MOLECULAR CHARACTERIZATION OF ALTERNARIA SPECIES AND INTEGRATED MANAGEMENT OF GREY BLIGHT DISEASE OF MUSTARD

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December, 2021

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Registration No.: 19- 10138

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

DOCTOR OF PHILOSOPHY
IN
PLANT PATHOLOGY

SEMESTER: JULY - DECEMBER, 2021

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BIOGRAPHICAL SKETCH

The author was born in the district of Jashore, Bangladesh on 1st January 1977. He is the only son of Mr. Md. Anisur Rhaman and late Rokeya Sultana.

He passed Secondary School Certificate (SSC) and Higher Secondary Certificate (HSC) from Jashore Education Board, Bangladesh in 1992 and 1994, respectively. He obtained the first division in both the examination. He also passed B.Sc. (Hons.) in 1998 (Examination held in 2001) and got First Class. He passed MS in Plant Pathology in 2003 from Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh funded by PETRRA Project under Bangladesh Rice Research Institute (BRRI), Bangladesh through IRRI (International Rice Research Institute), Philippines and obtained First class (GPA of 3.41 out of 4.0). Already he published 6 (Six) articles in the International Journal based on research work. He also visited India, Nepal and Thailand for academic purpose.

1n 2019, he was awarded NST (National Science and Technology) Fellowship under the Ministry of Science and Technology, Government of People's Republic of Bangladesh to pursue PhD Program in Plant Pathology at Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh. He successfully completed 24 credit hours course work and 2 credit hours' seminar securing the GPA of 4.00 out of 4.0. He joined in Bangladesh Civil Service (Agriculture), 27th batch as Agriculture Extension Officer in the Department of Agricultural Extension (DAE), Ministry of Agriculture on 30 November 2008. At present he is working as an Additional Deputy Director (ADD) at Department of Agricultural Extension (DAE), Khamarbari, Farmgate, Dhaka, Bangladesh. He is happily married to Razia Kabir, Lecturer (Economics), 33th batch in BCS (General Education), Govt. M.M. City College, Khulna, Bangladesh and blessed with one lovely daughter Fattahiya Zannat Kabir (Alif) and two sons Saifan Arham Kabir and Saiham Aiyan Kabir.

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CERTIFICATE

This is to certify that the thesis entitled, " **MOLECULAR CHARACTERIZATION OF ALTERNARIA SPECIES AND INTEGRATED MANAGEMENT OF GREY BLIGHT DISEASE OF MUSTARD**'' submitted to the Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY IN PLANT PATHOLOGY,** embodies the result of a piece of bona fide research work carried out by **Md. Humayun Kabir**, bearing Registration no: 19-10138 under my supervision and guidance. No part of the thesis has been submitted anywhere for any other degree or diploma.

I further certify that such help or sources of information, as have been availed of during the program of this investigation have been duly acknowledged.

Dated:
Place: Dhaka, Bangladesh

Prof. Dr. Md. Belal Hossain
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Dedicated to
My Beloved Parents

ACKNOWLEDGEMENT

All the compliments and appreciation to the Omnipotent, Omniscient, Ubiquitous Almighty 'Allah Rabbul Al-Amin,' the utmost ruler of the universe who enabled the author to pursue his higher study and to complete the research work as well as to submit the thesis for the degree of Doctor of Philosophy (PhD) in Plant Pathology from Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.

The author unaffectedly desires to express his earnest sense of gratefulness, respect, deep indebtedness and gratitude to his research Supervisor and Chairman of Advisory Committee, Dr. Md. Belal Hossain , Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for his benevolent and academic guidance, tireless exertion, valuable suggestions, stimulation, co-operation and constructive criticisms throughout the ample period of research work and the preparation of the manuscript.

The author expresses deepest gratitude and thankfulness to the Member of the Advisory Committee, Professor Dr. Md. Rafiqul Islam, Professor Dr. F. M. Aminuzzaman, Department of Plant Pathology, SAU, Dhaka and Prof. Dr. Md. Jamilur Rahman, Department of Genetics and Plant Breeding, SAU, Dhaka for their valuable guidance, instructions, motivations and cordial co-operation to complete the courses and the research work successful.

Special thanks and acknowledgment to the Chairman and all the respective teachers of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for their educational teaching, compassionate co-operation and stimulation throughout the period of the study.

The author fortuitously remembers the students of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka especially Yobaida Afrin Ara (Eti) and Ariful Islam, MS student of Plant Pathology department for their support in the entire period of study. The author also disburses his thanks to all the staffs of the

Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for their support during the exploration work.

The author loves to give thanks to all of his friends especially Dr. Borhan Ahmed (SSO), Bangladesh Jute Research Institute (BJRI), Dhaka and Dr. Md. Habibur Rahman (PhD Fellow) for their holistic and mental support throughout his study period in Sher-e-Bangla Agricultural University, Dhaka.

The author also likes to express his heart full gratitude to the authority of Bangladesh Agricultural Research Institute (BARI), Gazipur for giving the opportunity to use Plant Pathology Laboratory under Plant Pathology Division to purse throughout his study period and specially thanks to Dr. Md. Bazlur Rashid (SSO), Dr. Moinul Hossain (LAB In - charge) and Mr. Abul Kalam Azad, lab assistant of the Division for their cordial cooperation in the study period.

The author would like to acknowledge the Ministry of Science and Technology, Government People's Republic of Bangladesh for funding the entire research project through NST fellowship to complete the work fruitfully.

The author prays for his mother who sacrificed comfort in her life, nurtured the development and motivated and prayed for every goodness in life of her children but she passed away during the Ph. D program and his father who unconditionally supported every academic and learning endeavor for contribution in natural sciences. The care, support, and inspiration from his sister for keeping a balance between family and work. Especially, the author expresses his gratefulness to his wife Razia Kabir for being the one to motivate him for the drive. The author expresses his thanks to his relatives whose blessings and constant inspiration helped him to complete this study successfully.

The author likes to express his thankfulness to the authority of the Ministry of Agriculture (MOA), Government People's Republic of Bangladesh for giving the opportunity to pursue higher education by

granting deputation for the Ph. D program in Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.

Finally, the author grateful to his roommates and colleagues as well as the authority of the Department of Agricultural Extension for their support and inspiration throughout his study period. They were constant source of encouragement, fervor and enthusiasm in the critical moment of his studies.

July 2022 *The author*

MOLECULAR CHARACTERIZATION OF ALTERNARIA SPECIES AND INTEGRATED MANAGEMENT OF GREY BLIGHT DISEASE OF MUSTARD

ABSTRACT

Mustard (*Brassica* spp.) is the most important oil seed crop in Bangladesh and the major constraint is grey blight disease which caused significant yield losses. The prime aim of the study was to characterize the Alternaria species on the basis of morpho-molecular variation and integrated management of the grey blight disease. The present study was carried out in combination of six sequential experiments. The experiments were conducted in Molecular Biology and Plant Virology Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka and Plant Pathology Laboratory, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur. For characterization study, fungal DNA was extracted from the pure culture of *Alternaria* and PCR amplification was done using the ITS primer which designed to amplify at 700 bp. For the management study to evaluate the effectiveness of selected bio-agent, chemical fungicides, botanicals and their combined effects were assessed. In total 14 treatments including control were considered *viz.* T_1 = Control, T_2 = *Trichoderma harzianum* suspension, T_3 = Autostin 50 WDG, T_4 = Rovral 50 WP, T_5 = Dithane M-45, T_6 = Amistar Top 325 SC, T_7 = Neem leaf extract, T_8 = Allamanda leaf extract, T_9 = Lantana leaf extract, T_{10} = Datura leaf extract, T_{11}= Neem leaf extract+ Rovral, T_{12} = Datura leaf extract + Amistar Top, T_{13} = Lantana leaf extract + Dithane M- 45 and T_{14} = Allamanda leaf extract + Autostin. From the morphological characterization of *Alternaria* species, it was found that the highest radial mycelial growth (43 mm) was recorded in Manikganj isolate and the lowest (18.13 mm) in Satkhira isolate at 14 DAI and others morphological characters were varied significantly. All *Alternaria* isolate was found to be pathogenic in nature that confirmed in the pathogenicity test. From the molecular characterization study, the nucleotide sequences of *Alternaria* isolates were showed 99% similarity with the existing nucleotide sequences and closely related to isolates of *Alternaria brassicae, Alternaria brassicicola and Alternaria alternata* that globally found in GenBank data based. Among the treatments, there was no radial mycelial growth of *Alternaria* was recorded in T_4 (Rovral 50 WP), T_6 (Dithane M-45) and T_{11} (Neem leaf extract+ Rovral) at all observations. From the results of field management study, it was observed that all the treatments showed significant effect on different assayed parameters. In case of botanicals, the lowest disease incidence (%), disease severity (%), disease severity index (%) and pod infection (%) was found in T_9 treatment (Lantana leaf extract) which was 65.05%, 28.89%, 37.96% at 70 DAS respectively and 13.79%, at 75 DAS. In case of chemicals, the lowest disease incidence (%), disease severity (%), disease severity index (%) and pod infection (%) was found in T_5 treatment (Dithane M-45) which was 55.91%, 22.09%, 32.16% at 70 DAS, respectively and 6.72% at 75 DAS. In case of combined treatments, the lowest disease incidence (%), disease severity (%), disease severity index (%) and pod infection (%) was found in T_{13} treatment (Lantana leaf extract + Dithane M-45) which was 59.14%, 26.32%, 38.20% at 70 DAS, respectively and 12.30% at75 DAS. From the findings on different parameters studied in both management experiments, Dithane M-45, Lantana leaf extract and their combined treatment could be used in further field trials and then may be recommended for management of grey blight mustard.

CONTENTS

LIST OF ABBREVIATIONS

Abbreviation	Full name
%	Percent
@	At the rate of
˚C	Degree Celsius
µm	Micrometer
ANOVA	stands for Analysis of Variance
BADC	Bangladesh Agricultural Development Corporation
BARI	Bangladesh Agricultural Research Institute
CDA	Carrot Dextrose Agar
CRD	Complete Randomized Design
CV%	Co-efficient of Variance
DAE	Department of Agricultural Extension
DAI	Days After Inoculation
DAS	Days After Sowing
DHA	Dhaka
DIN	Dinajpur
DMRT	Duncan's Multiple Range Test
DNA	Deoxyribonucleic Acid
DSI	Disease Severity Index
EC	Emulsifiable Concentrate
et al.	And others
etc	Etcetera
FYM	Farmyard Manure
J.	Journal no
JAS	Jashore
KHU	Khulna
LSD	Least Significant Difference
MAN	Manikganj
ml	Milliliter
mm	Millimeter
MOA	Ministry of Agriculture
NCBI	National Center for Biotechnology Information
NST	National Science and Technology
PCDA	Potato Carrot Dextrose Agar
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
RCBD	Randomized Complete Block Design
RNA	Ribonucleic acid
SAT	Satkhira
SAU	Sher-e-Bangla Agricultural University

SDG	*Sustainable Development Goals*
SIR	Sirajganj
spp.	Species
TAN	Tangail
WDG	Water Dispersible Granule
WP	Wettable Powder

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CHAPTER –I

INTRODUCTION

INTRODUCTION

Mustard (*Brassica* spp.) belongs to the family Brassicaceae (or Cruciferae) is an important oil crop. It is second most important edible oil next to Soybean in the world. It is cultivated almost all over the world. China is the largest producer of mustard in the world. China, India and Pakistan grow about 90% of the world mustard production. The average production of the world is 0.87 ton/ha (FAO, 2020), whereas in Bangladesh is 0.5 ton/ha. Mustard is one of the major oil seed crops in Bangladesh which is widely cultivated during the winter season from the month of October to February and its contribution in total oil seed production is approximately 70%. Among the oilseed brassicas, yellow var., yellow brown var., brown toria var., Indian mustard Karan rai and oilseed rape are grown for edible oil, whereas black mustard is used as condiment and for pickle making, food preserving and spices to improve flavour, also as fodder for live-stocks. The leaves of young plants are used in the human diet as green vegetable. Mustard seed and oil has multiple uses in health care system. It improves the body complexion because of its antifungal property. It is used as a very good massage oil, which brings vitality and strength to the body and improves the circulatory system and cures bodyache. It also kills various microbes and thus, keeps skin infections away. Oral doses of oil help in strengthening the teeth and cure various mouth related diseases. It helps in healing wounds by stopping the pus formation and in curing various skin disorders by removing unwanted fluids from the body (Kumar and Chauhan, 2005). Erucic acid and glucosinolate are the two major deterrents of oil and seed meal in oilseed brassica, respectively (Singh *et al.*, 2013). The oilseed brassicas usually contain 38-57% of erucic acid, 4.7-13% linolenic acid and 27% of oleic and linoleic acid, which are of high nutritive value required for human health (Singh *et al.*, 2012). Mustard oil of plant origin constitute important component of human diet, ranking third after cereals and animal products and are nutritionally superior to animal oil (Singh *et al.*, 2002). Rapeseed and mustard are rich source of oil and contains 44% to 46% good quality oil (Rashid, 2013). The different varieties of mustard seed contain 40-44% oil and mustard oil cake contains 40% protein (Chowdhury and Hassan, 2013).

Oil cake is a nutritious food items for cattle and fish. It is also a good organic fertilizer for crops. Dry mustard plants may be used as fuel. Mustard seeds contain 40-45% oil and 20-25% protein. Using local ghani average 33% oil may be extracted. The crop is well adapted to almost all agro-climatic zones of the country. It is grown in tropical as well as temperate agro-climatic zones and well adapted to areas having a relatively cool, moist climate during the growing season. (Kumar *et al.,* 2014). It is a cold loving crop that is mainly cultivated in Rabi season in Bangladesh. The weather condition suitable for this crop is low temperature and scanty rainfall. At present about 0.24 million hectares of land are used to mustard cultivation in Bangladesh with yield of mustard oil in the order of 0.19 million MT per year (BBS, 2020). Yield of mustard is very low in Bangladesh in comparison to other countries. Total production and per hectare seed yield of this crop may be increased by using high yielding variety (HYV) and improved production technologies. Out of 64 districts, Chittagong, Sylhet, Dhaka, Tangail, Jashore, Bogra, Sirajganj and Pabna have comparatively higher acreage of land for cultivation of this Rabi crop but area coverage for the production of mustard is very low in Rangamati region. Its yielding is 36.83% of total oilseed production from 64.6% of the total area coverage (BBS, 2020).

Biotic and abiotic factors are responsible with the poor yield of mustard in Bangladesh. Among the biotic factors, diseases have been identified as one of the major factors. Mustard suffers from about 14 diseases (fungus 9, virus 2, bacteria 1, nematode 1 and parasitic plant 1) in Bangladesh (Bakr *et al.,* 2009). Among these diseases grey leaf blight, downy mildew and the parasitic plant are the most important (Anonymous, 2007). Of these disease, Alternaria leaf blight caused by *Alternaria* species is one of the major diseases of mustard (Meena *et al.,* 2016, Selvamani *et al.,* 2014, Jha *et al.,* 2013, Aneja and Agnihotri, 2013). Alternaria blight caused by *Alternaria brassicae* has been reported to inflict heavy yield losses to the tune of 35-60% in mustard crop (Karthikeyan *et al.,* 2021). This disease reduces mustard yield upto 47% in India (Sharma, 2009) and 30-60% in Bangladesh. It is a prominant disease in India, Australia, Canada, Africa, England, Germany, France, SriLanka, Spain and Sweden. (Ghasemi *et al.,* 2013).

Alternaria leaf blight caused by *A. brassicae and A. brassicicola* is a devastating seed brone disease of mustard. Grey leaf blight caused by *Alternaria sp* is widely distributed and the most serious and devastating disease of rapeseed-mustard (Fakir, 2008). Most of the *Alternaria* species are saprophytes and ubiquitous in the environment (Simmons, 1992). The pathogens are greatly influenced by weather with the highest disease incidence reported in wet seasons and areas with relatively heavy rainfall (Meena *et al*., 2010).

Symptoms are appeared on lower leaves with appearance of black point at first, at later the point enlarge to develop into prominent, round, concentric spots of various sizes. Later on, siliqua and stem round black conspicuous spots appear. These spots may coalesce resulting complete blacking of silliqua or weakling of the stem with formation of elongated lesions (Meena *et al.,* 2010). Spors are produced in chains or in branching fashions which are multicellular pigmented. The spores are broadest near the base and elongate beak taper gradually. Around the initial site of host leaf Alternaria morphologically produces a series of concentric rings (Anju *et al.,* 2013). The pathogen infects all aerial plant parts, reducing photosynthetic area and accelerating senescence and defoliation. *Alternaria* species can affect plant species in all growth stages, including seed. At seedling stages the disease is characterized by dark stem lesions just after germination that leads damping off, or stunted seedlings. The symptoms may vary with host and environment. *Alternaria* species is a necrotrophic pathogen produce lesion on leaves, stem and siliquae which affect seed quantity, quality by reducing oil content, seed size and seed colour (Duczek *et al.,* 1999). This disease may cause significant losses in both temperate and tropical Brassica crops (Mathpal *et al.*, 2011) The pathogen remained viable in diseased plant debris and seeds of infected plants (Ansari., 1989). The disease may cause 25% yield reduction at severe condition of infection (Anonymous, 2001). Grey blight causing yield loss which is approximately 30-40% in Bangladesh. The yield losses of mustard due to grey blight disease affect the market price of edible oil in the country. The market price closely depends on the local oilseed production.

There are many practices used for the controlling of grey blight diseases of mustard like cultural method, applying biological agent, botanicals, chemicals as well as integrated disease management (IDM). Date of planting or sowing is an important factor for incidence and severity of crop diseases. There are reports that *Alternaria* blight of mustard can be avoided to a large extent by adjusting sowing time (Ayub, 2001). Protection of seedpod from *Alternaria* blight infection should, therefore, be the most important aim for higher yield of mustard. The most economical and environmentally safe method of controlling the disease is use of resistant varieties. Information on resistance source is not available, although some sort of tolerance may be available. Foliar spray of *Trichoderma harzianum* against Alternaria leaf blight of mustard most effective treatment have shown resulting increasing the yield. The maximum plant height (cm) was recorded at *Trichoderma harzianum* (foliar spray) @ 2% (Yogita *et al.*,2017). The botanicals namely Neem, Eucalyptus, Datura, Pudina, Tulsi, Lantana were evaluated under crude and boiled forms against *A. brassicae* under *in- vitro* condition (Sasode *et al.*, 2012). Chemicals are being successfully used in controlling the disease (Meah *et al.,* 1988 and Howlider *et al*., 1985). Non-chemical methods of disease control may include use of biological agents, botanicals, adjustment in cultural practices etc. But using excessive chemicals is discouraged as it is harmful for the environment and the residual effect can cause decay to human health. Non-chemical methods of disease control may include use of biological agents, botanicals, adjustment in cultural practices etc. Different medicinal leaf extract which has antifungal effect such as neem, alamanda, lantana, datura etc can be used as botanicals and *Trichoderma harzianum* can be used as biological agent. They can be successfully applied as an ecofriendly alternative of chemicals for the management of grey blight of mustard. Using the botanicals as foliar spray and biological agent as soil treatment can successfully control the pathogen attack. Six fungicides *e.g.*, Dithane M-45 (0.2%), Dithane Z-78 (0.2%), Iprodione (Rovral) (0.2%), Blitox 50 (0.3%), Baycor (0.2%) and Mancozeb (64%) been applied to be effective against the spread of against *Alternaria* under different field conditions (Das R. 2015). But chemeical treatment is costly and not

ecofriendly. Four fungicides viz. Rovral 50 WP (0.2%), Dithane M-45 (0.3%), Ridomil 68 WP (0.2%), Bavistin DF (00.15) and two plant extracts viz. garlic clove extract, allamanda leaf extract were used in the field experiment. Among the fungicides and plant extracts tested, Rovral WP (0.2%) gave the best performance in reducing disease incidence and disease severity as well as increasing seed yield against grey blight of mustard. Seed infection by *Alternaria* spp. was reduced by 64.90% and seed yield was increased by 48.19% over control by the application of Rovral 50 WP (Alam, 2007). So, the combined treatment of chemicals and botanicals also shows satisfying result which also reduces the use of chemicals only in case of grey blight disease management in mustard.

The major aspects of biology of an organism is the cultural, morphological and physiological characters of an individual within a species. Although it is not frequent in asexually produced individuals of the progeny. Variability studies are important to document the changes occurring in populations and individuals as variability in morphological and physiological traits indicate the existence of different pathotypes (Meena *et al.*, 2016). Anamorph form of this pathogen shows great variability in morphology, physiology and pathogenicity. Several researchers have reported existence of variability based on morphology, sporulation, growth and cultural characteristics. Although, studies on pathogenic variability are important for the development of pre-breeding populations (Meena *et al.*, 2010). However, informations are lacking regarding differences in aggressiveness/virulence in the pathogenic population isolated from different Brassica species. Proper understanding in the variation of pathogen population is highly crucial in the process of breeding for resistance against a particular disease. Considering the above fact this research was undertaken to find out the morphological and Molecular variation among different isolates of *Alternaria* spp.

The prime aim of the present study was to find out the molecular variation among the isolates of *Alternria*. The disease intensity of mustard in selected survey areas and to evaluate the efficacy of selected botanical, chemical and their combined effect to manage grey blight disease of mustard caused by *Alternaria* spp.

Considering the above facts this research were undertaken with the following specific objectives.

- To assess the infection level of grey blight disease of mustard in the different selected areas of Bangladesh,
- To characterize *Alternaria* isolates associated with grey blight disease of mustard on the basis of morphological and molecular variation, and
- To formulate approaches for controlling the grey blight disease of mustard.

CHAPTER -II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Grey blight disease of Mustard caused by *Alternaria* spp. is an important and most devastating disease in the world including Bangladesh. Severe yield loss was occurred due to this disease of Mustard. Researchers all over the world have carried out intensive investigation on the grey blight of mustard. Literature in relation to disease intensity, severity, yield loss, morphological, molecular characterization and integrated management of grey blight of mustard is reviewed and presented in this chapter.

2.1. Oil seeds crop

Mustard (*Brassica* spp.) is the major oil seed crop of Bangladesh yielding 36.83% of total oilseed production from 64.6% of the total area coverage. At present about 0.31 million hectares of land are covered to mustard cultivation in Bangladesh with yield of mustard seed in order of 0.35 million tons per year (BBS, 2020).

