum* (wilt pathogen of pigeon pea) responsible for pigeonpea wilt. After incubation period fungal colony was developed ranging from 80-90 mm with smooth to serrated colony margin. The size of macroconidia varied from 18.47×2.98−30.24×3.52µm and in case microconidia, it was between 8.10×2.98−12.94×4.0µm. The shape of chlamydospores varied from round to oval.

Maina *et al.* (2017) stated that cultural and morphological variations exist among the 8 isolates of *Fusarium oxysporum* f. sp. *phaseoli* causing wilt of French bean. Luxuriant, moderately luxuriant and scanty aerial mycelial growth was observed among the isolates with either fluffy or fibrous texture. The radial mycelial growth of the isolates varied from 85 mm−71 mm recorded after seven days of incubation. Different types of colony color like purple, pink or white was also observed.

Raghu *et al.* (2016) studied variability among 44 isolates of *Fusarium* spp. causing wilt of Chili collected from different locations of South India. They found that the colony diameter ranged from 60 mm to 90 mm after 8 days of inoculation which were incubated at 27±1°C temperature. The colony color varied from white, cream to violet with yellow colored pigmentation on PDA

media. They also stated that the best media for growth and abundant sporulation of *Fusarium* spp. was PDA followed by oat meal agar and V-8 juice agar. The color of the colony also varied from white, cottony, cream and greyish in different media.

Patra and Biswas (2016) isolated eleven isolates of *Fusarium oxysporum* f.sp. *ciceri* to study variability among them collected from different locations of West Bengal, India. The radial growth of isolates ranged from 72 mm to 87 mm at seven days after inoculation on PDA medium. Sporulation of isolates was profuse to moderate. The size of macro-conidia was ranged from 13-15×2-3 µm to 15-19×3-4 µm, in micro-conidia it was from 3-4×1-2 µm to 5-6×2-3 µm. The number of septa in macroconidia was mostly 2-3 and in microconidia no septation or rarely single septation was found.

Chopada *et al.* (2014) collected 10 isolates of *Fusarium oxysporum* f. sp. *lycopesici* from wilted field of South Gujrat, India. According to their report the mycelia of the isolates produced moderate, profuse fluffy, thin, flat to slight fluffy and submerged growth with white, yellow, light pink, dark pink, orange and purple-orange pigmentation. The sporulation varied from $2.77\times10^6 - 21.68\times10^6$ spores/ml spore suspensions. Variation was also found in dry weight of the mycelia varied from 120.67 mg to 193.33 mg and the size of macro and microconidia was ranged from 15.46−21.8×4.91-5.45 to 21.42−44.28×7.35–9.14 µm and 3.57−14.28×2.68−4.46 to7.14−14.28×3.57−5.35µm, respectively.

Nirmaladevi and Srinivas (2012) studied colony color, mycelial growth pattern, radial growth, sporulation etc. of 114 isolates of *Fusarium oxysporum* f. sp. *lycopercisici* causing wilt of Tomato. The color and pigmentation of the isolates on PDA medium varied between white, creamish white to cream, light pink to pink and light purple to violet. They categorized the isolates into two groups based on mycelial growth pattern, i.e., fluffy growth and adherent smooth growth. On basis of colony diameter, the isolates were categorized into 3 groups viz., Fast growing (more than 70 mm), moderate growing (50-70 mm) and slow

growing (less than 50mm) and out of the 114 isolates, 85 isolates were rated as fast growing, 19 as moderate growing and 10 isolates as slow growing.

Nath (2011) isolated nine isolates of *Fusarium oxysporum* from wilted chickpea plants and studied cultural, physical & physiological variability among them. She reported that 25°C temperature was optimum for mycelial growth and sporulation with pH 6.0 for all isolates. According to this study oat meal agar medium was recommended for culturing *Fusarium* on the basis of the highest radial growth of Mycelia in order to get higher number of spores. The highest number of spores (both macro & micro) were produced in PDA media.

Ahmad (2010) studied morphological features of 27 isolates *Fusarium oxysporum* f. sp. *ciceris* collected from different region of Pakistan and among those isolates Foc-14 had the largest microconidia (3.7× 4.5 µm) and the smallest size was obtained from isolates Foc-21 (3.0×3.7 µm). The biggest size of macroconidia (7.5×20.10 µm) was obtained from Foc-25 whereas Foc-11 had the smallest size of macroconidia (3.5×22.5 µm).

Cha *et al.* (2007) characterized *Fusarium oxysporum* isolated from rotten stems and roots of paprika (*Capsicum annuum* var. *grossum*). They reported that, the fungal species produced white aerial mycelia with dark violet pigment on PDA media with room temperature and neutral pH. Unbranched and monophialides conidiophores was observed under microscope. The size of the microconidia was 3−11×1.5−3.5 µm with was oval-ellipsoidal shape and no septation and the size of macroconidia was 15-20×2-3.5 µm with slightly curved or slender shape and 2-3 septation.

Kulkarni (2006) studied *Fusarium oxysporum* f. sp. *gladioli* causing wilt of Gladiolus The results of the cultural studies indicated that the radial growth was maximum on PDA media (89.70 mm) ten days after inoculation which was significantly superior over all other media. The average size of the microconidia and microconidia was 4.3×1.75 µm and average 20.0×3.65 µm respectively.

2.11. Molecular identification and characterization of *Fusarium* spp.

Singha *et al.* (2016) isolated eight *Fusarium* spp. isolates from Assam, North India and molecular identification and characterization was performed by amplification of ITS region and RAPD analysis. Through RAPD images three cluster was identified among those isolates. They observed differences between the results when *Fusarium* isolates were identified morphologically and molecularly based on ITS. Morphologically *Fusarium* isolates F4 and F6 were identified as *Fusarium oxysporum* but based on ITS (molecular) identification F4 isolates was identified as *Fusarium equiseti* and F6 as *Fusarium proliferatum*.

Chehri (2016) collected 25 isolates of *Fusarium* spp. from infected roots of Tomato grown in different fields in Western Iran. After DNA sequence analysis using the *tef1* and *tub2* gene, the obtained sequences were compared with previous sequence in "NCBI-BLAST" database. Based on comparison of the sequence, fifteen isolates were identified as *Fusarium oxysporum*, three as *Fusarium redolens*, five as *Fusarium proliferatum* and two as *Fusarium verticillioides*.

Hafizi *et al.* (2013) studied genetic diversity of 51 isolates of *Fusarium solani* and 40 isolates of *Fusarium oxysporum* associated with crown disease of palm oil collected from nine states of Malaysia. Through molecular characterization based on IGS-RFLP analysis they found 27 haplotypes (a set of genetic determinants located on a single chromosome) among the *F. solani* isolates and 33 haplotypes among *F. oxysporum* isolates, which is the indication of higher intraspecific variations exists among *Fusarium* spp. Further UPGMA cluster analysis was also performed and from these results the isolates in both *Fusarium* species were divided into two main clusters with the percentage of similarity from 87%−100% for *F. solani*, and 89%−100% for *F. oxysporum* isolates.

Okungbowa and Shittu (2012) reviewed several articles about genome sequence of *Fusarium* spp. causing wilt disease of a wide range of plants. These include genomes of the cereal pathogen, *Fusarium graminearum,* which had a genome size of 36.2 Mb (Mega base), organized into 4 chromosomes. Genome size of maize pathogen, *Fusariun verticillioides* was 41.7 Mb which was organized into 12 chromosomes. Tomato pathogen, *Fusariun oxysporum* f. sp. *lycopersici* had 9.9 Mb genomic size which was organized into 15 chromosomes.

Ma *et al.* (2010) reported that the genomes of pathogenic *Fusarium* species consist of a larger number of proteins belongs to pathogenicity related protein families, compared to non- pathogenic fungi. This region encodes transcription factors, hydrolytic enzymes, and transmembrane transporters which play significant roles in pathogenicity of the fungi.

Saharana and Naef (2008) worked with 27 isolates of three *Fusarium* spp. viz., *Fusarium graminearum, Fusarium verticillioides and Fusarium oxysporum* causing Fusarium head blight (FHB) of wheat which were isolated from different states of India. They isolated genomic DNA from fresh mycelia by CTAB method and those isolates analyzed with four newly developed microsatellite markers and six previously published microsatellite marker and after study they found considerable genotypic variability among *Fusarium* spp. isolates causing Fusarium head blight of wheat in India.

Cha *et al.* (2007) isolated *Fusarium oxysporum* from rotten stems and roots of paprika (*Capsicum annuum* var. *grossum*) and identified them based on morphological characteristics. Those isolates were further identified at molecular level through amplification of fungal DNA by PCR at ITS region and comparing the sequence in “NCBI-BLAST” database showed that there were 100% similarities in DNA sequence between isolated pathogen and known DNA sequence of *Fusarium oxysporum.*

Mes *et al*. (1999) screened a collection of race 1 and race 2 isolates of *Fusarium oxysporum* f. sp. *lycopersici* for vegetative compatibility and characterized those isolates by random amplified polymorphic DNA (RAPD) analysis to establish the identity of the isolates. Two main groups were identified after comparison of RAPD profiles coincide with vegetative compatibility groups (VCGs). In addition, they identified several single member VCGs that could not be grouped those two main RAPD clusters. So they suggested that *Fusarium oxysporum* f. sp. *lycopersici* is a polyphyletic (a set of organisms, or other evolving elements, that have been grouped together but do not share an immediate common ancestor) taxon.

2.12. Pathogenic variation of *Fusarium* spp.

Maina *et al.* (2017) reported that, although all the 8 isolates of *Fusarium oxysporum* f. sp. *phaseoli* were pathogenic on 'Amy' (a variety of French bean), their pathogenic potentiality was significantly different. The most pathogenic isolate was Fop03, followed by Fop06 and Fop07 at means of having 97.0, 92.4 and 92.0%, pathogenicity, respectively. The least pathogenic isolate was Fop05 with a mean of 65.9% pathogenicity.

Raghu *et al.* (2016) showed that, there is a significant difference among 44 isolates of *Fusarium* spp. that cause disease in different varieties of chilli. Based on disease incidence these isolates were clustered into three groups i.e. highly pathogenic (91-100 % disease incidence), moderately pathogenic (81-90 % DI) and slightly pathogenic (< 80 % DI).

Teixeira *et al.* (2016) stated that all four isolates of *Fusarium oxysporum* isolated from Triângulo Mineiro, Brazil were pathogenic to passion fruit and cause wilt passion fruits. Among different species of passion fruits, *Passiflora edulis* was completely susceptible to *F. oxysporum* and *Passiflora alata* as wll as *P. setacea* were more resistant to *F. oxysporum* than *P. edulis*.

Chehri (2016) tested pathogenicity of different identified species of *Fusarium* from tomato in Western Iran. Test showed that three isolates of *Fusarium oxysporum* were highly pathogenic, whereas *F. proliferatum* and *F. redolens* were found to be weakly virulent against tomato plant. The result of pathogenicity test also demonstrated that four of 12 isolates of *F. oxysporum* and *F. verticillioides* were found to be nonpathogenic to tomato plants.

Nirmaladevi and Srinivas (2012) reported that 46 isolates of *Fusarium oxysporum* f. sp. *lycopercisici* was proven as highly virulent with more than 75% wilt incidence in tomato plants among 114 tested isolates of the pathogen.

Ahmad (2011) showed that, isolates Foc-2 (AZRI, Bahawalpur) had the highest pathogenic capacity, whereas, the least virulence isolates was Foc-4 (Chakwal) among 27 isolates of *Fusarium oxysporum* f. sp. *ciceris*.

Nath (2011) tested pathogenicity of nine Bangladeshi isolates of *Fusarium oxysporum* isolated from wilted chickpea plant. Among them only one isolate (FOC-1) found to be highly virulent (HV) type on reaction to chickpea variety BARI Chola-1.

Padwick (1940) categorized three hundred isolates of *Fusarium orthoceros* var. *ciceris* isolated from wilted chickpea plant into three groups on the basis of their pathogenic behavior, i.e. non-pathogenic (with 0 % DI), pathogenic (with 1-70% DI) and highly pathogenic (with > 70% DI).

CHAPTER III

MATERIALS AND METHODS

CHAPTER III
MATERIALS AND METHODS

The experiment was performed with a view to identifying and characterizing different isolates of wilt pathogen (*Fusarium* spp.) collected from different vegetable growing regions of Bangladesh. In this chapter, the materials used and the applied methods for conducting this research work were discussed.

3.1. The experimental site

The experiment was divided mainly into two parts i.e. lab experiment and pot experiment.

1. **Lab Experiment:** Performed in two different laboratories:
   - Isolation, identification, and characterization (cultural & morphological) of the pathogen was performed at "Plant Pathology Laboratory" located in Wazed Miah central laboratory, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh.
   - DNA isolation and sequencing for molecular identification was performed at Prof. RNDr. Karel Petrzik, CSc, Lab, Department. of Plant virology, UMBR, Biology Center, Czech Republic.

2. **Pot Experiment:** Pot experiment for determining pathogenic variability among different isolates was performed in "Net House", Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.

3.2. Duration of the experiment

The experiments were conducted during the period of "December, 2018 to March, 2020".

3.3. Design of the experiment

Both the lab and pot experiment was laid out in Complete Randomized Design (CRD) with three replications.

3.4. Collection of diseased sample

The roots of solanaceous crops viz. tomato, potato, chili & eggplant having typical symptoms (plate 1) of fungal wilt were collected with rhizosphere soil from different vegetable growing districts of Bangladesh viz. Dhaka, Gazipur, Cumilla, Manikganj, Gazipur, Faridpur, Jashor, Meherpur, Bogura and Panchagarh (Appendix I). The diseased roots were cut with distinct part of stem with a sharp knife and were put into a zip lock poly bag along with rhizosphere soil. Then those bags were brought to laboratory for further isolation procedure.

A. Plant having fungal wilt symptom

B. Separation of diseased root

C. Bagging of diseased root samples

Plate 1. Collection of diseased samples from infected fields

3.5. Lab Experiment

3.5.1. Equipment and Chemicals used

Equipment/ Materials used

- ✓ Autoclave
- ✓ Compound microscope
- ✓ Cover slip, slide
- ✓ Cotton
- ✓ Electronic balance
- ✓ Glassware viz. conical flask, wash glass, Petri dishes, test-tube
- ✓ Hot air oven
- ✓ Hot plate
- ✓ Laminar air flow cabinet
- ✓ Needle, scissors, forceps, knife, blades, scale
- ✓ Refrigerator
- ✓ Scotch tape, aluminum foil, blotter paper
- ✓ Spirit lamp

Chemicals used

- ✓ Agar powder (Lab graded)
- ✓ Cotton blue
- ✓ Dextrose powder (Lab graded)
- ✓ Distilled water
- ✓ Ethanol
- ✓ Formalin
- ✓ Lactic acid
- ✓ Mounting fluid
- ✓ Sodium hypochlorite

3.5.2. Preparation of Potato Dextrose Agar (PDA) media and culture plates

Protocol described by Ricker and Ricker (1936) was followed for media preparation. Media composition is given below:

Ingredients	Quantities/L
Peeled potato	200 g
Dextrose powder	20 g
Agar Powder	20 g
Distilled water	1 L

At first, fresh and disease free potato tuber was cleaned under running tap water. After peeling 200 gm sliced potato was measured and boiled with 1 L distilled water in a sterile vessel for 20-30 minutes in order to get potato infusion. It was then filtered by using cheesecloth and poured into a conical flask. Agar and dextrose was measured carefully and then mixed with potato infusion by stirring with a glass rod. Additional water was added to make volume one liter. The mixture was then sealed with a cotton plug and aluminum foil and was sterilized by autoclaving at 121°C temperature under 15 lbs. pressure for 15-20 minutes.

After autoclaving, the prepared media was kept 20-25 minutes for cooling and then 15-20 ml media was poured into 9 cm petriplates were sterilized in a hot air oven at 160 ℃ temperature for 1.5-2 hours. Before pouring, 25-30 drops of lactic acid and phenoxymethyl Penicillin tablet were added into the conical flask in order to maintain slightly acidic condition for the growth of fungi as well as for preventing bacterial contamination.

3.5.3 Isolation and identification of the pathogen

After bringing the diseased sample in the laboratory, the roots were washed thoroughly in running tap water. Tissue planting methods was followed to isolate the pathogen (Mohsin, 2013).

Firstly, the working surface was disinfected by 70% ethanol and then the root samples were cut into small pieces (.5-1 cm). Then the cut pieces were dipped into 1% Sodium hypochlorite (NaClO), 2-3 minutes for surface sterilization. After that, the cut pieces were washed in sterile water repeatedly for three times and then were placed on a sterilized tissue paper to soak extra water. The pieces were then placed on PDA media (Petriplates) with sterile forceps inside the laminar airflow cabinet and incubated at 25℃ ±1 temperatures for 5-7 days. After the incubation period the pathogen was observed under compound microscope (Model: Olympus CH20i) and was identified by CMI description.

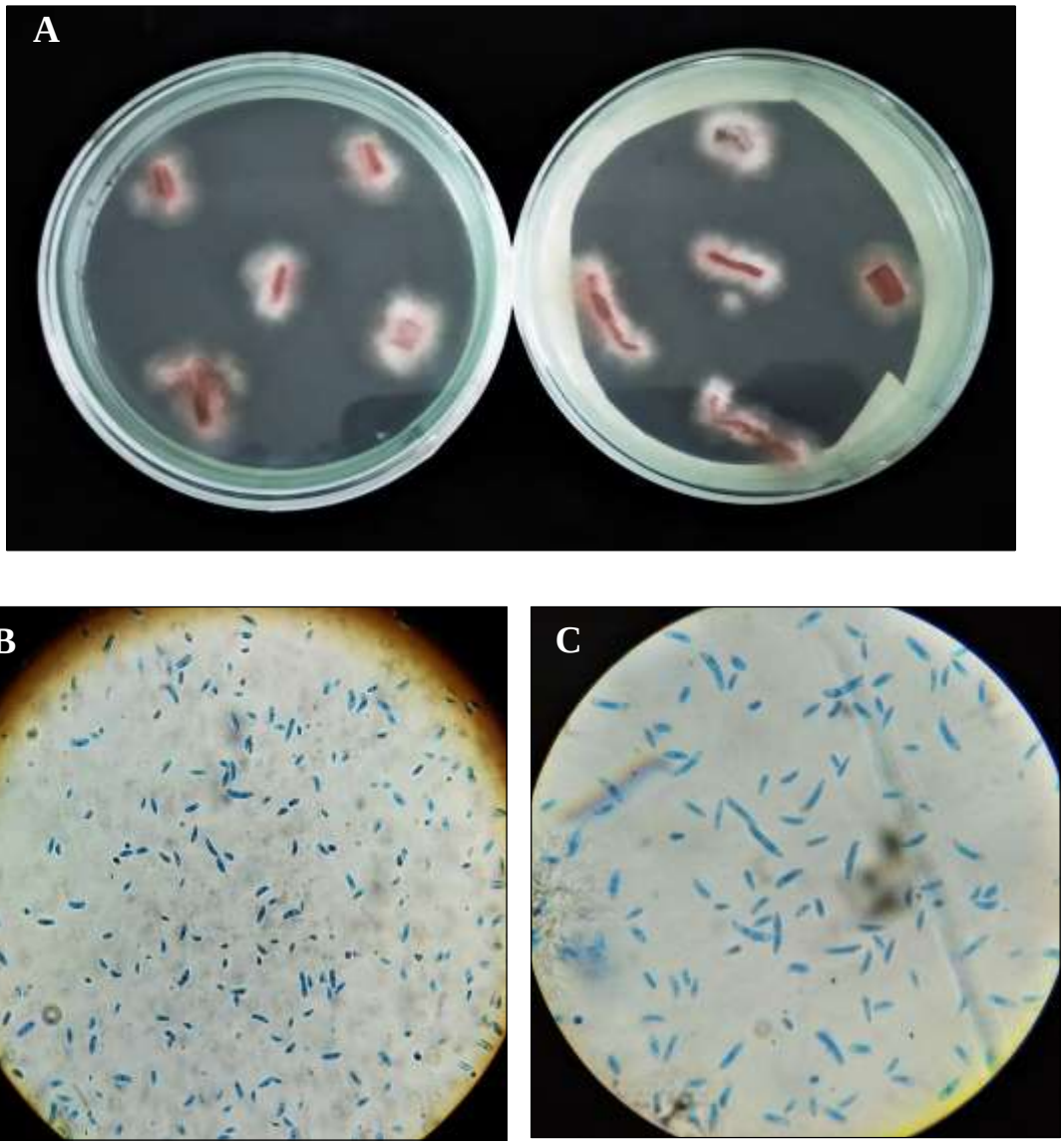


Plate 2. Isolation and identification of *Fusarium* spp. (A) Isolation by tissue planting method in PDA media (B) Conidia of *Fusarium* spp. under compound microscope (10X) and (C) Conidia under compound microscope (40X)

3.5.4. Purification and preservation of the pathogen

In order to get pure culture of *Fusarium* spp., 2-3 repeated sub-culture of mycelium was done by hyphal tip culture method. Hyphal tip was transferred into sterilized PDA plates for getting pure culture. The obtained pure culture was preserved at 4±0.5 ℃ temperature for further use.

Plate 3. Hyphal tip culture of *Fusarium* spp. inside of laminar air flow cabinet

3.5.5. Nomenclature of the Isolates

Total fifteen isolates of *Fusarium* spp. were collected from ten districts of Bangladesh and those were designated based on its locations and sources of isolation following the procedure of Aminuzzaman *et al.* (2010) (Table 1). For example, an isolate named by CChB represents that this isolate was collected from district-Cumilla (C), upazilla- Chandina (Ch) and the host plant was Brinjal (B).

Table 1. List of Isolates of *Fusarium* spp. based on locations and host plants

Sl. no.	Isolates	Area of collection & Host plant		
		District	Upazila/ Organization	Host plant
01	DSAUT	Dhaka	SAU	Tomato
02	DSAUB	Dhaka	SAU	Brinjal
03	GBARIB	Gazipur	BARI	Brinjal
04	MMsB	Manikganj	Manikganj sadar	Brinjal
05	MMsP	Manikganj	Manikganj sadar	Potato
06	MMsC	Manikganj	Manikganj sadar	Chili
07	CChB	Cumilla	Chandina	Brinjal
08	CChC	Cumilla	Chandina	Chili
09	BGT	Bogura	Gabtali	Tomato
10	BGP	Bogura	Gabtali	Potato
11	FAB	Faridpur	Alfadanga	Brinjal
12	JaJhB	Jashore	Jhikargacha	Brinjal
13	MeGP	Meherpur	Gangni	Potato
14	JhKT	Jhenaidah	Kaliganj	Tomato
15	PBC	Panchagarh	Boda	Chili

3.6. Cultural and morphological variability of *Fusarium* spp. isolates

3.6.1. Cultural variability among isolates of *Fusarium* spp.

Due to determination of cultural variability of *Fusarium* spp. all isolates were grown on PDA media and incubated at 25±2 ℃ temperature for 9 days (Nirmaladevi and Srinivas, 2012) and then observation was made on:

- ✓ Colony Color
- ✓ Colony Texture
- ✓ Shape
- ✓ Margin

3.6.2. Measurement of mycelial (radial) growth of the isolates

The radial mycelial growth of the isolates was started to measure after three days of inoculation in the petriplates and it was continued upto nine days after inoculation (DAI).

The isolates were categorized into three groups based on colony diameter of the isolates viz. fast growing (more than 70mm), moderate growing (50- 70mm) and slow growing (less than 50mm) according to Nirmaladevi and Srinivas, (2012)

The average mycelial growth per day was recorded and calculated by the following formula:

$$** \textbf{Growth/day} = \frac{\textbf{Growth at}((\textbf{4DAI}-\textbf{3DAI})+(\textbf{5DAI}-\textbf{4DAI})+(\textbf{6DAI}-\textbf{5DAI})+(\textbf{7DAI}-\textbf{6DAI})+(\textbf{8DAI}-\textbf{7DAI})+(\textbf{9DAI}-\textbf{8DAI}))}{\textbf{6}}$$

3.6.3. Determination of time (days) required for sporulation of the isolates

Microscopic slide of all isolates was prepared with 24 hours' interval starting from three days after inoculation and observed under compound microscope to determine the time (days) required for sporulation.

3.6.4. Counting of sporulation rate (conidia/ml suspension) of the isolates

Nine days old culture was used to determine the sporulation rate of the isolates using haemocytometer. 5 mm mycelial block of *Fusarium* spp isolates was cut from culture media using sterilized block cutter, kept into a test-tube and then one ml distilled water was added into the test-tube. Then it was stirred using a glass rod. After that, one drop of suspension was taken to haemocytometer for counting the spore under compound microscope. In case of each replication, the spore counting process was repeated for 10 times.

3.7. Molecular identification of *Fusarium* spp. isolates

In order to extract fungal DNA and sequencing, the pure mycelial culture of the fungal isolates was sent to Molecular laboratory. The name and address of the laboratory was mentioned in this chapter (3.1).

3.7.1. Extraction of genomic DNA, amplification and sequencing

The fungi were molecularly identified by comparison of the internal transcribed spacer (ITS) sequence. Fungal mycelium from colonies that had been selected to get the most complete coverage of the diverse fungal material was carefully scraped from the PDA plates and grounded by using mortar and pestle. Genomic DNA was extracted from all isolates using a traditional, chloroform-based protocol (Solis *et al.*, 2015). The protocol was mentioned in appendix II.

After the extraction of the DNA, amplification of the internal transcribed spacer (ITS) region was performed with primers ITS1 and ITS4 (White *et al.*, 1990) on an Eppendorf Mastercycler. (appendix III)

Table 2. Primer pair used in PCR amplification

Primer Name	Primer Sequence (5'-3')
ITS1	TCCGTAGGTGAACCTGCGG
ITS4	TCCTCCGCTTATTGATATGC

Source: White *et al.* (1990)

The obtained product was purified by using a spin column (Petrzik and Siddique, 2019) and then the purified amplicons were shifted to Eurofins (Testing Laboratories company) for sequencing.

After sequencing the sequenced data were received as ".ab1" file format via "e-mail" for further analysis and identification.

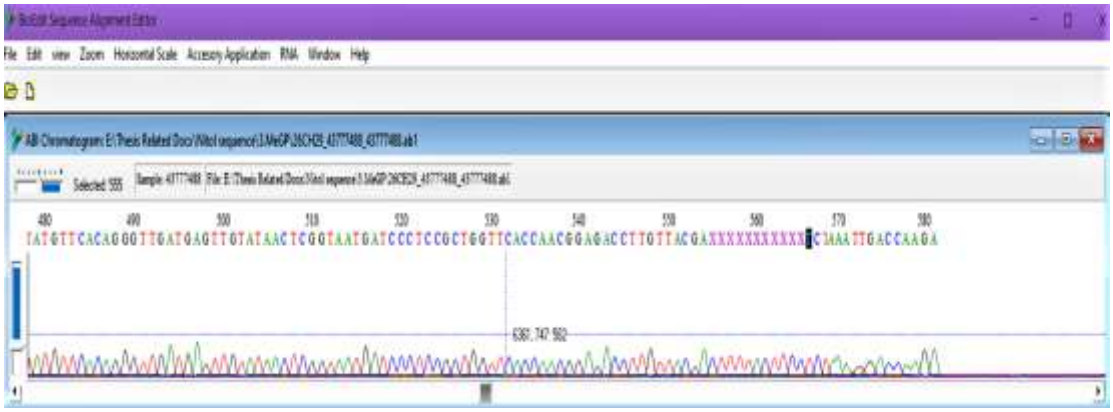
3.7.2. Analyzing the sequence for molecular identification and phylogenetic tree construction

3.7.2.1. Performing similarity search in "GenBank" using BLAST

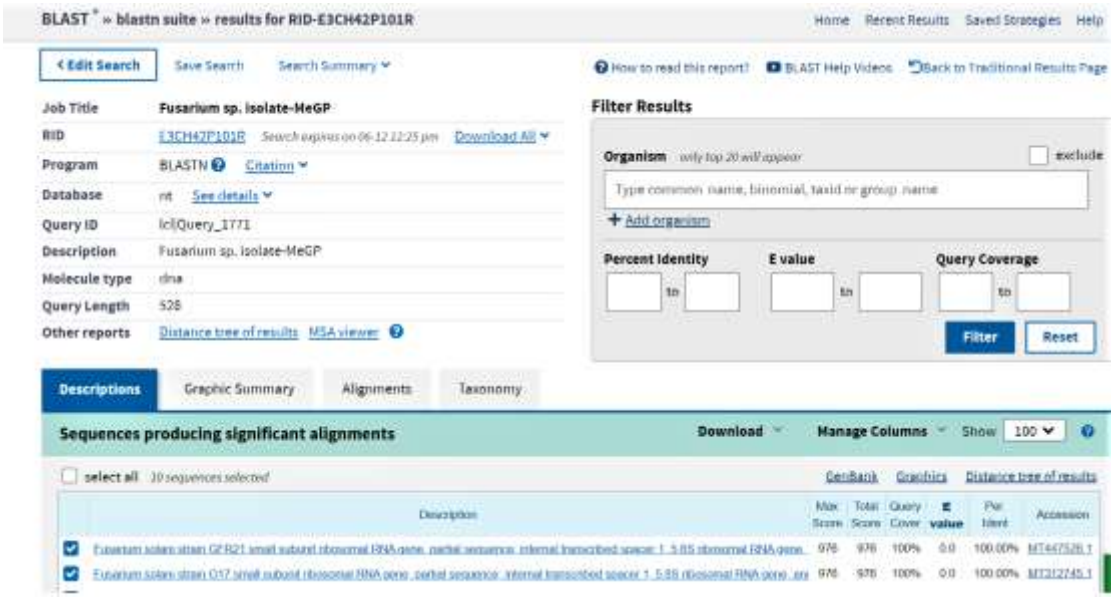
Each of the sequenced file received as ".ab1" file format was converted to "FASTA" file format using "BioEdit" computer program and then the sequence was blasted against NCBI-Database (http://www.ncbi.nlm.nih.gov/) inorder to identify and compare the sequence with the previously submitted sequences (Ahmmed, 2016).

3.7.2.2. Construction of "Phylogenetic Tree"

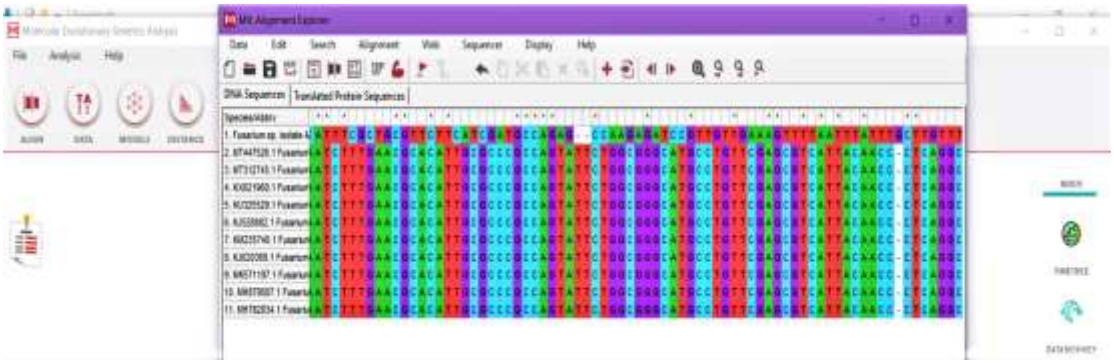
After Blast search, ten representative sequence results were selected and complete sequence were downloaded as "FASTA" format and then sequence of target fungi was aligned with those of related sequence using CLUSTALW and a neighbor-joining (NJ) tree was constructed in MEGA X (Kumar *et at.*, 2018) with 1000 bootstrap replications to determine ancestral relationship among them.



a) Conversion of ".ab1" file format into " FASTA" File format



b) Performing "BLAST" search for molecular identification and comparison



c) Performing sequence alignment for phylogenetic tree construction

Plate 4. Performing of sequence analysis for molecular identification and phylogenetic tree construction

3.8. Pot Experiment (Pathogenicity test)

Pot experiment was designed to determine pathogenic variability of all collected isolates of *Fusarium* spp.

3.8.1. Preparation of soil mixture

Soil preparation for pot experiment was done by thoroughly mixing of air dried loamy soil, sand and organic fertilizer maintaining ratio 2:1:1. Chemical fertilizers were not used in the pot soil.

3.8.2. Soli sterilization

Before inoculation of the pathogen and transplanting, soil sterilization was done to prevent contamination of other pathogen. For sterilization 0.4% formalin solution was added in the soil @ 200 ml/cft and was mixed thoroughly (Tasnin, 2018). After mixing, the soil was wrapped by a polythene sheet and kept for 48 hours. Then, after pulverization the soil was exposed for 7 days in open air and then it was ready for planting.

3.8.3. Preparation of inoculum and inoculation in the soil

The pure culture of total fifteen collected isolates of *Fusarium* sp. were sub-cultured on PDA medium and incubated for 10 days at 25℃ temperature with a view to getting a huge amount of inocula. For each isolate, at least nine plates were prepared and after incubation period, a plastic scrapper was used to scrap the inocula (mycelial mat and spores) for inoculation (Nath, 2011).

After potting the previously sterilized soil in sterilized earthen pot, the scrapped inocula of each isolate was incorporated in the soil with three replications (inocula collected from three pots were used in each replication) and three pots were used as control. Immediately after inoculation watering was done in each pot for giving proper growing environment of the fungus.

Plate 5. Soil Preparation for pathogenicity test; (A) Preparation of soil through mixing of air dried soil, sand and organic fertilizer (B) Soil sterilization through .4% formalin solution (C) Wrapping the sterilized soil through polythene (D) Potting of sterilized soil (E) Inoculation of mycelium

3.8.4. Collection and transplantation of seedlings

Fifteen days old seedling of BARI Brinjal-5 was collected from Horticulture Centre, FalBithi, Asadgate, Dhaka and was transplanted into the inoculated soil in order to perform pathogenicity test. Transplanting was done seven days after inoculation and after transplantation the pots were kept in the net house for observation of symptoms.

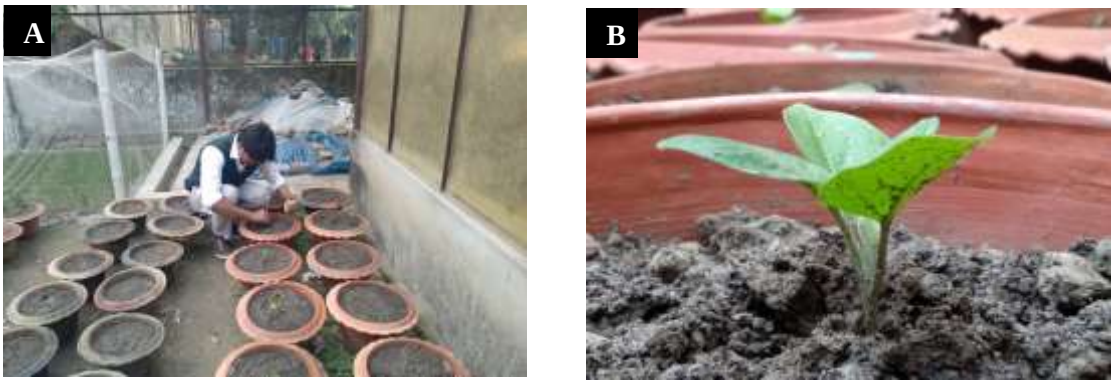


Plate 6. Seedling transplantation in inoculated pot for pathogenicity test (A&B)

3.8.5. Confirmation of the disease

After appearance of disease symptoms, it was necessary to confirm the disease and that is why Koch's postulates (re-isolation and identification of the pathogen) was performed following the standard procedure (Mitchell *et al.*,1997).

3.8.6. Assessment of wilt incidence

In order to assess the level of the virulence of the pathogen, the data of wilt incidence was assessed and recorded in case of each isolates with control pot at 30 DAT, 45 DAT and 60 DAT (days after transplanting) by the following formula:

$$\textbf{\% Wilt incidence} = \frac{\textbf{No of Plant wilted}}{\textbf{Total no of plant observed}} \times 100$$

The virulence level of each isolates was determined by using 1-5 rating scale developed by Nath (2011).

Where,

1= Highly virulent (HV), 76-100% plants wilted

2= Virulent (V), 51-75% plants wilted

3= Moderately virulent (MV), 26-50% plants wilted

4= Low virulent (LV), 1-25% plants wilted and

5= Avirulent (AV), no plants wilted

Though the data of wilt incidence was recorded at three different times i.e. 30 DAT, 45 DAT and 60 DAT, the aggressiveness of all the fifteen isolates was measured by considering the wilt incidence at 60 DAT.

3.9. Statistical analysis

Statistical analysis of data regarding cultural, morphological and pathogenic variability was performed by following standard procedure (Gomez and Gomez, 1984) and the treatment means were compared by Tukey's test. Statistix 10 computer package was used for performing analytical process.

CHAPTER IV

RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

Variability among different isolates of *Fusarium* spp. causing wilt disease of solanaceous vegetables were studied in this experiment. The findings of the research work were discussed in this chapter.

4.1. Symptomological study of Fusarium wilt

Fungal wilt was identified by observing the slow and stunted growth of the plant followed by yellowing of leaves from base to upward. At advance stage, leaf epinasty, formation of adventitious root, wilting of leaves and young stems, defoliation and marginal necrosis of the remaining leaves were also observed. Internal brownish discoloration was observed after longitudinal sectioning the infected stem. The present findings were similar to wilt symptoms described by Agrios (2005) and Miller *et al.* (2011).

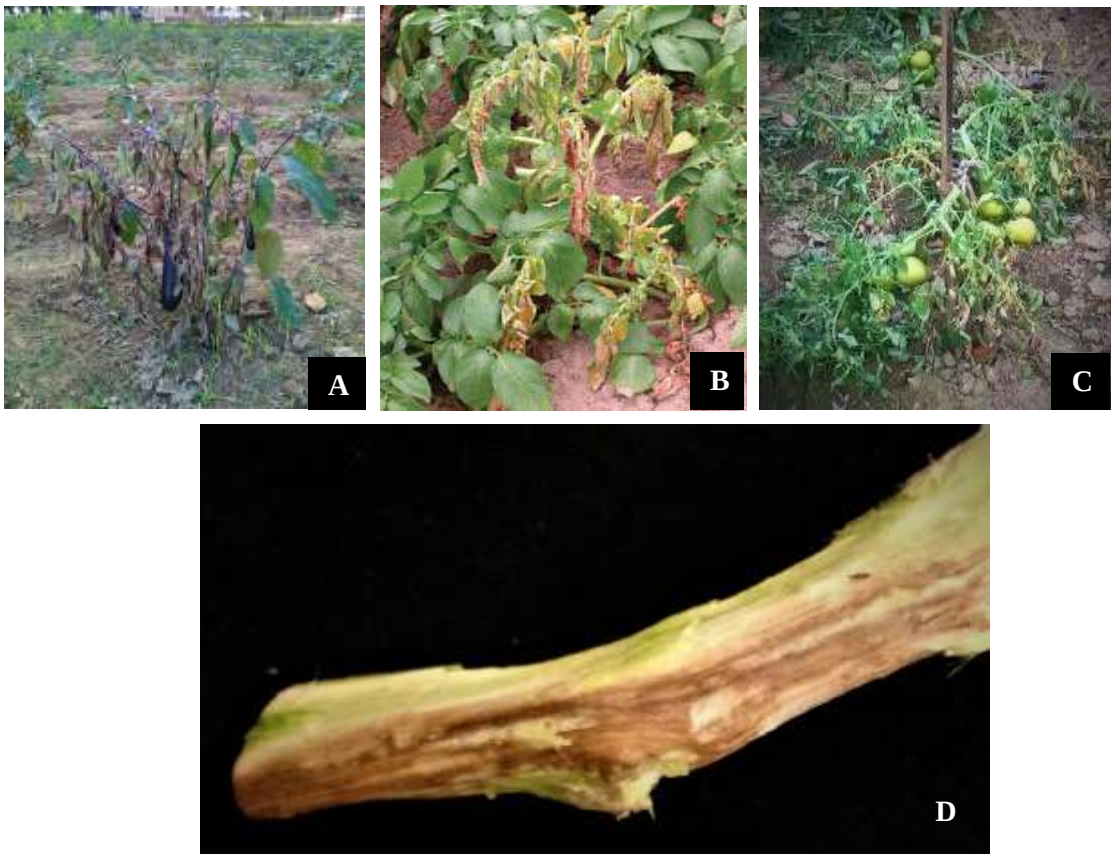


Plate 7. Fungal wilt symptoms in solanaceous vegetables; A) Wilted eggplant B) Wilted potato plant C) Wilted Tomato Plant D) Internal brownish discoloration of stem

4.2. Identification of *Fusarium* spp.

The fungus isolated by tissue planting method was observed under compound microscope and was identified as *Fusarium* sp. based on cultural appearance and conidial characteristics. Cottony white, pinkish to purplish mycelia were observed in case of different isolates (plate 8).

Hyaline, fusiform to sickle shaped and two to several celled macroconidia were observed as well as fusiform to ovoid, one to two celled hyaline microconidia were also observed (Figure 1). Thick walled chlamydospore was rarely observed. Among all, the most prevalent observed structure was micro conidia. The conidial characteristics found in the present investigation were similar to the description of Barnett and Hunter (1972).

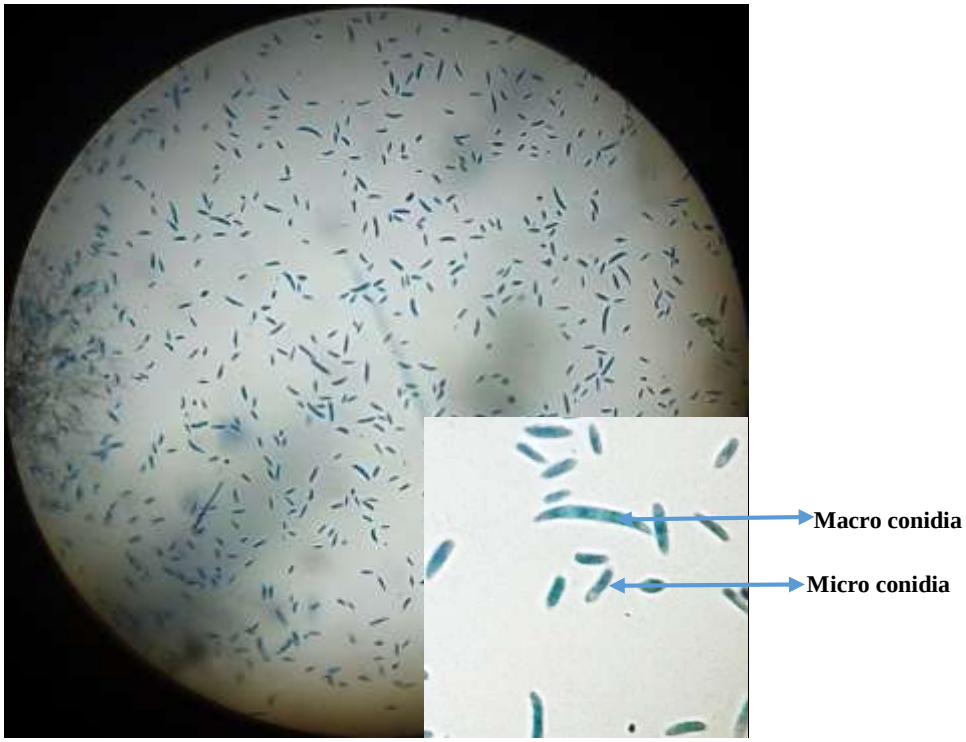


Figure 1. Macro and Micro conidia of *Fusarium* sp. under compound microscope (40X)

4.3. Cultural and morphological variability among isolates of *Fusarium* spp.

4.3.1. Colony characteristics of *Fusarium* spp. isolates

A significant variation was observed in colony characteristics in terms of colony color, shape, margin and texture among all the fifteen isolates of *Fusarium* spp. grown on PDA media (Plate 8 and Table 3).

Cottony white colony color was observed in case of isolates CChB, CChC and MeGP. Cottony white with brownish center colony color was found in isolates DSAUT and DSAUB, cottony pinkish white colony color was observed in isolate GBARIB, pinkish white colony color was found in isolates BGT and JhKT, whitish purple colony color was in isolate MMsB, purplish colony color was recorded in isolates FAB, MMsP and MMsC, creamy colony color was reported in isolate PBC and pinkish colony color was found in isolates in BGP and JaJhB (Plate 8 and Table 3).

Remarkable variation was also observed in shape of the fungal colony. Regular colony shape was found in isolates GBARIB, MMsP, CChB, FAB, JaJhB, MeGP and PBC. On the other hand, the isolates DSAUT, DASUB, MMsB, BGP showed regular colony shape with concentric ring. Irregular colony shape was observed in isolates MMsC, CChC, BGT, JhKT (Plate 8 and Table 3).