Mustard is cultivated almost all over the world. Among these, China is the largest producer of Mustard in the world. China, India and Pakistan grow about 90% of the world production of mustard. The average production of the world is 0.87 ton/ha (FAO, 2020), whereas in Bangladesh is very low. i.e. 0.5 ton/ha.

Mustard, however, it is production is mostly restricted by drought, which can become critical in climate change (EL Sabagh *et al.,* 2019; Islam *et al.,* 2019).

Mustard (*Brassica* spp.) is one of the important edible oilseed crop across the worldwide including Bangladesh. Other than the use of mustard oil for industrial and edible purposes, its cake is a nutritious feed for cattle because of up to 40% protein (Wanasundara *et al.,* 2016; Rahman *et al.,* 2018).

Ajala and Adelcke (2014) reported that Oilseed crops have occupied an important place in human nutrition as they remain the major sources of calories and proteins for a large proportion of the world population. Oilseeds are those seeds that contain considerably large amounts of oil. The most commonly known oilseeds

(conventional oil seeds) are groundnut, soybean, palm kernel, cotton seed, olive, sunflower seed. rapesecd. sesame seed. linseed. safflower seed etc.

Kumar (2014) conducted an experiment where edible oilseed plants are those whose seeds bear fixed nonvolatile oil and oilseed crops are grown primarily for the oil contained in the seeds. The oil content of small grains (e.g., wheat) is only 1%–2%, and that of oilseeds ranges from about 20% for soybeans to over 40% for sunflowers and canola rapeseed. Crops like rapeseed–mustard, peanut, and sunflower have oil recovery ratio of 45%, 40%, and 30%, respectively, whereas cottonseed and soybean have oil recovery ratio of 11.5% and 17% only.

Kumar *et al.* (2014) estimated that the oilseed brassicas usually contain 38-57 per cent of erucic acid, 4.7-13 per cent of linolenic acid and 27 per cent of oleic acid and linoleic acids, which are of high nutritive value required for human health.

Kumar *et al.* (2014) reported that mustard is cultivated almost all over the world. It is grown in tropical as well as temperate agroclimatic zones and is best adapted to areas having a relatively cool, moist climate during the growing season.

Aminpanah *et al.* (2013) analyzed that Rapeseed- mustard contains ample amount of unsaturated fatty acid i.e. oleic acid (8-40 %), omega-6 fatty acid (lenolenic acid) (5-10 %), omega-3 fatty acid (linoleic acid) (10-29 %), eicosinoic acid (5-12 %), erucic acid (40-55 %) and the low concentration of saturated fatty acid (7%).

Chowdhury and Hassan (2013) found that the different varieties of mustard seed contain 40- 44% oil and mustard oil cake contains 40% protein.

Rapeseed-mustard is the third most important oilseed commodity in the world after soybean (*Glycine max*) and palm (*Elaeis guineensis* Jacq) in world agriculture and India is the third largestproducer with global contribution of 28.3 per cent acreage and 19.8 per cent production (Shekhawat *et al.*, 2012; Bandopadhyay *et al.*, 2013).

Rashid (2013) conducted an experiment where Rapeseed and mustard are rich source of oil and contains 44% to 46% good quality oil.

Arif *et al.* (2012) carried out an experiment to evaluate potential nutrients of oil seeds and tile observed highest amount of crude oil in *B. juncea* (Purr-e-NIFA) and B. *napus* (NIFA Raya) i.e. 45.67% and 43.87% respectively.

Salisbury and Barbetti (2011) stated that the Brassicaceae family contains a large number of crops including oilseed rape (*Brassica napus* and *B. rapa*, *B. campestris*) and oilseed Mustard including Indian Mustard (*B. juncia*), Ethiopian Mustard and brown Mustard (*B. nigra*).

Choi *et al*. (2007) reported that mustard possesses high levels of plant sterols, like β-sitosterol and campesterols which competitively inhibit cholesterol absorption and reduces cholesterol level by 10% to 15%. It is rich in natural antioxidants and Vitamin A, C and E.

Schranz *et al.* (2007) reported that the oilseed Brassicas include *Brassica napus, Brassica juncea, Brassica carinata* and *Brassica campestris (or Brassica rapa)* is also used as condiment.

Agnihotri A and Kumar S (2004) reported that Rapeseed-mustard contains protein (20-25 %), carbohydrate (14-16 %), fiber (10-15 %), moisture (6-8 %), mineral (3-4 %), vitamins (0.7-0.9 %) glucosinolate (2-3 %), phytic acid (3-6 %), sinapine (1-1.5 %) and tannin (1.6-3.1 %) .

Anita and Gowthaman (2003) studied that rapeseed - mustard which is an important oilseed crop among different constraints in the production of mustard, diseases are the most important limiting factors which restrict the cultivation and decrease the productivity of these crops. In Indian context, fungal diseases are rated as one of the most important factor contributing to yield losses in oilseed crops.

Rahman (2002) studied that Brassica oil crop is the most important group that supplies major edible oil in Bangladesh. Bangladesh is running a short of 60-75% of the demand of edible oil.

Singh *et al.* (2002) reported that Rapeseed/Mustard oil of plant origin constitute important component of human diet, ranking third after cereals and animal products and are nutritionally superior to animal oil.

A good number of oilseed crops like mustard, sesame, groundnut, linseed, niger, safflower, sunflower and soybean are being cultivated in Bangladesh. Among the oilcrop seeds, the first three are considered as the major oilcrops. From our internal production, one-third of the total requirement can be met up. The shortfall is met up by import at the cost of about US $160 million per year (Anonymous, 2001).

2.2. Disease of mustard

Karthikeyan *et al.* (2021) reported that *Alternaria* blight caused by *Alternaria brassicae* has been reported to inflict heavy yield losses to the tune of 35-60% in mustard crop.

Krishan *et al*. (2017) described among all the infectious fungal diseases affecting crucifers (on the basis of yield losses and wide distribution) *Alternaria* blight, white rust, sclerotinia stem rot, downy mildew and powdery mildew are considered to be most devastating.

Krishan *et al.* (2017) found that damping-off is caused by the fungus *Rhizoctonia solani. Besides R. solani* other fungi like *Pythium spp*., *Phytophthora spp*., *Fusarium spp*., *Alternaria spp*. and *Leptosphaeria maculans* are also responsible for causing damping-off in brassicas. All these pathogens are grouped together and are common residents of soil therefore, symptoms and preventive measures are also similar for all of them. Damping off fungi have a wide range of host and they can even survive on alternative non-brassica hosts.

S. sclerotiorum causing *Sclerotinia* stem rot or white stem rot in rapeseed-mustard is worldwide in distribution and is pathogenic to more than 400 plant species and more than 500 plant species (Sharma *et al.*, 2015) at various developmental stages of plants.

Kumar *et al.* (2014) reported that the pathogens have a wide spectrum of hosts including almost all crucifers. Host specificity varies in different species of *Alternaria* on *Brassica* species. *Alternaria* pathogens are soil-borne, airborne and seed-borne.

Meena *et al.* (2014) described that Sclerotinia stem rot in rapeseed mustard starts as elongated, water-soaked lesions on stem especially at base or at internodes and later white mycelial growth covers these lesions and affected plants look whitish from distance. The disease becomes air borne and spread through infected flower petals which fall and become lodged between the main stem and side branches. Large oval to round shaped holes are also formed on leaves due to air borne infection. Under severe infection, defoliation, shredding of stem, wilting and drying of plants occurs. Infected plants will ripe earlier and stand out among green plants.

It is thus evident that continuous increasing trend of charcoal rot is alarming for farmers and authorities in sunflower business not only in Pakistan (Khan *et al.*, 2003) but also in neighboring Iran Rafiei *et al.* (2013).

Khatun *et al.* (2011) reported that *Alternaria* blight of mustard has been recognized as the most serious and devastating disease in this country.

Meena *et al.* (2010) studied that more than thirty diseases are known to occur on *Brassica* crops in India. These include Alternaria blight, white rust, downy mildew, powdery mildew etc. Among these, Alternaria blight caused by *Alternaria brassicae* (Berk.) Sacc. has been reported from all the continents of the world affecting most cruciferous crops and is one among the important diseases of rapeseed-mustard causing severe yield losses with no proven source of transferable resistance in any of the hosts.

Bakr *et al.* (2009) conducted an experiment where mustard plant suffers from about 14 diseases (fungus-9, virus-2, bacteria-1, nematode-1 and parasitic plant-1) in Bangladesh.

Choi *et al.* (2009) described that the most widely recognized fungal species, *Albugo candida* (Pers.) Roussel, had been thought to be the exclusive white rust pathogen of the Brassicaceae, infecting as many as 63 genera and 241 plant species.

Kirk (2008) reported that *Alternaria* leaf spot and blight in *Brassica* plants are mostly caused by *Alternaria* species (*Alternaria brassicae*, *Alternaria brassicicola*, *Alternaria raphani*, *Alternaria alternata*). Almost 299 species are listed in this genus.

Kohire *et al.* (2008a) observed 40% yield loss due to powdery mildew in Indian mustard. Considering the differences in disease intensity from year to year, it appears that the loss is proportional to the disease intensity, which varies considerably depending on the stage at which it occurs.

Sclerotinia rot is also a serious threat to oilseed rape production with substantial yield losses worldwide including Australia, Europe, India and North America (Hind *et al.*, 2003, Koch *et al.*, 2007, Malvarez *et al.*, 2007, Singh *et al.*, 2008).

Among these diseases grey leaf blight, downy mildew and the parasitic plant are the most important for Mustard (Anonymous, 2007).

Koefet A and Fink M (2007) reported that *Alternaria* blight disease caused by *A. brassicae* (Berk) Sacc. had been reported from all the continents of the world and was one of the important disease of Indian mustard causing up to 60 per cent yield losses.

Sangeetha and Siddaramaiah (2007) described that white rust, downy mildew and *Alternaria* blight caused by *Albugo candida* (Pers.) Kuntze *Peronospora parasitica* (Pers.) (de Bary) and *Alternaria brassicicola* (Schw.) Wiltshire respectively are important diseases on Indian mustard causing considerable loss.

Eimori *et al.* (2005) stated that Bottom rot of *B. campestris* L. caused by R. solani has also been reported in Japan.

Rot of mustard caused by Sclerotinia sclerotiorum (Lib.) de Bary has become important in recent times with high (up to 66%) DI and severe yield losses (up to 39.9%) leading to discouragement of growers of the crop (Chattopadhyay *et al.*, 2003), although reports of even 100% yield loss due to the disease are available (Saharan *et al.*, 2005).

Shrestha *et al.* (2005) determined that average yield losses in the range of 32-57 per cent due to Alternaria blight have been reported from Nepal.

White rust caused by *A. candida* (Pers. ex. Fr.) Kuntz. can result in yield loss up to 47% on oilseeds *Brassica* and 89.9% in *B. juncea* (Varshney *et al.,* 2004) with each percent of disease severity and staghead formation causing reduction in seed yield of about 82 and 22 kg/ha, respectively (Meena *et al.,* 2002).

This stem rot disease of *Brassica* is intermittent as infection occurs mostly in favorable environmental conditions. In some parts of India approx. 80 percent of disease incidence have been recorded (Sharma *et al.,* 2001).

Shrestha *et al.* (2000) studied that *Alternaria brassicae* is known to cause seed infection and the infected seeds have already been shown to act as main source of recurrence of the disease in the field.

The *Alternaria* blight disease has been reported to reduce the seed and oil yields by 27%–80% and 17%–33%, respectively, in India. A negative correlation between increase in disease intensity (25%–96%) and yield components and oil content has been established (Kolte 1985, Chattopadhyay 1999).

Vanniasingham and Gilligan (1989) experimented with the increase in temperature, light intensity and leaf wetness the rates of production and maximum number of sporulating bodies produced per leaf also increases, thereby increasing the risk and severity of the infection in *Brassica.*

2.3. Symptoms of grey blight

Karthikeyan *et al.* (2021) described that typical symptoms of Alternaria blight disease are the formation of spots on leaves, stems and siliquae. The concentric black spots produced were usually gray coloured and their characters varied with host and environmental factors. Initially symptoms appeared on the lower leaves

as black points, later which enlarged to develop into prominent, round, concentric spots of various sizes. As disease progressed, the lower leaves defoliated and disease appeared on middle and upper leaves. At the later stage of the plant growth, spots appear on siliquae and stem. The spots were round black and quite conspicuous and the siliquae turn completely black. On the stem, black, elongated spots became visible in the form of black streaks with or without necrotic gray centres.

Meena *et al*. (2016) reported that Foliar diseases are one of the most important limiting factors for cultivation of oilseed *Brassica* in tropical and sub-tropical areas in India. *Alternaria brassicae* (Berk.) Sacc. causes heavy economic losses to oilseed *Brassica* (Latin name) in terms ofseed yield.

Meena *et al.* (2015) stated Alternaria Blight (AB) caused by *A. brassicae* and *A. brassicicola* is a major constraint in rapeseed-mustard production in India. To study the effect of nutrients and lower leaf removal in Indian mustard (*Brassica juncea* L.) on AB disease an experiment was conducted during 2009-10 and 2010-11. Results indicated that maximum AB reduction (26%) was observed by soil application of Potash at 40 kg haG1+Zinc sulphate at 25 kg haG1+Copper sulphate at 40 kg haG1+Sulphur at 10 kg haG1+Ridomil-MZ 72 as foliar spray at 0.25% was better when applied as foliar spray (21%).

Selvamani *et al.* (2014) described Black leaf spot caused by *A. brassicae* (Berk.) Sacc. is an important disease of crucifers. Under field conditions environmental factors influenced the progression of *Alternaria* leaf spot. There was periodical increase in lesion number and per cent disease index (PDI). Maximum and minimum temperature was positively correlated with disease development but average temperature showed high degree of correlation than the minimum (1.5-15.3˚C) and maximum temperature (10-28˚C). The laboratory study indicated that optimum conditions for spore germination were (20-24˚C) with more than 90 percent relative humidity.

Aneja *et al.* (2013) observed that in the world oilseed brassicas grown is second largest. The production of this crop is greatly hampered by the fungal diseases.

Alternaria blight being one of the most devasating fungal disease. This pseudo-fungi, by causing foliar damage to the crop leads to yield reduction and severely deteriorates the oil quality.

Anju (2013) said that Alternaria morphologically produces a series of concentric rings at the initial site of attack of host plant. It has a destructive effect on Cucarbitaceae, Brassicaceae and Solanaceae. Due to Alternaria blight 32-57% average yield loss occure.

Ghasemi *et al.* (2013) mentioned that *Alternaria* blight disease severely damages to oil-producing species of Brassica spp. all over the world and reduces the quality and quantity of the oil.

Jha *et al.* (2013) satted that *Alternaria* blight caused by *A. brassicae* is a major devastating disease of Indian mustard, causing significant reduction in seed yield. Maximum Alternaria blight severity on leaves and siliqua was observed during 12- 18 February, 2013.

Nowocki (2012) found that Alternaria pathogens usually cause black spot disease, leading to damping-off of seedlings, spotting of leaves of cabbages, blackleg of heads of cabbages, and spotting/browning of cauliflower curds and broccoli florets.

Meena *et al.* (2011) examined in India, Indian mustard attacked by various diseases viz., *Alternaria* blight (A. brassicae), white rust (*Albugo candida*), powdery mildew (*Erysiphe cruciferarum*) and Sclerotinia rot (*Sclerotinia sclerotiorum*). It 6 is a challenged for plant pathologiests to identify the ecofriendly disease management.

Mathpal *et al.* (2011) conducted an experiment that *A. brassicae* is a necrotrophic pathogen produce lesion on leaves, stem and siliquae which affect seed quantity as well as quality by reducing oil content, seed size and seed colour. This disease may cause significant losses in both temperate and tropical Brassica crops.

Kohl *et al.* (2011) described that *A. brassicicola* and *A. brassicae* (dark leaf spot) can infect leaves of Brussels sprouts resulting in yield losses. Infections of outer leaves of sprouts cause severe losses in quality. Crop residues can be a major primary inoculum source of the pathogens. *A. brassicae* population increased in stalks exposed on the soil surface. The observed variation in population sizes of the pathogens between individual pieces of crop residues indicates a stochastic spread of pathogens.

Meena *et al*. (2010) found that Alternaria blight disease caused by *A. brassicae* (Berk.) Sacc. has been reported from all the continents of the world affects most cruciferous crops and is one among the important diseases of rapeseed mustard causing severe yield losses. The pathogen is greatly influenced by weather, the highest disease incidence reported in wet seasons and in areas with relatively high rainfall. The disease is characterized by formation of spots on leaves, stem and siliqua. At seedlings dark stem lesions appear which results damping-off.

Khol *et al.* (2010) studied that infections by *A. brassicicola and A. brassicae* can cause severe losses of yield and seed quality in organic seed production of Brassica vegetables. On pod tissues development of *A. brassicicola and A. brassicae* and developing seeds was followed and seed quality was assessed. Seed colonization by the pathogen increased slowly until maturation but sharply 7 increased during maturation. They were concluded that *A. brassicicola* and A. brassicae have the potential to infect pods and seeds soon after flowering. For the production of high quality seeds, producers must prevent such early infections. Therefore, new control measures are needed for use in organic cropping systems.

Meena *et al*. (2010) carried out an experiment that symptoms of the *Alternaria* blight disease of mustard are characterized by the formation of spots on leaves, stem, and siliquae. The pathogen has been reported to affect seed germination and quality and quantity of oil.

Chauhan (2009) described that the best time for the diseased sample collection was January to April. Infected leaf samples were collected in cellophane bag.

Samples were collected from the different sites of Srinagar Garhwal and brought to the laboratory for further studies.

Fakir (2008) stated that Alternaria leaf blight caused by *A. brassicae and A. brassicaecola* is a devastating seed brone disease of mustard. The pathogen generally infects initially the leaves. From the infected leaves it spreads to stem and siliqua.

Atwal *et al*. (2005) conducted an experiment on Alternaria blight of mustard in which the phase of infection on silique adversely affects the normal seed development, weight, color, oil content, and the quality of seed. Alternaria blight–infected leaves of Indian mustard showed significant decrease in oil, triglyceride, 18:2, and 22:1 fatty acid content and also in the level of different lipid classes (phospholipids, glycolipids, and sterols).

Mehta *et al.* (2005) studied that these lesions rapidly multiply and spread to other plant parts like stems and siliques etc. The black or brown spot contains visible circular, concentric rings. The circular spots often merge to form large patches causing the leaf blight. In severe attacks plant shows damping off of seedling, wilting and rotting at foot and root like symptoms also.

Meena *et al. (*2004) conducted an experiment on Alternaria blight of Mustard, on seedlings, symptoms include dark stem lesions immediately after germination that can result in damping-off or stunted seedlings. Generally, disease appeared at 40–45 days after sowing (DAS), and most critical stages have been reported at 75 and 45 days of plant growth.

Saharan and Mehta (2002) described that Alternaria blight attacks all the green aerial parts of the plant reducing its photosynthetic area and vigour. The disease, in northern parts of India is usually seen during mid December to early January as chlorotic specks, later turning into minute dark-brown or black spots on lower leaves of young plants. On older leaves, the spots turn into circular, dark-brown, sunken necrotic lesions surrounded by light yellow halo and bear conidiophores and conidia in concentric rings, at the grayish white centre, giving them a target

board effect. Under congenial weather conditions, lesions enlarge and coalesce fast, resulting in mild to severe defoliation due to the production of senescence promoting compounds.

Duczek *et al.* (1999) reported Alternaria black spot, caused by *A. brassicae* (Berk.) Sacc, is found worldwide and is endemic in the northern canola growing areas of the Canadian prairie provinces, where it causes major yield reductions of up to 36%. This disease also reduces seed quality by increasing the green seed count, reducing seed weight, and decreasing the percent germination in harvested seed.

Bansal *et al.* (1990) reported in western Canada Alternaria leaf spot caused by A. brasscae is an economically important disease. Thirty-five cultivars/strains belonging to six *Brassica* species were evaluated for their reaction to *Alternaria brassica*e under laboratory conditions. Detached leaves were wounded and inoculated with a spore suspension, and incubated at room temperature for 4 days.

Conn (1990) stated the causal agent of Blackspot disease of rapeseed and mustard is *A. brassicae.* It is an economically important pathogen in western Canada, in several countries of Europe and Southeast Asia.

2.4. Disease-cycle

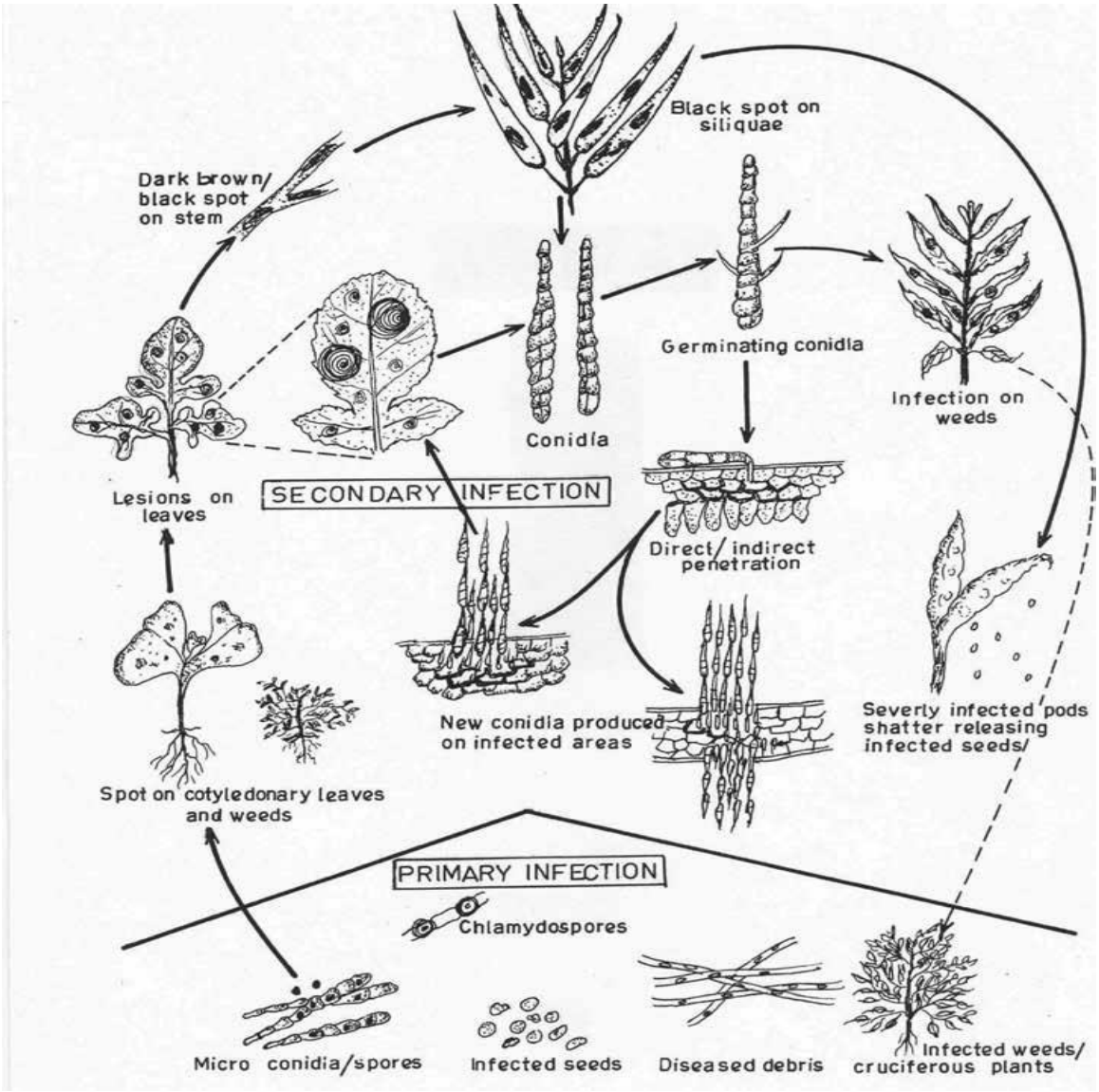


Figure 1. Disease cycle of *Alternaria* blight of rapseed –mustard (Mehta, 2020)

Recently, Goyal *et al.* (2013b) found that conidia of *A. brassicae* germinated on the upper epidermis of *B. juncea* leaf, by producing one or several germ tubes and penetrating the host directly without the formation of appressorium. The mycelia ramified, colonized mesophyll and palisade tissue caused necrosis of the cells by producing toxins or metabolites that resulted in the formation of necrotic spots and reduction in photosynthetic area of different plant parts. This infection also decreased the amount of all the cell constituents like lignin, lipids, suberin, and

protein, except phenolic compound in all the tissues of Alternaria infected *B. juncea* leaves as compared to healthy leaves.