Variability was also recorded in colony margin of the fungal isolates. Entire (regular and smooth) colony margin was found in isolates DSAUT, DSAUB, GBARIB, MMsB, MMsP, CChB, BGP, JaJhB, MeGp, and PBC. Wavy colony margin was found in isolates MMsC and CChC. Curled, filiform and undulated colony margin were found in isolates BGT, FAB and JhKT, respectively (Plate 8 and Table 3).

Different types of colony texture were also observed in different isolates of *Fusarium* spp. i.e. Crateriform (saucer shaped) texture was found in isolates DASUT and DSAUB, fluffy texture was found in isolates GBARIB, CChC,

BGT, BGP, JaJhB and MeGP, flat colony texture was visible in isolates MMsB, MMsP, MMsC, CChB, FAB and JhKT. Convex type of colony texture was found in isolates PBC (Plate 8 and Table 3).

Table 3. Colony characteristics of *Fusarium* spp. isolates grown on PDA media

SL No.	Isolates	Colony Characteristics			
		Color	Shape	Margin	Texture
01	DSAUT	Cottony white with brownish center	Regular with concentric ring	Entire (Regular & smooth)	Crateriform (Saucer shaped)
02	DSAUB	Cottony white with brownish center	Regular with concentric ring	Entire	Crateriform
03	GBARIB	Cottony pinkish white	Regular	Entire	Fluffy
04	MMsB	Whitish purple	Regular with concentric ring	Entire	Flat
05	MMsP	Purplish	Regular	Entire	Flat
06	MMsC	Purplish	Irregular	Wavy	Flat
07	CChB	Cottony white	Regular	Entire	Flat
08	CChC	Cottony white	Irregular	Wavy	Fluffy
09	BGT	Pinkish white	Irregular	Curled	Fluffy
10	BGP	Pinkish	Regular with concentric ring	Entire	Fluffy
11	FAB	Purplish	Regular	Filiform	Flat
12	JaJhB	Pinkish	Regular	Entire	Fluffy
13	MeGP	Cottony white	Regular	Entire	Fluffy
14	JhKT	Pinkish white	Irregular	Undulated	Flat
15	PBC	Creamy	Regular	Entire	Convex

From the above results it can be depicted that, the variability among different isolates of *Fusarium* spp. in terms of colony characteristics was remarkable. This present results are in agreement with several past studies (Nirmaladevi and Srinivas (2012), Nath (2011) and Dubey *et al.* (2010)). Nirmaladevi and Srinivas (2012) found significant variation among one hundred fourteen isolates of *Fusarium oxysporum* f. sp. *lycopersici* in respect of colony color, texture, margin and shape. They found colony color varied between white, creamy white to cream, light pink to pink and light purple to violet and they also found fluffy to flat colony texture. The present findings were also supported by Nath (2011) and Dubey *et al.* (2010).

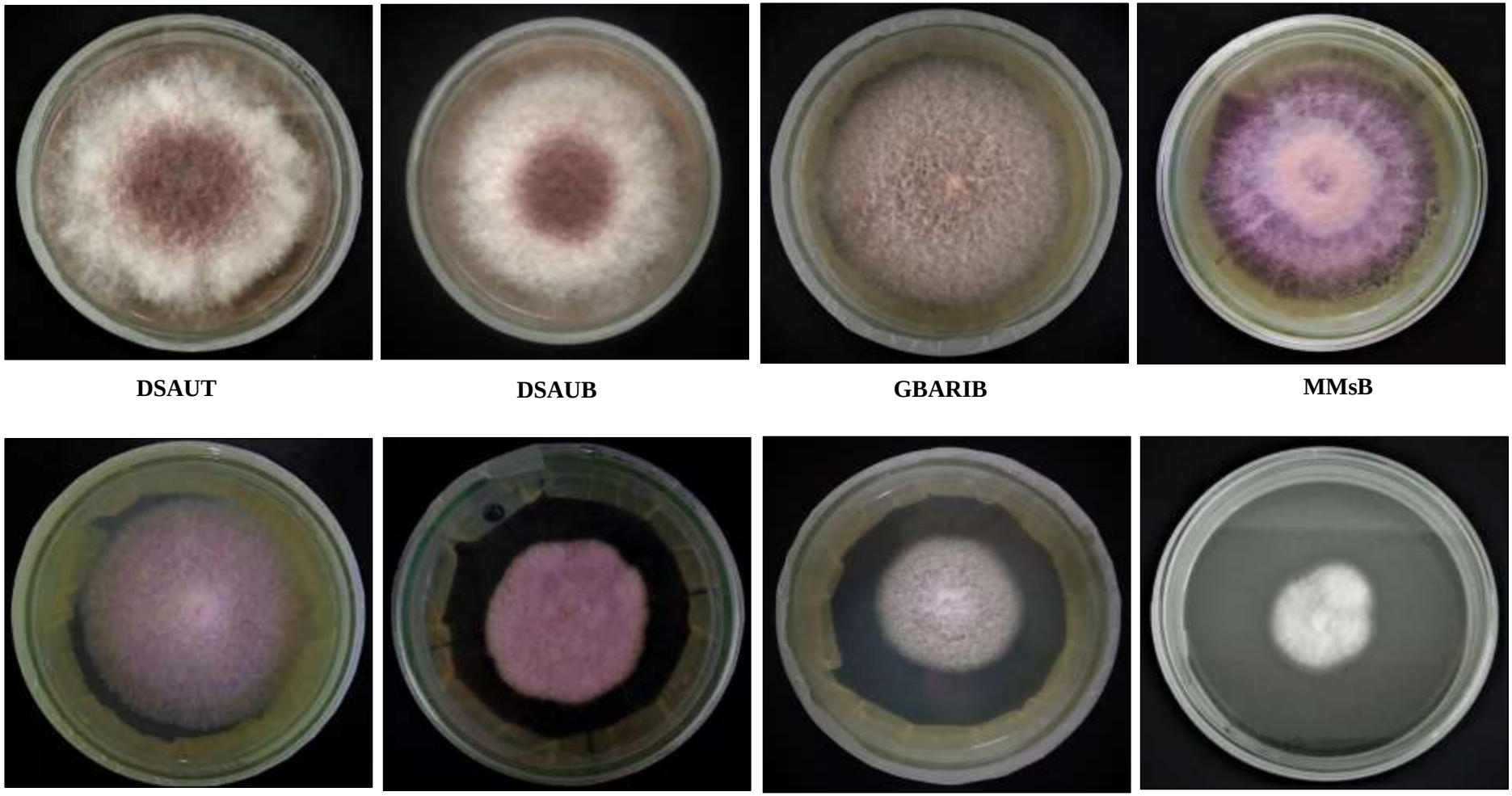


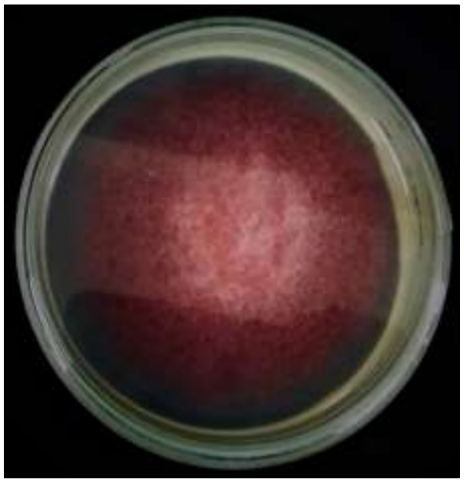
Plate 8. Colony characteristics *Fusarium* spp. isolates grown on PDA Media

Plate 8. Continued

BGT

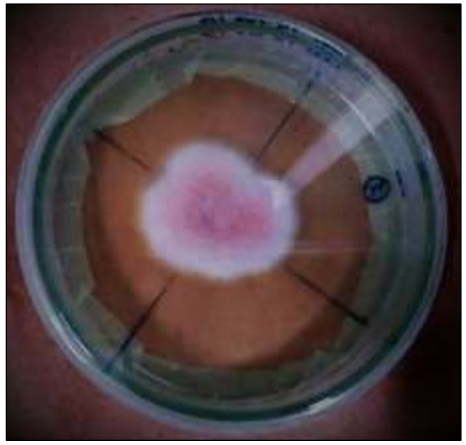
BGP

FAB



JaJhB

MeGP



JhKT

PBC

4.3.2. Radial mycelial growth of isolates of *Fusarium* spp.

Radial mycelial growth of fifteen isolates of *Fusarium* spp. varied significantly in PDA media (Table 4).

After three days of inoculation, the maximum increase of colony diameter (27.90 mm) was recorded in isolate DSAUT and it was statistically similar to isolate DSAUB (27.83mm). The mycelial growth of isolate MMsB (27.13 mm) was also statistically similar to isolate DSAUB. The minimum increment of colony diameter was found in case of isolate JhKT (8.33 mm).

After four days of inoculation, the highest increase of colony diameter was measured in isolate MeGP (38.77 mm). The second highest mycelial growth was observed in isolates MMsB (37.23 mm) which were statistically similar to isolate DSAUB (37.17mm) as well as isolate DSAUT (36.67 mm). The isolates JhKT showed the lowest (10.26 mm) mycelial growth.

The maximum increase of colony diameter was recorded in isolates DSAUB (48.17 mm) at five days after inoculation and the growth of isolate DSAUT (47.33 mm) was statistically similar to DSAUB isolates. The minimum increment of colony diameter was recorded in isolate JhKT (12.43 mm).

After six days of inoculation, the maximum radial growth of mycelium was observed in isolate DSAUB (58.00 mm) followed by DSAUT (57.50 mm) and isolate MMsB (56.16 mm). The radial mycelial growth of DSAUT was statistically similar to DSAUB and the radial mycelial growth of DSAUB was statistically similar to MMsB. The minimum increment of colony diameter was observed in isolate JhkT (14.33 mm) preceded by CChC (18.36mm).

At seven days after inoculation, the maximum colony diameter (67.50 mm) was observed in isolate DSAUB which was statistically similar to isolates DSAUT (66.83 mm) and MMsB (66.13 mm). The lowest colony diameter (16.20mm) was observed in case of JhKT.

After eight days of inoculation, the maximum colony diameter (78.33 mm) was found in isolates DSAUB which was found statistically non-significant to isolate DSAUT (77.33 mm) followed by isolate MMsB (75.63 mm). The minimum increment of colony growth (18.17 mm) was observed in isolate JhKT.

After nine days of inoculation, it was found that the isolate DSAUB almost covered the petriplates and mycelial growth was the highest (89.73). The second highest colony diameter (87.66 mm) was observed in isolate DSAUT. The minimum mycelial growth was recorded in isolate JhKT as it covered only 20.33 mm of petriplates.

The maximum average mycelial growth/day was observed in isolate DSAUB (10.32 mm) followed by isolate DSAUT (9.96 mm), MMsB (9.68 mm), MeGP (9.05 mm), GBARIB (9.03 mm) and isolate PBC (8.89 mm). The lowest average colony growth/day (2.00 mm) was recorded in isolate JhKT, which differed statistically compared to any other isolates and preceded by isolate CChC (3.37 mm), CChB (4.08 mm), MMsC (5.23 mm), MMsP (7.60 mm), BGP (7.67 mm), FAB (7.96 mm) and isolate JaJhB (8.68 mm). (Table 4).

Based radial mycelial growth at nine days after inoculation the isolates DSAUT, DSAUB, GBARIB, MMsB, BGP, FAB, JaJhB and PBC were categorized as fast growing fungi. Three isolates viz. MMsP, MMsC and BGT were grouped as moderate growing and the isolates CChB, CChC and JhKT were grouped as slow growing isolates (Table 4 and Figure 2).

Table 4. Radial mycelial growth of *Fusarium* spp. isolates grown on PDA media

SL No.	Isolates	Radial mycelial growth (mm)								
		3DAI	4DAI	5DAI	6DAI	7DAI	8DAI	9DAI	Average growth/day	Category based on growth
01	DSAUT	**27.90 a**	36.67 b	47.33 ab	57.50 ab	**66.83 a**	77.33 ab	87.66 b	9.96 b	Fast growing
02	DSAUB	27.83 ab	37.17 b	**48.17 a**	**58.00 a**	67.50 a	**78.33 a**	**89.73 a**	**10.32 a**	Fast growing
03	GBARIB	25.77 cd	34.66 c	43.67 cd	52.57 cd	61.67 b	70. 76 c	79.93 d	9.03 de	Fast growing
04	MMsB	27.13 b	37.23 b	46.67 b	56.16 b	66.13 a	75.63 b	85.23 c	9.68 c	Fast growing
05	MMsP	24.33 f	31.33 f	38.60 f	45.73 f	53.53 e	61.53 g	69.96 i	7.60 h	Moderate growing
06	MMsC	20.36 h	25.67 g	30.70 g	36.23 g	41.70 f	46.93 h	51.67 j	5.23 i	Moderate growing
07	CChB	17.10 i	21.33 h	25.40 h	29.60 h	33.53 g	37.63 i	41.60 k	4.08 j	Slow growing
08	CChC	9.26 j	12.40 i	15.60 i	18.86 i	22.23 h	25.66 j	29.53 l	3.37 k	Slow growing
09	BGT	20.96 h	26.20 g	31.33 g	36.67 g	41.90 f	47.30 h	52.66 j	5.28 i	Moderate growing
10	BGP	25. 26 de	32.80 e	40.57 e	48.07 e	55.93 de	63.60 f	71.30 h	7.67 h	Fast growing
11	FAB	24.96 ef	32.93 de	40.96 e	49.10 e	57.00 d	64.96 ef	72.73 g	7.96 g	Fast growing
12	JaJhB	25.43 cde	33.97 cd	42.63 d	51.27 d	60.03 bc	68.86 d	77.57 e	8.68 f	Fast growing
13	MeGP	26.10 c	**38.77 a**	44.63 c	53.87 c	62.67 b	71.66 c	80.40 d	9.05 d	Fast growing
14	JhKT	**8.33k**	**10.26 j**	**12.43 j**	**14.33 j**	**16.20 i**	**18.17 k**	**20.33 m**	**2.00 l**	Slow growing
15	PBC	22.33 g	31.23 f	40.07 e	48.93 e	57.80 cd	66.73 e	75.67 f	8.89e	Fast growing
hsd (.05)		0.749	1.06	1.414	1.602	2.680	1.83	1.294	.153	—
CV(%)		1.12	1.20	1.29	1.22	1.75	1.04	.65	.70	—

From the result on mycelial growth, it can be said the that the isolates studied in the present experiment showed some sort of difference in their growth rate. Average per day growth rate varied from 2.00 to 10.32 mm. Thus, there is a significant variation among all the fifteen isolates in terms of mycelial growth. Nirmaladevi1 and Srinivas (2012) recorded mycelial growth of 119 isolates of *Fusarium oxysporum* f.sp. *lycopersici* and found colony diameter was ranged from 14.56 mm to 87.97 mm after nine days of inoculation which supported the findings of the present study. Kulkarni (2006) also found variability among *Fusarium oxysporum* f. sp. *gladioli* isolates that supported the results of the present study.

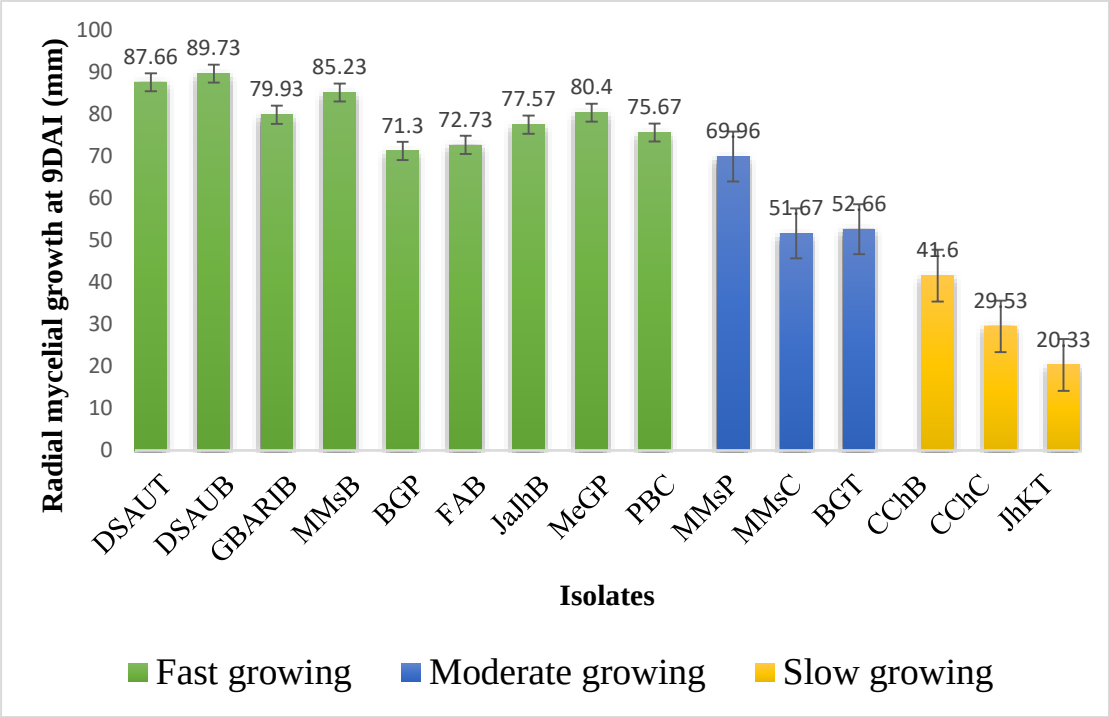


Figure 2. Grouping of *Fusarium* spp. isolates based on colony diameter

4.3.3. Sporulation rate and sporulation time of the isolates of *Fusarium* spp.

4.3.3.1. Rate of sporulation

Variation in sporulation rate of different isolates of *Fusarium* spp. was recorded and it was varied from 2.66×10^6 /ml – 12.33×10^6 /ml spore suspension (Table 5).

The highest sporulation of ml^{-1} suspension was recorded in isolates DSAUB (12.33×10^6 /ml) which was statistically similar to isolate DSAUT (12.00×10^6 /ml**)**. This was followed by isolate MMsB (**(**10.67×10^6 /ml**)**, GBARIB (9.67×10^6 /ml), MeGP (9.33×10^6 /ml**)**, PBC (8.66×10^6 /ml**)**, JaJhB (8.33×10^6 /ml), FAB (8.33×10^6 /ml**)** and isolate BGP (**(**7.33×10^6 /ml**)**. The lowest sporulation rate was recorded in isolate JhKT (2.66×10^6 /ml) which was statically similar to isolate CChC (3.33×10^6 /ml). The sporulation rate of CChC was followed by the isolate CChB (3.67×10^6 /ml), MMsC (5.00×10^6 /ml), BGT (5.33×10^6 /ml) and isolate MMsP (6.00×10^6 /ml). The present findings on sporulation rate were supported by Chopada *et al.* (2014) who found that the sporulation rate was varied from $2.77 \times 10^6 - 21.68 \times 10^6$ spores/ml spore suspensions in *Fusarium oxysporum* isolates from wilted tomato plant in India. The present findings were also supported by Ahmad (2010).

4.3.3.2. Time required for sporulation

The maximum sporulation time (7.33 days) was recorded in case of isolate JhKT which was statistically similar to isolate BGT (6.67 days) followed by isolates MMsC (6.66 days) and CChC (6.66 days). The minimum time required for sporulation in isolate DSAUT (3.67 days) which was statistically indifferent to isolates DSAUB (3.33 days) followed by isolate MMsB (4.00 days) (Table 5). This finding are in agreement with Patra and Biswas (2016). They also found variation of sporulation time among different isolate of *Fusarium* spp.

Table 5. Sporulation rate and time required for sporulation of *Fusarium* spp. isolates

SL no.	Isolates	No. of spore/ml suspension ×(10^6)	Sporulation time (days)
01	DSAUT	12.00 ab	**3.67 e**
02	DSAUB	**12.33 a**	**3.33 e**
03	GBARIB	9.67 bcd	4.67cde
04	MMsB	10.67 bc	4.00 de
05	MMsP	6.00 efgh	6.33 ab
06	MMsC	5.00 ghij	6.66 ab
07	CChB	3.67 hij	6.66 ab
08	CChC	3.33 ij	7.33a
09	BGT	5.33 fghi	6.67 ab
10	BGP	7.33 defg	6.00 abc
11	FAB	7.67 def	5.66 bc
12	JaJhB	8.33 cde	5.67 bc
13	MeGP	9.33 cd	4.67 cde
14	JhKT	**2.66 j**	**7.33 a**
15	PBC	8.66 cd	5.33 bcd
hsd (.05)		2.46	1.617
CV (%)		10.84	9.60

4.3.4. Correlation and Regression study between mycelial growth and mycelial growth and sporulation rate

Correlation and Regression between mycelial growth and mycelial growth and sporulation rate was studied. The regression line suggested that, there is a positive relationship between radial mycelial growth and number of spore/ml suspension. It means, spore production was increased with the increase of mycelial growth rate. The regression equation was Y = 1.161x - 0.8892 where x denotes average mycelial growth and Y denotes no. of spore/ml suspension. The value of correlation co-efficient R^2 was 0.8974, which was statistically significant (Figure 3).

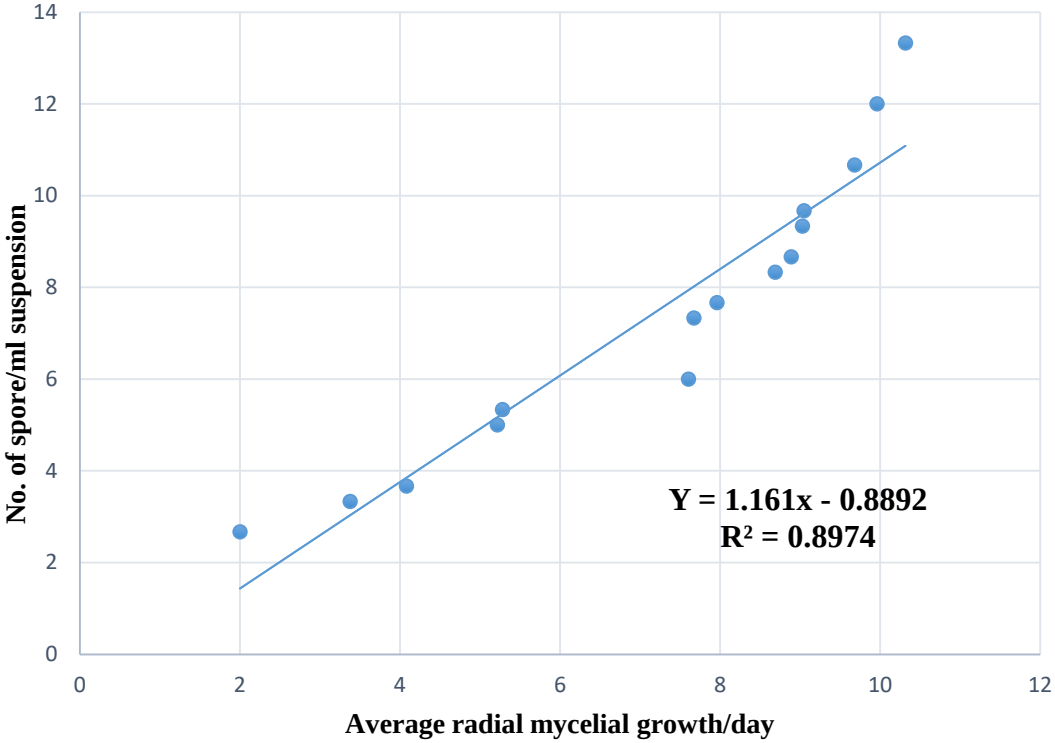


Figure 3. Relationship between average mycelial growth/day and No. of spore/ ml suspension

4.3.5. Correlation and Regression study between mycelial growth and mycelial growth and sporulation time

The regression line suggested that there is a negative relationship between radial mycelial growth and sporulation time that means, the isolate with higher mycelial growth rate required less time for spore production. The regression equation was Y = -0.4437x + 8.8853 where x denotes average mycelial growth and Y denotes sporulation time. Co-relation co-efficient R^2 was 0.767, which was statistically significant. (Figure 4).

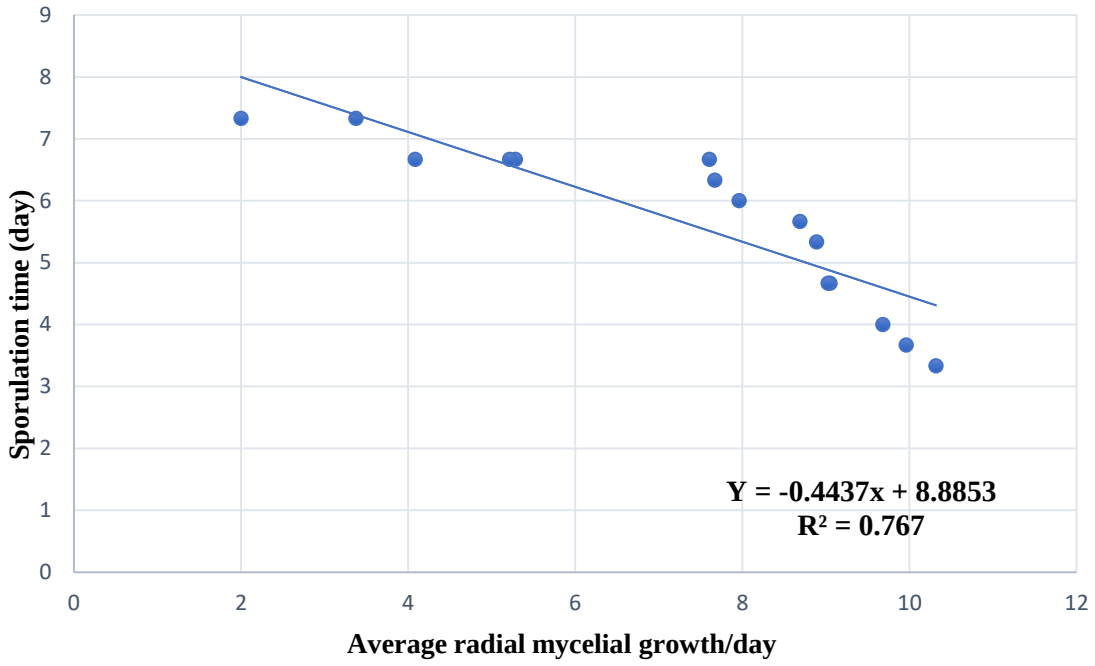


Figure 4. Relationship between average mycelial growth/day and sporulation time (day)

The findings of the correlation and regression study were supported by Mohsin (2013). He also found positive relationship between mycelial growth rate and sporulation rate and negative relationship between mycelial growth rate and sporulation time in case of different *Alternaria porri* isolates.

4.4 Molecular identification and characterization

The isolates of *Fusarium* spp. from different locations of Bangladesh were molecularly identified through comparing the nucleotide sequences of the queried isolates against "NCBI-BLAST" database. Phylogenetic tree was also constructed to reveal the ancestral relationship among the identical isolates.

Nine isolates viz. DSAUT, GBARIB, MMsB, MMsC, BGT, BGP, JJHB, MeGP and PBC among fifteen were sent to molecular lab for DNA extraction and sequencing. The sequenced data received as ".ab1" file format that were converted into "FASTA" file format (Appendix IV) in order to search them in "NCBI-BLAST" database for comparison.

The results of molecular identification revealed that, though all those nine isolates were under the genus *Fusarium,* they did not belong to same species. Six isolates were identified as *Fusarium oxysporum* and three were reported as *Fusarium solani*, *F. commune* and *F. fujikuroi* respectively. The detail results of molecular identification in each isolates were discussed below.

4.4.1. Molecular identification and phylogenetic tree construction of isolate-DSAUT

The "NCBI-BLAST" database showed that, DSAUT isolates of *Fusarium* sp. isolated from wilted tomato root cultivated in Sher-Bangla Agricultural University (SAU) showed more than 90% nucleotide (nt) similarity with different *Fusarium oxysporum* isolates reported worldwide. (Table 6).

DSAUT isolate of *Fusarium* spp. shares 94.24% sequence identity with *Fusarium oxysporum* isolate JUF0016 (accession no. MH368097.1). This related fungal species was isolated from Jahangir Nagar University, Bangladesh, that was responsible for leaf infection of Aloe Vera (GenBank [1]).

Another isolate *Fusarium oxysporum* strain GENF003 bearing accession no. KX196809.1 causing stalk rot disease of maize in South-East China (GenBank [2]) shares 95.55% sequence identity with our queried one.

Fusarium oxysporum isolate F20 (accession no. MF069180.1) associated with stem rot of *Medinilla myriantha* (semi-epiphytic flowering Plant) in Florida also shared 94.47% nucleotide identity with target isolate (GenBank[3]), where the query coverage was 87%.

Two strain of *Fusarium oxysporum* i.e. UACH-137 (accession no. KU056819.1) and UACH-227 (accession no. MG557859.1) isolated from wilted root of tomato plant in Mexico responsible for 50-80% yield loss (Isaac *et al.,* 2018), shared 93.98% and 94.73% identical sequence respectively with the target isolates i.e. isolate DSAUT. Query coverage was 96% in case of each above mentioned isolates.

Nucleotide sequence of *Fusarium oxysporum* isolate NJ3256 with accession no. MK120427.1 was 94.63% resembled with the queried (DSAUT) isolate and this related fungal strain was isolated from sesame (*Sesamum indicum*) root having wilt symptoms in South Korea (GenBank [4])

The above table also showed that, three another isolates of *Fusarium oxysporum* viz. F84-Kr1t6 (accession no. KC304812.1), F4-TK4 (accession no. KC304799.1) and MB1C (accession no. KC282839.1) which were associated to citrus wilt in Tunisia found to be 94.31%, 93.89% and 94.12% identical to the queried isolate respectively in relation to nucleotide sequence. The lower expected value (E-value) and higher bit-score of the related sequence also denotes the higher similarity among the sequences (Table 6).

Table 6. Percentage of nucleotide identities of DSAUT isolate with selected *Fusarium* fungi reported worldwide

Worldwide reported relevant *Fusarium* spp. isolates	Accession No.	Max Score	Total Score	Query Coverage (%)	E-Value	Identity (%)
Fusarium oxysporum isolate JUF0016	MH368097.1	797	797	96%	0.00	94.24%
Fusarium oxysporum strain GENF003	KX196809.1	797	797	96%	0.00	94.55%
Fusarium oxysporum isolate UACH-137	KU056819.1	780	780	96%	0.00	93.98%
Fusarium oxysporum strain UACH-227.	MG557859.1	773	773	96%	0.00	94.73%
Fusarium oxysporum isolate F84-Kr1t6	KC304812.1	730	730	88%	0.00	94.31%
Fusarium oxysporum strain 170231	MF069180.1	728	728	87%	0.00	94.47%
Fusarium oxysporum isolate NJ3256.	MK120427.1	726	726	87%	0.00	94.63%
Fusarium oxysporum isolate F20	MF993097.1	726	726	87%	0.00	94.46%
Fusarium oxysporum isolate MB1C	KC282839.1	725	725	88%	0.00	94.12%
Fusarium oxysporum isolate F4-TK4	KC304799.1	715	715	88%	0.00	93.89%

Phylogenetic tree of DSAUT

The phylogenetic tree constructed by creating alignment with the complete sequence of related isolates revealed the ancestral relationship between *Fusarium* sp. isolate-DSAUT and *Fusarium oxysporum* isolate MB1C (accession no. KC282839.1) as they were in same clade of the tree (Figure 5).

This isolate MB1C was the first reported pathogen in Tunisia causing wilt of matured (10-15 years old) citrus tree (Hannachi, *et al.*,2014). The boot strap values of this clade is 92 which denotes the 92% accuracy of this result.

The phylogeny also revealed that, both the isolate DSAUT and *F. oxysporum* isolate MB1C shares a common ancestor with *F. oxysporum* isolate UACH-137 (accession no. KU056819.1) which was a wilt pathogen of tomato field in Mexico. As well as all those three isolates were ancestrally related to another wilt pathogen in Mexico viz. *F. oxysporum* isolate UACH-227 (accession no. MG557859.1) because of creating a common clade in the phylogeny. All other relevant isolates in this phylogeny have created different clades, which means those isolates are distantly related to *Fusarium* sp. isolates-DSAUT (Figure 5).

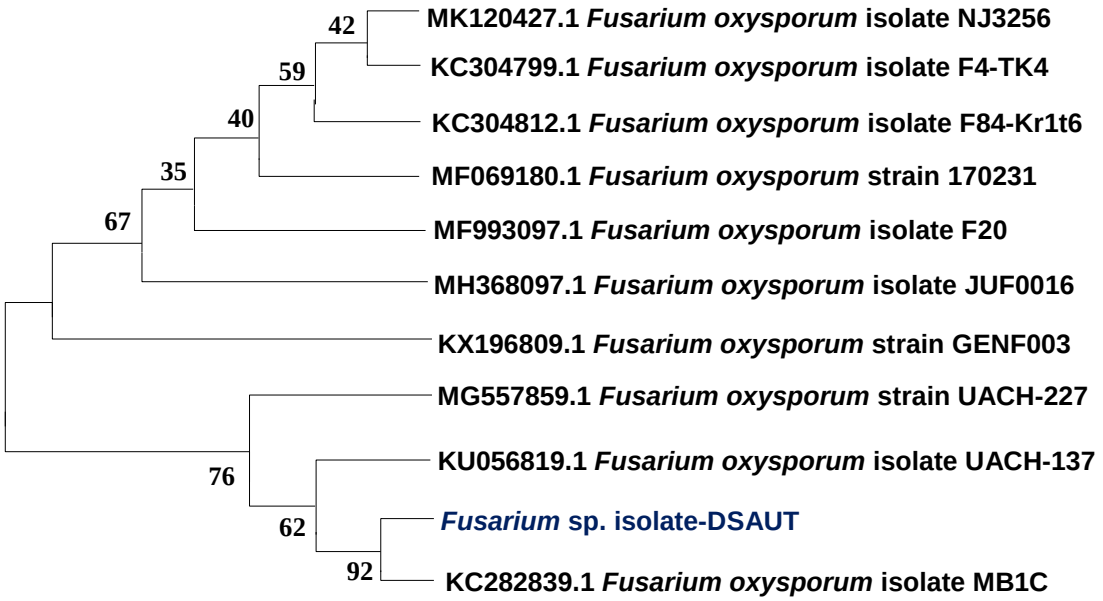


Figure 5. Neighbor joining (NJ) Phylogenetic tree of DSAUT isolate in relation to other *Fusarium* isolates generated with 1000 bootstrap replicates

4.4.2. Molecular identification and phylogenetic tree construction of isolate-GBARIB

Through comparing the sequence of *Fusarium* sp. isolate-GBARIB, isolated from a wilted brinjal collected from Bangladesh Agricultural Institute in "NCBI-BLAST" database, it was revealed that two species of *Fusarium* viz. *F. oxysporum* and *F. fujikuroi* (mainly perfect stage of *Fusarium oxysporum*) were found to be identical in relation to nucleotide sequence to our queried one (GBARIB isolate). It was also revealed that, at least three forma specialis (f. sp.)

of *Fusarium oxysporum* i.e. f. sp. *cubens*; f. sp. *lentis* & f.sp. *lycopersici* were identical to our target isolate(GBARIB).

The table (Table 7.) described below showed that, four strain of *Fusarium oxysporum* f. sp. *cubense* named as strain A51 (accession no. MF630984.1); strain A14 (accession no. MF540558.1); strain A13 (accession no. MF540559.1) and strain A39 (accession no. MF630975.1) had 90.77%, 90.77%, 90.37% & 90.35% sequence identity with the queried isolate respectively and those for isolates covered 93% query only. Those all four strain of *Fusarium oxysporum* was identified as causal agent of panama disease (banana wilt) of banana in Pakistan (GenBank[5,6,7&8]).

A wilt pathogen of isolated from a wilted tomato root in Raipur, Chattishgarh, India named as *Fusarium oxysporum* f. sp. *lycopersici* strain FWT67 bearing accession no. KC478636.1 (GenBank[9]) and our queried isolates i.e. *Fusarium* sp. isolate-GBARIB were 89.60% identical in relation to nucleotide sequences with 95% query coverage.

Three isolates of *Fusarium oxysporum* f. sp. *lentis* named as isolate FLSC26 (accession no. KY678295.1); isolate FLSC4 (accession no. KY678273.1) &; isolate FLSC18 (accession no. KY678287.1) shared 90.37%, 90.18% & 89.80% sequence identity with 93% query coverage. All this three identical pathogen was reported to cause wilt of lentil in India (GenBank[10,11&12])

On the contrary, two strain of *Fusarium fujikuroi* (synonym: *Gibberella fujikuroi*) designated as *F. fujikuroi* strain CBS 130402 (accession no. KU604034.1) *F. fujikuroi* strain CBS 221.76 (accession no. KR071666.1) share the maximum sequence identity and that was 94.64% in each cases with only 70% query coverage. The expected value (E-value) was quite higher also i.e. 5e-157 (Table 7).

Table 7. Percentage of nucleotide identities of GBARIB isolate with selected *Fusarium* fungi reported worldwide

Worldwide reported relevant *Fusarium* spp. isolates	Accession No.	Max Score	Total Score	Query Coverage (%)	E-Value	Identity (%)
Fusarium oxysporum f. sp. *cubense* strain A51	MF630984.1	647	647	93%	0.00	90.77%
Fusarium oxysporum f. sp. *cubense* strain A14	MF540558.1	647	647	93%	0.00	90.77%
Fusarium oxysporum f. sp. *cubense* strain A13	MF540559.1	638	638	93%	1e-178	90.37%
Fusarium oxysporum f. sp. *cubense* strain A39	MF630975.1	634	634	93%	1e-177	90.35%
Fusarium oxysporum f. sp. *lentis* isolate FLSC26	KY678295.1	632	632	93%	5e-177	90.37%
Fusarium oxysporum f. sp. *lycopersici* strain FWT67	KC478636.1	630	630	95%	2e-176	89.60%
Fusarium oxysporum f. sp. *lentis* isolate FLSC4	KY678273.1	627	627	93%	2e-175	90.18%
Fusarium oxysporum f. sp. *lentis* isolate FLSC18	KY678287.1	616	616	93%	5e-172	89.80%
Fusarium fujikuroi strain CBS 130402	KU604034.1	566	566	70%	5e-157	94.64%
Fusarium fujikuroi strain CBS 221.76	KR071666.1	566	566	70%	5e-157	94.64%

Phylogenetic tree of GBARIB

The phylogenic analysis based on other identical sequence (Figure 6.) showed that, a common clade was formed by the isolate GBARIB and *Fusarium fujikuroi* strain CBS 221.76 (accession no. KR071666.1), which indicate that, those two isolates are closely related to each other and share a common ancestor though the accuracy of the result is 72% (based on bootstrap values). This two isolates

are also sharing a common ancestor with another *Fusarium fujikuroi* strain CBS 130402 bearing accession no. KU604034.1 (Figure 6).

Though *Fusarium fujikuroi*, is known to occur bakanae disease of rice worldwide, especially in Asian region, it has been also reported as a causal agent of fungal wilt of different vegetable and pulse crops. Choi *et al.* (2019) isolated and identified three isolates of *Fusarium fujikuroi* from three different soybean growing region of South Korea which was responsible for Fusarium wilt on *Glycine max* (soybean). All those three isolates were submitted to NCBI-Gene Bank with accession no. MH577990.1; MH577991.1 and MH577995.1.

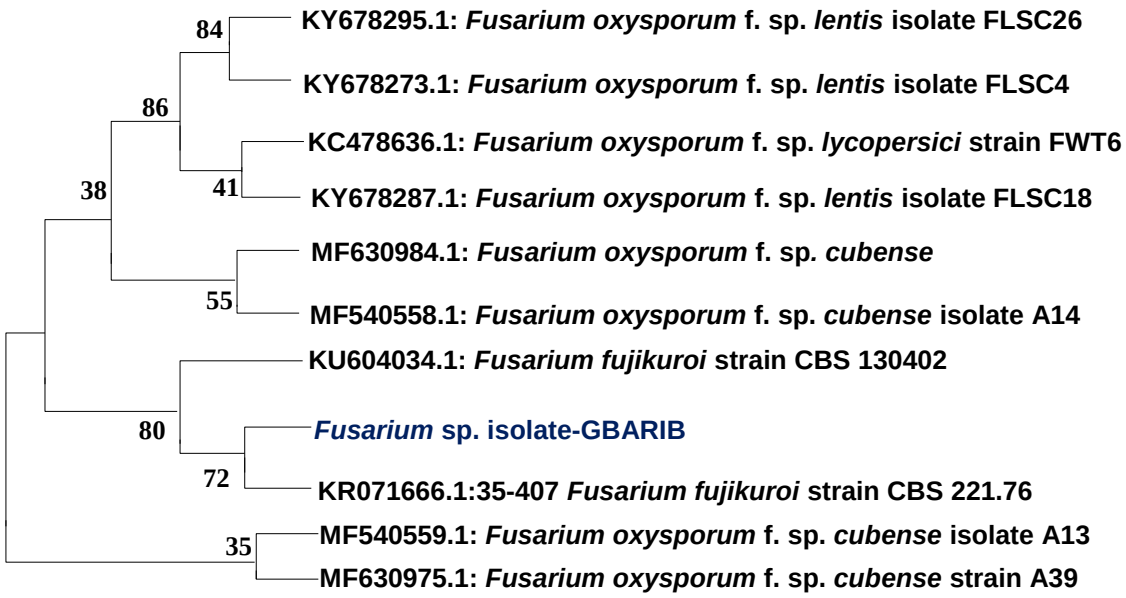


Figure 6. Neighbor joining (NJ) Phylogenetic tree of GBARIB isolate in relation to other *Fusarium* isolates generated with 1000 bootstrap replicates

4.4.3. Molecular identification and phylogenetic tree construction of isolate-MMsB

Through comparing the nucleotide sequence of *Fusarium* sp. isolate-MMsB against "NCBI-BLAST" database, highly similar sequences to our queried one were identified. Almost all the isolates obtained from BLAST search showed

higher sequence identity i.e. 97-100% and the details of the ten most relevant isolates are presented (Table 8).

Fusarium oxysporum strain VL203 with accession no. JF440593.1 was the most identical sequence having 100% sequence identity which also covered 99% query. This strain was isolated from stem and stumps of mountain pine (*pinus mugo*) grown in Baltic sea region mainly in Lithuania (Lygis and Vasiliauskaite, 2014).

Most of the selected isolates (six isolates out ten) were highly identical to the queried isolate and showed 99.81% similar sequence and 100% query coverage to MMsB. Those were *Fusarium oxysporum* strain GFR32 (accession no. MT447537.1), *F. oxysporum* isolate FUS-33 (accession no. MH879861.1), *F. oxysporum* isolate AFIC12 (accession no. KU872818.1*)*, *F. oxysporum* strain EECC-643(accession no. KP942940.1), *Fusarium* sp. HJAG7. (accession no. KM005084.1) and *F. oxysporum* strain F-H.6.5-030318-02 (accession no. EU364842.1).

Among them the isolate with accession no. EU364842.1 is reported to cause fungal wilt of cucumber, melon, water melon and bottle gourd in China(GenBank[13]) On the other hand, *F. oxysporum* isolate FUS-33 (accession no. MH879861.1) is responsible for Fusarium wilt of *Citrus reticula* (Mandarin orange) in Pakistan (GenBank[14])

Another three isolates of *Fusarium* spp. i.e. *Fusarium oxysporum* isolate Fo07 (accession no MK571185.1), *Fusarium oxysporum* f. sp. *lentis* isolate AP_FOL (accession no. MK452341.1) and *Fusarium oxysporum* strain G405 (accession no. KR094464.1) showed also 99.81% sequence with the isolate MMsB but in that case the query coverage was 99%.

The above table also showed that, the expected value (E-value) was found as 0.00 in case of isolates which is the indication of a better similarity of all isolates (Table 8).

Table 8. Percentage of nucleotide identities of MMsB isolate with selected *Fusarium* fungi reported worldwide

Worldwide reported relevant *Fusarium* spp. isolates	Accession No.	Max Score	Total Score	Query Coverage (%)	E-Value	Identity (%)
Fusarium oxysporum strain VL203	JF440593.1	970	970	99%	0.00	100%
Fusarium oxysporum strain GFR32	MT447537.1	968	998	100%	0.00	99.81%
Fusarium oxysporum isolate FUS-33	MH879861.1	968	998	100%	0.00	99.81%
Fusarium oxysporum isolate AFIC12	KU872818.1	968	998	100%	0.00	99.81%
Fusarium oxysporum strain EECC-643	KP942940.1	968	998	100%	0.00	99.81%
Fusarium sp. HJAG7.	KM005084.1	968	998	100%	0.00	99.81%
Fusarium oxysporum strain F-H.6.5-030318-02	EU364842.1	968	998	100%	0.00	99.81%
Fusarium oxysporum isolate Fo07	MK571185.1	966	966	99%	0.00	99.81%
Fusarium oxysporum f. sp. *lentis* isolate APFOL	MK452341.1	966	966	99%	0.00	99.81%
Fusarium oxysporum strain G405	KR094464.1	966	966	99%	0.00	99.81%

Phylogenetic tree of MMsB

Phylogenetic tree constructed with all selected isolate suggested that, there is an ancestral relationship between *Fusarium sp.* isolate-MMsB and *Fusarium oxysporum* f. sp. *lentis* isolate AP_FOL (accession no. MK452341.1) because in the tree they are in same clade. Bootstrap values of this clade also suggest that, the accuracy of this clade is 98%.