Alternaria blight of rapeseed-mustard has been reported to be predominantly seed borne (Parajuli, 2005).

Mehta *et al*. (2002) carried out an experiment in Indian subcontinent, oilseeds Brassica are sown from late August to November depending on the crop, prevailing temperature, and availability of soil moisture for seed germination. Crop harvest occurs from February to May. Off-season crops are grown in nontraditional areas from May to September and this, coupled with harboring of the fungal pathogen by vegetable *Brassica* crops and alternative hosts (*Anagallis arvensis, Convolvulus arvensis*), could be a reason for carryover of the *A. brassicae* from one crop season to another.

Shrestha *et al*. (2000) conducted an experiment where *A. brassica*e was observed predominantly in the seed coat and rarely in embryos of *Brassica campestris* var. toria and *B. juncea.*

2.5. Pathogen and their morphological and cultural study

Shiwangi *et al.* (2019) reported of conidia and conidiophores of *Alternaria brassicae* and *A. brassisicola* on the basis of mean morphology and per cent frequency was done in fourteen entries of Indian mustard namely, NDYR32, NDRE8-16, NDRS2010, PRB2004-3, Ashirwad, NDRE8213, NDR8501, NDRE4, NDYR8, JD6, NDRE7, NDRE22, NDRE2011 and Varuna. Both the species i.e. *A. brassicae* and *A. brassicicola* were isolated from infected leaves, stems and pods. *A. brassicae* and *A. brassicicola* isolates obtained from leaves, stem and pods showed variability in colony diameter, size of conidiophores and conidia, septation in Review of Literature 14 conidiophores and conidia. On mean basis, the maximum colony diameter of 35.00 mm in NDRE8-16 and 35.33 mm in NDRE4 were noted with *A. brassiceae* and *A. brassicicola* isolates, respectively. The maximum length (160.45 mm) and width (21.48 mm) were noted in Ashirwad with conidia of *A. brassicae* as compared to *A. brassicicola*

isolate. In conidia, higher number of transverse (15.00) and longitudinal septa (4.00) and maximum per cent frequency (73.33) were also recorded in Ashirwad with *A. brassicae* while in case of conidiophores, the maximum number of septa (7.00) found in Ashirwad and NDR8501.

Kumar and Singh (2018) twenty isolates of *A. brassicae* were found from different divisions of Madhya Pradesh to identify the morphological variation. All the isolates showed high level of variability in vitro in respect of conidial length, width, beak length and number of septa. The conidial length and width ranged from 61.6 to 126.3 µm and 9.7 to 16.9 µm respectively and beak length of conidia ranged from 43.0 to 74.3 µm in different isolates of *A. brassicae.* The number of horizontal septa and vertical septa ranged from 4.6 to 7.6 and 0.3 to 2.6 respectively.

Mehra *et al.* (2017) the present investigation was carried out to study the cultural, morphological, pathogenic and molecular variability among A. *brassicae* isolates collected from different *Brassica* spp. (Pantnagar) and from different geographical locations of India. Substantial variations were found in spore morphology in respect to conidial length, width and number of septa. Average conidial length and width were varied from 29.0-6.6 x 185.3-28.2µm. Maximum spore length and width was in AB-B. carinata isolate (185.3 x 25.6 µm), while minimum in AB-B. caulorapa (29.0 x 6.6µm). Number of horizontal and vertical septa ranged between 3.50-14.75 and 0.75-5.0 respectively.

Monowara *et al.* (2017) was conducted an experiment on morphological variation on growth and sporulation of *Alternaria* species of *Alternaria* leaf blight of mustard. All the isolates showed high level of variability in in-vitro in respect of radial mycial growth, colony colour, sub surface colour, colony shape, colony texture, zonation (surface and sub surface), length and width of conidia, beak length and number of septa. The maximum and minimum radial mycial growth was recorded 90 mm in isolate NATAb and 83.67 mm in isolate GAZAb, respectively at 14 days after incubation. Significant variation in conidial length, width, beak and no. of conidia observed in all isolates. The length of conidia

ranged from 41.56 to 117.54µm with 3 to 11 transverse and 0 to 3 vertical septa. The width and beak length varied from 10.34 to 23.12 µm and 16.78 to 72.65 µm, respectively. Surface colour were olivacious green to black and circular shaped colonies were observed in all isolates on PDA medium. Colony texture were cottony to velvety. Subsurface colour varied from light brown to black and pinkish. Zonation found in some isolates and some did not produce on both surface and subsurface. All conidia were murifrom and light brown to deep brown in colour. Potato Carrot Dextrose Agar medium (PCDA) and 25 o C temperature were found optimum for different isolates for mycelia growth and sporulation.

Monowara *et al.* (2017) carried out an experiment to find out the Morphological variation on growth and sporulation of *Alternaria* species of *Alternaria* leaf blight of mustard from 10 representative geographical locations of Bangladesh, at Plant Pathology Laboratory, Oilseed Research center, Bangladesh Agricultural Research Inistitute (BARI), Joydevpur, Gazipur, Bangladesh. All the isolates showed high level of variability in in-vitro in respect of radial mycial growth, colony colour, sub surface colour, colony shape, colony texture, zonation (surface and sub surface), length and width of conidia, beak length and number of septa. The maximum and minimum radial mycial growth was recorded 90 mm in isolate NATAb and 83.67 mm in isolate GAZAb, respectively at 14 days after incubation. Significant variation in conidial length, width, beak and no. of conidia observed in all isolates. The length of conidia ranged from 41.56 to 117.54µm with 3 to 11 transverse and 0 to 3 vertical septa. The width and beak length varied from 10.34 to 23.12 µm and 16.78 to 72.65 µm, respectively. Surface colour were olivacious green to black and circular shaped colonies were observed in all isolates on PDA medium. Colony texture were cottony to velvety. Subsurface colour varied from light brown to black and pinkish. Zonation found in some isolates and some did not produce on both surface and subsurface.

Saha (2016) collected 23 isolates of *A. brassicae* from different cultivars in Uttar Pradesh and characterized for cultural, morphological, pathogenic and molecular variations. The colony colour is white, dark brown to light brown and pinkish in

11 white. The maximum length of conidia ranged from 150-122 µm with 8-9 transverse and 2 vertical septation.

Shaharan *et al.* (2016) described *A. brassicae* mycilium septate, brownish grey, conidia brownish black, obclavete, murifrom, produced singly or in chain 2-3. Length of conidia varied from 96 µm-114 µm, bredth varied from 17 µm-24 µm and beck length varied from 45 µm-65 µm. The transverse and longtidunal septation varied from 10-11 and 0-6 respectively.

Mohsin *et al.* (2016) evaluated twenty-seven (27) isolates of *Alternaria porri* for characterization of cultural, morphological and pathogenic variabilities, were isolated from diseased leaf samples collected from different onion growing regions of Bangladesh. *A. porri* colonies colony colour ranged between light to dark olivacious and grayish white with irregular, regular with concentric ring and regular without concentric ring shape. Margin of colonies were entire, irregular and wavy with effuse, fluffy and velvety texture. Morphological variation in conidia production was between 7.720×103 to 47.02×103 per mm2 with sporulation time 3.33 to 11.00 days.

Singh *et al.* (2016) tested fungal colonies of different isolates of *Alternaria brassicae* (Berk.) Sacc. varied in their cultural behavior. Cultures were found fluffy to compressed, with wavy, smooth to rough margins. Colonies colour varied from black, brown, light brown to dark brown and growth was either slow, medium or fast on different culture media. Among the media in general, the maximum growth of each fungal isolates were recorded on PDA as compare to others; while slowest growth was recorded on radish root agar medium. The maximum mycelial growth of 89.93 mm was recorded in the isolate obtained from PNUK on PDA while, minimum growth (32.40 mm) was noted on radish root agar medium of JBMP isolate.

Soo-Sang *et al.* (2016) observed that a fungus was isolated from the initial lesion, and cultured on potato dextrose agar. Colony color on upper surface of plate varied from olive gray to charcoal gray. Size of conidia mostly extend to 19–50 µm × 5–9 µm in nature and 20–59 µm × 8–13 µm in culture, with 3–8 transverse

septa and usually no longitudinal septa or only 1 longitudinal septa in 1–3 of the transverse compartments and also have a short or long beak.

Yadav *et al.* (2016) were identified the morphological variations on the basis of its morphological and cultural characters of *A. brassicae* isolates from four different locations. Colonies of all the isolates were moderately fast growing, amphigenous, ashy grey, fluffy, circular. Conidia were obclavate to muriform, ovate elongated singly on conidiophore, sometimes in short acropetal chain. Highest average conidial size (140×20.7 µm and 138.6×22.9 µm) with 8.2 and 6.8 average transverse septa and 2.0 and 1.2 average longitudinal septa respectively. Highest average beak length was recorded 54.2 µm. The maximum mean radial growth of fungus at temperatures of 25°C.

Ginoya *et al.* (2015) reported cultural and morphological variability of *Alternaria* spp on seven different media viz., potato dextrose agar, host leaf extract agar, host fruit extract agar, oatmeal agar, Richards' agar, Czapek's Dox agar and Rose Bengal agar revealed considerable variation among the isolates of A. alternata indicated the existence of variability in the pathogen. Oatmeal agar and potato dextrose agar were found as an excellent media to support the growth and spore formation of isolates of *A. alternata,* respectively. Distinct differences in terms of conidial length, breadth, beak length and number of septa were recorded among eight isolates of *A. alternata.* The average conidial length varied from 16.93 to 59.24 µm and breadth ranges from 6.90 to 14.98 µm with beak length of 3.25 to 44.07 µm. The transverse septa varied from 2 to 10 and longitudinal septa varied from 0 to 4.

Saharan *et al.* (2015) stated the pathogenic variability in four species of *Alternaria* is reported to be governed by determinant attributes viz., pathological, symptomatological, morphological, cultural, nutritional, biochemical, genetical, molecular, proteome level, thermo, and fungicidal sensitivity. Three races of *A. brassicae* viz., RM-1, RM-2 and V-3 virulent on rapeseed-mustard group of crops were identified. While race RM-1 was avirulent only on *B. oleracea* var. *Capitata*, race RM-2 was avirulent on both *B. oleracea* var. *Capitata* and *B. oleracea* var. 13 *Botrytis*. Race V-3, from vegetable crops was most virulent on the all host

differentials. Three *A. brassicae* isolates designated as A, C and D differed in their morphology, growth, sporulation, and cultural characteristics along with virulence on B. carinata. Four *A. brassicae* pathotypes from B. juncea were identified and designated as Bj-4, Bj-5, Bj-6 and Bj-7.

Singh *et al.* (2015) conducted an experiment to study the cultural and pathogenic variations of *A. brassicae,* affected leaf samples were collected from ten different places for the isolation and purification. Ten isolates of *A. brassicae* grown on five different culture media viz. Potato dextrose agar, Oatmeal agar, Host leaf extract agar, Czapek - dox agar and Carrotjuice agar *A. brassicae* varied in their cultural behaviour ranging from fluffy to compressed, with wavy, smooth to rough margins. Colonies colour varied from black, brown, light brown to dark brown and growth varied from slow, medium to fast on different culture media. Variation in zonation and sporulation on different medium were also observed in the isolates. Among the media in general, the fastest growth of each fungal isolates were recorded on PDA (83.77 mm) as compare to others; while slowest growth was recorded on Czapek - dox agar (79.54 mm) medium.

Singh *et al.* (2015) examined ten isolates of *A. brassicae* for studied morphological, physiological and cultural variation collected from different geographical region in India. All the isolates showed variability in vitro in respect of size and septation of conidiophores and conidia, beak length, effect of temperature on radial growth and liquid medium on mycelia fresh weight. The length and width of conidiophores varied from 36.8-36.4 µm and 4.73-6.58 µm respectively. Conidiophores septation varied from 4.53-6.25 in different isolates of *A. brassicae*. Conidial length and width varied from 104.02-142.47 µm, 11.62-216.95 µm respectively. Beak length varied from 43.35-70.57 µm, transverse and longitudinal septa varied from 6-8.3 µm and 0.25-2.75 µm respectively. Carrot Potato Agar was better for all the cultures.

Pramila *et al*. (2014) found 10 isolates of *A. brassicae* for morphological, cultural, pathogenic and molecular variation. Colour of colonies ranged between white, off white to light brown. Conidia length and width varied from 105-135 µm and 10 -

20 µm respectively. Horizontal septa varied from 6.9-9.4. Colony was circular in shape.

Aneja (2014) examined 55 isolates of *Alternaria* species collected from infected leaf samples. Among them 32, 20 and 3 isolates were *A. brassicae*, *A. brassicaecola* and *A. alternata* respectively. Septation space pattern 3-6, 2-6, 2-4, transverse and 0-2, 0-1, 2-3 longitudinal septation in *A. brassicae*, *A. brassicaecola* and *A. alternata* respectively. The spore size varies from 36.7-257.6 µm in A. brassicae. Mycelial growth varied between 30-80 mm in A. brassicae.

Chand (2014) described the conidia of *A. brassicicola* were muriform, olivaceous brown colour with nonexistent beaks. The size of conidia recorded 13-120 µm in length and 6-16µm in width. The shape of the conidia was cylindrical to obclavate with cross and longitudinal septa. Isolates showed light black and grey white colours in Host Extract Agar media. The temperature between 25-30oC was found optimum for the growth and sporulation of *Alternaria*.

Kumar *et al.* (2014) reported Conidia are brownish black, obclavate, borne singly or sparingly in chains of 2-4, muriform with long beak and the overall conidial size 15 ranges between 148-184 × 17-24 µm with 10-11 transverse and 0-6 longitudinal septa. Sporulation occurs between the temperatures of 8-24°C but optimum temperatures range between 16-24°C.

Swati *et al.* (2014) carried out an experiment in India to found the morphological, cultural, pathogenic and genetic variability in thirty-two *A. brassicicola* (Schwein) Wiltshire isolates infecting cauliflower (*Brassicae oleracea* var *botrytis*) from different parts. Dark olivaceous black fungal colonies were observed with small, obpyrifom, septate, brown colored spores forming in chain having no beak. A 16 significant (p200 µm). The breadth was observed in range of 13.5-36 µm. The number of horizontal septa varied between 5–13. The number of beak septa varied from 0-6.

Goyal *et al.* (2011) worked on morphological and cultural variability among the oilseeds *Brassica* isolates of *A. brassicae* from different geographical regions of

India were reported from across the rapeseed-mustard growing region of India Variation in conidial morphology, mycelial growth, sporulation of thirteen isolates of *A. brassicae* collected from different geographical zones were dependent on temperature and geographical origin.

Variability in the morphological characteristics in *A. brassicae* isolates of different regions of India have been reported (Meena *et al.,* 2005; Kaur *et al.,* 2007; Singh *et al.,* 2007; Goyal *et al.,* 2011).

Vishunavat and Kolte (2008) found for inoculation, spore suspension was prepared from freshly developed conidial growth of *Alternaria* spp. using sterile distilled water and then strained through muslin cloth. The spore concentration was adjusted to $1x10^5$ conidia/ml distilled water using hemocytometer. The plants were sprayed with freshly prepared spore suspension using an atomizer.

Kaur *et al.* (2007) reported that variability of *Alternaria* spp. on the basis of morphology, sporulation, growth and other cultural characteristics also have been reported earlier.

Simmons (2007) worked to recognize *Alternaria* sp. by the morphology of their large conidia. They are catenate, formed in chains or solitary, typically ovoid to obclavate, often beaked, pale brown to brown, multicelled, and muriform.

Some researchers have worked on cultural variability in *Alternaria* species in respect of mycelial growth and sporulation (Ansari *et al.*, 1989; Patni *et al.*, 2005; Kaur *et al.*, 2007).

Meena *et al.* (2005) found that mycelial growth, sporulation of *A. brassicae* is also affected by relative humidity and showed variation in its requirement.

Different temperatures were found optimum for growth and sporulation of *A. brassicae* in a range of 20–25ºC (Singh *et al.,* 2007) and 20–30ºC (Meena *et al.,* 2005), respectively.

Chohan and Saharan (2003) observed that *Alternaria* blight is a destructive disease of rapeseed-mustard throughout the world and appears each year in crop

fields. The disease is caused by different species of *Alternaria* viz. A. *brassicae* (Berk) Sacc, *A. brassicicola* (Schw.), *A. raphani* Grows and Skolo and *A. alternata* (Fr) Keisskr. Among than *A. brassicae* is much more destructive and occurs more frequently than A. *brassicicola* on *B. juncea*.

Barry *et al.* (2002) evaluated 308 isolates of *Alternaria* spp. were collected from the five sample sites. Based on characteristics of single-spored colonies, all isolates were grouped into approximately four colony types. Group 1 consisted of colonies contain lettuce green to olive green and usually had a prominent (2 to 5 mm) white margin. Colony texture was felty to woolly. Group 2 isolates produced colonies whch were pale olive gray to olive gray, often with a very thin (1 to 2 mm) white margin. Colony texture was generally woolly to cottony. Group 3 isolates produced colonies visible with typically dark olive gray to iron gray to castor gray in color. The colony margin of these isolates was often wavy or torn. Colony texture was generally felty to woolly presentative culture. Group 4 isolates 24 produced colonies that were either white to pale gray or apricot orange in color. Colonies generally had a cottony texture. The undersurfaces of the colonies were usually orange or dark orange. No diffusible pigments or crystals were produced by these isolates. Isolates typically produced colonies over 70 mm in diameter after 7 to 10 days.

Simmons (1995) expanded concepts from both Elliot and Neergaard in loosely organizing the genus into *Alternaria* 14 species-groups based upon characteristics of conidia and catenulation. Additional species groups discussed in other work include the *A. arborescens*, *A. brassicicola*, *A. porri*, and *A. radicina* groups (Roberts *et al.* 2000; Simmons, 1995; Pryor and Gilbertson, 2000, 2002).

The mycelium and conidiophores of the *A. brassicae* are septate and the colour of mycelium and conidiophores are yellowish brown and chestnut brown to grayish brown respectively. Conidia of A. brassicae found single or in chains (upto four) and originated from the small pore of the conidiophore wall, straight or slightly curved, obclavate, 75-350 µ in overall length, 20-30 µ thick in broadest part, the beak about 1/3 to 1/2 the length of conidium and 5-9 µ thick. 10-11 transverse and

0-6 longitudinal septation are found in conidia. The growth of *A. brassicae* is slow and less sporulation. Conidia of *A. brassicicola* are mostly in chains of 20 or more, sometimes branched arising through small pore of conidiophore wall, straight or curved, more or less cylindrical but often slightly swollen at the base, septate, pale to mild olivaceous brown, 18-130 µ long, 8-30 µ thick in broadest part, the beak usually non-existent or 1/6 the length of conidium and 6-8 µ thick with less than 6 transverse septa and few upto 6 longitudinal septa. The growth pattern of *A. brassicicola* is fast, well developed black sooty colony and abundant sporulation. Conidia of *A. raphani* are commonly in chains of 2-3, straight or slightly curved, generally with short beak, dark golden brown or olivaceous brown, 50-130 µ long, 14-30 µ thick in the broadest part with 3-7 transverse and often a number of longitudinal septa. The cottony growth of mycelium and abundant sporulation are found in *A. raphani*. The colonies of *A. alternata* are usually black or olivaceous black, conidiophores arising solitary or in small groups, simple or branched, pale to mild olivaceous or golden brown. Conidia of *A. alternata* are branched, ellipsoidal, often with a short conical or cylindrical beak, overall length 20-63 µ, 9-18 µ thick in broadest part, beak pale, 2-5 µ thick with upto 8 transverse and usually several longitudinal septa. The study on cultural variability in *Alternaria* species in respect of mycelial growth and sporulation on different temperature, relative humidity, hydrogen ion concentration (Ansari *et al.,* 1989), media (Patni *et al.,* 2005) and light (Ansari *et al.,* 1989) have been done. Variability on the basis of morphology, sporulation, growth and other cultural characteristics also have been reported earlier.