The pathogen ancestrally related to the target pathogen is mainly a forma specialis (f. sp.) of *Fusarium oxysporum* which was isolated from the root of a wilted lentil plant (*Lens culinaris*) plant in Uttarakhand, India (GenBank[15]). On the other hand, the fungi bearing accession no. JF440593.1 has a common

ancestor with both of previously mentioned pathogen and the queried isolate and the accuracy of this portion of tree is 85% (Figure 7).

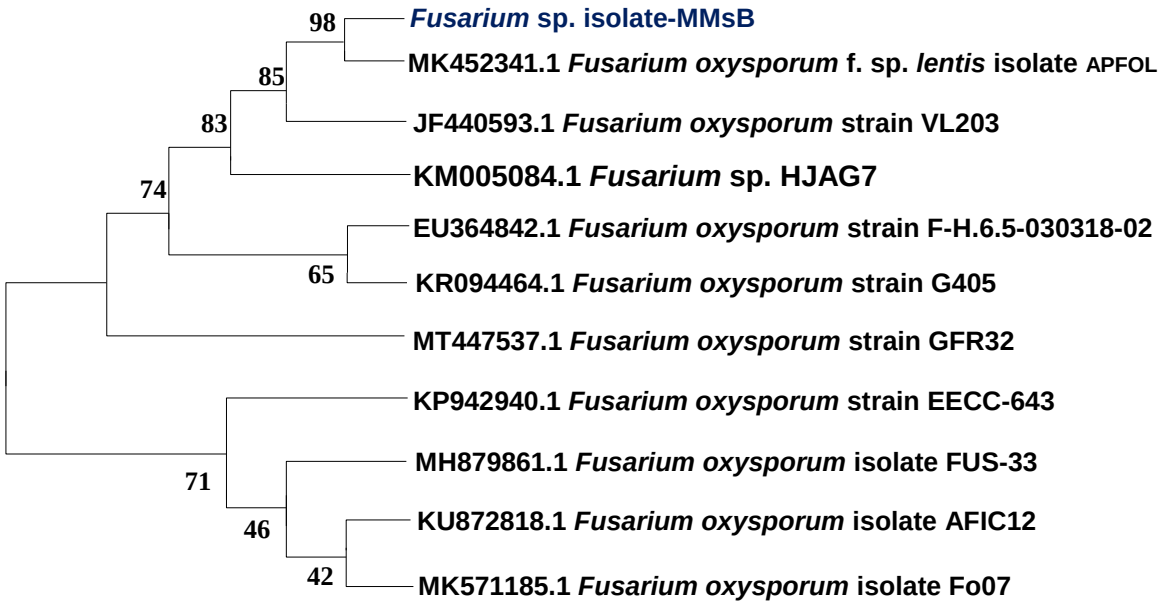


Figure 7. Neighbor joining (NJ) Phylogenetic tree of MMsB isolate in relation to other *Fusarium* isolates generated with 1000 bootstrap replicates

4.4.4. Molecular identification and phylogenetic tree construction of isolate-MMsC

MMsC isolates of *Fusarium* sp. which was isolated from wilted root chili showed higher sequence similarity with other Fusarium isolates reported worldwide after completion of the sequence comparison in "NCBI-BLAST" database.

Uncultured *Fusarium* sp. clone C6406 (accession no. KF718226.1) & C1106 (accession no. KF718222.1) which was isolated from the decayed rhizome of *Alpinia officinarum* in China (Shubin *et al.*, 2014) shares 99.81% sequence identity and 100% query coverage with our target isolate where the E-value was 0.00. Another isolate named as *Fusarium oxysporum* isolate FPV-32. (accession no. HG423346.1) showed also the same result.

Though three clone of *Fusarium oxysporum* viz. clone SF_789 (accession no. MT530065.1); clone SF_269 (accession no. MT529545.1) and clone SF_993

(accession no. MT530269.1) as well as *Fusarium oxysporum* strain VL203 with accession no. JF440593.1 had also 99.81% identical sequence, this time query coverage was 99%.

99.62% identical sequence with targeted once was overserved in case of *Fusarium oxysporum* isolate FCO3 (accession no. LR535799.1); *Fusarium oxysporum* f. sp. *vanillae* strain HJAG2 (accession no. KM005080.1) and *Fusarium oxysporum* isolate FOFB62 (accession no. HQ651161.1) and in each case the query coverage was 99%. (Table 9.)

The isolates bearing accession KM005080.1 was associated with stem and root rot of Vanilla (*Vanilla plantifilia)* in Mexico (Adame-García *et al.,* 2015)

Table 9. Percentage of nucleotide identities of MMsC isolate with selected *Fusarium* fungi reported worldwide

Worldwide reported relevant *Fusarium* spp. isolates	Accession no.	Max Score	Total Score	Query Coverage (%)	E-Value	Identity (%)
Uncultured *Fusarium* sp. clone C6406	KF718226.1	961	961	100%	0.00	99.81%
Uncultured *Fusarium* sp. clone C1106	KF718222.1	961	961	100%	0.00	99.81%
Fusarium oxysporum isolate FPV-32.	HG423346.1	961	961	100%	0.00	99.81%
Fusarium oxysporum clone SF_789	MT530065.1	957	957	99%	0.00	99.81%
Fusarium oxysporum clone SF_269	MT529545.1	957	957	99%	0.00	99.81%
Fusarium oxysporum strain VL203	JF440593.1	957	957	99%	0.00	99.81%
Fusarium oxysporum clone SF_993	MT530269.1	957	957	99%	0.00	99.81%
Fusarium oxysporum isolate FCO3.	LR535799.1	953	953	99%	0.00	99.62%
Fusarium oxysporum f. sp. *vanillae* strain HJAG2	KM005080.1	953	953	99%	0.00	99.62%
Fusarium oxysporum isolate FOFB62	HQ651161.1	953	953	99%	0.00	99.62%

Phylogenetic tree of MMsC

The phylogenetic tree built on the basis of some identical isolates of *Fusarium* sp. showed that, there is an ancestral relationship between the target isolate and other selected isolates. It can be predicted that, both the queried isolate and *Fusarium oxysporum* isolate FPV-32 (accession no. HG423346.1) were sharing a common ancestor with 100% bootstrap values indicating higher accuracy of the result. Curtis *et al.* (2014) described those isolate of *F. oxysporum* as a causal agent of root and crown rot in Southern Italy responsible for upto 100% yield loss of Chickpea (Figure 8).

The tree also revealed that, there was also a common ancestor between this two isolates and *Fusarium oxysporum* strain VL203 (accession no. JF440593.1) was isolated from stem and stumps of *Pinus mugo* in Lithuania (Lygis and Vasiliauskaite, 2014).

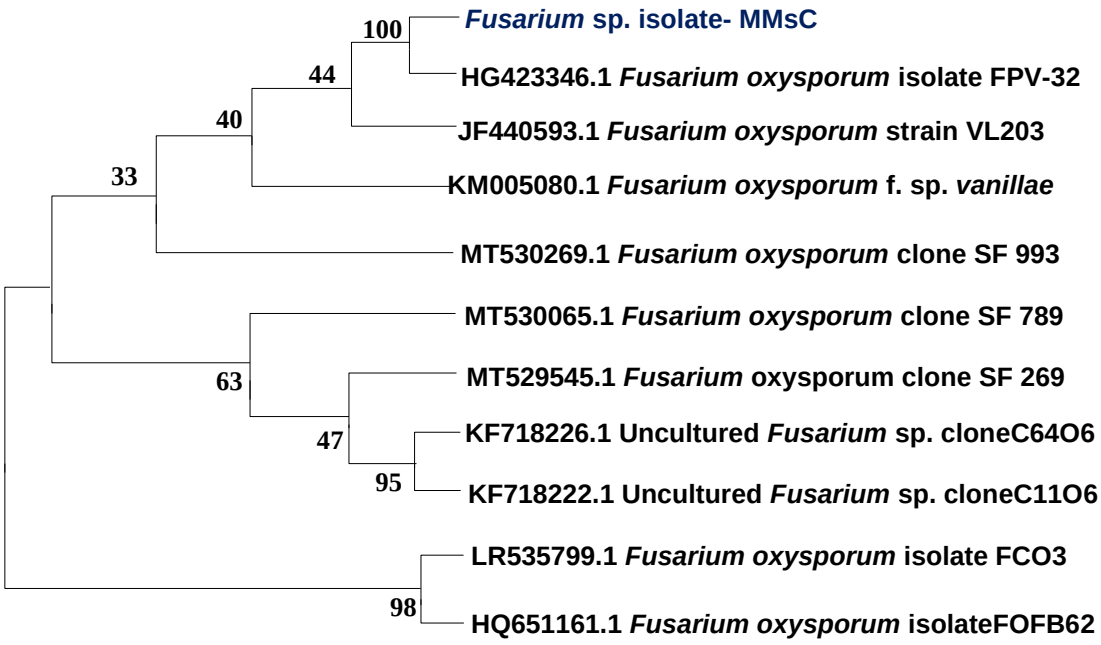


Figure 8. Neighbor joining (NJ) Phylogenetic tree of MMsC isolate in relation to other *Fusarium* isolates generated with 1000 bootstrap replicates

4.4.5. Molecular identification and phylogenetic tree construction of isolate-BGT

Almost all isolates identified as similar to the target sequence (BGT) after searching in the "NCBI-BLAST" database showed very higher nucleotide identity. Ten most identical sequences were presented below (Table 10) The zero expected values (E-Value) in case of all isolates was the indication of higher sequence similarity.

Wood tissue declining pathogen i.e. *Fusarium oxysporum* isolate MH139Trs with accession no. MK817045.1 of young (2-3 years old) grape vine in Turkey, (Akgul and Ahioglu, 2019) showed 99.80% sequence identity and 100% query coverage with our target isolates. Those isolates of *Fusarium* was able to produce 2.2 ± 0.7 mm lesion in wood tissue of grapevine.

Four clone of *Fusarium oxysporum* designated as clone SF_993 (accession no. MT530269.1); clone SF_907 (accession no. MT530183.1); clone SF_416 (accession no.MT529692.1) and clone SF_284 (accession no. MT529560.1) had shared 99.61% sequence identity with our queried isolate where the query coverage was 100%.

Fusarium oxysporum isolate FT (accession no. MT020427.1) isolated from rhizosphere soil of tomato in India (GenBank[16]) had also 99.61% sequence identity and 99% query coverage with our target sequence and same results were obtained in case of *Fusarium oxysporum* strain UACH-217 (accession no. MG557869.1); *Fusarium oxysporum* strain LS1677 (accession no. KT896661.1) as well as *Fusarium* sp. KR4 (accession no. KC623568.1)

Most interestingly, *Fusarium* sp. isolate-BGT had also shared 99.61% sequence identity with *Humicola* sp. strain A791 bearing accession no. MN121400.1. collected from decayed root of *Callistemon viminalis* (weeping bottlebrush) in China (GenBank[17]) (Table 10).

Humicola sp. is a fungal species, in perfect/sexual stage which is under the subdivision Ascomycotina and family Chaetomiaceae (Global catalogue of microorganism). On the other hand, in case of imperfect stage/asexual stage this fungus is under the subdivision Deuteromycotina and family Dematiaceae (Bertoldi *et al.,* 1972).

Table 10. Percentage of nucleotide identities of BGT isolate with selected *Fusarium* fungi reported worldwide

Worldwide reported relevant *Fusarium* spp. isolates	Accession No.	Max Score	Total Score	Query Coverage (%)	E-Value	Identity (%)
Fusarium oxysporum isolate MH139Trs	MK817045.1	931	931	100%	0.00	99.80%
Fusarium oxysporum clone SF_993	MT530269.1	926	926	100%	0.00	99.61%
Fusarium oxysporum clone SF_907	MT530183.1	926	926	100%	0.00	99.61%
Fusarium oxysporum clone SF_416	MT529692.1	926	926	100%	0.00	99.61%
Fusarium oxysporum clone SF_284	MT529560.1	926	926	100%	0.00	99.61%
Fusarium oxysporum isolate FT	MT020427.1	926	926	99%	0.00	99.61%
Humicola sp. strain A791	MN121400.1	926	926	99%	0.00	99.61%
Fusarium oxysporum strain UACH-217	MG557869.1	926	926	99%	0.00	99.61%
Fusarium oxysporum strain LS1677	KT896661.1	926	926	99%	0.00	99.61%
Fusarium sp. KR4	KC623568.1	926	926	99%	0.00	99.61%

Phylogenetic tree of BGT

On the basis of those ten identical isolates phylogenetic tree was constructed to show the ancestral relationship among them. The result revealed that, our queried isolates i.e. *Fusarium* sp. isolates-BGT and *Fusarium oxysporum* strain UACH-217 (accession no. MG557869.1) were derived from a common ancestor and the accuracy of this portion of tree was 95%. This isolate of *Fusarium* named as

UACH-217 was reported to cause wilt of tomato in Mexico with upto 85% disease incidence (Isaac *et al.*, 2018)

The isolate named as *Fusarium oxysporum* isolate MH139Trs was also has also an ancestral relationship with both the two isolates mentioned above. The phylogeny also suggests that, *Fusarium* sp. KR4 (accession no. KC623568.1) isolated from *Clerodendrum inerme* (GenBank[18]) formed a separate clade means this isolate is distantly related to all other isolate (Figure 9).

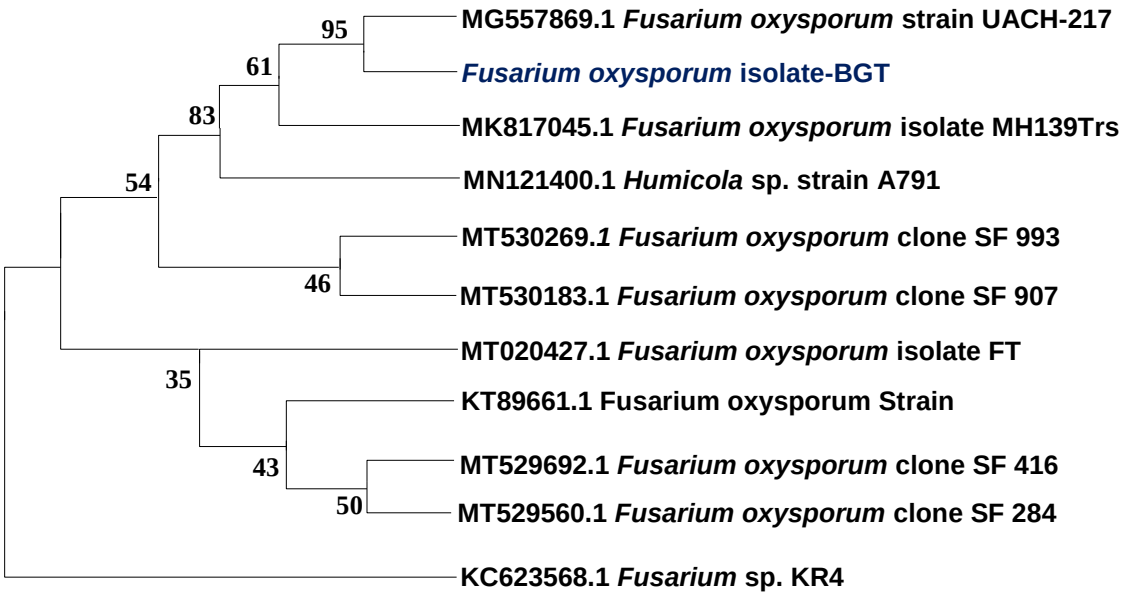


Figure 9. Neighbor joining (NJ) Phylogenetic tree of BGT isolate in relation to other *Fusarium* isolates generated with 1000 bootstrap replicate

4.4.6. Molecular identification and phylogenetic tree construction of isolate-BGP

In case of isolate BGP, almost all isolates similar to the target sequence shared higher sequence identity after searching in the "NCBI-BLAST" database and in all cases it above 99% as well as the expected value (E-value) was zero denotes higher similarity among them.

Fusarium oxysporum PKNU1022 (accession no. LC428050.1) showed the highest sequence similarity with the queried sequence i.e. 99.86% sequence identity and 100% query coverage.

Fusarium oxysporum clone SF_35 with accession no. MT529311.1 and *Fusarium* sp. isolate P1754 (accession no. KT269026.1) showed the second highest sequence identity i.e. 99.81% with the isolate- BGP, in that case the query coverage was 100%.

Fusarium oxysporum clone SF_266 (accession no. MT529542.1); *Fusarium oxysporum* isolate FUS-29-1(accession no. MH879586.1); *Fusarium oxysporum* f. sp. *lentis* isolate AP_FOL (accession no. MK452341.1) and *Fusarium oxysporum* strain UFSMQ12 (accession no. KX496876.1) also shared 99.63% identical sequence to the queried one with 100% query coverage.

Fusarium oxysporum strain A19 (accession no. KT898585.1) shared 99.62% sequence identity with the isolate-BGP and in that query coverage was 99%. Wherever, 99.44% identical nucleotide was found in case two isolates designated as *Fusarium oxysporum* strain G405 (accession no. KR094464.1) and *Fusarium oxysporum* isolate AFIC4 (accession no. KU872815.1) (Table 11).

Table 11. Percentage of nucleotide identities of BGP isolate with selected *Fusarium* fungi reported worldwide

Worldwide reported relevant *Fusarium* spp. isolates	Accession No.	Max Score	Total Score	Query Coverage (%)	E-Value	Identity (%)
Fusarium oxysporum clone SF_35	MT529311.1	983	983	100%	0.00	99.81%
Fusarium oxysporum clone SF_266	MT529542.1	979	979	100%	0.00	99.63%
Fusarium oxysporum isolate FUS-29-1	MH879586.1	979	979	100%	0.00	99.63%
Fusarium oxysporum f. sp. *lentis* isolate AP_FOL	MK452341.1	979	979	100%	0.00	99.63%
Fusarium sp. isolate P1754	KT269026.1	979	979	99%	0.00	99.81%
Fusarium oxysporum strain UFSMQ12	KX496876.1	977	977	100%	0.00	99.63%
Fusarium oxysporum strain G405	KR094464.1	976	976	100%	0.00	99.44%
Fusarium oxysporum PKNU1022	LC428050.1	972	972	100%	0.00	99.86%
Fusarium oxysporum isolate AFIC4	KU872815.1	972	972	100%	0.00	99.44%
Fusarium oxysporum strain A19	KT898585.1	972	972	99%	0.00	99.62%

Phylogenetic tree of BGP

The phylogenetic tree obtained from alignment of the related sequence showed that, the isolate-BGP and *Fusarium oxysporum* PKNU1022 (accession no. LC428050.1) were in the same clade, denote that both of them are sharing a common ancestor and bootstrap value suggests that, the accuracy of this clade is 93%. This related isolate was reported as damping off pathogen of Larch (*Larix kaempferi*) in Korea (GenBank[19]).

This two *Fusarium* isolates have also formed a clade with the isolates *Fusarium oxysporum* strain A19 (accession no. KT898585.1) which was isolated from

ancient parchment deposited in the archive of University of Coimbra, Portugal (GeneBank[20]) (Figure 10).

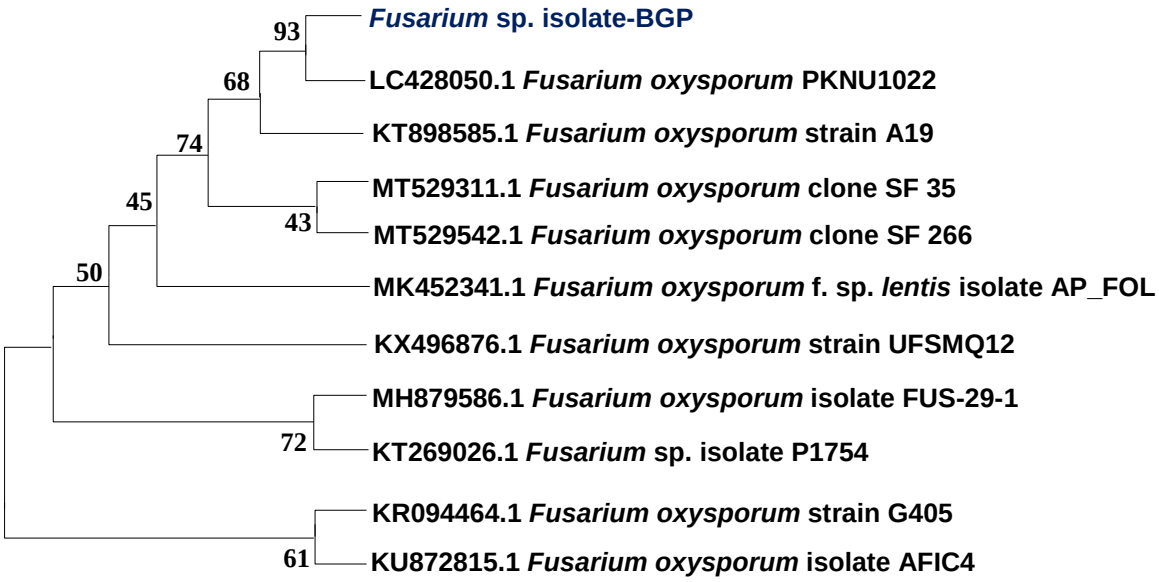


Figure 10. Neighbor joining (NJ) Phylogenetic tree of BGP isolate in relation to other *Fusarium* isolates generated with 1000 bootstrap replicates

4.4.7. Molecular identification and phylogenetic tree construction of isolate-JJhB

After completion of the nucleotide sequence of JJhB isolates in "NCBI-BLAST" database, it was revealed that the *Fusarium oxysporum* strain GENF003 (accession no. KX196809.1) known as stalk rot pathogen of maize in south-east China (GenBank[2]) was 95.28% identical to the nucleotide sequence of JJhB with 100% query coverage.

Fusarium oxysporum isolate cucumeri (accession no. MG670446.1) reported to cause wilt of cucumber in Iran (GenBank[21]) was 95.27% similar to the queried sequence in respect to sequence identity with 99% query coverage.

Fusarium oxysporum strain MIAE01291 (accession no. KC787032.1) and *Fusarium oxysporum* strain UACH-217 (accession no. MG557869.1) shared 95.39% and 95.54% % sequence similarity with the JJhB isolate, where the query coverage was 98%.

94.89% sequence identity was found in case of *Fusarium oxysporum* isolate L3 (accession no. MG670445.1) was responsible for causing severe wilt of tomato in Iran (GenBank[22]) with 99% query coverage.

Fusarium sp. YXN18. (accession no. KC139479.1) isolated from decayed tobacco leaf (GenBank[23]); *Fusarium oxysporum* f.sp. *gladioli* isolate FOG Pune (accession no. KU721005.1) reported to cause wilt of gladiolus in India (Genbank[24]) and *Fusarium oxysporum* strain UACH-221 (accession no. MG557869.1) shared 95.22%, 95.00% and 95.54% identical sequence with JJhB isolate.

Fusarium oxysporum isolate AM1(accession no. MN560102.1) isolated from *Solanum tuberosum* in Russia (Genbank[25]) and *Fusarium oxysporum* f. sp. *cubense* isolate BMAE87Foc shared 94.50% and 94.74% similar sequence respectively where they covered 95% query. The Expected value (E-value) was zero in all cases indicated the good alignment of the sequence (Table 12).

Table 12. Percentage of nucleotide identities of JJhB isolate with selected *Fusarium* fungi reported worldwide

Worldwide reported relevant *Fusarium* spp. isolates	Accession No.	Max Score	Total Score	Query Coverage (%)	E-Value	Identity (%)
Fusarium oxysporum strain GENF003	KX196809.1	819	819	100%	0.00	95.28%
Fusarium oxysporum isolate cucumeri	MG670446.1	815	815	99%	0.00	95.27%
Fusarium oxysporum strain MIAE01291	KC787032.1	808	808	98%	0.00	95.39%
Fusarium oxysporum strain UACH-217	MG557869.1	804	804	98%	0.00	95.54%
Fusarium oxysporum isolate L3.	MG670445.1	804	804	99%	0.00	94.89%
Fusarium sp.YXN18.	KC139479.1	804	804	96%	0.00	95.22%
Fusarium oxysporum strain UACH-221.	MG557865.1	793	793	96%	0.00	95.32%
Fusarium oxysporum f.sp. *gladioli* isolate FOG Pune	KU721005.1	763	763	94%	0.00	95.00%
Fusarium oxysporum isolate AM1	MN560102.1	761	761	95%	0.00	94.50%
Fusarium oxysporum f. sp. *cubense* isolate BMAE87Foc	MN396267.1	761	761	95%	0.00	94.74%

Phylogenetic tree of JJhB

The phylogenetic tree was constructed after comparing the sequence in "NCBI-BLAST" database with a view to showing ancestral relationship with among the isolates.

The phylogeny showed that, the queried isolate –JJhB and *Fusarium oxysporum* f. sp. *cubense* isolate BMAE87Foc (accession no. MN396267.1) were derived from a common ancestor as they are in the same clade of the phylogeny. Bootstrap value (84) suggested the accuracy of the result in percentage. This isolate of *Fusarium oxysporum* was under the race 4 and was the first reported pathogen causing panama disease of banana in Turkey (GenBank[26])

The tree also revealed that, those two isolates were sharing a common ancestor with *Fusarium oxysporum* isolate cucumeri (accession no. MG670446.1) which was responsible for causing wilt of cucumber (GenBank[21]) (Figure 11).

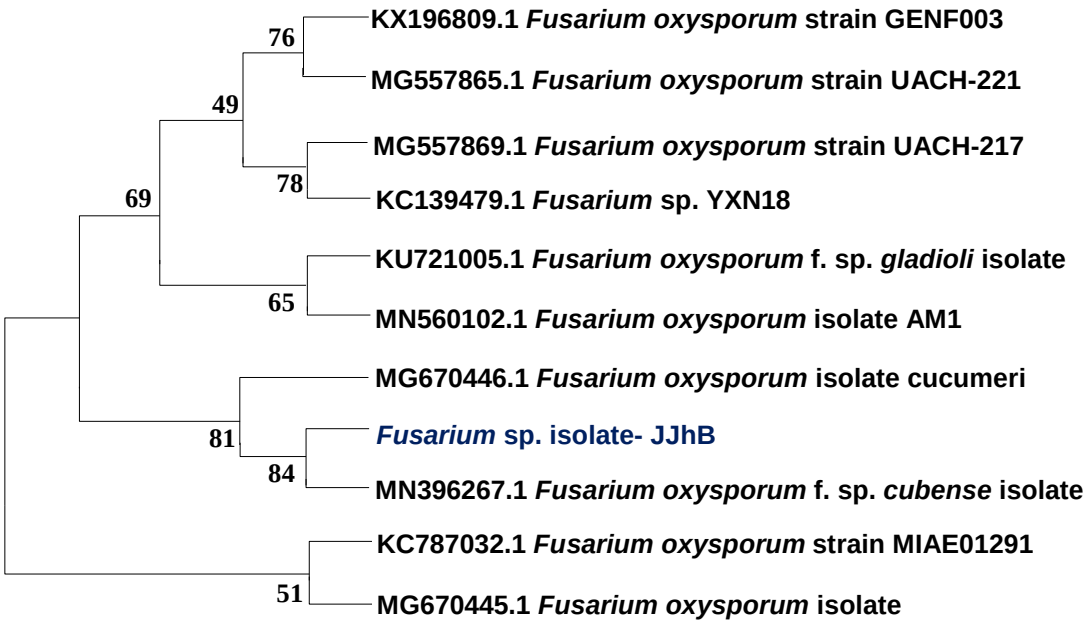


Figure 11. Neighbor joining (NJ) Phylogenetic tree of JJhB isolates in relation to other *Fusarium* isolates generated with 1000 bootstrap replicates

4.4.8. Molecular identification and phylogenetic tree construction of isolate-MeGP

The nucleotide sequence of isolate- MeGP was compared in the "NCBI-BLAST database and identified as *Fusarium solani.* Though *Fusarium oxysporum* is the main causal agent of fungal wilt, *Fusarium solani* were also reported to cause fungal wilt. Dwivedi *et al.* (2018) described *Fusarium solani* as a causal agent of fungal wilt of eggplant. In USA *Fusarium solani* was reported to cause wilt of pima cotton (Zhu *et al.*, 2019).

Seven isolates of *Fusarium solani* viz. *F. solani* strain GFR21 (accession no. MT447526.1); *F. solani* strain G17 (accession no. MT312745.1); *F. solani* isolate VOR6 (accession no. KX621960.1); *F. solani* (accession no. KU325529.1); *F. solani strain* ZB073 (accession no. KJ528882.1); *F. solani*

isolate ZK004 (accession no. KM235740.1) and *F. solani* strain T-ICA06NP (accession no. KJ620369.1) showed 100% sequence identity with the isolate MeGP and also covered 100% query.

Among them, the isolate bearing accession no. KX621960.1 was associated with wilt *Viola odorata* (GenBank[27]) and isolate ZK004 was associated with wilt of Tomato in China (GenBank[28]) Another three isolates *Fusarium solani* isolate Fs03 (accession no. MK571197.1) isolated from egg and males of *Meloidogyne* spp. associated with roots of tomato (GenBank[29]); Fusarium *solani* isolate FUS-17 (accession no. MH879587.1) associated with wilt of *Citrus reticulate* (*Mandarin* orange) in Pakistan (GenBank[30]) and Fusarium *solani* isolate BC-3 (accession no. MH782034.1) responsible for decaying of marijuana leaves in Canada (Genbank[31]) showed 99.81% sequence identity with 100% query coverage (Table13).

Table 13. Percentage of nucleotide identities of MeGP isolate with selected *Fusarium* fungi reported worldwide

Worldwide reported relevant *Fusarium* spp. isolates	Accession No.	Max Score	Total Score	Query Coverage (%)	E-Value	Identity (%)
Fusarium solani strain GFR21	MT447526.1	976	976	100%	0.00	100%
Fusarium solani strain G17	MT312745.1	976	976	100%	0.00	100%
Fusarium solani isolate VOR6.	KX621960.1	976	976	100%	0.00	100%
Fusarium solani	KU325529.1	976	976	100%	0.00	100%
Fusarium solani strain ZB073	KJ528882.1	976	976	100%	0.00	100%
Fusarium solani isolate ZK004	KM235740.1	976	976	100%	0.00	100%
Fusarium solani strain T-ICA06NP	KJ620369.1	976	976	100%	0.00	100%
Fusarium solani isolate Fs03	MK571197.1	970	970	100%	0.00	99.81%
Fusarium solani isolate FUS-17	MH879587.1	970	970	100%	0.00	99.81%
Fusarium solani isolate BC-3	MH782034.1	970	970	100%	0.00	99.81%

Phylogenetic tree of MeGP

The phylogenetic tree constructed after comparing the sequence in Gene Bank showed that the queried sequence formed a separate clade, so there was no direct ancestral relationship among other similar isolates but three isolates i.e. *Fusarium solani* (accession no. KU325529.1); *F. solani* strain T-ICA06NP (accession no. Kj620369.1) and *F. solani* strain ZB073 (accession no. KJ528882.1) were distantly related to isolate MeGP (Figure 12).

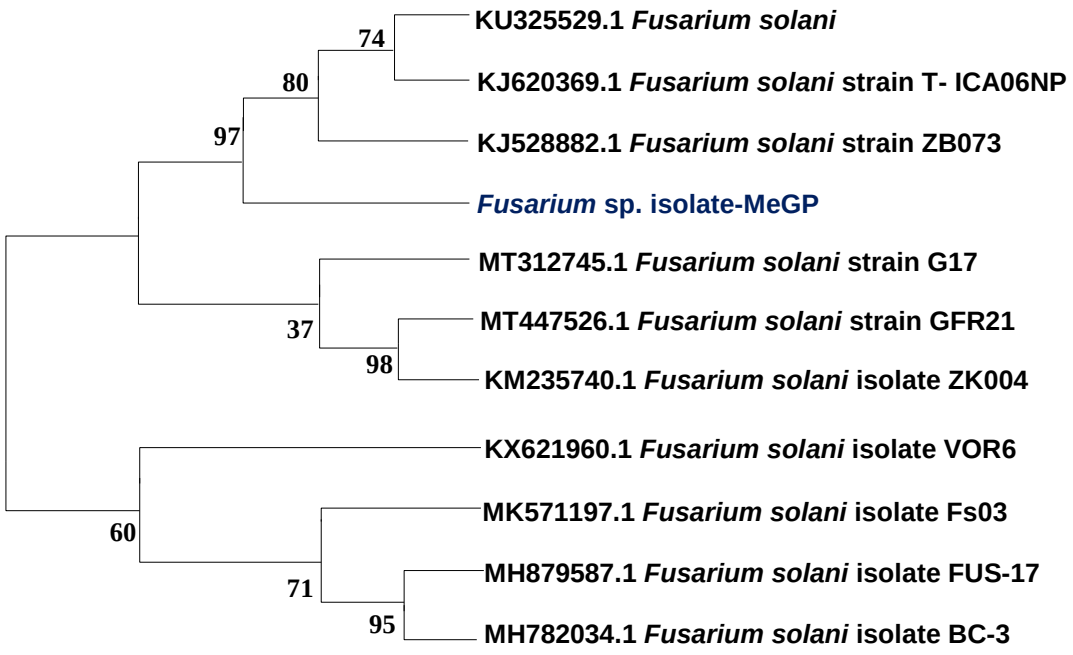


Figure 12. Neighbor joining (NJ) Phylogenetic tree of MeGP isolates in relation to other *Fusarium* isolates generated with 1000 bootstrap replicates

4.4.9. Molecular identification and phylogenetic tree construction of isolate-PBC

Morphologically the isolate PBC was identified as *Fusarium oxysporum* but it was identified as *Fusarium commune* after identifying the species at molecular level. It is a newly identified species under the genus *Fusarium* and closely related to *F. oxysporum* species complex isolated from different plants and soil (Kovgaard *et al.,* 2003). It was also reported to cause wilt and root-rot disease of rice in Malaysia (Husna *et al.,* 2020).

All the identified species similar to the isolate PBC showed 99.80 % sequence identity and 99% query coverage (Table 16.) Among them the first three identified sequence i.e. *Fusarium commune* isolate W_MNSO2_6_24B. (accession no. MN452698.1); *Fusarium commune* isolate W_ARSO2_4_17 (accession no. MN452696.1) *Fusarium commune* isolate ILSO1_3_8_1B (accession no. MN452682.1) were associated with soybean seedling disease in the US Midwest (GeneBank[31,32&33])

Fusarium commune isolate LD6-2 (accession no. MN121503.1) was associated with leaf decaying of lily in China (GenBank[34])

Two isolate was also identified as *Fusarium oxysporum* f. sp. *raphani* strain: MAFF 240304 (accession no. AB586993.1) and *Fusarium oxysporum* f. sp. *rapae* strain: MAFF 240321. (accession no. AB586994.1) which were mainly used to create genetic marker inorder to identify different isolates of *Fusarium* (GenBank[35]) (Table 14).

Table 14. Percentage of nucleotide identities of PBC isolate with selected *Fusarium* fungi reported worldwide

Worldwide reported relevant *Fusarium* spp. isolates	Accession No.	Max Score	Total Score	Query Coverage (%)	E-Value	Identity (%)
Fusarium commune isolate W_MNSO2_6_24 B.	MN452698.1	900	900	99%	0.00	99.80%
Fusarium commune isolate W_ARSO2_4_17	MN452696.1	900	900	99%	0.00	99.80%
Fusarium commune isolate ILSO1_3_8_1B	MN452682.1	900	900	99%	0.00	99.80%
Fusarium commune isolate y-004.	MN560023.1	900	900	99%	0.00	99.80%
Fusarium commune isolate 2016_1190	MN521627.1	900	900	99%	0.00	99.80%
Fusarium commune isolate KSSO1_1_22_2.	MK594070.1	900	900	99%	0.00	99.80%
Fusarium commune isolate x-028	MN209933.1	900	900	99%	0.00	99.80%
Fusarium commune isolate LD6-2	MN121503.1	900	900	99%	0.00	99.80%
Fusarium oxysporum f. sp. *rapae* strain: MAFF 240321.	AB586994.1	900	900	99%	0.00	99.80%
Fusarium oxysporum f. sp. *raphani* strain: MAFF 240304	AB586993.1	900	900	99%	0.00	99.80%

Phylogenetic tree of PBC

The phylogenetic tree that was constructed after comparing the sequence showed that the isolates-PBC formed a common clade with *Fusarium commune* isolate WMNSO26 24B (accession no. MN452698.1) So, there was a close ancestral relationship among those two isolates. These two isolates also formed a common clade with *Fusarium commune* isolate WARSO26 24B means they were togetherly related with this isolate. Those two related isolates were responsible for seedling disease of soybean (GeneBank[31&32]) (Figure 13).

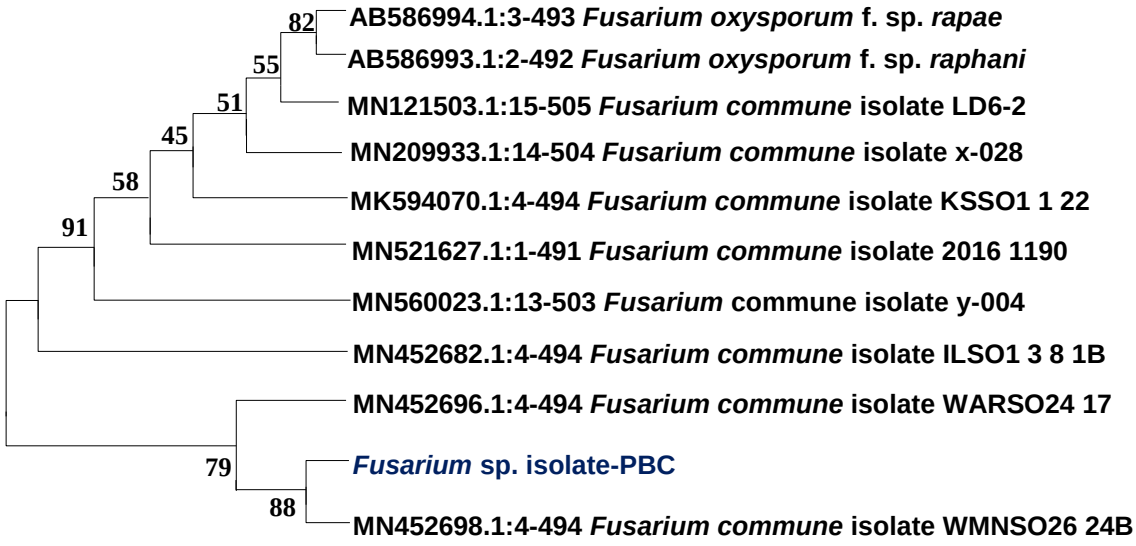


Figure 13. Neighbor joining (NJ) Phylogenetic tree of PBC isolates in relation to other *Fusarium* isolates generated with 1000 bootstrap replicates

4.5. Pathogenic variability of *Fusarium* spp. isolates

Pathogenicity test of the isolates of *Fusarium* spp. was performed in net house against fifteen days old eggplant seedling after inoculation. Data of wilt incidence was recorded at 30, 45 and 60 days after transplanting. Significant variation was recorded among different isolates of *Fusarium* spp. in terms of disease reaction (Table 15).

After thirty days of transplanting wilt incidence varied from 0-66.66%, though maximum isolates didn't show disease reaction at this early stage. In case of isolates DSAUT, DSAUB, BGT and MeGP wilt incidence were 33.33% where 66.66% disease incidence was observed in case of isolate MMsB (Table 15).

After forty-five days of transplanting 100% wilt incidence was occurred by isolates DSAUB, DSAUT, BGP, MMsC whereas, the isolates GBARIB, MMsP, JaJhB showed 66.66% wilt incidence. CChB and CChC showed only 33.33% disease incidence. The rest of the isolates showed 0.00% disease incidence in this experiments (Table 15.)

a) Healthy plant in control (un inoculated) pot b) Wilted plant after inoculation

Plate 9. Pathogenicity test for *Fusarium* spp. isolates

Table 15. Wilt incidence and virulence level of *Fusarium* spp. isolates at 30, 45 and 60 DAT

SL No.	Isolates	Wilt incidence (%) at different days after transplanting (DAT)			Virulence level
		30 DAT	45 DAT	60 DAT	
01	DSAUT	33.33	100	100	HV
02	DSAUB	33.33	100	100	HV
03	GBARIB	0.00	66.66	100	HV
04	MMsB	66.66	100	100	HV
05	MMsP	0.00	66.66	66.66	V
06	MMsC	0.00	100	100	HV
07	CChB	0.00	33.33	33.33	MV
08	CChC	0.00	0.00	33.33	MV
09	BGT	33.33	33.33	66.66	V
10	BGP	0.00	100	100	HV
11	FAB	0.00	0.00	0.00	AV
12	JaJhB	0.00	66.66	100	HV
13	MeGP	33.33	100	100	HV
14	JhKT	0.00	0.00	0.00	AV
15	PBC	0.00	0.00	66.66	V
16	Control	0.00	0.00	0.00	—

At sixty days after inoculation eight isolates named as DSAUT, DSAUB, GBARIB, MMsB, MMsC, BGP, JaJhB and MeGP showed 100% disease incidence and these were categorized as highly virulent (HV) pathogen. The isolates MMsP, BGT and PBC were categorized as virulent (V) pathogen as they showed 66.66% disease incidence whereas the isolates CChB and CChC showed 33.33% disease incidence and these were grouped under moderately virulent (MV) pathogen. On the other hand, no disease incidence was shown by FAB and

JhKT like as control pot and these were categorized as avirulent (AV) pathogen. The present results were supported by previous reports (Teixeira *et al.*, 2016 & Raghu *et al.*, 2016). Teixeira *et al.* (2016) reported that found pathogenic variability was found among different isolates of *Fusarium* spp. causing wilt of passion fruits. Raghu *et al.* (2016) worked with 44 isolates of *Fusarium oxysporum* causing wilt of chilli and found pathogenic variability among them.

CHAPTER V

SUMMARY AND CONCLUSION

CHAPTER V
SUMMARY AND CONCLUSION

Fungal wilt caused by *Fusarium* spp. is considered as one of the most problematic plant diseases around the globe as well as in Bangladesh that causes a huge amount of economic loss. Almost all the crops and vegetables having economic importance are associated with at least one disease caused by *Fusarium* spp. Adoption of a successful disease management strategy against wilt disease caused by *Fusarium* spp. is difficult due to presence of a wide range of variability among different isolates of this fungus. The present research was aimed to study the cultural, morphological, molecular and pathogenic variability among different isolates of *Fusarium* spp. causing wilt disease of solanaceous vegetables collected from major vegetables growing regions of Bangladesh.

A remarkable variability was observed among all the collected isolates of *Fusarium* spp. in respect of colony characteristics, radial mycelial growth, sporulation rate and time, molecular characteristics and pathogenic behavior. Cottony white, cottony white with brownish center, cottony pinkish white, pinkish white, whitish purple, purplish and creamy colored colony were found among different isolates where the colony shape varied from regular, regular with concentric ring to irregular. Colony margins were mostly entire, wavy, curled, filiform and undulated with crateriform, fluffy, flat and convex colony texture.

In PDA media, average radial mycelial growth of the isolates varied from 2.00−10.32 mm day^{-1} at 25±2°C temperature. Isolate DSAUB exhibited the highest mean growth per day, where the lowest growth rate was observed in isolate JhKT. The isolates were clustered into three groups based on colony diameter at nine days after inoculation viz. fast growing (DSAUT, DSAUB, GBARIB, MMsB, BGP, FAB, JaJhB and PBC), moderate growing (MMsP, MMsC and BGT) and slow growing (CChB, CChC and JhKT) fungi.

The number of conidia in ml^{-1} spore suspension was varied from 2.66×10^6 -12.33×10^6 ml^{-1} suspension and it was the highest in isolate DSAUB and lowest in isolate JhKT. A positive relationship was observed between mycelial growth rate and conidial production that means conidia production increased with the increase of mycelial growth rate. The highest time required for sporulation was recorded in isolate JhKT (7.33 days) where the lowest time required in case of isolate DSAUT (3.33 days). The relationship between growth rate and sporulation time was inversely proportional. The isolate having higher growth rate required less time for sporulation.