2.6. Pathogenicity

Singh and Singh (2019) evaluated ten isolates of *A. brassicae* from various agro-climatic location of India viz., Uttar Pradesh (Ab1), Madhya Pradesh (Ab2), Uttarakhand (Ab3), Bihar (Ab4), Jharkhand (Ab5), West Bengal (Ab6), Haryana (Ab7), Rajasthan (Ab8), Chhattisgarh (Ab9) and Gujarat (Ab10) and characterized for pathogenic variations. All the isolates showed high level of variability. The incubation period of the isolates was recorded on *B. juncea* 3 to 4

days, *B. carinata* 6.17 to 6.83 days, B. napus 5.17 to 6.00 days, *B. nigra* 4.17 to 5.17 days and in *B. campestris* it was ranged from 3.17 to 4.00 days. The results revealed that the maximum PDI was noted on *Brassica juncea* followed by *B. campestris* var. yellow sarson, *B. nigra, B. napus* and *B. carinata*. Based on PDI ten isolates could be classified into three groups in which group one consist of isolates Ab3, Ab6, Ab7 and Ab5. Isolates Ab8, Ab2 and Ab4 fall in second group; while group three include isolates Ab1, Ab9 and Ab10.

Manika *et al. (*2013) conducted an experiment that all the *A. brassicae* isolates both from cauliflower and mustard were found to be pathogenic in nature.

Akhter *et al.* (2012) evaluated that, eight mustard varieties (SAU-1, BINA-6, TORI-7, BARI-9, BARI-6, SOFOL, AGRANI and SS-75) were evaluated for their reaction against *Alternaria* blight (*Alternaria brassicae*) under natural condition at the experimental field of Sher-e-Bangla Agricultural University, Dhaka during winter season from November 2007 to February 2008. At 60 days after sowing (DAS) disease severity did not exceed 5% and no symptoms were observed in the siliqua. Results revealed that, among the varieties the lowest disease severity was observed in Agrani in all stages of plant growth. Maximum disease severity (97.17%) was found in SAU Sarishsa 1 giving lowest yield (1266.55kg/ha).

Craven *et al.* (2008) studied that another major work pertaining to the studies related to the identification of virulence factors was the disruption of Aso-1, a gene required for hyphal fusion (anastomosis) which was also found to be required for pathogenicity in *Alternaria* species.

Eventually, over a hundred genes have been functionally analyzed through various techniques like gene knockout and overexpression experiments making *A. brassicicola* the species of choice for functional genomics research to define conserved virulence mechanisms for this important genus of fungi (Oide *et al.*, 2006; Cho *et al.*, 2006, 2007; Kim *et al.*, 2007).

Cho *et al*, (2006, 2007) carried out an experiment that another area ripe for exploration in the *A. brassicicola*. Brassicaceae pathosystem is fungal signal transduction mechanisms. Disruption of the Fus3/Kss1 MAP kinase homolog (Amk1) in *A. brassicicola* resulted in a complete loss of pathogenicity as observed in other fungi. Interestingly, in the latter study it was shown that addition of long polypeptide nutrients partially restored pathogenicity to the mutants.

Kim *et al.* (2007) observed that in another study, a non-ribosomal peptide synthase gene (AbNPS2) was found to be important for cell wall integrity, conidial viability, and virulence of aged spores of *A. brassicicola.*

2.7. Molecular diversity of Mustard (Oil crop seed)

Priyanka *et al.* (2020) reported partial resistance was observed in varieties GSL-5, Pusa aditya, Kiran, RH-479, Sheetal, GSL-2, GSL-1 against alternaria blight as they are recorded with a smaller number of spots/10 cm2, small size of spot, less sporulation, less per cent disease severity, less infection rate, less leaf defoliation and higher yield. The last appearance of disease was noted on genotype GSL-5, (53 DAS) followed by Kiran (52 DAS). Significantly lower per cent disease severity on leaves was recorded on cultivar GSL-5 (12.35%) followed by Kiran (14.12%), Pusa aditya (14.16%) and GSL-2 (15.37%), respectively. The minimum infection rate on leaves was recorded on cultivar GSL-5 (0.214) followed by Pusa aditya (0.218). The maximum infection rate was recorded on leaves on cultivar KBS-3 (0.229) followed by PS-66 and T-27 (0.228) respectively.

Singh *et al.* (2020) screened Indian mustard 47 genotypes, these screening was not found highly resistant, resistant and moderately resistance against Review of Literature 28 *Alternaria* blight of rapeseed-mustard six genotypes viz., Kiran, TMR-14-6, TMR14-1, TMR-14-5, TMR-14-3, TMR-14-4 with disease severity of 5-10 per cent respectively were rated as moderately susceptible were found nine genotypes with disease severity 11-25 percent. 31genotypes were marked as susceptible in which disease severity was found to be 26 to 50%.

Patial *et al.* (2019) studied genetic diversity among 34 linseeds (*Linum usitatissimum* L.) genotypes 18 agro-morphological traits as per the standard descriptors of DUS, UPOV 2011 at the experimental farm of the Department of Crop Improvement, CSKHPKV, Palampur. Six genotypes were found to be distinctive on the basis of morphological traits. Results revealed that sufficient genetic variability was observed for all the characters studied based on various genetic variability parameters. Principal component analysis (PCA) indicated that, out of total principal components, three PCs contributed 71.60% to the total variance amongst the genotypes assessed for nine agronomic traits. PC I contributed maximum towards the variability (36.91%) followed by PC II (22.15%) and PC III (12.54%). Cluster analysis clearly differentiated 34 genotypes into three clusters with cluster III having highest 15 genotypes. Sufficient variability was observed in the genotypes studied based on phenotypic and genotypic variance, principal component analysis and cluster analysis which could be utilized by researchers, in breeding programme and the genetic distinctiveness in the genotypes can be protected under PPVFRA.

Summuna *et al.* (2018) carried out an experiment, of the twenty seven different genotypes screened for their reaction against Alternaria blight, two genotypes viz. RH- and JM8113 and PC-1 were found to be moderately susceptible, eighteen genotypes -5 showed moderate resistance, four genotypes viz. GM-3, RH-1359, RH-819viz. Geeta, PusaBahar, Rohini, RH-30, Shivani, RH-781, RGN-13, GM-2, RRN-505, GM-1, PusaJaganath, Vaibhav, RSPN-602, DGS-1, RSPN-25, RSPN-2 and RSPR-69 recorded susceptible reaction.

Summuna *et al.* (2018) conducted an experiment that twenty-seven different genotypes of rapeseed-mustard germplasm were sown to test their reaction against Alternaria blight. Leaves and pods of the genotypes under test were scored for disease severity at 75 and 100 DAS using the scale proposed by (Conn *et al.*, 1990). Of the germplasm tested, two genotypes *viz.* RH8113 and PC-5 showed moderate resistance at 75 and 100 DAS (>10-20% disease severity), four genotypes *viz*. GM-3, RH-1359, RH-819 and JM-1 were found to be moderately

susceptible (>20-30% disease severity) while eighteen genotypes *viz.* Geeta, PusaBahar, Rohini, RH-30, Shivani, RH-781, RGN-13, GM-2, RRN-505, Krishna, GM-1, PusaJaganath, Vaibhav, RSPN-602, DGS-1, RSPN-25, RSPN-2 and RSPR-69 were found to be susceptible recording a disease severity ranging from 30 to 50 per cent in leaves and pods at 75 and 100 DAS. Three genotypes *viz.* Kranti, Varuna and CS-54 were found to be highly susceptible to Alternaria blight recording a disease severity of more than 50 per cent at both 75 and 100 DAS.

Singh *et al.* (2018a) evaluated two hundred genotypes for resistance against *Alternaria* blight of rapeseed-mustard. None of the entry was found resistant, twenty-two genotypes namely DLSC-1, DRMR-261, DRMR-270, GSC-101, GSL-1, NPC20 and PHR-2, CNH-11-13, CNH-11-7, EC-552608, HNS-1001, PAB 04-10, PAB 05-16, PAB 05-19, PAB 09-05, PAB-2004-4, PAB-2005-16, PPBJ 5, PPBJ-2, PPBJ-3, PPBN 3 and PPBN-2 were moderately resistant and 63 genotypes moderately susceptible.

Singh *et al.* (2018b) conducted field experiment during 2013-14 and 2014-15 with 200 genotypes for evaluation of their resistance to *Alternaria* blight as well as to develop effective management strategies for this disease. None of the genotypes were found disease-free or highly resistant, only 7 genotypes namely DLSC-1, DRMR-261, DRMR-270, GSC-101, GSL-1, NPC-20 and PHR-2 were found resistant, 15 genotypes were rated as moderately resistant. Rest of the genotypes was either recorded susceptible or highly susceptible.

Tiwari *et al.* (2017) described Indian mustard [*Brassica juncea* (L.) Czern & Coss.] varieties were identified through distinctiveness, uniformity and stability (DUS) testing protocol on the basis of experiment conducted during 2013-14 and 2014-15 consecutive years. The experimental procedure was carried as per official guideline of Indian mustard. The selected mustard varieties were identified based on six morphological characters that had capability to separate varieties into groups. Further they were classified based on three qualitative characters viz., leaf hairiness, time of 50% flowering and maturity period, three

quantitative characters viz., number of siliqua on main shoot, seeds per siliqua, and weight of 1000 seeds. The varieties PCR-7, Pusa Jai kisan, RH-781 and CS-52 were identified separately on the basis of hairiness, number of siliqua on main shoot and number of seeds per siliqua respectively. All the remaining varietieswere classified into groups based on time of 50% flowering, maturity period and weight of 1000 seeds. Thus the present study concludes that morphological characters of DUS protocol prove to be very useful and convenient for varietal identification which in turn helps in maintenance of genetic purity of varieties.

Bhusal *et al.* (2017) carried out an experiment to characterize 20 genotypes of sorghum {*Sorghum bicolor* (L.) moench} on the basis of 33 morphological characters provided by Protection of Plant Variety & Farmer's Right Act (PPV&FRA) for Distinctiveness Uniformity and Stability (DUS) testing in sorghum. Experimental results revealed that maximum variation was found on the basis of glume colour among the genotypes i.e. G 46, HC 308, HJ 513 had green white, IS 3237, SSG 9, HC 171 had yellow white, SSG 59-3, COFS 29 had grayed purple, S 437-1, SGL-87, S 540-S, SSG (PSSG) had grayed yellow and remaining seven genotypes had grayed orange glume colour. The studied traits showed five genotypes had distinct state of expression. Genotype S-540 showed very high plant height upto the base of flag leaf, HC 136 had compact panicle density at maturity, COFS 29 had very long glume length, SSG 59-3 had distinct expression for days to panicle emergence (50 % of the plants with 50 % of anthesis) and COFS 29 and IS 18551 had short and very long leaf width of blade, respectively. The Principal Component Analysis (PCA) revealed principal Factor (PFI) and Principal Factor (PFII) with maximum variability (64.99 %). Classification of genotypes on the basis of DUS traits provided identification of key characteristics of various genotypes.

Bisht *et al*. (2015) conducted an experiment, screening of *Brassica* germplasm revealed that among 240 *Brassica* germplasm none was found highly resistant against *Alternaria* blight only 08 Brassica germplasm viz. IC-255498, IC-

296685, IC-326253, IC-335847, IC339589, IC-339597, IC- 360723, and IC-417020 were found to be moderately resistant with 11-25% disease severity against *Alternaria* blight, IC-296705, IC-328316 and IC-338523, were susceptible and showed 26-50% disease severity while 234 were found highly susceptible with more than 50% disease severity.

Banga *et al.* (2013) observed the seed yield in different genotypes was mainly governed by seed yield per plant. The 1000-seed weight was the maximum in RL-1359 (5.15g) whereas it was at par in other genotypes. The experiment consisted of four genotypes of Indian mustard (RI.-l359. TAC-437. K. ranti and NRCI)R-60 I).

Formation of chlamydospores is reported in *A. brassicae* and *A. raphani*, while microsclerotia are found to be produced by the former. Although the use of speciesgroup designation does not resolve definitive species boundaries within *Alternaria*, advantages of its use are that it organizes at the subgeneric level the morphologically diverse assemblage of *Alternaria* species and permits the generalized discussion of morphologically similar species without becoming over restricted due to nomenclatural uncertainty. Cultural, morphological, pathogenic, and molecular variation in isolates of *A. brassicae* has been indicated by several workers (Goyal *et al.*, 2011a, 2013a, Khulbe *et al.*, 2011, Kumawat *et al.*, 2011, Sharma *et al.*, 2013a, b).

Various technologies, namely, embryo rescue, somatic hybridization, somaclonal variations, genetic transformation, molecular markers, and signal transduction, have been used for incorporation of resistance against this pathogen in oilseed Brassicas by Aneja and Agnihotri (2013).

Chhikara *et al*. (2012) described that transgenics of Indian mustard with barley 156 Diseases of Edible Oilseed Crops antifungal genes class II chitinase and type I ribosome-inactivating protein, which coexpressed in plants, showed some resistance against *A. brassicae* infection through delayed onset of the disease and restricted number, size, and expansion of lesions as compared to wild plants.

Benali *et al*. (2011) reported that the molecular approaches have been used increasingly in taxonomy and systematics of filamentous fungi including phyto-pathogens at the species and subspecies level.

DRMR (2011) screened rapeseed-mustard cultivars for resistance to *Alternaria* blight diseases. None of the genotype showed resistant reaction to *Alternaria* blight. However, EC 338997, EC 339000, EC 414293, NPJ 154 and RH 345 were found moderately resistant to *Alternaria* blight.

Meena *et al*. (2011) reported that *S. Alba,* GSL 1 and T-27 were highly resistant against thirteen *Alternaria brassicae* isolates.

Pedras *et al.* (2009a) reported that *A. brassicicola* produce some detoxifying enzymes, namely, brassinin hydrolases, which are dimeric protein of 120 kDa and catalyze the detoxification of brassinin, a phytoalexin produced in crucifers after fungal infection.

Pedras *et al.* (2009b) showed the most selective phytotoxic metabolite, namely, *brassicicolin A*, and the major phytoalexin, namely, spirobrassinin, were produced by *A. brassicicola* in liquid cultures and in infected leaves of *B. juncea*, respectively.

Labuda *et al*. (2008) clearly separated a new species, A. jesenskae from the other related large-spored and filament beaked *Alternaria* species on the basis of sequences of the ITS1, 5.8S and ITS2 region as well as by its distinctive morphology. RAPD and RFLP markers were used for genetic variation in *Alternaria brassicae, A. brassicicola,* and *A. raphani* collected from geographically diverse regions of the world. UPGMA analysis of RAPD data of isolates of three *Alternaria* species showed four groups in which intra-regional variation between isolates was less apparent Variation was, however, higher in *A. brassicicola,* as based on RAPD analysis. Two isolates (from Canada and France) of *A. raphani* also showed variability with different RAPD profiles generated by all five primers tested. Five distinct polymorphic RAPD products were used as hybridization probes for RFLP analysis to detect interand intra-specific variation. Variation among *A. brassicae, A. brassicicola*, and *A. raphani* was evident. Non-

radioactive probes were also used to hybridize with Southern blots of *A. brassicae, A. brassicicola, Leptosphaeria maculans, Rhynchosporium secalis* and *Brassica juncea* for the selection of A. brassicaespecific probe(s).

Mondal *et al.* (2007) explained that the pathogenesis-related (PR) proteins are toxic to invading fungal pathogens but are present in plant in trace amount. Thus, overexpression of these proteins may increase resistance to pathogenic fungi in several crops. Indian mustard plants transformed with class l basic glucanase gene showed restricted number, size, and spread of lesion caused by *A. brassicae.* This gene produces a PR protein glucanase that hydrolyzes a major cell-wall component, glucan, of pathogenic fungi and acts as a plant defense barrier.

Atwal and Sangha (2004a) reported that some extracellular enzymes, namely, cellulase and pectinases (polygalacturonase [PG] and pectin methyl esterase), are produced by *A. brassicae* under different cultural conditions.

Insight has been gained into genes being expressed during *Alternaria* infection of Brassica (Cramer and Lawrence 2004). The authors used suppression subtractive hybridization between RNA isolated from the spores of *A. brassicicola* incubated in water and on the leaf surface of an ecotype of Arabidiopsis thaliana followed by cloning and sequencing of cDNA clones that were differentially expressed. One gene (P3F2), only expressed during infection, was identified, although its function remains to be determined. A similar approach with other pathogens could lead to advances in the understanding of pathogenicity.

Bhowmik (2003) examined to ensure high disease development, screening nursery is inoculated once or twice from a 10 day-old-cutture of grown in nutrient medium or from washings of infected leaves.

Mondal *et al.* (2003) studied that the chitinase enzyme when overexpressed degrades the cell wall of invading fungal pathogens and plays an important role in plant defense response. Indian mustard, which has been transformed with chitinase gene tagged with an overexpressing 35S cauliflower mosaic virus

(CaMV) promoter, showed delay in the onset of disease as well as reduction in number and size of lesions.

Bock *et al.* (2002) studied the genetic variation within and between eighteen isolates of *Alternaria brassicicola,* five isolates of *A. alternata,* and a single isolate of Rhynchosporium secalis using AFLP. The AFLP analysis distinguished consistently within and between *A. brassicicola, A. alternata* and *Rhynchosporium secalis*, however multiple isolates from a particular location tended to cluster together. Despite the absence of an identified sexual stage, *A. brassicicola* would appear to have a means for generating and maintaining significant variation.

Kanrar *et al.* (2002) studied with rapid advances in techniques of tissue culture, protoplast fusion, embryo rescue, and genetic engineering make transfer of disease resistance traits across wide crossability barriers possible. A cDNA encoding hevein (chitin-binding lectin from Hevea brasiliensis) was transferred into B. juncea cv. RLM-198. Southern analysis of the putative transgenics showed integration of the transgene. Northern and Western analyses proved that the integrated transgene is expressed in the transgenics. In whole plant bioassay under glasshouse conditions, transgenics were found to possess parameters that are associated with resistance such as longer incubation and latent period, smaller necrotic lesion size, lower disease intensity, and delayed senescence.

Molecular relationships amongst *Alternaria* species based on nuclear ribosomal DNA and hostsp ecific toxins (Kusaba and Tsuge, 1994, 1995) or with other related fungi have been analyzed (Pryor and Gilbertson, 2002; Chou and Wu, 2002).

Prasad *et al.* (2002) screened 71 rapeseed and mustard genotypes for resistance to *Alternaria* blight disease. They reported that 15 genotypes were moderately resistant to *Alternaria* blight.

Kolte *et al*. (2001) reported that genotypes PR8988 and PR-9024 showed high degree of resistance to *Alternaria* blight and genotypes PR-9301 and PR-9650 showed high degree of susceptibility.

Mora and Earle (2001) studied, pollen culture, and sensitivity test to destruxin B. While in studies on the mechanism of tolerance to *Alternaria* blight some have indicated the effect of additive genes or polygene or cluster gene (Krishnia *et al.*, 2000) with resistance being controlled by nuclear genes of partial dominance, there has also been indication of components of resistance being significantly correlated to each other regarding slow blighting (Kumar and Kolte 2001) and dominance (h) having a predominant role in genetic control of time of appearance; additive × dominance predominant for other disease progression factors, namely, area under the disease progress curve (Meena *et al.*, 2011a).

Dang *et al.* (2000) reported that genotypes *viz.* DIR-1507 and DIR-1522 of *Brassica juncea* had stable resistance against *Alternaria* blight.

Molecular methods have been used not only in differentiating between species (Roberts *et al.,* 2000), but also for assessing intra-specific variation (Morris *et al.*, 2000).

Morris *et al.* (2000) reported that were able to identify a high level of genetic diversity within *alternata* populations on tomato using RAPD within and between regions in California.

Pryor and Gilbertson (2000) reported that phylogenetic analysis of ITS and mitochondrial small subunit (SSU) rDNA sequences revealed that the *Stemphylium* spp. were distinct from *Alternaria* and *Ulocladium* spp.

Since resistance to *Alternaria* blight is governed by additive or polygenes, breeding for resistance to these diseases could involve pyramiding of minor genes, introgression of genes from material found resistant, reciprocal recurrent selection or diallel selective mating (Krishnia *et al.,* 2000), wide hybridization (*B. alba*), molecular breeding (viz., from *C. sativa* by somatic hybridization; transgenic expressing *Trichoderma harzianum* endochitinase gene.

Variation at DNA level among *A. brassicae* (Berk.) Sacc., *A. brassicicola* (Schwein) Wiltshire, *A. raphani* J.W. Groves & Skolko and *A. alternata* (Fr.) Keissl have been established (Jasalavich *et al.,* 1995) by restriction fragment length polymorphism (RFLP) (Botstin *et al.,* 1980).

2.8. Management

a) Biological

Yogita *et al.* (2017) was conducted an experimented that foliar spray of *Trichoderma harzianum* against *Alternaria* leaf blight of mustard most effective treatment have shown results at par with the foliar spray of *Trichoderma harzianum* in increasing the yield. The maximum plant height (cm) was recorded at *Trichoderma harzianum* (foliar spray) @ 2%.

Meena *et al.* (2011) observed that foliar application of soil inhabitants isolates of *T. harzianum* and *P. fluorescens* were found effective in the management of *Alternaria* blight.

Spray of soil with isolates of *Trichoderma viride* at 45 and 75 days after sowing could manage Alternaria blight of Indian mustard (*Brassica juncea*) as effectively as mancozeb (Meena *et al.,* 2004), which have been confirmed later in multilocation trials (AICRP-RM, 2007).

T. harzianum is a biocontrol agent in control of *A. alternata* (Roco and Perez, 2001; Monte, 2001; Sempere and Santamarina, 2007).

Dubey (2003) workedon some of the isolates of *Trichoderma* spp. included in the present study significantly inhibited several pathogens. However, some reports indicate possibility of biological management of the disease. Phyllosphere residents Aureobasidium pullulans and Epicoccum nigrum reduced the infection by *A. brassicicola,* especially when they were inoculated 14 h before the pathogen.

McMullen, M.P. and Lamey, H.A. (2000) described antagonistic activity of *Trichoderma* species grew much faster than the tested fungi and inhibited the

growth of pathogen. But both the species of *Trichoderma* differed with each other in their ability to suppress the growth of the pathogen. Among two, *Trichoderma harzianum* showed high percentage growth inhibition of test pathogen when compared to *Trichoderma viride.* Maximum inhibition 43.81% was recorded from *Trichoderma harzianum*, 30% was recorded from *Trichoderma viride.* Antagonistic fungi or bacteria are a promising agent treat seeds, such as Trichoderma sp. (an antagonist fungus).

b) Botanicals

Sasode *et al.* (2012) evaluated with the botanical viz., Neem, Eucalyptus, Datura, Pudina, Tulsi, Lantana were evaluated under crude and boiled forms against A. brassicae under in vitro condition.

Shinde and Dhale (2011) stated the results proved that Garlic bulb extract in reducing the *Alternria* leaf blight of mustard.

Sharma *et al*. (2007) reported that the Neem leaf extract showed high efficacy to inhibit the radial growth of *Alternaria* spp.

Botanicals viz., bulb extract of *Allium sativum* has been reported to effectively manage *Alternaria* blight of Indian mustard (Meena *et al.,* 2004; Patni and Kolte, 2006).

Chattopadhay *et al*. (2005) reported that Neem leaf extract was best followed by Garlic bulb extract in reducing the *Alternaria* leaf blight of mustard in leaf, silique.

Chand and Singh (2004) described the effects of extracts of oak (*Calotropis procera*), eucalyptus (*Eucalyptus globulens* [E. globulus]), jatropha (*Jatropha multifida*), neem (*Azadirachta indica*) and bulbs of garlic (*Allium sativum*) on *Alternaria* blight (*Alternaria brassicae*) of Indian mustard showed best performance.

Meena *et al*. (2004) was carried out an experiment by using different botanical viz., Neem, Eucalyptus, Datura, Pudina, Tulsi, Lantana under crude and boiled

forms against *Alternaria brassicae* under *in- vitro* where bulb extract of *Allium sativum* has been reported to effectively manage *Alternari*a blight of Indian mustard.

c) Chemical

Ahmed *et al.* (2018) found that seed treatment as well as spraying with Rovral 50 WP was found to be best in reducing *Alternaria* blight incidence and severity and increasing quality seed of mustard.

Das (2015) reported that a number of fungicides have been reported to be effective against the spread of against *Alternaria* under different field conditions *e.g.* Dithane M-45 (0.2%), Dithane Z-78 (0.2%), Iprodione (Rovral) (0.2%), Blitox 50 (0.3%), Baycor (0.2%) and Mancozeb (64%)

Prasad and Lallu (2006) found that first spray of carbendazim (0.1%) + mancozeb (0.2%) followed by two sprays of mancozeb (0.2%) at early date of sowing was the best combination in reducing the grey blight of mustard disease severity on leaves (18.7%) and pods (10.4%) higher realization yield (1295.8 kg/ha), 1000 seed weight (5.12 g) and oil content (42.6%).

Singh *et al.* (2006) observed that six seed dressing fungicides, i.e. Metalaxyl, Carbendazrm, Mancozeb, Thiophanate-methyl, Iprodione and BAS 38601 F (a seed dressing fungicide containing 40% Carbendazim + 32% Mancozeb), in combination with spray of Mancozeb (0.25%) were tested for the control of foliar diseases, *Alternaria* leaf spot (*Alternaria brassicae*) and white rust (*Albugo candida*) of Indian mustard. All the seed treatments improved germination and reduced disease intensity. Seed treatment with Mancozeb and spray of same fungicide was most effective against Alternaria leaf spot controlling up to 58.8 to 74.7 % disease. The highest yield was recorded with Iprodione (16.0-17.36 q/ha) and Mancozeb (26.0-31.12 q/ha).

Singh and Singh (2006) was conducted a field experiment in India during 2002-03 and 2003-04 to develop spray schedule(s) for the management of blight caused by *Alternaria brassicae* and *A. brassicicola*, and white rust caused by *Albugo*

candida, using Indian mustard (*Brassica juncea*) cv. Narendra Rai sown on 15 and 30 October, and 15 November. Mancozeb and Ridomil MZ-72 were sprayed in spray schedule combinations. Sowing on 15 October resulted in the lowest incidence of leaf blight, pod blight and white rust intensity and the highest 1000-seed weight and yield. Three consecutive sprays of Mancozeb 75 WP (0.2%) at fortnightly intervals, beginning at the disease initiation resulted in the lowest leaf blight incidence and pod blight intensity. Seed yield and 1000-seed weight under all the dates of sowing were highest with 2 consecutive sprays of Mancozeb followed by a third spraying with Ridomil MZ 72 (0.25%).

Singh *et al.* (2006) evaluated with six seed dressing fungicides, i.e. metalaxyl, carbendazim, mancozeb, thiophanate-methyl, iprodione and BAS 38601 F (a seed dressing fungicide, 40% carbendazim+32% mancozeb), in combination with spray of mancozeb (0.25%) were tested for the control of foliar diseases, *Alternaria* leaf spot (*Alternaria brassicae*) and white rust (blister) [*Albugo candida*] of Indian mustard. All the seed treatments improved germination and reduced disease intensity. Seed treatment with mancozeb and spray of same fungicide was most effective against *Alternaria* leaf spot controlling up to 58.8 to 74.7% disease. The most effective seed treatment fungicides for white rust were carbendazim reducing 75.8-80.8% infection at first location and mancozeb controlling 62.0-81.8% disease. Results with iprodione were at par with mancozeb. Thiophanate-methyl was very effective against both the diseases. The highest yields were recorded with iprodione (16.0-17.36 q/ha) and mancozeb (26.0-31.12 q/ha). The seed treatment with mancozeb or carbendazim and three spray of mancozeb was therefore, recommended for management of foliar diseases of Indian mustard.

Singh and Singh (2005a) was carried out an experiment in India for controlling *Alternari*a blight (AB) caused by *Alternaria brassicae* and *A. brassicicola* and observed that seed treatment combined with three foliar sprays of Mancozeb 75% WP (0.2%) at 15-day intervals, beginning at 45 days after sowing, resulted in the lowest AB incidence and the highest seed yield and cost-benefit ratio of 1:5.2. It

was followed by foliar sprays of Mancozeb 75% WP alone in all cases. Highest avoidable losses due to the combined effect of these diseases in seed yield, seed test weight and oil content were 34.7, 13.1 and 4.2%, respectively.

Shrestha *et al.* (2005) observed that mancozeb and iprodione had effectively reduced grey blight disease in the sprayed plots and increased seed yield by 48% and 130%, respectively. The correlation between disease severity and yield, and yield components was negative and highly significant. Average yield loss was estimated to be in the range of 32 to 57%. Seed infection was also significantly higher in non sprayed treatment than sprayed one. The disease showed a negative effect on oil content causing losses on oil between 4.2 to 4.5%.

Ansari *et al.* (2004) observed that Amistar @ 0.1% (2 spray) showed marked effect in reducing the *Alternaria* blight of mustard over control and increased 43.48% yield over control.

Meena *et al*. (2004) obtained that Mancozeb was the best among all the treatments, resulting in the lowest disease severity on leaves of mustard.

Chattopadhyay and Bhunia (2003) evaluated seven fungicides viz; mancozeb 0.2%, captan 0.2% metalaxyl M.Z 0.25%. iprodione 0.2%, bayletan 0.05% (triadimefon), copper oxychloride 0.3% and antracol 0.2% (propineb) aganist *Alternaria* leaf blight of rapseed-mustard (*Brassica campestris* cv. Yellow Sarson) caused by *Alternaria brassicae.* Best control of the disease was observed by iprodione followed by mancozeb. Higher seed yield and significant increase of 1000-seed weight were also recorded from single spray of iprodione followed by mancozeb. Highest seed yield and significant increase of 1000-seed weight were also recorded from single spray of iprodione at post flowering stage. But maximum economic return was obtained from two spraying of mancozeb at 45 DAS and 60 DAS.

Hossain (2003) conducted an experiment where Seed health regarding incidence of *Alternaria brassicae* were different due to application of different treatments. The lowest seed infection (3.5%) by *Alternaria brassicae* was found in the seed

lot obtained from treated plot with Rovral-50 WP in BARI-6 plot compared to control.

Mukherjee *et al.* (2003) studied the efficiency of iprodione against *Alternaria* blight (*Alternaria brassicae*) infecting Indian mustard cv. Pusa Bold in New Delhi, India, during 1998-2000. Iprodione was sprayed to plants at 500 g a.i. /ha during the early pod stage. Iprodione was more effective than mancozeb (control) in the reduction of *Alternaria* blight incidence. The increase in Indian mustard yield in iprodione-treated plots was higher by 24-59% than that in the control plots.

Mukherjee *et al*. (2003) observed that the yield of mustard in Iprodione treated plot was higher by 24% - 59% than that in the control plots. Spraying of Rovral (Iprodione) was reported more effective than other fungicides and the highest yield were recorded with Rovral.

Prasad *et al.* (2003) investigated an experiment in Kanpur, Uttar Pradesh, India, during the 1999/2000 and 2000/01 rabi seasons on Indian mustard genotypes PAB 9534, PAB 9511, JMM 915, RN 490 and Varuna to determine the losses due to *Alternaria* blight (*Alternaria brassicae*) under protected and unprotected conditions. Varuna and PAB 9511 were used as the susceptible and resistant controls, respectively. The protected plots were sprayed with 0.25% mancozeb starting from 40 days after sowing and 3 subsequent sprays at 15-day intervals. The disease appeared 45 days after sowing. The highest disease intensity was recorded at flowering and pod formation. Treatment with mancozeb reduced disease incidence in all the genotypes. There was a 72.6 and 59.0% reduction in disease severity for RN 490 and the lowest disease intensity (17.8 and 16.1%) was recorded in the protected plots compared to the unprotected plots (39.6 and 32.5%) in both the years. The highest seed yield loss (20.8 and 21.9%) was observed in Varuna under unprotected conditions; however, it also gave the highest seed yield (20.3 and 19.5 q/ha) followed by RN 490 (18.5 and 18.3 q/ha) in the protected plots. Pooled analysis of data revealed that Varuna had the highest disease intensity (22.0 and 44.0%) and yield performance (19.9 and 15.7 q/ha) in

protected and unprotected plots, respectively. The 1000-seed weight of RN 490 in protected (5.2 g) and unprotected (4.8 g) plots was similar with Varuna.

Rovral (Iprodione) 50WP when applied as fungicide significantly decreased *Alternaria* infection of mustard. Rovral has been reported to be the most effective fungicide against *Alternaria* leaf blight of mustard (Mathur, S.B. and Kongsdal, O. 2003).

Singh and Maheshwari (2003) carried out a study during the rabi seasons in Haryana, India, to determine the effect of Baycor (bitertanol), Blitox-50 (copper oxychloride), Akomin-40 (phosphoric acid salt), Contaf 5E (hexaconazole), Validicin (validamycin), Bavistin (carbendazim) and Dithane M-45 (mancozeb) sprays twice at 15-day intervals on *Alternaria* leaf spot (*Alternaria brassicae*) of *Brassica juncea* cv. PR-45 (Pusa Raya). The disease caused 71 and 44% average leaf and pod infection, respectively. Among the fungicides, Contaf exhibited the most effective control of the disease on leaves and pods. The disease index was lowest (16.08) in Contaf-sprayed plots whereas it was 59.09 in unsprayed control plots. The average yield was higher by 23, 10 and 9% in Contaf, Dithane M-45 and Blitox-50 sprayed plots, respectively, over the control. Two sprayings of 0.5% Contaf at 15-day intervals was effective for the control of the disease.

Godika and Pathak (2002) described the efficacy of 0.2% mancozeb, 0.2% Antracol [propineb], 0.25% Ridomil MZ [mancozeb+metalaxyl], 0.05% Bayleton [triadimefon] and 0.3% copper oxychloride in controlling blight disease (*Alternaria brassicae*) and white rust (*Albugo candida*) in Indian mustard in a field experiment conducted during 1997-2000. Antracol spraying resulted in the lowest Alternaria blight severity, whereas Ridomil MZ resulted in the lowest white rust severity. The highest yield (13.47 q/ha) and cost benefit ratio were recorded with Ridomil MZ spraying.

Amistar has been reported to control fungal and bacterial infection in cabbage (Robak, 1998), oilseed rape (Weber and Karolewski, 2000), barley and cucumber and in rice and chilli (Firoz and Hossain, 2000 and Rahman, 2002).

Singh and Singh (2002) described on timely sown (15-20 october) of mustard crops during 1995/96-2001-02 revealed *Alternaria blight* (AB-*Alternaria brassicae*), white rust (WR-*Alugo candida*), downy mildew (DM- *Peronospora parasitica*) were the major mustard diseases in mid eastern India and together caused 44.06% avoidable yield loss. In trails conducted in the same field during 2001-02 and 2002-03 crop seasons, 3 spray of Iprodione 50 WP (Rovral @ 0.20%). Follwed by mancozeb 75 WP (Indofil M 45 @ 0.2%) and propineb 70 WP (Antracol @ 0.2%) gave the most effective AB control and yield gain. Significantly superior WR control was obtained by 2 sprays of metalaxyl+ mancozeb 72 WP (Ridomil MZ @ 0.25%) followed by 3 sprays of captan 50 WP (Captaf @ 0.20%).

Godika *et al.* (2001) was conducted an experiment from 1994/95 to 1996/97 in Rajasthan, India to evaluate the efficacy of different fungicides, named Mancozeb, Ridomil MZ, (mancozeb+metalaxyl), Captan, Rovral (iprodione), Bayletan (tridimefon), and copper oxycloride, against *Alternaria* blight (Alternaria brassicae) and white rust (*Albugo candida*) of Indian mustard. All the fungicides significantly controlled both diseases, but their efficacy varied. Rovral was the most effective in controlling of *Alternaria* blight; mean disease intensity in leaf and pod was 8.75 and 5.6%, respectively. On the other hand, Ridomil MZ was the most effective in controlling white rust; mean disease intensity in leaves and staghead were 8.5 and o.5 %, respectively. Yield was highest with Rovral (2.1 t/ha), followed by Mancozeb and Ridomil MZ, each recording a yield of 1.9 t/ha.

Anwar and Khan (2001) used Indian mustard. Indian mustard cv. RL-18 seeds these were treated with four fungicides: Benlate [benomyl], Vitavax [carboxin], Ridomil [metalaxyl] or Thiovit [sulfur] at 2 g/kg seed. All the fungicides reduced the disease incidence. Benlate showed the best performance and reduced the disease incidence by 76.6%, followed by Vitavax, Ridomil and Thiovit which reduced disease incidences by 70.0, 63.3 and 53.5%, respectively. The maximum increase in yield i.e. 51.4% was observed in plots treated with Benlate followed by Vitavax, which recorded increased yield (44.6%). Ridomil and Thiovit were

the least effective in reducing the disease incidence and increasing the yield. Benlate was the most effective in reducing the disease incidence and in improving the yield.

Study conducted by Godika *et al.* (2001) from 1994/95 to 1996/97 in Rajasthan, India to evaluate the efficacy of different fungicides, mancozeb, Ridomil MZ (mancozeb+metalaxyl), captan, Rovral (iprodione), Bayletan [triadimefon], and copper oxychloride, against *Alternaria* blight (*Alternaria brassicae*) and white rust (*Albugo candida*) of Indian mustard. All the fungicides significantly controlled both the diseases, but their efficacy varied. Rovral was the most effective in controlling of *Alternaria* blight; mean disease intensity on leaf and pod was 8.75 and 5.6%, respectively. On the other hand, Ridomil MZ was the most effective in controlling white rust; mean disease intensity in leaves and staghead were 8.5 and 0.5%, respectively. Yield was highest with Rovral (2.1 t/ha), followed by Mancozeb and Ridomil MZ, each recording a yield of 1.9 t/ha.

Pandya *et al.* (2000) determined that four sprays of iprodione (0.2%) gave the maximum control of *Alternaria* blight. Maximum yield was obtained in the treatment Ridomil 0.5% (3392 kg/ha), while it was minimum in the control (2896 kg/ha).

Panja *et al.* (2000) worked with four different fungicides: Indofil M-45 [mancozeb + thiophanate-methyl], Mancozeb, 75% WP at 0.25%, Fytolan (copper oxychloride, 50% WDP) at 0.4%, Bavistin (carbendazim, 50% WP) at 0.1% and Ridomil MZ, (metalaxyl + mancozeb 72% WP) at 0.15% and their two specific combinations viz., Ridomil at 0.075% + Fytolan at 0.2% and Ridomil at 0.075% + Bavistin at 0.05% were tested against *Alternaria* leaf blight (*Alternaria brassicae* and/or *A. brassicicola*) and white rust diseases of mustard. Fytolan alone or in combination with Ridomil was superior to other treatments with respect to the reduction of leaf blight incidence and increase of crop yield. However, Fytolan + Ridomil treatment was better than Fytolan alone because of its effectiveness against white rust of mustard aside from a lower blight incidence and increased crop yield. A positive correlation existed between the reduction of

leaf blight incidence and the increase in seed yield, which was absent in case of white rust. Reduction of disease incidence of leaf blight by fungicides was found to be associated with inhibition of mycelial growth and spore germination.

Rahman (2000) observed that Rovral at 1000 ppm sprayed for 3 times was the best treatment for reducing the disease intensity and increasing yield. Percent leaf area diseased, % siliqua infection and number of spots per siliqua were reduced by 64.9%, 57.1% and 70.5% with 3 sprays.

This view is also strengthened from economic point of view and also from the findings obtained disease reduction and yield increase by 115% and 147% over control with 2 sprays of Rovral starting at 50 days age and also (Pandya *et al.*, 2000) who obtained lowest disease and highest yield with Rovral spray starting at siliqua filling stage.

Meah *et al.* (1999) was carried out a field experiments in Bangladesh during October 1997 to February 1998 to determine the effect of some management practices on mustard (cultivars Sampad and BINA) seed infection. Fungicide (Rovral 50WP, iprodione at 0.2%) applications include no fungicides, once at 40 days after sowing, and twice at 40 and 55 days after sowing. Weeding, and spraying of insecticides and fungicides, on mustard resulted in 9.5 to 7.3%, 12.7 to 3.6% and 8.3 to 4.1% reduction of infected seeds, respectively. In the control, 36-39% was infected by *A. brassicae*, while among the seeds under the various treatments; only 19-31% was affected. A greater percentage of healthy seeds were taken from treated crops.

Mridula *et al*. (1994) evaluated five fungicides, Blitox-50 [copper oxychloride], Bavistin [carbendazim], Dithane M-45 [mancozeb], Topsin-M [thiophanate-methyl] and thiram, were tested *in -vitro* against *A. brassicae,* which causes leaf blight in [Indian] mustard. Mancozeb was the most effective fungicide for inhibiting growth.

e) Integrated management

Chattopadhyay (2008) observed that different bioagents, plant extracts and fungicides are used as seed treatments and foliar spray against *Alternaria* blight of mustard in India mustard to find out effective and economical control.

Alam (2007) was conducted an experiment that the efficacy of some selected fungicides and plant extracts against *Alternaria brassicae* and *Alternaria brassicicola* causing grey blight of mustard (var. SAU Sarisha-1, Brassica campestris). Experiments were conducted at the Farm of Sher-e-Bangla Agricultural University, Dhaka and in the laboratory of Regional Agricultural Research Station, Bangladesh Agricultural Research Institute, Rahmatpur, Barishal during rabi season during the month of November, 2006 to February, 2007. Four fungicides viz. Rovral 50 WP (0.2%), Dithane M-45 (0.3%), Ridomil 68 WP (0.2%), Bavistin DF (00.15 and two plant extracts viz. Garlic clove extract, Allamanda leaf extract were employed in the experiment. Among the fungicides and plant extracts tested, Rovral WP (0.2%) showed the best performance in reducing disease incidence and disease severity as well as increasing seed yield against gray blight of mustard. Seed infection by Alternaria spp. was reduced by 64.90% and seed yield was increased by 48.19% over control by the application of Rovral 50 WP.

Prasad (2006) was carried out a field trial during rabi 2002/03 and 2003/04, in India to evaluate the efficacy of different spraying combinations of three fungicides (ridomil [metalaxyl], carbendazim and mancozeb) and five plant extracts (*Datura stramonium, Eucalyptus globosus, Azadirachta indica, Allium sativum and Allium cepa*) against *Alternaria* blight (*Alternaria brassicae*) of Indian mustard cv. Varuna. Comparative analysis of various spraying schedules revealed that first spray of carbendazim (0.1%) + mancozeb (0.2%) followed by two sprays of mancozeb (0.2%) at early sowing (20 October) was the best combination in reducing the disease severity on leaves (18.7%) and pods (10.4%) and in increasing yield (1295.8 kg/ha), 1000-seed weight (5.12 g) and oil content (42.6%). Sowing on 20 October also gave higher seed yield and reduced disease

intensity on leaves and pods in comparison to later sowing. Among the botanicals integrated with the standard fungicide (mancozeb), 5% aqueous extract of *D. stramonium, E. globosus* and *Allium sativum* reduced the disease intensity by 21.7, 23.3 and 25.5% on leaves, respectively. However, mancozeb provided the highest reduction (20.9%) of the disease on leaves and was statistically at par to these plant extracts. Apart from mancozeb, D. stramonium was found to be most effective in increasing seed yield.

Kumar *et al*. (2004) described that the efficacy of different fungicides (Emisan 6 [2-methoxyethylmercury chloride], wettable sulfur, Ridomil MZ-72 [mancozeb + metalaxyl], Blitox-50 [copper oxychloride], Dithane M-45 [mancozeb], Kitazin [iprobenfos], Bavistin [carbendazim] and Baynate [thiophanate-methyl]) and neem products (Furpume, Bioneem, Nimbicidine and Achook) were tested against 15 isolates of *Alternaria brassicae* collected from different locations in Haryana, India. Kitazin was highly effective against all the isolates in inhibiting spore germination. It was followed by Dithane M-45 and Ridomil MZ-72 but was statistically at par. Similarly, Achook and Bioneem were also effective compared to furpume and nimbicidine. Variations were also observed among isolates in their sensitivity against these fungicides.

Ferdous *et al.* (2002) was carried out an experiment to investigate the effect of three plant extracts and one fungicide on the incidence of *Alternaria* blight (caused by *Alternaria brassicae*) of mustard (*Brassica* sp.) cv. Sonali Sarisha under neutral field conditions in Gopalgonj, Bihar, India, during 1997-98. Young leaves of neem (*Azadirachta indica*), mustard (*Brassica* sp.) cv. Sambal (30-35 days old) and garlic cloves were macerated in tap water and 1% spray solution was prepared using the crude extracts. The fungicide Rovral (iprodione) at 0.1% was also used. All the 4 treatments were used at 1 litre/10m2 areas. Two sprays at flowering (35-45 days) and fruiting (45-55 days) were given at 7 days' interval. The fungicide treatment was the best in reducing *Alternaria* blight intensity and in increasing yield. Among the non- fungicidal treatments, the spray of garlic and neem leaf crude extracts proved promising. Spray of these 2 extracts at flowering stage suppresed disease incidence and increased yield.