The isolates of *Fusarium* spp. was also varied markedly in respect of their level of virulence. All the isolates except FAB and JhKT were able to create disease symptoms after inoculation in pot. That is why, these two isolates were grouped as avirulent pathogen. The remaining isolates viz. DSAUT, DSAUB, GBARIB, MMsB, MMsC, BGP, JaJhB and MeGP were grouped as highly virulent pathogen, where the isolates MMsP, BGT, PBC were denoted as virulent and the isolates CChB, CChC were clustered as moderately virulent pathogen.

In case of molecular identification after analyzing the sequenced data of nine isolates in "NCBI-BLAST" database, six isolates viz. DSAUT, MMsB, MMsC, BGT, BGP and JJHB were identified as *Fusarium oxysporum* and another three isolates viz. GBARIB, MeGP and PBC were identified as *Fusarium fujikuroi*, *Fusarium solani* and *Fusarium commune*, respectively. Phylogenetic tree of queried isolates constructed with 1000 bootstrap replicates in relation to other *Fusarium* isolates reported worldwide revealed that a strong ancestral relationship was present among the native isolates and the worldwide reported isolates of *Fusarium* spp.

Considering the above results and discussion it can be concluded and recommended that:

- ✓ Pathogenic variability of *Fusarium* spp. exists in Bangladesh environment
- ✓ Other *Fusarium* spp. (viz. *F. fujikuroi*, *F. solani* and *F. commune*) than *F. oxysporum* were also found to be involved in developing wilt disease of solanaceous vegetables in Bangladesh.
- ✓ Further study with more isolates of *Fusarium* spp. from the rhizosphere of solanaceous vegetables is suggested covering all the "Agro-Ecological Zones" of Bangladesh.

CHAPTER VI

REFERENCES

CHAPTER VI
REFERENCES

Adame-García, J., Rodríguez-Guerra, R., Iglesias-Andreu, L.G. and Luna-Rodríguez, M. (2015). Molecular Identification and Pathogenic Variation of *Fusarium* Species Isolated from *Vanilla planifolia* in Papantla Mexico. *Bot. Sci.* **93**(3): 669-678.

Agrios, G.N. (2005). Vascular wilts caused by Ascomycetes and Deuteromycetes (Mitosporic Fungi). **In:** Plant Pathology (5th edition). Elsevier Acad. Press. Burlington, Mass, USA. p. 523.

Ahmad, M.A. (2010). Variability in *Fusarium oxysporum* f. sp. *ciceris* for Chickpea Wilt Resistance in Pakistan. Ph.D. thesis, Quad-i-Azam University, Islamabad, Pakistan.

Ahmmed, A.N.F. (2016). Manual for Molecular Diagnosis of Seed Borne Fungi of Rice. David Cahill Lab, Agricultural Biotechnology group, Deakin University, Waurn Ponds, VIC 3216, Australia. pp. 29-30.

Akgul, D.S. and Ahioglu, M. (2019). Fungal pathogens associated with young grapevine decline in the Southern Turkey vineyards. BIO Web of Conferences **15**, 01027: 42nd World Congress of Vine and Wine, July 15-18. Geneva, Switzerland.
DOI: https://doi.org/10.1051/bioconf/20191501027

Aminuzzaman, F.M., Hossain, I. and Ahmed, F. (2010). Cultural variation and pathogenicity of *Bipolaris sorokiniana* on wheat in Bangladesh. *Int. J. Agric. Environ. & Biotech*. **3**: 93-98.

Armstrong, G.M. and Armstrong, J.K. (1948). Non-susceptible hosts as carriers of wilt Fusaria. *Phytopathology*. **38**: 808-826.

Armstrong, G.M. and Armstrong, J.K. (1981). Formae speciales and races of *Fusarium oxysporum* causing wilt disease. **In**: *Fusarium*: Disease, biology, and taxonomy. P.E. Nelson, T.A. Toussoun and R.J. Cook (ed.). University Park, Pennsylvania State University Press, USA. pp. 391–399.

Barnett, H.L. and Hunter, B.B. (1972). Illustrated Genera of Imperfect Fungi (4th edition). American Phytopathological Society Press. St. Paul, Minnesota, USA. p.130.

BBS (2018). Yearbook of Agricultural Statistics of Bangladesh, Bangladesh Bureau of Statistics, Statistical Division, Ministry of Planning, Government of the People's Republic of Bangladesh, Dhaka.

Beckman, C.H. (1987). The Nature of Wilt Diseases of Plant. American Phytopathological Society Press. St. Paul, Minnesota, USA. p.175.

BER (2019). Bangladesh Economic Review, Economic Advisory Section, Department of Finance, Ministry of Finance, Bangladesh. p.15.

Bertoldi, M.D., Lepidi, A.A. and Nuti, M.P. (1972). Classification of the genus *Humicola* spp. traaen preliminary reports and investigations. *Mycopathologia et Mycologia applicata*. **46**(4):289-304.

Bishop, C.D. and Cooper, M.R. (1983). An ultrastructural study of vascular colonization in three vascular wilt diseases I. Colonization of susceptible cultivars. *Physiological Plant Pathol*. **23**(3): 323-343.

Booth, C. (1971). *Fusarium*: Laboratory guide to the identification of the major species. Commonwealth Mycological Institute. Surrey, Kew, UK. p. 58.

Carmona, S.L., David, D.B., Gomez, M.R., Lopez, W., Ceballos, N., Zapata, J.C., Simbaqueba, J. and Soto-Suare, M. (2020). Characterization of Pathogenic and Nonpathogenic *Fusarium oxysporum* Isolates Associated with Commercial Tomato Crops in the Andean Region of Colombia. *Pathogens*. **70:** 1-23.

Cha, S.D., Jeon, Y.J., Ahn, G.R., Han, J.I., Han, K.H. and Ki, S.H. (2007). Characterization of *Fusarium oxysporum* isolated from Paprika in Korea. *Mycobiology.* **35**(2): 91-96.

Charoenporn, C., Kanokmedhakul, S., Lin, F.C., Poeaim, S., and Soytong, K. (2010). Evaluation of bio-agent formulations to control Fusarium wilt of tomato. *Afr. J. Biotechnol*. **9:** 5836-5844.

Chaudhary, R.G., Dhar, V. and Singh, R.K. (2009). Association of fungi with wilt complex of lentil at different crop stages and moisture regimes. *Archives of Phytopathology and Plant Protection*. **42:**340-343.

Chehri, K. (2016). Molecular identification of pathogenic *Fusarium* species, the causal agents of tomato wilt in western Iran. *J. Plant Protection Research*. **56** (2):144-148.

Chi, C. and Hanson, E.W. (1964). Relation of temperature, pH and nutrition to growth and sporulation of *Fusarium* spp. from red clover. *Phytopathology*. **54** (9): 1053-1058.

Choi, H.W., Hong, S.K. and Lee, Y.K. (2019). First Report of *Fusarium fujikuroi* Causing Fusarium wilt on *Glycine max* in Korea. *Plant Disease*. **103**(9): DOI: 10.1094/PDIS-09-18-1602-PDN

Chopada, G.B., Singh, P. and Chandulal, K. (2014). Cultural and morphological variability among *Fusarium oxysporum* f.sp. *lycopersici* causing wilt of tomato in south Gujarat region. *Archives of Phytopathology and Plant Protection*. **7:** 1-10.

Colyer, P.D. (2007). "Current status of Fusarium wilt in the United States and future challenges," In Proceedings: World Cotton Research Conference" 4, pp.10-14.

Curtis, F.D., Palmieri., Vitullo, D. and Lima, G. (2014). First report of *Fusarium oxysporum* f.sp. *pisi* as causal agent of root and crown rot on chickpea (*Cicer arietinum* L.) in Southern Italy. *Plant Dis*. DOI: 10.1094/PDIS-09-13-0941-PDN.

Dwivedi, S.K. and Ganesh, P. (2018). Biological and Chemical Management of *Fusarium solani* Causing Wilt in Eggplants. *IJSSR*.**7**(4): 1914-1925.

Dikkar, M. and Deshmukh, V.V. (2003). Nutritional requirement of Fusarium wilt of chickpea. *Crop Res.* **25**(1): 197-199.

Dubey, S.C., Singh, S.R. and Singh, B. (2010). Morphological and pathogenic variability of Indian isolates of *Fusarium oxysporum* f. sp. *ciceris* causing chickpea wilt. *Archives of Phytopathology and Plant Protection.* **43**(2): 174 -190.

Elliot, M.L. (2009). Fusarium wilt of canary island date palm. Fact Sheet, University of Florida, Plant Pathology. **215**:1-7.

Epp, M.D. (1987). Somaclonal variation in bananas: a case study with Fusarium wilt. *Banana and Plantain Strategies*. (Cairns, Australia: ACIAR Proceedings). **21**: 140-150.

Farooq, S., Iqbal, S.M. and Rauf, A. (2005). Physiological studies of *Fusarium oxysporum* f. sp. *ciceri*. *Int. J. Agri. Biol.* **7**(2): 275-277.

Flood, J. (2006). A review of Fusarium wilt of oil palm caused by *Fusarium oxysporum* f. sp. *elaeidis*. *Phytopathology*. **96**(6): 660-662.

Francis, R.G. and Burgess, L.W. (1975). Surveys of *Fusarium* and other fungi associated with stalk rot of maize in eastern Australia. *Australian Journal of Agricultural Research*. **26**: 801-807.

Gangadhara, N.B., Nagaraja, R., Basavaraja, M.K. and Krishna N. R. (2010). Variability studies of *Fusarium oxysporum* f. sp. *vanillae* isolates. *Int. J. Science and Nature*. **1**(1):12-16.

Global Catalogue of Microorganism (1st july,2020). Taxonomy: *Humicola* sp. Retrieved from: http://gcm.wfcc.info/speciesPage.jsp?strain_name=Humicola%20minima

Gomez, K.A. and Gomez, A.A. (1984). Statistical Procedure for Agricultural Research. John Wiley and Sons, New York, USA. p. 187

Gordon, T.R. (2017). *Fusarium oxysporum* and the Fusarium wilt syndrome. *Annual review of phytopathology*. **55:** 23-29.

Gunua, T. (2010). A review of sweet potato scab and studies on *Fusarium* wilt of sweet potato (*Ipomoea batatas* (L.) Lam.) and approaches to its management. M. Phil Thesis, The University of Queensland, Brisbane, Australia.

Hafizi, R., Salleh, B, and Lattifa, Z. (2013). Morphological characterization of *Fusarium solani* and *Fusarium oxysporum* associated with crown rot disease of oil palm. *Brazilian J. Microbiol*. **44**(3): 959-968.

Hannachi, I., Cherif, M. and Spadaro, D. (2014). First report of mature citrus trees being affected by Fusarium wilt in Tunisia. *Plant Disease*. **98**(4): 566-566. DOI: 10.1094/PDIS-12-12-1134-PDN

Haware, M.P., Nene, Y. L., Pundir, R. P. S. and Narayana, J. (1992). Screening of world chickpea germplasm for resistance to Fusarium wilt. *Field Crops Res*. **30:** 147-154.

Hayes, W.A. (1978). Nutrition, substrates and principles of disease control. **In:** The Biology and Cultivation of Edible Mushrooms. Academic press. New York. pp. 202-220.

Huisman, O.C. (1982). Interactions of root growth dynamics to epidemiology of root invading fungi. *Annual Review of Phytopathology*. **20**: 303-327.

Husna, A., Latiffah., Z and Noor, N.M.I.M. (2020). *Fusarium commune* associated with wilt and root rot disease in rice. *Plant pathology*. DOI: https://doi.org/10.1111/ppa.13270

Hutmacher, B., Davis M.R., and Kim, Y. (2003). *Fusarium* Information. *University of California Cooperative Extension*. p 7.

Imam, S.H. (2018,16 January). Bangladesh 3rd in veg. growth. *The Financial Express*. Retrieved from: https://today.thefinancialexpress.com.bd/print/bangladesh-3rd-highest-in-veg-growth-1516038300 . Access Date: 12 April, 2020

Inoue, I., Namiki, F. and Tsuge, T. (2002). Plant colonization by the vascular wilt fungus *Fusarium oxysporum* Requires FOW1, a gene encoding a mitochondrial protein. *The Plant Cell*. **14**:1869-1883.

Isaac, M.R., Leyva-Mir, S.G., Sahagun-Castellanos, J., Camara-Correia, K., Tovar-Pedraza, J.M. and Rodriguez-Perez, J.E. (2018). Occurrence, identification, and pathogenicity of *Fusarium* spp. Associated with tomato wilt in Mexico. *Not Bot Horti Agrobo*. **46**(2): xxx-xxx. DOI:10.15835/nbha46211095

Jamaria, S.L. (1972). Nutritional requirement of *Fusarium oxysporum* f. sp. *niveum*. *Indian Phytopathology*. **25:** 29-32.

Joshi, R. (2018). A review of *Fusarium oxysporum* on its plant interaction and industrial use. *J. Medicinal Plant Studies.* **6**(3): 112-115.

Khan, A. A. (1991). Present status of vegetable disease in Bangladesh. **AGRIS:** *International research system for agriculture & technology.* p. 123. Retrieved from: http://agris.fao.org/agrissearch/search.do?recordID=BD9125071

Khilare, V.C. and Ahmed, R. (2012). Effect of different media, pH and temperature on the growth of *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt. *Int. J. Advance Biological Research.* **2**(1): 99-102.

Kistler, H.C. (1997). Genetic diversity in the plant pathogenic fungus *F. oxysporum*. *Phytopathology*. **87:** 474 -479.

Kovgaard, K., Rosendahl, S., O'Donnell, K. and Nirenberg, H.I. (2003). *Fusarium commun*e is a new species identified by morphological and molecular phylogenetic data. *Mycologia*. **95:**630-636

Kulkarni, S.P. (2006). Studies on *Fusarium oxysporum* Schlecht Fr f. sp. *gladioli* (Massey) Snyd. & Hans. Causing wilt of gladiolus. M.S. Thesis, University of Agricultural Science, Dharwad, India.

Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K. (2018) MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol Biol Evol*. **35(**6):1547-1549.

Kumari, S., Anokheand, A. and Kuma, R. (2017). Nutritional quality and health benefits of solanaceous vegetables. *Progressive Research-an*. **12**(2): 1942-45.

Lesile, J.F. and Summerell, B.A. (2006). The *Fusarium* laboratory manual. Blackwell publishing professional. State Avenue, Ames, Iowa, USA. p. 2.

Lević, T., Slavica, S., Vesna, K. and Aleksandr, S.B.S. (2009). *Fusarium* species: the occurrence and the importance in agriculture of Serbia. *Zbornik Matice Srpske za Prirodne Nauke*. **116:** 33-48.DOI: 10.2298/ZMSPN0916033L.

Lygis, V. and Vasiliauskaite, I. (2014). Fungi in Living and Dead Stems and Stumps of *Pinus mugo* on Coastal Dunes of the Baltic Sea. *Plant Prot. Sci.* **50** (4): 221-226.

Ma, L., Does, V.D., Brokovich, K.A., Coleman, J.J., Dabousi, M.J. Grabherr, M., Gale, L.R., Brown, D.W. (2010). Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium*. *Nature.* **464**: 367-373.

Maina, P.K., Wachira, P. M., Okoth, S. A. and Kimenju, J. W. (2017). Cultural, morphological and pathogenic variability among *Fusarium oxysporum* f. sp. *phaseoli* causing wilt in French Bean (*Phaseolus vulgaris* L.). *J. Advances in Microbiology*. **2**(4): 1-9.

Mandeel, Q.A. (2007). Modeling competition for infection sites on roots by nonpathogenic strains of *Fusarium oxysporum*. *Mycopathologia* .**163** (1): 9-20.

Mes, J.J., Weststeijn, E.A., Herlaar, F., Lambalk, J.J.M., Wijbrandi, J., Haring, M.A., and Cornelissen, B.J.C. (1999). Biological and molecular characterization of *Fusarium oxysporum* f. sp. l*ycopersici* divides race 1 isolates into separate virulence groups. *Phytopathology*. **89**:156-160

Miller, S.A., Rowe, R.C. and Riedel, R.M. (2011). Fusarium and Verticillium wilts of tomato, potato, pepper, and eggplant. Fact Sheet. Columbus, Ohio State University. pp. 1-4.

Mina, U. and Dubey, S.C. (2010). Effect of environmental variables on development of *Fusarium* wilt in chickpea (*Cicer arietinum*) cultivars. *Indian J. Agric. Sci*. **80**(3):231-234.

Mitchell, J.K., Orsted, K. M. and Warne, C.M. (1997). Fun Microbiology: Using a Plant Pathogenic Fungus to Demonstrate Koch's Postulates. *The American Biology Teacher*. **59**(9): 574-577.

Mohsin, S.M. (2013). Cultural, morphological, pathogenic and molecular characterization of *Alternaria porri* causing purple blotch of onion. M.S. Thesis, Sher-e-Bangla Agricultural University (SAU), Dhaka, Bangladesh.

Morrell, J. and Bloom, J. (1981). Influence of *Meloidogyne incognita* on Fusarium Wilt of Tomato at or below the Minimum Temperature for Wilt Development. *J. Nematol.***13**(1): 57-60.

Moss, M.O. and Smith J. E. (1984). The Applied Mycology of *Fusarium.* Cambridge University Press, New York, USA. p. 9.

Mostert, D., Molina, A.B., Daniells, J., Fourie, G., Hermanto, C. and Chao, C. P. (2017). The distribution and host range of the banana Fusarium wilt fungus, *Fusarium oxysporum* f. sp. *cubense*, in Asia. *PLoSONE*. **12**(7): 1-24.

Nath, N. (2011). Morphological and physiological variation of *Fusarium oxysporum* f. Sp. *ciceri* isolates causing wilt disease in chickpea. M.S. Thesis, Sher-e-Bangla Agricultural University (SAU), Dhaka, Bangladesh.

Nirmaladevi, D. and Srinivas, C. (2012). Cultural, morphological, and pathogenic variation in *Fusarium oxysporum* f. sp. *lycopersici* causing wilt of tomato. *Batman Uni. J. Life Sci.* **2**(1): 1-16.

Okungbowa, F.I. and Shittu, H. O. (2012). Fusarium Wilts: An Overview. *Environmental Research journal*. **6**(2): 83-102.

Ortiz, E., Cruz, M., Melgarejo, M.L., Marquínez, X. and Hoyos-Carvajal, L. (2014). Histopathological features of infections caused by *Fusarium oxysporum* and *Fusarium solani* in purple passion fruit plants (*Passiflora edulis* Sims). *Summa Phytopathologica*. **40**(2):134-140.

Padwick, G.W. (1940). The genus *Fusarium*, 111. A critical study of pathogens causing wilt of gram (*Cicer arietinum* L.) and released species of the subsection orthocera with special relation to the variability of key characteristics. *Indian J. Agric. Sci.* **10:** 241-284

Patra, S. and Biswas, M.K. (2016). Studies On Cultural, morphological and pathogenic variability among the isolates of *Fusarium oxysporum* f. sp. *ciceri* Causing wilt of chickpea. *Int. J. Plant, Animal and Environmental Sci.* **7**(1): 11-16.

Pattison, A.B., Wright, C.L., Kukulies, T.L. and Molina, A.B. (2014). Ground cover management alters development of Fusarium wilt symptoms in Ducasse bananas. *Aust. J. Plant Pathol*. **43**: 465-476.

Peng, H.X., Sivasithamparam, K., and Turner, D.W. (1999). Chlamydospore germination and Fusarium wilt of banana plantlets in suppressive and conducive soils are affected by physical and chemical factors. *Soil Biol. Biochem.* **31**(4): 1363-1374.

Pennington, J.A.T. and Fisher, R.A. (2009). Classification of fruits and vegetables. *J. Food Composition and Analysis*. **22**(1): 23-31.

Petruzzello, M. (2015). Solanaceae: Encyclopedia online Britannica. Encyclopaedia Britannica, Inc. Retrieved from: https://www.britannica.com/plant/Solanaceae.

Petrzik, K. and Siddique, A.B. (2019). A mycoparasitic and opportunistic fungus is inhabited by a mycovirus. Retrieved from: https://doi.org/10.1007/s00705-019-04359-x

Ploetz, R.C. (2005). Panama disease: An old nemesis rears its ugly head Part 1. **In:** The beginnings of the banana export trades. American Phytopathological Society Press, online, St. Paul, Minnesota, USA. pp. 1-13. Retrieved from: https://www.apsnet.org/edcenter/apsnetfeatures/Pages/PanamaDiseasePart1.aspx

Prasad, G. and Dwivedi, S.K. (2019). Biological and chemical management of *Fusarium solani* Causing Wilt in Eggplants. *IJSRR.* **7**(4): 1914-1925.

Raghu, S., Benagi, V.I. and Nargund, V.B. (2016). Cultural, morphological and pathogenic variability among the isolates of *Fusarium solani* causing wilt disease of Chilli (*Capsicum annuum* L.). *J. Pure and Appl. Microbiol.* **10**(1): 1-5.

Ramaiah, A.K. and Garampalli, R.K.H. (2013). Screening of indigenous potential antagonistic *Trichoderma* spp. from tomato and brinjal rhizospheric soil against *Fusarium* spp. *IOSR J. Agric.& veterinary Sci***.** **4**(3): 42-47.

Riker, A.J. and Riker, R.S. (1936). Introduction to research on plant diseases. **In:** a guide to the principles and practices for studying for various plant diseases problems. University of Wisconsin press, Madison, USA. p. 117.

Rishbeth, J. (1957). Fusarium wilt of bananas in Jamaica. II. Some aspects of host parasite relationships. *Ann. Bot.* **21:** 215–245.

Rubatzky, V.E., Yamaguchi, M. (1997). Tomatoes, Peppers, Eggplants, and other Solanaceous Vegetables. **In:** World Vegetables. Springer, Boston, USA. p. 224. Retrieved from : https://doi.org/10.1007/978-1-4615-6015-9_23

Saharana, M.S. and Naef, A. (2008). Detection of genetic variation among Indian wheat head scab pathogens (*Fusarium* spp. isolates) with microsatellite markers. *Crop Protection*. **27:** 1148-1154.

Sahu, S., Koshale, K. and Tiwari, R.K.S. (2017). Variability Among Isolates of *Fusarium oxysporum* f. sp. *udum* Causing Pigeonpea Wilt. *Int. J. of Microbiology Research*. **9**(2): 850-853.