CHAPTER -III

MATERIALS AND METHODS

MATERIALS AND METHODS

Different constraints are involved to reduce the quality and quantity of mustard cultivation and among the constraints, diseases play an important role. The present study was conducted entitled Molecular Characterization of *Alternaria* Isolates Associated with Grey Blight Disease of Mustard and Its Integrated Management. This chapter deals with six (6) experiments throughout the study period. The experiments were as follows-

Experiment-1: Survey on the Major Diseases of Mustard in Selected Districts of Bangladesh

Experiment-2: Identification and Characterization of *Alternaria* Isolates.

Experiment-3: Pathogenicity Test

Experiment- 4: Study on Genetic Variation among the Selected *Alternaria* Isolates through Molecular Analysis.

Experiment- 5: Evaluation the Efficacy of Selected Fungicides, Botanicals, Bio - agent and their Combined Effect in Controlling *Alternaria* (*In -vitro*).

Experiment- 6: Integrated Management of Grey Blight of Mustard through Selected Fungicides, Botanicals, Bio - agent and their Combined Effect (*In – vivo*).

3.1. Experiment-1: Survey on the Major Diseases of Mustard in Selected Districts of Bangladesh

3.1.1. Location of surveyed areas

The survey study was conducted in eight selected districts of Bangladesh namely Jashore, Khulna, Satkhira, Dhaka, Manikganj, Tangail, Sirajganj and Dinajpur. In total three upazilas /sub-stations from each of the district was considered for sampling i.e., total 24 (Twenty-four) upazilas were selected for sampling. The survey data was collected followed by the pre-structure questionnaire (Appendix – I). 5 (Five) farmers were selected to collect the data from each of the upazilla. It means total 120 (One hundred twenty) farmers were selected to collect the survey data**.** Selected Districts and Upazilas from where data collected of the survey study present in Table 1 and Geographical Map presented in Appendix – II.

Table 1. Selected districts and upazilas of the surveyed study

Sl No	Name of the District	Name of the Upazila	Sample collection and survey date	Number of farmers
1	Jashore	Sadar	26.12.2019	5
		Jhikargacha	24.12.2019	5
		Abhynagar	28.12.2019	5
2	Khulna	Phultala	19.01.2020	5
		Dumuria	29.12.2019	5
		Digholia	19.01.2020	5
3	Satkhira	Sadar	20.01.2020	5
		Tala	20.01.2020	5
		Kalaroa	23.12.2019	5
4	Dhaka	Savar	15.01.2020	5
		Dhamrai	08.01.2020	5
		Keraniganj	15.01.2020	5
5	Manikganj	Sadar	08.01.2020	5
		Shibaloy	08.01.2020	5
		Ghior	08.01.2020	5
6	Tangail	Mirzapur	22.01.2020	5
		Sadar	22.01.2020	5
		Basail	22.01.2020	5

7	Sirajganj	Sadar	22.01.2020	5
		Ullapara	21.01.2020	5
		Raiganj	21.01.2020	5
8	Dinajpur	Sadar	26.01.2020	5
		Ghoraghat	26.01.2020	5
		Hakimpur	26.01.2020	5

3.1.2. Survey period

Surveys were done during the robi season period from December 2019 to February, 2020.

3.1.3. Data collection

Data were collected from farmer's field during mustard cultivation to record the disease incidence. Data was collected by following process.

1) Non - Random sampling

Non-probability sampling is a sampling technique where the samples are gathered in a process that does not give all the individuals in the population equal chances of being selected (Appendix -III).

2) Random sampling

Random sampling is a part of the sampling technique in which each sample has an equal probability of being chosen. A sample chosen randomly is meant to be an unbiased representation of the total population (Appendix – IV).

3.1.5. Processing of the data collection

Data was collected according to the prescribed questionnaire in the selected areas of Bangladesh from the Mustard growers or farmers. The data collection is represented in district wise in the appendix – V.

3.1.6. Observation of the symptoms

Symptoms of the diseases were studied by visual observation. Sometimes hand lens was used for critical observation of the disease and sometimes a disease was identified based on matching the observed symptoms in the infected leaves, stem

and pod with the symptoms. Identification of all the fungal diseases was finally confirmed by identification of the associated fungal organism through isolation.

3.1.7. Collection of diseased specimen and processing

Leaves were collected from infected disease plant. The specimens were collected and preserve into gipped poly bag and then kept in the laboratory room following standard procedure of preservation. The disease specimen was preserved until the isolation was performed. The collected disease specimens ware arranged in district wise that represented in the Plate 1.

A

B

C

D

E

F

G

H

Plate 1. Specimens collected from selected districts A – Jashore, B - Khulna, C- Satkhira, D- Dhaka, E- Manikganj, F-Tangail, G - Sirajganj and H - Dinajpur

3.1.8. Data analysis

The collected data was arranged then put in the excel sheet and analysis using the computer basis software SPSS.

3.2. Experiment- 2: Identification and characterization of *Alternaria* Isolates

3.2.1. Experimental site

The experiment was conducted in Molecular Biology and Plant Virology Laboratory under the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka -1207 and Plant Pathology Laboratory under Plant Pathology Division, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur, Bangladesh.

3.2.2. Experimental period

The experiment 2 was carried out from January to June 2020.

3.2.3. Collection of diseased leaf samples

Mustard leaves having typical symptoms were collected from 8 (Eight) mustard growing districts of Bangladesh namely Jashore, Khulna, Satkhira, Dhaka, Manikganj, Tangail, Sirajganj and Dinajpur. The diseased leaves were cut from the plants grown in the field and put into a brown paper envelope. Then the brown paper envelopes of each collection were taken to the laboratory to isolate the causal organism (Plate 2 - 3).

Plate 2. Symptoms of *Alternaria* leaf blight of mustard

Plate 3. Collected diseased leaf samples

3.2.4. Designation of collected isolates

The collected isolates were designed as JAS, KHU, SAT based on their collected location. For example, an isolate collected from Jashore and recognized as first three letters of the area.

Table 2. Designation of collected isolates of *Alternaria* species

District	Isolates designation	Village/Place
Jashore	JAS	Fatepur
Khulna	KHU	Digolia
Satkhira	SAT	Sadar
Dhaka	DHA	Savar
Manikganj	MAN	Shibaloy
Tangail	TAN	Sadar
Sirajganj	SIR	Sadar
Dinajpur	DIN	Sadar

3.2.5. Isolation, identification, purification and preservation of the isolates

3.2.5.1. Preparation of PDA medium

Potato dextrose agar (PDA) media was prepared according to standard protocol and autoclaved at 121^0 C for 30 minutes under 15 psi. After autoclaved the media

was kept few minutes for cooling and added 25-30 drops of lactic acid then poured into sterile petriplates.

3.2.5.2. Isolation and Identification of *Alternaria* spp.

The pathogen was isolated by tissue planting method. The surface area of the clean bench was strilized with 70% eathanol. Then the infected leaf samples were taken into the clean bench and cut into small pices, the cut pices were then strilized with 70% eathanol by dipping for 1 minute. Surface sterilized leaf pices were taken out with the help of strile forceps and put on sterile distiled water to remove the steriliant for 3 consecutive wash.

After washing the cut pieces were placed on sterilize moist blotter paper (Whatman No. 1) in petriplates (Plate - 4) and incubated at 25±1˚ C for 5-7 days. After incubation the fungal mycelia grew over the infected leaf pieces were transferred on fresh PDA medium with the help of a sterilize needle and incubated at 25±1˚C for 7 days. Reculture were done to get pure culture and then observe under after incubation the fungus mycelia were examined under stereomicroscope and compound microscop. A semi permanent slide was prepared and examined under identification of the pathogen. The fungus was identified following the keys of Eills (1971).

Plate 4. Surface sterilized leaf pieces placed over moistened filter paper

3.2.5.3 Purification and preservation

The pure culture of Alternaria species from the PDA was transferred to PDA slants and allowed to grow at $25_{\pm}1^{0}$C for 7 days. After incubation PDA slants were preserved in refrigerator at 4^{0}C for further study.

3.2.5.4. Measurement of fungal sporulation

To determine conidial concentration of each isolate, cultures grown in the seven different media plates were considered. 10 ml of sterile distilled water was added to culture plate and using a sterile glass slide, the culture surface was gently scrapped to make a conidial suspension. Conidial concentration was determined using a haemocytometer (Figure 4).

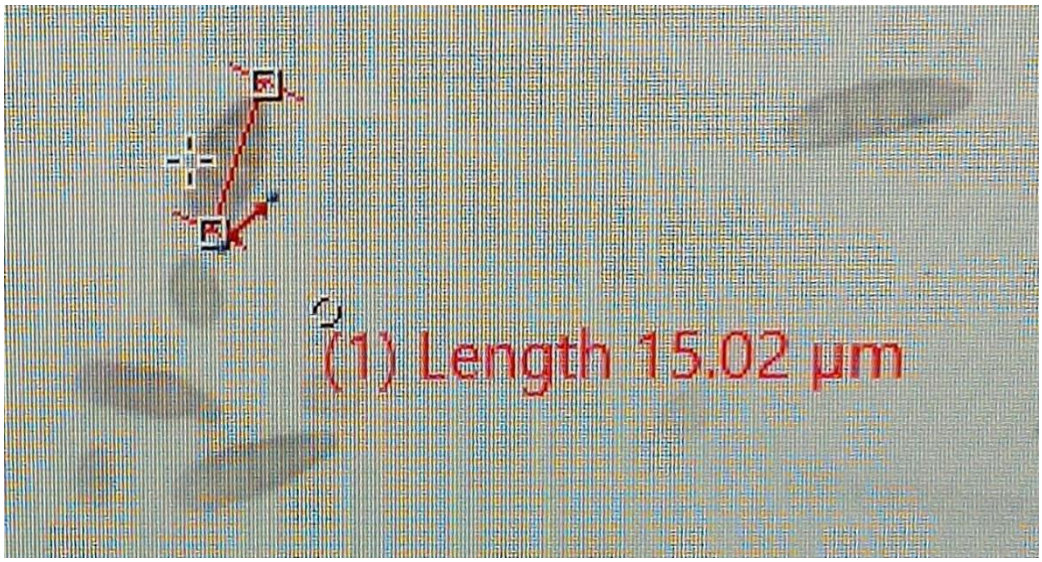


Figure 2. Conidial length, breath and beck (40X)

3.2.5.5. Data Analysis

The data were statistically analyzed using computer-based software Statistix-10. Treatment means were compared by DMRT (Duncan's Multiple Range Test).

3.3. Experiment- 3: Pathogenicity Test

3.3.1. Experimental site

The experiment was conducted in Molecular Biology and Plant Virology Laboratory under the Department of Plant Pathology and net house under the Department of plant pathology of Sher-e-Bangla Agricultural University, Dhaka-1207.

3.3.2. Experimental period

The experiment was conducted from November – December 2020.

3.3.3. Re-culture of the pathogen

Re-culture was done using the pure culture storage for other study.

3.3.4. Inoculum preparation

The purification of fungal isolates was done single spore isolation technique. A dilute spore suspension was poured on solid agar Petri-dishes to make a very thin layer on it and spores were allowed to settle down on the agar surface. Settled spores were separated out from each other, selected under the microscope and encircled with the help of dummy cutter in Petri-dishes. They were lifted along with agar blocks and transferred to another Petri-dish containing sterilized 2% PDA medium. After proper growth of fungus obtained by single spore culture.

3.3.5. Spore counting

To determine the conidial concentration of each isolate, 5 ml of distilled water (dH_2O) and 2.5 ml of ethanol was added in each petri plate containing 14 days old pure culture. The surface of the fungal growth on PDA was brushed gently with a toothbrush to disperse the spores. 3 ml of suspension and 22 ml of dH_2O were taken in a test tube and mixed well by shaking. The final suspension (30 ml) was used in a haemocytometer and the numbers of conidia/ml were counted under a compound microscope and number of conidia/ml was estimated (Nasreen *et al.*, 2017). The procedure was repeated for thrice and a mean number of conidia/ml was estimated.

3.3.6. Pathogenicity Test

The pathogenicity tests of the fungus were made on healthy leaves of host plant separately in order to establish the pathogenic nature of the fungus isolated according to Koch's postulates. To test the pathogenicity of the fungus isolated from the diseased leaves of Brassica campestris L. a series of experiments were conducted in net house of plant pathology Dept, SAU, Dhaka under natural environmental conditions. Firstly, the earthen pots of 10" diameter were prepared by filling soil and farmyard manure (FYM) in 3:1 ratio, respectively and seeds of local rapeseed variety, were sown at the rate of 5 seeds per pot. The fresh plants

at the age of 15 days were sprayed by sterilized distilled water followed by spraying of conidial suspension @ $3x10^4$. Control was sprayed with plain sterilized distilled water only. The pots were covered by fresh polythene bags and put on the wooden benches overnight. After a week the symptoms commenced to appear. The typical symptoms were identical to the symptoms in the collected diseased material. The diseased leaves from the pots were put to isolation. They yielded the same pathogen which was sprayed on the potted plants, thus proving the Koch's postulates (Koch, 1876) and the pathogenicity of *Alternaria* got confirmed.

3.4. Experiment- 4: Study on Molecular Variation among the Selected *Alternaria* isolates.

3.4.1. Experimental site and period

The experiment was conducted in Molecular Biology and Plant Virology Lab. Under the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207 and Plant Pathology Laboratory under Plant Pathology Division, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur, Bangladesh.The experiment 4 was carried out from January 2021 to December 2021.

3.4.2. Extraction of fungal genomic DNA

A. Preparation of plant leaf samples with a mechanical bead-beating device

This preprocessing protocol required a mechanical bead-beating device with a bead and tube combination or a bead and sealable deep-well plate combination.

Materials supplied by the user

• bead-beating device (e.g., MP Bio Medicals Fast Prep®-24 Instrument)

• sterile, aerosol-resistant pipette tips for sample transfer into prefilled reagent cartridges

• micro centrifuge or plate-specific centrifuge

Sample processing notes

The total yield of genomic DNA from plant materials depended on the volume of material processed and the amount of genomic DNA in the plant material used. Each cartridge supplied in the Maxwell® RSC Plant DNA Kit was designed to purify genomic DNA from 310µl of plant lysate with up to 20mg of plant material. The reagents required to generate plant lysates were supplied in the kit. Nuclease-Free Water was provided to dilute the binding buffer in the first well of the cartridge to optimize binding of genomic DNA.

1. Placed up to 20mg of leaf tissue in the bottom of each tube or well.
2. Placed a bead (or beads, as recommended by manufacturer) into each tube or well.
3. Added 300µl of Tail Lysis Buffer (TLA) to each tube or well.
4. Added 10µl of RNase A to each well (optional, to eliminate RNA).
5. Run the bead-beating device using the time and speed recommended by the manufacturer. Some optimization required to generate sufficient sample lysis for the desired DNA yield.
6. Placed the extraction tubes or plates into a centrifuge and spin for 2 minutes at maximum speed to pellet any solid particulates from the sample lysate.
7. Proceed to Section 3.D to set up the deck tray(s) and cartridges.

B. Preparation of plant leaf samples with a microtube, pestle and liquid nitrogen

This preprocessing protocol used a microtube and pestle for tissue grinding and liquid nitrogen to freeze the sample.

Materials supplied by the user

• Pellet Pestles (Sigma Aldrich Cat.# Z359947)

• Click Fit Micro tubes, 1.5ml

• liquid nitrogen • sterile, aerosol-resistant pipette tips for sample transfer into prefilled reagent cartridges

• micro centrifuge

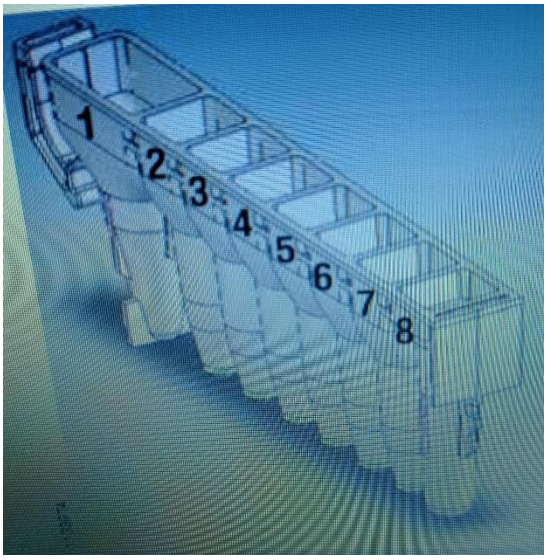
Sample processing notes

The total yield of genomic DNA from plant materials depends on the volume of material processed and the amount of genomic DNA in the plant material used. Each cartridge supplied in the Maxwell® RSC Plant DNA Kit is designed to purify genomic DNA from 310µl of plant lysate with up to 20mg of plant tissue. The reagents required to generate plant lysates are supplied in the kit. Nuclease-Free Water is provided to dilute the binding buffer in the first well of the cartridge to optimize binding of genomic DNA.

1. Placed up to 20mg of leaf tissue in the bottom of a ClickFit Microtube, 1.5ml.
2. Added liquid nitrogen to the plant tissue sample. Allow the liquid to evaporate, freezing the sample.
3. Used a pellet pestle, grind the frozen plant tissue against the tube wall as thoroughly as possible.
4. Added 300µl of Tail Lysis Buffer (TLA) to each tube.
5. Added 10µl of RNase A to each tube (optional, to eliminate RNA).
6. Vortex tubes for 10 seconds.
7. Placed tubes with lysate into a micro centrifuge and spin for 2 minutes at maximum speed to pellet solid particulates from the lysate.
8. Proceed to Section 3.D to set up the deck tray and cartridges.

C. Maxwell® RSC plant DNA cartridge preparation

Changed gloves before handling cartridges, Plungers and Elution Tubes. Placedeach cartridge in the deck tray(s) with the labeled side facing away from the Elution Tubes. Pressed down on the cartridge to snap it into position. Carefully peeled back the seal so that all plastic came off the top of the cartridge. Ensure that all sealing tape and any residual adhesive were removed before placing cartridges in the instrument. Placed a Plunger into well #8 of each cartridge. Well #8 is the well closest to the Elution Tubes. (Figure 3)



User adds to wells

1. 300µl of Nuclease-Free Water + preprocessed samples
8. Plunger

Figure 3. Maxwell® RSC cartridge

3.4.3. Instruments used

DNA isolation:

1. Automated DNA extractor, Model: Maxwell 16, Origin: Promega, USA.
2. Homogenizer, Pro Scientific,

DNA quantification:

1. Nano Drop Spectrophotometer, Model: ND2000, Origin: Thermo Scientific, USA.

PCR:

1. Gene Atlas, Model: G2, Origin: Astec, Japan.

Gel electrophoresis system:

1. Horizontal, Model: Mini, Origin: CBS Scientific, USA.

Gel Documentation:

1. Alpha Imager, Model: mini, Origin: Protein Simple, USA.

PCR clean-up:

1. Centrifuge, Model: Kitman24, Origin: Tomy-Japa

3.4.4. Reagents used

DNA isolation:

1. Maxwell® 16 LEV Plant DNA Kit, Model: AS1420, Origin: Promega, USA.

PCR:

1. Hot Start Green Master Mix, (dNTPs, Buffer, MgCl2, Taq Pol), Cat: M7432, Origin: Promega, USA.
2. Primer ITS4: TCC TCC GCT TAT TGA TAT GC
3. Primer ITS5: GGA AGT AAA AGT CGT AAC AAG G

Gel:

1. Agarose, Cat: V3125, Origin: Promega, USA.
2. 100 bp DNA Ladder, Cat: G2101, Origin: Promega, USA.
3. 1kb DNA Ladder, Cat: G5711, Origin: Promega, USA.
4. Ethidium Bromide Solution, Cat: H5041, Origin: Promega, USA.
5. TAE Buffer: Cat: V4251, Origin: Promega, USA.

PCR clean up:

1. SV Gel and PCR Clean Up System, Cat: A9281, Origin: Promega, USA.

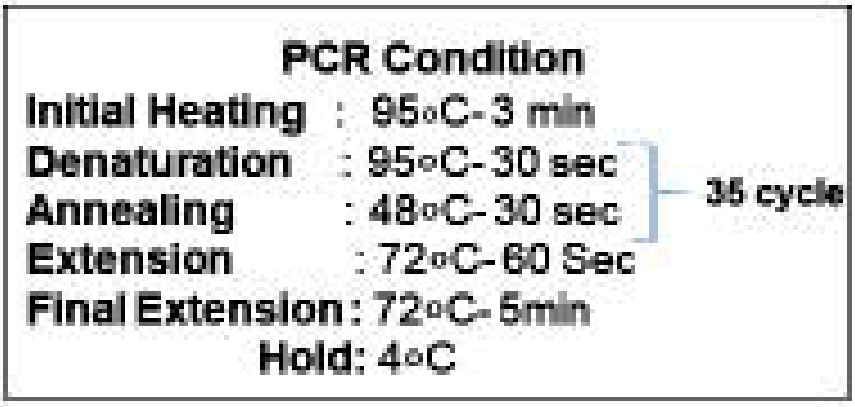
3.4.5. PCR for amplification

The following wing ITS primer pairs was used for amplification-

Primer ITS4: TCC TCC GCT TAT TGA TAT GC

Primer ITS5: GGA AGT AAA AGT CGT AAC AAG G

3.4.6. PCR Condition



Following chemicals were used in the PCR reaction 0f 25 µl

Sl	Items	Volume	Reaction Number	Total Volume
1	Master Mix	12.5 µl	X1	12.5
2	T DNA (Concentration 25-65 ng/ul)	1 µl	X1	1
3	Primer F (Concentration 10-20 pMol)	1 ul	X1	1
4	Primer R (Concentration 10-20 pMol)	1 ul	X1	1
5	Water	9.5 ul	X1	9.5
Total		25 ul	Total	25

**Equation for Annealing Temperature:

$$\text{Annealing Temp} = \frac{(Tm\ F + Tm\ R)}{2} - (3\ to\ 5)$$

Primer sequence:

PCR Product Size was around 700 bases. And extension time was directly related with product size. Our Promega Taq Polymerase amplified 1000 base per min. 60 Sec extension time needed for PCR Work.

Thermal Profile for our Primers with M7431 Master Mix:

Number of Cycle	Step Name	Temp	Time.
1	Pre Heat	95°C	3 min
35 Cycle	Denaturation	95°C	30 sec
	Annealing	48°C	30 sec
	Extension	72°C	60 Sec
1	Final Extension	72°C	5 min
1	Hold	4°C	Over Night.

3.4.7. Agarose gel electrophoresis and DNA purification

Gel electrophoresis of the genomic DNAs was carried out for qualitative estimation of samples prepared. A good DNA preparation appeared as a single band. A submarine horizontal agarose slab gel apparatus (Bangalore Genei) as described by Sambrook *et al.* (1989) was used. Agarose (1.0% W/V) was suspended in 1.0X TAE buffer and boiled for 3-5 min, to dissolve it. After cooling, it was poured in an electrophoresis tray and allowed to solidify at room

temperature. Slots were made by fixing a slot former (comb) over the tray, prior to pouring the agarose. The comb was removed after the agarose and gel was transferred to an electrophoresis tank containing 1X TAE buffer. Mixed 2 µl of loading dye with 4 µl DNA sample. DNA sample was loaded in slot. Gel was run at constant voltage (40 V) for three hours. After completion of electrophoresis, DNA bands were visualized in gel documentation and photographed has been taken on gel documentation system (Alpha Digidoc) USA.

The genomic DNA isolated by the above methods which contained some amounts of RNA and proteins as contaminants. The RNA contamination was removed by treating isolated DNA with DNase free RNase. Thereafter additional de-proteinization steps were followed to remove RNase. These steps eliminated most of the part of protein contamination.

Procedure:

Bovine pancreatic ribonuclease A was prepared at a concentration of 10 mg/ml in NaCl + Tris-HCl, 2 µl RNase added in each vial. DNA was heated for 40 min at 37°C. Equal volume of chloroform was mixed gently and centrifuged at room temperature for 15 min. at 13000 rpm. Upper aqueous layer was taken out in eppendorf tubes. Added equal volume of chloroform and phenol (1:1) mixed gently with inversion and centrifuged for 15 min at 13000 rpm at room temperature. Materials and Methods 42 Upper aqueous layer was taken out in eppendorf tubes mixed equal volume of phenol and shaked vary gently, centrifuged at room temperature 13000 rpm for 15 min. Upper aqueous layer was taken out in new eppendorf tube. Added 0.7 volume of isopropanol mixed by inversion and incubated at - 20°C for 4 hours, centrifuged 13000 rpm for 15 min at 4°C. The DNA pellets were washed with 70% ethanol for 5 min and pelleted by centrifugation and kept for drying at 37°C. 100 µl of ddh_20 water were added after proper drying and left overnight at 4°C to dissolve.

3.4.8. Internal transcribed spacer (ITS) region analysis

Eight Alternaria isolates were analyzed by amplifying the regions of the rDNA repeat from the 3’ end of the 18s and 5’ end of the 28s gene using PCR conditions with the two universal primers, Primer ITS4: TCC TCC GCT TAT TGA TAT

GC and Primer ITS5: GGA AGT AAA AGT CGT AAC AAG G which were synthesized on the basis of conserved regions of the eukaryotic rRNA gene (White *et al.*, 1990; Jasalavich *et al.*, 1995). The PCR- amplification reactions were performed in a 25 µl mixture containing 50 mM KCl, 20 mM Tris HCl (pH 8.0), 2.0 mM MgCl2, 20 µM of each of the four deoxynucleotide triphosphates, 20 pmol of each primer, 50 ng/µl of template and 2.5 U of Taq polymerase. These reactions were subjected to an initial denaturation hot start at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 30 s, primer annealing at 48°C for 30 s and primer extension at 72°C for 1 min and a final extension for 5 min at 72°C in a thermal cycler. Aliquots (4 µl) of the amplified products were analysed by electrophoresis in 1.5% (wt/vol) agarose gel in 1X TAE buffer (40 mM Tris, 20mM acetic acid, 1mM EDTA [pH8]), stained with ethidium bromide (1 µg/ml) and electrophoresis was carried out at 70 volts for 2 h in TAE buffer. The molecular marker was 1kb DNA ladder (Biomatrix Co. Ltd.). The desired bands were cut from the gel with minimum quantity of gel portion and the amplified PCR product was eluted using QIAGEN DNA gel extraction kit.

3.4.9. Nucleotide sequencing and in silico analysis

The sequencing of the PCR product was carried out in automated Sequencer at Xcelris Lab., Malaysia. Related sequences were searched for homology using BLAST bioinformatic search tool available at the Gen-Bank database (http://www.ncbi.nlm.nih.gov/blast/) (Altschul *et al.*, 1997). Sequences used for comparison were obtained from NCBI database (http://www.ncbi.nlm.nih.gov) and the details are given in the results. The multiple sequence alignment and pair wise alignment were performed using the Clustral W algorithm in Bioedit (Hall, 1999) and Phylogenetic analysis was done using MEGA5.0 (Tamura *et al.*, 2011) software. To assess the possible phylogenetic relationship neighborhood-joining bootstrap tree was created using CLUSTAL W 1.6 matrix by the CLUSTAL X programme ver. 1.81 (Thompson *et al.*, 1997). The ITS sequences have submitted to GenBank. Sequences used for comparison were obtained from NCBI database (http://www.ncbi.nlm.nih.gov) and the details are given in the results.

3.4.10. Formation of phylogenetic tree/ Phylogenetic analysis

Phylogenetic analysis was performed using neighbor – joining methods (Saitou and Nel, 1987). Bootstrap analysis was performed using resampled 1000 times using the DNAMAN analysis system. All reference sequences were obtained from the national center for Biotechnology Information (NCBI) database.

3.5. Experiment- 5: Evaluation the efficacy of selected bio - agent, fungicides, botanicals and their combined effect against the *Alternaria* (*In - vitro*).

3.5.1. Experimental site

The experiment was conducted in Molecular Biology and Plant Virology Laboratory under the Department of Plant Pathology of Sher-e-Bangla Agricultural University, Dhaka-1207.

3.5.2. Experimental period

The experiment was conducted from June 2020 to February 2021.

3.5.3. Design and treatments of the experiment

The experiment was designed in Completely Randomized Design (CRD) with three replications. Fourteen (14) treatments was used for the experiments which was as follows -

T_1 = Control

T_2 = *Trichoderma harzianum* suspension (0.2%)

T_3 = Autostin 50 WDG (0.3%)

T_4 = Rovral 50 WP (0.2%)

T_5 = Dithane M-45 (0.3%)

T_6 = Amistar Top 325 EC (0.1%)

T_7 = Neem leaf extract (10%)

T_8 = Allamanda leaf extract (10%)

T_9 = Lantana leaf extract (10%)

T_{10} = Datura leaf extract (10%)

T_{11} = Neem leaf extract (10%) + Rovral (0.2%)

T_{12} = Datura leaf extract (10%) + Amistar Top (0.1%)

T_{13} = Lantana leaf extract (10%) + Dithane M- 45 (0.3%)

T_{14} = Allamanda leaf extract (10%) + Autostin (0.3%)

3.5.4. Collection of bio-agent

The Bio- agent *Trichoderma harzianum* was collected from the laboratory of Plant Pathology Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur for the study.

3.5.5. Multiplication of bio-agent

The *Trichoderma harzianum* suspension was prepared @ 20 ml/liter. The suspension was measured and mixed thoroughly with water then preserved in cool temperature and darkness in the refrigerator.

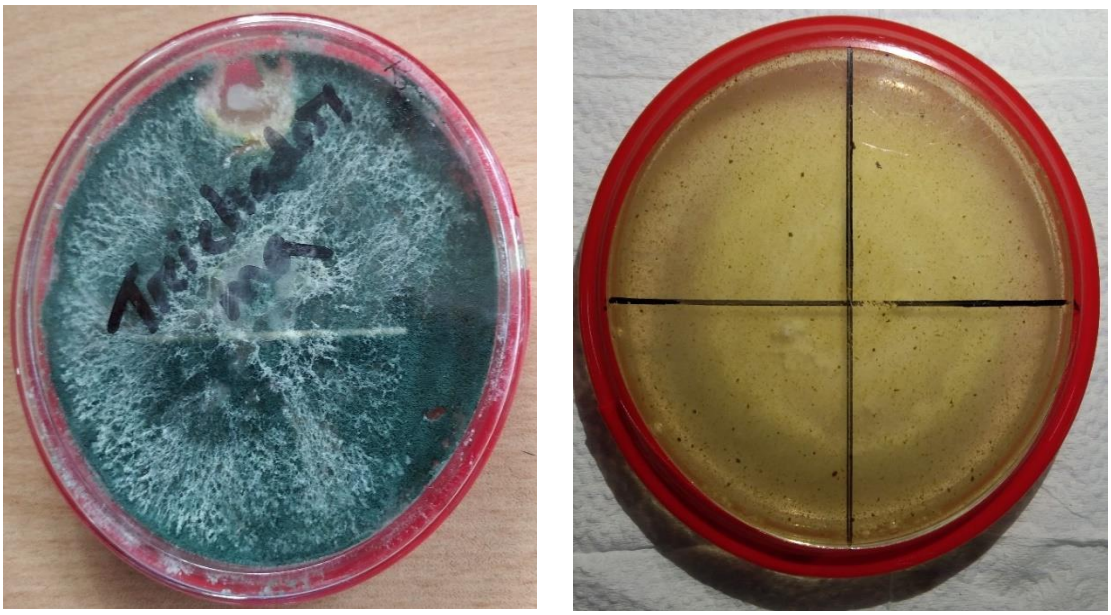


Plate 5. Showing pure culture of *Trichoderma harzianum*

3.5.6. List of selected fungicides with their formulation

The selected fungicides used in the *in-vitro* are as follows –

Table 3. Details of the fungicides used in the *in-vitro* study

Sl No	Trade Name	Chemical name	Active ingredients (a.i)	Doses Used (@)
1	Autostin	Carbendazim	WDG 50 %	0.3%
2	Rovral 50 WP	3-(3,5dichlorophenyl)-N-(Imethylethyl)-2,4 dioxoimidazolidene carboxamide (Cl3HI3)3N3CI2	Iprodione (50%)	0.2%
3	Dithane M-45	Manganous ethylene bisdithio carbamate-ion(C4H6N2S4)	Mancozeb (50%)	0.3%
4	Amistar Top	Azoxystrobin+Difenoconazol	EC 325	0.1%

3.5.7. Collection of fungicides

The selected fungicides viz. Autostin, Rovral 50 WP, Dithane M-45 and Amistar Top was collected from Siddik Bazar, Dhaka and preserved it in the Molecular Biology and Plant Virology Laboratory under the Department of Plant Pathology of Sher-e-Bangla Agricultural University, Dhaka-1207 for further study.

3.5.8. Preparation of fungicides

Fiungicides were prepared as per recommended dose.

A B C D

Plate 6. Showing selected fungicides of A – Autostin, B- Amistar Top, C - Dithane M -45 and D – Rovral

3.5.9. Collection of botanicals

Neem leaf, Allamanda leaf, Lantana leaf and Datura leaves were used as botanicals. Botanicals were collected from the campus of Sher-e-Bangla Agricultural University, Dhaka.

3.5.10. Preparation of botanical extract

Fresh leaves were collected and weighed 100 gm per botanical. The leaves were washed and chopped into small pieces. To prepare the extract the choppedmleaves were blended with electric blender and sieved through strainer. Hundred ml leaf extract of each botanical was added into one litre distilled water maintaining ratio 1:10 for foliar spray.

A B C D

Plate 7. Showing botanical leaves of A- Allamanda, B – Datura, C – Lantana and D – Neem

3.5.11. Inoculation of bio-chemicals and botanicals

Requisite quantity of each treatment (Biological, botanicals, fungicides and their combined effect) was incorporated in 100 ml PDA and mixed thoroughly by shaking, prior to pouring into petri plates. After the pouring of PDA in Petri plates, the medium was allowed to solidify and these plates were centrally inoculated with the 6 mm diameter disc of pathogen which is cut by sterilized cork borer, taken from the margin of actively growing 7 days old culture. Control was used as such without adding treatment in the medium. Four replications of each treatment incubated at 25±1° C for growth of the pathogen. The efficacy of various treatments (Biological, botanicals, fungicides and their combined effect) was observed by measuring radial growth of the fungal colony in millimeters (mm). The inhibition evaluation was evaluated in terms of per cent inhibition of fungal growth was compared to the check. The efficacy of various treatments (Biological, botanicals, fungicides and their combined effect) was assessed by measuring the linear growth of the fungus. Radial growth of the pathogen in control (mm) and colony characters were observed for the study. Percent inhibities was measured as follows –

$$P_1 = \frac{C - T}{C} \times 100$$

C = Control radial mycelial growth

T = Radial mycelial growth in treatment

3.5.12. Data analysis

The data were statistically analyzed using computer-based software Statistix-10.

3.6. Experiment- 6: Integrated management of grey blight of mustard through selected bio - agent, chemical, botanicals and their combined effect.

3.6.1. Experimental sites

The experiment was conducted at central research field of Sher-e-Bangla Agricultural University, Dhaka-1207. The location of the site was 23^{o} 74 N latitude and 90^{o} 35 longitude with an elevation of 8.2 meter from sea level.

3.6.2. Experimental period

The experiment was carried out during the Rabi season from November 2021 to March 2022. Seeds were sown on 19 November 2021 and were harvested on 11, February 2022.

3.6.3. Soil type

The experimental site was situated in the subtropical zone. The soil of the experimental site lies in agro-ecological regions of "Madhupur Tract" (AEZ No. 28). Its top soil is clay loam in texture and olive gray with common fine to medium distinct dark yellowish-brown mottles. The pH 4.47 to 5.63 and organic carbon contents is 0.82 (Appendix- XI).

3.6.4. Climate

The climate of the experimental field area was of sub-tropical in mature characterized by high temperature associated with heavy rainfall during Kharif season (April to September) and scanty rainfall with moderately low temperature during Rabi season (October to March).

3.6.5. Weather

The monthly mean of daily maximum, minimum and average temperature, relative humidity, monthly total rainfall and sunshine hours received at the experimental site during the period of the study have been collected from the surface synoptic Data card, Bangladesh Meteorological Department, Dhaka (Appendix-XII).

3.6.6. Variety

The mustard (*Brassica campestries*) variety BARI Sarisha-14 released from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur was used for the experiment. Seeds were collected from Bangladesh Agricultural Development Corporation (BADC), Gabtoli, Dhaka.

3.6.7. Treatments of the experiment

Multiple treatments i.e. 14 treatments were used in the experiment as follows: T_1 = Control (No spray applying) , T_2 = *Trichoderma harzianum* suspension @0.2%, T_3 = Autostin 50 WDG @0.3%, T_4 = Rovral 50 WP @0.2%, T_5 = Dithane M-45 @ 0.3%, T_6 = Amistar Top 325 SC @0.1%, T_7 = Neem leaf extract (10%), T_8 = Allamanda leaf extract (10%), T_9 = Lantana leaf extract @10%, T_{10} = Datura leaf extract @10%, T_{11} = Neem leaf extract+ Rovral, T_{12} = Datura leaf extract + Amistar Top, T_{13} (Lantana leaf extract + Dithane M- 45), T_4 = Allamanda leaf extract + Autostin.

3.6.8. Design and layout

The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. The whole plot was divided into three blocks each containing fourteen (14) plots of 3m x 1.5m size, giving 42 units plots. The space was kept 1m between the blocks and 0.5m between the plots (Appendix-XIII).

3.6.9. Land preparation

The land was ploughed with a power tiller in the first week of November 2021 and left exposed to sunlight for 7 days. Then the land was ploughed and cross-ploughed by a country plough until the soil had a good tilth. It required different times ploughing and every ploughing was followed by laddering to level the land and break up clods. After each ploughing, weeds and rubbish were removed. Finally spade (Kodal) was used to prepare plots and drains.

3.6.10. Application of manure and fertilizers

Manure and fertilizers were applied as per standard recommendation.

The following doses were used for carrying out the field study (Anonymous, 2001).

Manures /Fertilizers	Rate /ha
Urea	250 kg
TSP	170 kg
MP	85 kg
Gypsum	150 kg
Zinc oxide	5 kg
Boric acid	10 kg

3.6.11. Preparation and application of spray solution

All treatments were applied as foliar spray. Sprays were done at 50, 60, 70 days after sowing. Every time the fungicide was freshly prepared prior to application and the spray tank was thoroughly cleaned before filling with new spray materials. Special attention was given to complete coverage of the growing plants with the fungicides. Adequate precaution was taken to avoid drifting of spray materials from one plot to the neighboring ones (Figure 4).

Figure 4. Treatments spraying in the experiment al plot

Details of the fungicides used as spray materials are given in Table 4.

Table 4: Details of fungicides

Sl No	Trade Name	Chemical Name	Active ingredients (a.i)	Doses used (@)
1	Autostin	Carbendazim	WDG 50 %	0.3%
2	Rovral 50 WP	3-(3,5dichlorophenyl)-N-(Imethylethyl)-2,4 dioxoimidazolidene carboxamide (Cl3HI3)3N3CI2	Iprodione (50%)	0.2%
3	Dithane M-45	Manganous ethylene bisdithio carbamate-ion(C4H6N2S4)	Mancozeb (50%)	0.3%
4	Amistar Top	Azoxystrobin+Difenoconazol	EC325	0.1%

3.6.13. Intercultural operations

Weeding was done, when necessary, followed split doze fertilizer application. After weeding and fertilizer application flood irrigation was given by filling the drains surrounding the beds by pumping water in those drains with a water pump. After soaking the plots excess water was allowed to be drained out. Malathion 57 EC was applied three times at 10 days intervals to control aphid.

3.6.14. Tagging and data collection

Randomly fifteen plants were selected from each plot and red tagged was used for data collection and mean values were determined to get rating score of each treatment (Figure 5)

Figure 5. Red tagged of the selected diseased plant

3.6.15. Harvesting of crop

When 80% of the plants showed symptoms of maturity i.e. straw colored leave, stem, siliquae was noticed the crop was harvested as seed yield taken. At maturity, ten plants were harvested by uprooting and then they were tagged properly. Data were recorded on different parameters from these harvested plants

3.6.16. Collection of data

The following parameters were considered for data collection.

On diseases incidence

a. Percent leaf infection

b. Percent leaf area diseases (% LAD)

c. Percent pod infection

d. Number of spots/pod

On yield and yield contributing characters

a. Number of pods/plant

b. 1000-seed weight

c. Yield/plant (gm)

d. Yield (Mton/ha)

3.6.17. Identification of the disease and evaluating the efficacy of selected bio-chemicals

Identification of the disease was done mainly through visual observation of typical symptoms of grey blight like development of circular spots on leaves & pods with concentric ring and elongated lesion on stem. Later on spots coalesce and ultimately the leaves become blighted. The disease incidence (%), disease severity (%) and disease severity index (%) of grey blight of Mustard was recorded by counting infected plants, infected leaves and estimating infected leaf area at 50, 60 and 70 days after sowing (DAS). The pod infection (%) was recorded by counting the infected pods at 55, 65 and 75 (DAS). To evaluate the efficacy of the selected bio-chemical treatments for disease management, the following parameters were undertaken: number of infected plant/plot, number of infected leaf/plant, infected leaf area/ leaf, number of infected pod/ plant, fresh yield/plot (kg/ha), dry yield/plot (kg/ha), fresh grain weight (gm/plot), dry grain weight (gm/plot) and 1000-seed weight/plot (gm).

3.6.18. Procedure of data collection

3.6.18.1. Percent leaf infection

Fifteen plants per plot (4.5 m^2) were selected randomly and red tagged was used for indication. Data on percent leaf infection were recorded at 50, 60 and 70 days after sowing (SAS) by visual observation of symptoms. Percent leaf infection was calculated by the following formula –

Percent disease incidence was calculated by the following formula:

$$\% \text{ Disease Incidence} = \frac{\text{Number of infected plant}}{\text{Number of total inspected plant}} \times 100$$

3.6.18.2. Percent disease severity (leaf area diseased)

Data on disease severity or percent leaf area diseased (LAD) were recorded at 50,60, and 70 days after sowing by visual observation of symptoms. Percent leaf area diseased was calculated by the following formula.

Percent disease severity was calculated by the following formula:

$$\% \text{ Disease Severity} = \frac{\text{Number of infected leaf per plant}}{\text{Number of total inspected leaf per plant}} \times 100$$

3.6.18.3. Disease severity index

Data on percent Disease Severity Index (DSI) were recorded at 50, 60, and 70 days after sowing by visual observation of symptoms. Percent leaf area diseased was calculated by the following formula.

Percent disease severity index was calculated by the following formula:

$$\% \text{ Disease Severity Index} = \frac{\text{Leaf area infected}}{\text{Total leaf area}} \times 100$$

3.6.18.4. Percent pod infection

Data on percent pod infection were recorded at 55, 65 and 75 days after sowing by visual observation of symptoms. Percent pod infection was calculated by the following formula.

Percent pod infection was calculated by the following formula:

$$\% \text{ Pod Infection} = \frac{\text{Number of infected pods per plant}}{\text{Total number of pods per plant}} \times 100$$

3.6.19. Statistical analysis

The data were statistically analyzed using computer-based software Statistix-10. Treatment means were compared by DMRT (Duncan's Multiple Range Test). To determine the relationship between percent disease incidences, severity, severity index and pod infection with yield for each of the treatments correlation and regression study were done.

CHAPTER - IV

RESULTS AND DISCUSSION

RESULTS

Experiment-1: Survey on the Major Diseases of Mustard in Selected Districts of Bangladesh

Disease is one of the major constrains to successful crop production. Plant pathogenic microorganisms, which are among the most devastating causes of plant diseases from seedling to subsequent plants and storage, result in significant financial losses for producers and the human public health risks due to food shortage, too. Among the pathogenic microorganisms, an important group of fungi with negative economic, technological, and public health impacts that cause spoilage of food and animal feedstuff during production and distribution. Once the spores have germinated, the fungus can penetrate and infect plant tissue through a plant's cuticle, through natural openings (called stomata) in the plant's leaves or stems, or through wound. Mustard fungi mainly occur in leaves that causes rolling, curling, chlorosis, mottling, necrosis, stunting etc. of mustard leaves. The deformed leaves hamper in photosynthesis that finally cause hinder to the formation of quality seed production. This chapter includes the result of the study i.e. disease incidence level of mustard fungi in selected surveyed areas of Bangladesh and Mustard grower's information (gender, seed source, seed treatment, treatments name, variety type and transplanting date etc). Symptomology of the major mustard fungal diseases was also recorded.