Septembre-Malaterreb, A., Remizeb, F. and Pouchereta, P. (2018). Fruits and vegetables, as a source of nutritional compounds and phytochemicals: Changes in bioactive compounds during lactic fermentation. *Food Research International*. **104**: 86-99.

Shubin, L., Juan, H., RenChao, Z., ShiRu, X. and YuanXiao, J. (2014). Fungal Endophytes of *Alpinia officinarum* Rhizomes: Insights on Diversity and Variation across Growth Years, Growth Sites, and the Inner Active Chemical Concentration. *PLoS ONE* **9**(12): e115289. DOI: 10.1371/journal. Pone. 0115289

Singh, K.B. and Reddy, M.V. (1991). Advances in disease resistance in chickpea. *Advances in Agron.* **45**: 191-222.

Singha, I.M., Kakoty, Y., Unni, B.G., Das, J. and Kalita, M.C. (2016). Identification and characterization of *Fusarium* sp. using ITS and RAPD causing *Fusarium* wilt of tomato isolated from Assam, North East India. *J. Genetic Engineering and Biotechnology*. **14**: 99–105.

Smith, S.N., Ebbels, R.H., Garber, R.H. and Kappelman Jr, A.J. (1981). "Fusarium wilt of cotton," **In:** *Fusarium:* Diseases, Biology and Taxonomy. Nelson, P.E., Toussoun, T.A. and Cook, R.J.(ed.). University Park and London: Pennsylvania State University Press, pp. 29–38.

Solis, M.J.L., Cruz, T.E.D, Schnittler, M., and Unterseher, M. (2015). The diverse community of leaf inhabiting fungal endophytes from Philippine natural forests reflects phylogenetic patterns of their host plant species *Ficus benjamina*, *F. elastica* and *F. religiosa*. *Mycoscience*. **30**:1-11. Retrieved from: https://doi.org/10.1016/j.myc.2015.10.002

Steinkellner, S., Mammerler, R. and Vierheilig, H. (2008). Germination of *Fusarium oxysporum* in root exudates from tomato plants challenged with different *Fusarium oxysporum* strains. *European J. Plant Pathol.* **122**: 395-401.

Stover, R.H. (1956). Studies on Fusarium wilt of bananas. I. The behaviour of *F. oxysporum* f. sp. *cubense* in different soils. *Can. J. Bot.* **34**(2): 927-942.

Stover, R.H. (1962). Fusarial wilt (Panama Disease) of bananas and other *Musa* species. The Commonwealth Mycological Institute press, Surrey, Kew, UK. p. 117.

Su, H.J., Hwang, S.C. and Ko, W.H. (1986). Fusarial wilt of 'Cavendish' Bananas in Taiwan. *Plant Dis*. **70**: 814-818.

Tasnin, R. (2018). Integrated approaches for the management of wilt complex of tomato. M.S. Thesis, Sher-e-Bangla Agricultural University (SAU), Dhaka, Bangladesh.

Teixeira, L.M., Coelho, L. and Tebaldi, N.D. (2016). Characterization of *Fusarium oxysporum* isolates and resistance of passion fruit genotypes to fusariosis. *Jaboticabal*. **39**(3): 1-11

Walter, S., Nicholson, P. and Dooha, F.M. (2009). Action and reaction of host and pathogen during Fusarium head blight disease. *New Phytologist*. **185**: 54-66.

Wanjiru, W.M., Zhensheng, K. and Buchenauer, H. (2002). Importance of cell wall degrading enzymes produced *by Fusarium graminearum* during infection of wheat heads. *European J. Plant Pathol*. **108:** 803-810.

White, T.J., Bruns, T., Lee, S. and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. **In:** PCR protocols. A guide to methods and applications. Innis, M.A., Gelfand, D.H., Sninsky. J.J. and White, T.J. (ed.). Academic Press, New York, pp. 315-322.

Wong, M.Y. (2003). *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) WC Snyder and HN Hans. *Soilborne Plant Pathogen Class Project*. NC state university, USA. Retrieved from: https://projects.ncsu.edu/cals/course/pp728/profile.html.

Zhu, Y., Abdelraheem, A., Sanogo, S., Wedegaertner, S., Nichols, R. and Zhang, F. (2019). First Report of *Fusarium solani* Causing Fusarium Wilt in Pima Cotton (*Gossypium barbadense)* in New Mexico, U.S.A. *Plant Disease*. **103**(12): DOI: https://doi.org/10.1094/PDIS-05-19-1081-PDN

Zitter, T.A. (1998). Fusarium diseases of cucurbits. Fact sheet, New York State IPM program. pp. 1-4. Retrieved from : https://hdl.handle.net/1813/43275.

GenBank (URL):

1. https://www.ncbi.nlm.nih.gov/nuccore/MH368097.1
2. https://www.ncbi.nlm.nih.gov/nuccore/KX196809.1
3. https://www.ncbi.nlm.nih.gov/nuccore/MF069180.1
4. https://www.ncbi.nlm.nih.gov/nuccore/MK120427.1
5. https://www.ncbi.nlm.nih.gov/nuccore/MF630984.1
6. https://www.ncbi.nlm.nih.gov/nuccore/MF540558.1
7. https://www.ncbi.nlm.nih.gov/nuccore/MF540559.1
8. https://www.ncbi.nlm.nih.gov/nuccore/MF630975.1
9. https://www.ncbi.nlm.nih.gov/nuccore/KC478636.1
10. https://www.ncbi.nlm.nih.gov/nuccore/KY678273.1
11. https://www.ncbi.nlm.nih.gov/nuccore/KY678287.1
12. https://www.ncbi.nlm.nih.gov/nuccore/KY678295.1
13. https://www.ncbi.nlm.nih.gov/nuccore/EU364842.1
14. https://www.ncbi.nlm.nih.gov/nuccore/MH879861.1
15. https://www.ncbi.nlm.nih.gov/nuccore/MK452341.1
16. https://www.ncbi.nlm.nih.gov/nuccore/MT020427.1
17. https://www.ncbi.nlm.nih.gov/nuccore/MN121400.1
18. https://www.ncbi.nlm.nih.gov/nuccore/KC623568.1
19. https://www.ncbi.nlm.nih.gov/nuccore/LC428050.1
20. https://www.ncbi.nlm.nih.gov/nuccore/KC623568.1
21. https://www.ncbi.nlm.nih.gov/nuccore/MG670446.1
22. https://www.ncbi.nlm.nih.gov/nuccore/MG670445.1
23. https://www.ncbi.nlm.nih.gov/nuccore/KC139479.1
24. https://www.ncbi.nlm.nih.gov/nuccore/KU721005.1
25. https://www.ncbi.nlm.nih.gov/nuccore/MN560102.1
26. https://www.ncbi.nlm.nih.gov/nuccore/MN396267.1
27. https://www.ncbi.nlm.nih.gov/nuccore/KX621960.1
28. https://www.ncbi.nlm.nih.gov/nuccore/KM235740.1
29. https://www.ncbi.nlm.nih.gov/nuccore/MK571197.1
30. https://www.ncbi.nlm.nih.gov/nuccore/MH879587.1
31. https://www.ncbi.nlm.nih.gov/nuccore/MN452698.1
32. https://www.ncbi.nlm.nih.gov/nuccore/MN452696.1
33. https://www.ncbi.nlm.nih.gov/nuccore/MN452682.1
34. https://www.ncbi.nlm.nih.gov/nuccore/MN121503.1
35. https://www.ncbi.nlm.nih.gov/nuccore/AB586994.1

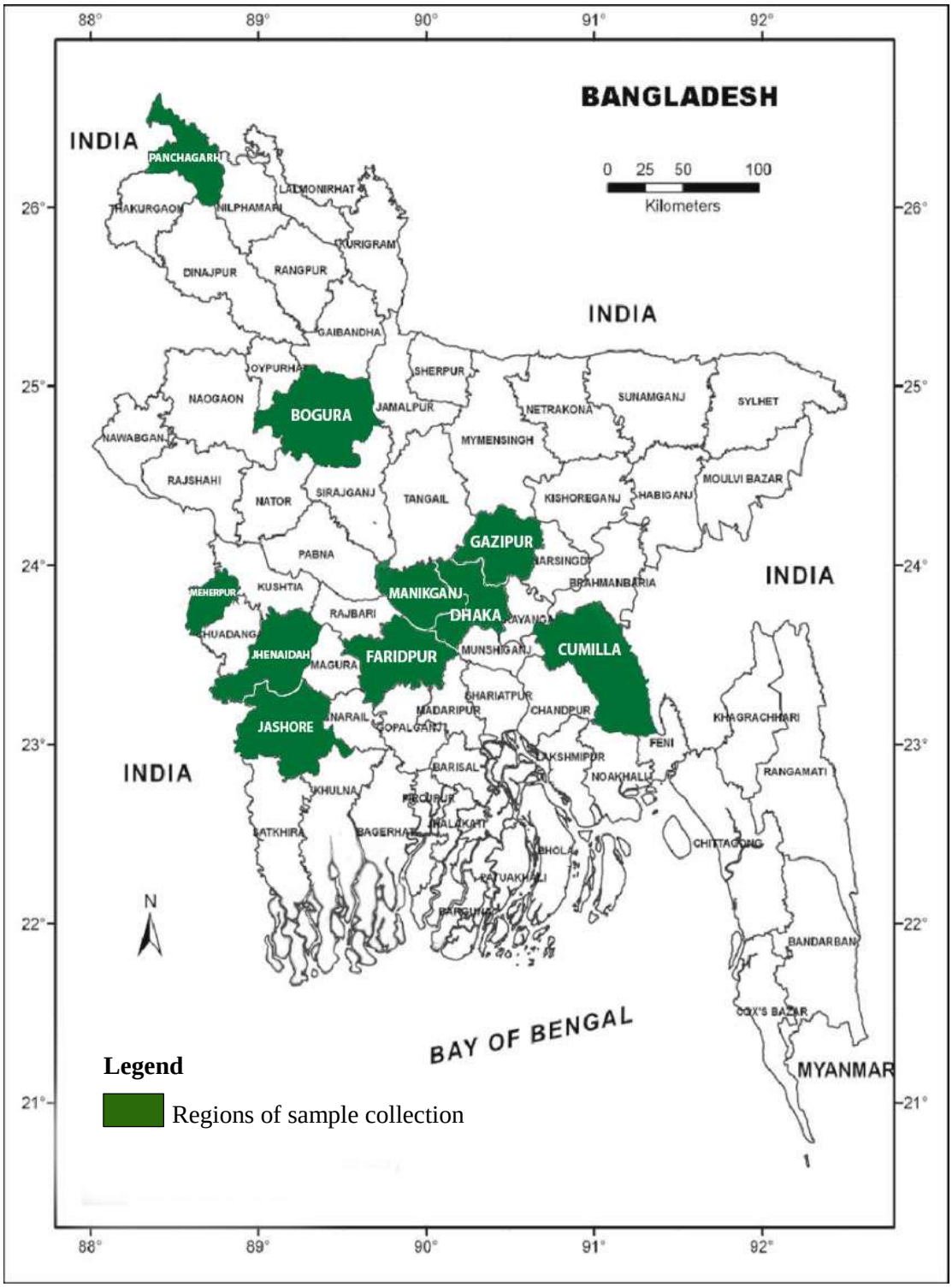
CHAPTER VII

APPENDICES

CHAPTER VII

APPENDICES

Appendix I. Map showing the sample collection regions under the study



Appendix II. Chloroform based protocol of fungal cDNA Extraction

- ✓ Grind 0.1-10 mg fungal mycelia by using mortar and pestle with glass beads and transfer into a 2 ml tube after grinding in mortar pestle with
- ✓ Add 1 ml 2 STE buffer, vortex shortly (30 sec) and put in 65 °C heat block for 15-30 minutes
- ✓ Add 400 µl chloroform+ 600 µl phenol+ 100 µl of 10% SDS, vortex shortly and rotate 30 min
- ✓ Centrifuge at 15000 rpm at 4 °C for 10 min took the supernatant (500µl) in the new tube
- ✓ Add 300 µl isopropanol (ice cold/keep in freezer)
- ✓ Centrifuge at 4 °C at 1500 rpm for 10 min, discard supernatant without disturbing pellet
- ✓ Wash with 700 µl ethanol (70%) by centrifuge at 4 °C for 1-3 min and discard the supernatant without disturbing pellet
- ✓ Add 700 µl of cold 95% ethanol, invert to mix and centrifuge at 4 °C for 1-3 min
- ✓ Dry the pellet in a vacuum centrifuge or on a heat block at 55°C
- ✓ Re-suspend pellet with 50 µl of water (ddH_2O/sterile)

Appendix III. PCR amplification protocol

1. Primers ITS 1 and ITS 4 (White *et al.*, 1990)

2. "Green Master Mix" Preparation:

	15 µl	**25 µl**
Green 2X plutonium master mix	7.5µl	12.5µl
Primer pair	0.5 + 0.5 µl	0.5+0.5µl
cDNA	1.5 µl	1.5 µl
H_2O	5 µl	10 µl

3. PCR reaction mixture (25 µl)
    a. 15.64 µl ddH2O,
    b. 5 µl Mango-Buffer,
    c. 1.7 µl MgCl2 (Bioline, 50 mM),
    d. 0.5 µl dNTP (10 mM),
    e. 0.5 µl of each primer (10 pM/µl),
    f. 0.16 µl (5 U/µl) of Taq DNA polymerase
    g. and 1 µl (template DNA);

4. Setup of the Mastercycler
    a. 5 min at 94 °C,
    b. 35 cycles:
        i. 35 s at 94 °C
        ii. 50 s at 52 °C
        iii. 1 min 30 s at 72 °C
    c. and 5 min at 72 °C.

5. PCR products were run on agarose gel (1%) for control and took the picture.
6. The purified amplicons were shipped to Eurofins for sequencing.

Appendix IV. DNA sequence (FASTA format) for BLAST

Isolates' Designation	DNA Sequence (FASTA Format)
DSAUT	AACTTGGATCTACTGATCCGAGGTCACATTCAGAAGTTGGGGTTTAACGGCGTGG CCGCGACGATACCAGTAACGAGGGTTTTACTACTACGCTATGGAAGCTCGACGTG ACCGCCAATCAATTTGAGGAACGCGAATTAACGCGAGTCCAACACCAAGCTGTGC TTGAGGGTTGAAATGACGCTCGAACAGGCATGCCCGCCAGAATACTGGCGGGCGC AATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTAT CGCATTTTGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAG ATTTGATTTATTTATGGTTTTACGTTTAGGGGTCCTCTGGCGGGCCGTCCCGTTT TACCGGGAGCGGGCTGATCCGCCGAGGCAACAAGTGGTATGTTCACAGGGGTTTG GGAGTTGTAAACTCGGTAATGATCCCTCCGCTGGTTCACCAACGGAGACCTTGTT ACGACTTTTACTTCCTCTAAATGCA
GBARIB	CATCTACTGATCCGAGGTCAACATTCAGAAGTTGGGGGTTTAACGGCTTGGCCGC GCCGCGTTCCAGTTGCGAGGGTTTTACTACTACGCAATGGAGGCTGCAGCGAGAC CGCCACTAGATTTCGGGGCCGGCTTGCCGCAAGGCCGATCCGAGGGCTTGAGGGT TGAAATGACGCTCGAACAGGCATGCCCGCCAGAATACTGGCGGGCGCAATGTGCG TTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTT GCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTGATT TTATGGTTTTACTCAGAAGTTACATATAGAAACAGAGTTTAGGGGTCCTCTGGCG GGCCGTCCCGATCCGCCGAGGCAACAAGTGGTATGTTCACAGGGGTTTGGGAGTT GTAAACTCGGTAATGATCCCTCCGCTGGTTCACCAACGGAGACCTTGTTAC GACTTTTACTTCCTCTAAT
MMsB	ATCCGAGGTCACATTCAGAAGTTGGGGTTTAACGGCGTGGCCGCGACGATTACCA GTAACGAGGGTTTTACTACTACGCTATGGAAGCTCGACGTGACCGCCAATCAATT TGAGGAACGCGAATTAACGCGAGTCCCAACACCAAGCTGTGCTTGAGGGTTGAAA TGACGCTCGAACAGGCATGCCCGCCAGAATACTGGCGGGCGCAATGTGCGTTCAA AGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTTGCTGC GTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTGATTTATTT ATGGTTTTACTCAGAAGTTACATATAGAAACAGAGTTTAGGGGTCCTCTGGCGGG CCGTCCCGTTTTACCGGGAGCGGGCTGATCCGCCGAGGCAACAAGTGGTATGTTC ACAGGGGTTTGGGAGTTGTAAACTCGGTAATGATCCCTCCGCTGGTTCACCAACG GAGACCTTGTTACGACTTTTACTTCCTCTAAA
MMsC	ATCGAGGTCACATTCAGAAGTTGGGGTTTAACGGCGTGGCCGCGACGATACCAGT AACGAGGGTTTTACTACTACGCTATGGAAGCTCGACGTGACCGCCAATCAATTTG AGGAACGCGAATTAACGCGAGTCCCAACACCAAGCTGTGCTTGAGGGTTGAAATG ACGCTCGAACAGGCATGCCCGCCAGAATACTGGCGGGCGCAATGTGCGTTCAAAG ATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTTGCTGCGT TCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTGATTTATTTAT GGTTTTACTCAGAAGTTACATATAGAAACAGAGTTTAGGGGTCCTCTGGCGGGCC GTCCCGTTTTACCGGGAGCGGGCTGATCCGCCGAGGCAACAAGTGGTATGTTCAC AGGGGTTTGGGAGTTGTAAACTCGGTAATGATCCCTCCGCTGGTTCACCAACGGA GACCTTGTTACGACTTTTACTTCCTCTA
BGT	ATCCGAGGTCACATTCAGAAGTTGGGGTTTAACGGCGTGGCCGCGACGATACCAG TAACGAGGGTTTTACTACTACGCTATGGAAGCTCGACGTGACCGCCAATCAATTT GAGGAACGCGAATTAACGCGAGTCCCAACACCAAGCTGTGCTTGAGGGTTGAAAT GACGCTCGAACAGGCATGCCCGCCAGAATACTGGCGGGCGCAATGTGCGTTCAAA GATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTTGCTGCG TTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGATTTGATTTATTTA TGGTTTTACTCAGAAGTTACATATAGAAACAGAGTTTAGGGGTCCTCTGGCGGGC CGTCCCGTTTTACCGGGAGCGGGCTGATCCGCCGAGGCAACAAGTGGTATGTTCA CAGGGGTTTGGGAGTTGTAAACTCGGTAATGATCCCTCCGCTGGTTCACCAACGG AGACCTTGTTAC

Isolates' Designation	DNA Sequence (FASTA Format)
BGP	ATCCGAGGTCACATTCAGAAGTTGGGGTTTAACGGCGTGGCCGCGACGATTACCA GTAACGAGGGTTTTACTACTACGCTATGGAAGCTCGACGTGACCGCCAATCAATT TGAGGAACGCGAATTAACGCGAGTCCCAACACCAAGCTGTGCTTGAGGGTTGAAA TGACGCTCGAACAGGCATGCCCGCCAGAATACTGGCGGGCGCAATGTGCGTTCAA AGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTTGCTGC GTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTGATTTATTT ATGGTTTTACTCAGAAGTTACATATAGAAACAGAGTTTAGGGGTCCTCTGGCGGG CCGTCCCGTTTTACCGGGAGCGGGCTGATCCGCCGAGGCAACAAGTGGTATGTTC ACAGGGGTTTGGGAGTTGTAAACTCGGTAATGATCCCTCCGCTGGTTCACCAACG GAGACCTTGTTACGACTTTTACTTCCTCTAATTGACCAAG
JJhB	ATCTACTGATCCGAGGTCACATTCAGAAGTTGGGGTTTAACGGCGTGGCCGCGAC GATACCAGTAACGAGGGTTTTACTACTACGCTATGGAAGCTCGACGTGACCGCCA ATCAATTTGAGGAACGCGAATTAACGCGAGTCAACACCAAGCTGTGCTTGAGGGT TGAAATGACGCTCGAACAGGCATGCCCGCCAGAATACTGGCGGGCGCAATGTGCG TTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTT GCTGCGTTCTTCATCGATGCCAGAACCAAGAGATTTATTTATGGTTTTACTCAGA AGTTACATATAGAAACAGAGTTTAGGGGTCCTCTGGCGGGCCGTCCCGTTTTACC GGGAGCGGGCTGATCCGCCGAGGCAACAAGTGGTATGTTCACAGGGGTTTGGGAG TTGTAAACTCGGTAATGATCCCTCCGCTGGTTCACCAACGGAGACCTTGTTACGA CTTTTACTTCC
MeGP	GGTCACATTCAGAAGTTGGGTGTTTTACGGCGTGGCCGCGCCGCTCTCCAGTTGC GAGGTGTTAGCTACTACGCAATGGAAGCTGCGGCGGGACCGCCACTGTATTTGGG GGACGGCGTTGTGCCCGCAGGGGGCTTCCGCCGATCCCCAACGCCAGGCCCGGGG GCCTGAGGGTTGTAATGACGCTCGAACAGGCATGCCCGCCAGAATACTGGCGGGC GCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTT ATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAGCCAAGAGATCCGTTGTTGAA AGTTTTAATTTATTTGCTTGTTTACTCAGAAGAAACATTATAGAAACAGAGTTAG GGGGTCCTCTGGCGGGGGCGGCCCGTGTTACGGGGCCGTCTGTTCCCGCCGAGGC AACGTTATAGGTATGTTCACAGGGTTGATGAGTTGTATAACTCGGTAATGATCCC TCCGCTGGTTCACCAACGGAGACCTTGTTACGA
PBC	CATCTACTGATCCGAGGTCAACATTCAGAAGTTGGGGGTTTAACGGCTTGGCCGC GCCGCGTTCCAGTTGCGAGGGTTTTACTACTACGCAATGGAGGCTGCAGCGAGAC CGCCACTAGATTTCGGGGCCGGCTTGCCGCAAGGGCTCGCCGATCCCCAACACCA AACCCGAGGGCTTGAGGGTTGAAATGACGCTCGAACAGGCATGCCCGCCAGAATA CTGGCGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTC ACATTACTTATCGCATTTTGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCC GTTGTTGAAAGTTTTGATTTATTTATGGTTTTACTCAGAAGTTACATATAGAAAC AGAGTTTAGGGGTCCTCTGGCGGGCCGTCCCGTTTTACCGGGAGCGGGCTGATCC GCCGAGGCAACAAGTGGTATGTTCACAGGGGTTTGGGAGTTGTAAACTCGGTA

Appendix V. Field visit for sample collection

Appendix VI. View of pot preparation for pathogenicity test