4.1. Identification of the major fungal diseases of Mustard on the basis of visible characteristics symptoms

4.1.1. Symptomology

Four diseases were identified on the basis visible symptoms. These were grey blight, white rust, powdery mildew and sclerotinia stem rot of Mustard were observed. The symptoms of these diseases are as follows-

Symptoms of Alternaria or Grey blight:

The fungus attacks the lower leaves as small circular brown necrotic spots and slowly increase in size. In severe cases many concentric spots coalesce to cover large patches showing blightening and defoliation. Circular to linear dark brown

spots also develop on stems and pods which becomes elongated at later stage. Infected pods produce small discolored and shriveled seeds (Plate -8)

Plate 8. Symptoms of Grey blight of Mustard on leaves and pods

Symptoms of white rust:

All aerials parts of the plant are attacked. White or creamy yellow pustules of various shapes and sizes were found on the surface of the leaves, mainly on the lower surface (Figure 6). Severely infected leaves were become thick, fleshy, inrolled and their reduced sizes. In few plants with symptoms of infection included chlorosis on leaf surfaces, white blister-like growths on the underside of leaves and on the stems of the plant, and swelling of the roots. In addition, abnormalities in the growth of the host can occur with more serious infections.

Figure 6. Symptoms of white rust of Mustard on leaves

Symptoms of powdery mildew:

Grayish white irregular necrotic patches were observed the lower surface of the leaves and the upper surface of the leaves yellow spots were there corresponding to necrotic spot-on lower surface (Figure 7). In some plants with Powdery mildew, symptoms appeared as dirty white, circular, floury patches on either sides of the leaves.

Figure 7. Symptoms of powdery mildew of mustard on leaves

Symptoms of Sclerotinia stem rot:

As disease progresses, the affected portions of stem were developed a bleached appearance at the internodes and eventually the tissues shreds. Infected plant become wilting and drying. Black sclerotial bodies were also seen on infected plant parts. Sclerotia were variously shaped bodies of tightly packed white mycelium covered with a dark, melanized protective coat (Figure 8).

Figure 8. Symptoms of Sclerotinia stem rot of mustard on stem

4.1.2. Mustard cultivated in selected districts of Bangladesh and its yield

Among the surveyed district, the highest Mustard cultivation was found in Sirajganj districts which was 52680 ha of land with yield 69011 MT and the lowest cultivated area was recorded in Khulna district which was 595 ha of land with the production of 744 MT. It was also recorded that almost similar yield (MT/ha) was found in all surveyed districts (Table 5).

Table 5. Mustard cultivation area with production in main mustard growing districts (FY: 2019-20)

Sl No	District	Area (ha)	Yield (MT)	Yield rate (MT/ha)
1	Jashore	13640	18005	1.32
2	Khulna	595	744	1.25
3	Satkhira	11385	14914	1.31
4	Dhaka	11465	14446	1.26
5	Manikganj	36555	47887	1.31
6	Tangail	41507	54789	1.32
7	Sirajganj	52680	69011	1.31
8	Dinajpur	12732	16806	1.32

Source: Department of Agricultural Extension, Khamarbari, Dhaka, Bangladesh, FY 2019-20

4.1.3. Mustard varieties cultivated seed used and yield in selected upazilas of Jashore district

It was noticed that both High Yielding Variety (HYV) and local varieties of mustard are cultivated in selecyted upazilas of Jashore district. From the survey study, it was also noticed that most of the farmers selected HYV varieties for commercial cultivation. Although the local varieties have the popularity of best quality. In Sadar upazila of Jashore district total cultivated area was 2400 ha of

land and production was 3230 MT (1.34 MT/ha). Out of this cultivated area mostly occupied by HYV varieties, namely BARI Sarisha – 14 (960 ha), BARI Sarisha -15 (360 ha), BARI Sarisha – 9 (260 ha). Among the local varieties, Tori - 7 (400 ha) was mostly cultivated in this upazila. In Jikargacha upazila of Jashore district total cultivated area was 1865 ha of land and production was 2518 Mton (1.35 Mton/ha). Out of this cultivated area mostly occupied by HYV varieties, namely BARI Sarisha – 14 (625 ha), BARI Sarisha - 9 (103 ha), BARI Sarisha - 15 (102 ha). Among the local varieties, Tori -7 (840 ha) was mostly cultivated in this upazila. In Abhoynagar upazila of Jashore district total cultivated area was 1200 ha of land and production was 1,624 (1.35 MT/ha). Out of this cultivated area mostly occupied by local varieties, namely Tori – 7 (843 ha). Among the HYV varieties, BARI Sarisha – 14 (325 ha) was mostly cultivated in this upazilla (Table 6).

Table 6. Mustard varieties, cultivated area and yield in selected upazilas of Jashore district (2019-2020)

Sl No	Upazilla	Mustard variety	Area (ha)	Yield (MT)	Yield rate (MT/ha)
1	**Sadar**	BARI Sarisha - 9	260	**3230**	**1.34**
		BARI Sarisha -11	120		
		BARI Sarisha -14	960		
		BARI Sarisha -15	360		
		BINA Sarisha - 4	10		
		BINA Sarisha - 9	10		
		Tori -7	400		
		Rye Sarisha -5	150		
		Jota Rye	130		
		Total	**2400**		
2	**Jikargacha**	BARI Sarisha - 9	103	**2518**	**1.35**
		BARI Sarisha -11	35		
		BARI Sarisha -14	625		
		BARI Sarisha -15	102		
		BINA Sarisha - 4	15		
		Tori -7	840		
		Rye Sarisha -5	10		

		Jota Rye	135		
		Total	**1865**		
3	**Abhoynagar**	BARI Sarisha - 9	20		
		BARI Sarisha -14	325		
		BARI Sarisha -15	7		
		Tori -7	843		
		Jota Rye	05	**1624**	**1.35**
		Total	**1200**		

Source: Department of Agricultural Extension, Khamarbari, Dhaka, Bangladesh, FY 2019-20.

4.1.4. Mustard varieties, cultivated area and yield in selected upazilas of Khulna district

In Digolia upazila of Khulna district total cultivated area was 45 ha of land and production was 61.8 ton (1.37 MT/ha). Out of this cultivated area mostly occupied by HYV. For example, BARI Sarisha -14 (25 ha), BARI Sarisha -15 (16 ha). In Fultala upazila of Khulna district total cultivated area was 37 ha of land and production was 52.2 ton (1.35 MT/ha). Out of this cultivated area mostly occupied by HYV, namely BARI Sarisha -14 (20 ha), BARI Sarisha -15 (12 ha). Among the local varieties, Tori -7 and Rye Sarisha – 5 are little bit cultivated in this upazila. In Dumuria upazila of Khulna district total cultivated area was 125 ha of land and production was 159.3 MT (1.27 MT/ha). Out of this cultivated area mostly occupied by HY, namely BARI Sarisha -14 (50 ha), BARI Sarisha -15 (36 ha). Among the local varieties, Rye Sarisha -5 (25 ha) was mostly cultivated in this upazila. From the study regarding the mustard varieties cultivated in Khulna district, it was observed that HYV are mostly cultivated in this district but most of the HYVs are highly susceptible to mustard fungi. The survey data are presented in Table 7.

Table 7. Mustard varieties, cultivated area and yield in selected upazilas in Khulna district (2019-2020)

Sl No	Upazilla	Mustard variety	Area (ha)	Yield (MT)	Yield rate (MT/ha)
1	**Digolia**	BARI Sarisha -11	1	**61.8**	**1.37**
		BARI Sarisha -14	25		
		BARI Sarisha -15	16		
		BINA Sarisha - 5	2		
		BINA Sarisha - 9	1		
		Total	**45**		
2	**Fultala**	BARI Sarisha -11	1	**52.2**	**1.35**
		BARI Sarisha -14	20		
		BARI Sarisha -15	12		
		BINA Sarisha - 5	1		
		BINA Sarisha - 9	1		
		Tori -7	1		
		Rye Sarisha -5	1		
		Total	**37**		
3	**Dumuria**	BARI Sarisha -11	1	**159.3**	**1.27**
		BARI Sarisha -14	50		
		BARI Sarisha -15	36		
		BINA Sarisha - 5	2		
		BINA Sarisha - 9	1		
		Tori -7	7		
		Rye Sarisha -5	25		
		Jota Rye	1		
		Sampad	2		
		Total	**125**		

Source: Department of Agricultural Extension, Khamarbari, Dhaka, Bangladesh, FY 2019-20.

4.1.5. Mustard varieties, cultivated area and yield in selected upazilas of Satkhira district

In Sadar upazila of Satkhira district total cultivated area was 3450 ha of land and production was 5245 MT (1.48 MT/ha). Out of this cultivated area mostly occupied by HYV, namely BARI Sarisha -14 (1650 ha), BARI Sarisha -9 (830 ha), BARI Sarisha -15 (200 ha). Among the local varieties, Tori -7 (380 ha) was mostly cultivated in this upazila. In Kalaroa upazila of Satkhira district total

cultivated area was 6500 ha of land and production was 8404 MT (1.29 MT/ha). Out of this cultivated area mostly occupied by local varieties. For example, Tori -7 (3455 ha). Among the HYVs, BARI Sarisha -14 (2605 ha) was mostly cultivated in this upazila. In Tala upazila. total cultivated area was 465ha of land and production was 669 MT (1.43 MT/ha). Out of this cultivated area mostly occupied by local varieties, namely Tori -7 (300 ha). Among the HYVs, BARI Sarisha -15 (70 ha) was mostly cultivated in this upazila. From the study regarding the mustard varieties cultivated in Satkhira district, it was observed that HYV and local varieties are cultivated in this district but most of the varieties are susceptible to mustard diseases. The survey data are presented in Table 8.

Table 8. Mustard varieties, cultivated area and yield in selected upazilas of Satkhira district (2019-2020)

Sl No	Upazilla	Mustard variety	Area (ha)	Yield (MT)	Yield rate (MT/ha)
1	**Sadar**	BARI Sarisha - 9	830	**5245**	**1.48**
		BARI Sarisha -14	1650		
		BARI Sarisha -15	200		
		BARI Sarisha -17	150		
		BINA Sarisha - 4	150		
		BINA Sarisha - 9	180		
		Tori -7	380		
		Total	**3540**		
2	**Kalaroa**	BARI Sarisha - 9	117	**8404**	**1.29**
		BARI Sarisha -14	2605		
		BARI Sarisha -15	107		
		BARI Sarisha -17	42		
		BINA Sarisha - 9	174		
		Tori -7	3455		
		Total	**6500**		
3	**Tala**	BARI Sarisha - 9	30	**669**	**1.43**
		BARI Sarisha -14	65		
		BARI Sarisha -15	70		
		Tori -7	300		
		Total	**465**		

Source: Department of Agricultural Extension, Khamarbari, Dhaka, Bangladesh, FY 2019-20

4.1.6. Mustard varieties, cultivated area and yield in selected upazilas of Dhaka district

In Savar upazila of Dhaka district total cultivated area was 700 ha of land and production was 896.19 MT (1.28 MT/ha). Out of this cultivated area mostly occupied by HYVs, namely BARI Sarisha -14 (315 ha), BARI Sarisha -9 (110 ha). Local variety like Tori -7 (240 ha) was mostly cultivated in this upazila. In Keraniganj upazila of Dhaka district total cultivated area was 2410 ha of land and production was 3022.5 MT (1.25 MT/ha). Out of this cultivated area mostly occupied by HYVs, namely BARI Sarisha -14 (1082 ha), BARI Sarisha -15 (220 ha). Local variety of Tori -7 (950 ha) are mostly cultivated in this upazila. In Dhamrai upazila of Dhaka district total cultivated area was 4820 ha of land and production was 6393 MT (1.32 MT/ha). Out of this cultivated area mostly occupied by local variety, namely Tori -7 (3463 ha). Among the HYVs, BARI Sarisha -14 (1224 ha) was mostly cultivated in this upazila. From the study regarding the mustard varieties cultivated in Dhaka district, it was observed that both HYV and local variety are cultivated in this district and both varieties are highly susceptible to mustard diseases. The survey data are presented in Table 9.

Table 9. Mustard varieties, cultivated area and yield in selected upazilas of Dhaka district (2019-2020)

Sl No	Upazilla	Mustard variety	Area (ha)	Yield (MT)	Yield rate (MT/ha)
1	**Savar**	BARI Sarisha - 9	110	**896.19**	**1.28**
		BARI Sarisha -14	315		
		BARI Sarisha -15	20		
		BARI Sarisha -17	10		
		BINA Sarisha - 4	2		
		BINA Sarisha -6	3		
		Tori -7	240		
		Total	**700**		
2	**Keraniganj**	BARI Sarisha - 9	110		
		BARI Sarisha -14	1082		
		BARI Sarisha -15	220		
		BARI Sarisha -17	25		

		BINA Sarisha - 4	1	**3022.5**	**1.25**
		BINA Sarisha - 9	2		
		Tori -7	950		
		Total	**2410**		
3	**Dhamrai**	BARI Sarisha - 14	1224	**6393**	**1.32**
		BARI Sarisha -15	12		
		BARI Sarisha -17	84		
		BINA Sarisha - 4	5		
		BINA Sarisha - 9	9		
		Tori -7	3463		
		Sampad	10		
		Total	**4820**		

Source: Department of Agricultural Extension, Khamarbari, Dhaka, Bangladesh, FY 2019-20.

4.1.7. Mustard varieties, cultivated area and yield in selected upazilas of Manikganj districts

In Sadar upazila of Manikganj district total cultivated area was 7150 ha of land and production was 8882.5 MT (1.24 MT/ha). Out of this cultivated area mostly occupied by HYVs, namely BARI Sarisha – 14 (4223 ha), BARI Sarisha -17 (133 ha). Local variety of Tori - 7 (2756 ha) was mostly cultivated in this upazila. In Ghior upazila of Manikganj district total cultivated area was 6210 ha of land and production was 8625 MT (1.29 MT/ha). Out of this cultivated area mostly occupied by HYVs, namely BARI Sarisha – 14 (3261 ha), BARI Sarisha - 17 (100 ha). Local variety of Tori -7 (2700 ha) was mostly cultivated in this upazila. In Shibaloy upazila of Manikganj district total cultivated area was 5149 ha of land and production was 6908.4 MT (1.34 MT/ha). Out of this cultivated area mostly occupied by HYVs, namely BARI Sarisha – 14 (3478 ha). Local variety of Tori - 7 (300 ha) was mostly cultivated in this upazila. From the study regarding the mustard varieties cultivated in Manikganj district, it was observed that HYV varieties are mostly cultivated in this district but most of the HYV and local varieties are highly susceptible to mustard diseases. The survey data are presented in Table 10.

Table 10. Mustard varieties, cultivated area and yield in selected upazilas of Manikganj district (2019-2020)

Sl No	Upazilla	Mustard variety	Area (ha)	Yield (MT)	Yield rate (MT/ha)
1	**Sadar**	BARI Sarisha -14	4223	**8882.5**	**1.24**
		BARI Sarisha -15	10		
		BARI Sarisha -17	133		
		BINA Sarisha - 4	17		
		BINA Sarisha - 9	11		
		Tori -7	2756		
		Total	**7150**		
2	**Ghior**	BARI Sarisha - 9	20	**8625**	**1.29**
		BARI Sarisha -14	3261		
		BARI Sarisha -15	25		
		BARI Sarisha -17	100		
		BINA Sarisha - 4	50		
		BINA Sarisha - 9	44		
		BINA Sarisha - 11	10		
		Tori -7	2700		
		Total	**6210**		
3	**Shibaloy**	BARI Sarisha - 14	3478	**6908.4**	**1.34**
		BARI Sarisha -15	1		
		BARI Sarisha -17	34		
		BINA Sarisha - 4	129		
		BINA Sarisha - 9	30		
		Tori -7	300		
		Total	**5149**		

Source: Department of Agricultural Extension, Khamarbari, Dhaka, Bangladesh, FY 2019-20.

4.1.8. Mustard varieties, cultivated area and yield in selected upazilas of Tangail district

In Sadar upazila of Tangail district total cultivated area was 49700 ha of land and production was 6445 MT (1.30 MT/ha). Out of this cultivated area mostly occupied by HYVs, namely BARI Sarisha -14 (2450 ha), BARI Sarisha - 9 (150 ha), BARI Sarisha -15 (20 ha). Among the local varieties, Tori -7 (2170 ha) was mostly cultivated in this upazila. In Basail upazila of Tangail district total

cultivated area was 4550 ha of land and production was 5788.8 MT (1.27 MT/ha). Out of this cultivated area mostly occupied by local varieties, namely Tori -7 (3200 ha). Among the HYVs, BARI Sarisha -14 (1240 ha) was mostly cultivated in this upazila. In Mirzapur upazila of Tangail total cultivated area was 8525 ha of land and production was 10607.9 MT (1.24 MT/ha). Out of this cultivated area mostly occupied by local varieties, namely Tori -7 (4945 ha). Among the HYVs, BARI Sarisha -14 (3421 ha) was mostly cultivated in this upazila. From the study regarding the mustard varieties cultivated in Tangail district, it was observed that HYV and local varieties are cultivated in this district but most of the varieties are susceptible to mustard diseases. The survey data are presented in Table 11.

Table 11. Mustard varieties, cultivated area and yield in selected upazilas of Tangail district (2019-2020)

Sl No	Upazilla	Mustard variety	Area (ha)	Yield (MT)	Yield rate (MT/ha)
1	**Sadar**	BARI Sarisha - 9	150	**6445**	**1.30**
		BARI Sarisha -14	2450		
		BARI Sarisha -15	20		
		Tori -7	2170		
		Total	**4790**		
2	**Basail**	BARI Sarisha - 9	10	**5788.8**	**1.27**
		BARI Sarisha -14	1240		
		BARI Sarisha -15	45		
		BARI Sarisha -17	55		
		BINA Sarisha - 9	174		
		Tori -7	3200		
		Total	**4550**		
3	**Mirzapur**	BARI Sarisha - 14	3421	**10607.9**	**1.24**
		BARI Sarisha -15	7		
		BARI Sarisha -17	85		
		BINA Sarisha - 4	5		
		BINA Sarisha - 9	12		
		Tori -7	4945		
		Rye Sarisha	50		
		Total	**8525**		

Source: Department of Agricultural Extension, Khamarbari, Dhaka, Bangladesh, FY 2019-20.

4.1.9. Mustard varieties, cultivatedarea and yield in selected upazilas of Sirajganj district

In Sadar upazila of Sirajganj district total cultivated area was 2025 ha of land and production was 2418.65 MT (1.20 MT/ha). Out of this cultivated area mostly occupied by local varieties, namely Tori – 7 (1100 ha). Among the HYVs, BARI Sarisha -14 (580 ha) was mostly cultivated in this upazila. In Raiganj upazila of Sirajganj district total cultivated area was 1300 ha of land and production was 1660.70 MT (1.28 MT/ha). Out of this cultivated area mostly occupied by local varieties, namely Tori -7 (690 ha). Among the HYVs, BARI Sarisha -14 (360 ha) was mostly cultivated in this upazila. In Ullapara upazila of Sirajganj total cultivated area was 18750 ha of land and production was 24592.75 MT (1.31 MT/ha). Out of this cultivated area mostly occupied by local varieties, namely Tori -7 (8915 ha). Among the HYVs, BARI Sarisha -14 (6975 ha) was mostly cultivated in this upazila. From the study regarding the mustard varieties cultivated in Sirajganj district, it was observed that both HYV and local varieties are cultivated in this district but most of the varieties are susceptible to mustard diseases. The survey data are presented in Table 12.

Table 12. Mustard varieties, cultivated area and yield in selected upazilas in Sirajganj district (2019-2020)

Sl No	Upazilla	Mustard variety	Area (ha)	Yield (MT)	Yield rate (MT/ha)
1	**Sadar**	BARI Sarisha - 9	170	**2418.65**	**1.20**
		BARI Sarisha -14	580		
		BARI Sarisha -15	40		
		BARI Sarisha -17	10		
		BINA Sarisha - 4	1		
		BINA Sarisha - 9	15		
		Tori – 7	1100		
		Maghi	109		
		Total	**2025**		
2	**Raiganj**	BARI Sarisha -14	360		
		BARI Sarisha -15	235		
		BARI Sarisha -17	5		

		BINA Sarisha - 9	10	**1660.70**	**1.28**
		Tori -7	690		
		Total	**1300**		
3	**Ullapara**	BARI Sarisha -14	6975	**24592.75**	**1.31**
		BARI Sarisha -15	970		
		BARI Sarisha -17	30		
		BINA Sarisha - 4	30		
		BINA Sarisha - 9	20		
		SS -75	1750		
		Sampad	60		
		Tori -7	8915		
		Total	**18750**		

Source: Department of Agricultural Extension, Khamarbari, Dhaka, Bangladesh, FY 2019-20.

4.1.10. Mustard varieties, cultivated area and yield in selected upazilas of Dinajpur district

In Sadar upazila of Dinajpur district total cultivated area was 502 ha of land and production was 803 MT (1.59 MT/ha). Out of this cultivated area mostly occupied by HYV, namely BARI Sarisha -14 (277 ha), BARI Sarisha -15 (175 ha). In Hakimpur upazila of Dinajpur district total cultivated area was 820 ha of land and production was 1192.8 MT (1.45 MT/ha). Out of this cultivated area mostly occupied by HYV, namely BARI Sarisha -14 (405 ha), BARI Sarisha -15 (210 ha). Among the local varieties, Tori -7(150 ha) is mostly cultivated in this upazila. In Ghoraghat upazila of Dinajpur district total cultivated area was 810 ha of land and production was 1200 MT (1.54 MT/ha). Out of this cultivated area mostly occupied by HYV, namely BARI Sarisha -14 (524 ha), BARI Sarisha -15 (225 ha). Among the local varieties, Sampad (35 ha) was mostly cultivated in this upazila. From the study regarding the mustard varieties cultivated in Dinajpur district, it was observed that HYV are mostly cultivated in this district but most of the HYVs are highly susceptible to mustard diseases. The survey data are presented in Table 13.

Table 13. Mustard varieties, cultivated area and yield in selected upazilas in Dinajpur district (2019-2020)

Sl No	Upazilla	Mustard variety	Area (ha)	Yield (MT)	Yield rate (MT/ha)
1	**Sadar**	BARI Sarisha - 9	29	**803**	**1.59**
		BARI Sarisha -14	277		
		BARI Sarisha -15	175		
		BARI Sarisha -17	18		
		BINA Sarisha - 4	3		
		Total	**502**		
2	**Hakimpur**	BARI Sarisha -14	450	**1192.8**	**1.45**
		BARI Sarisha -15	210		
		BARI Sarisha -17	8		
		BINA Sarisha - 4	2		
		Tori -7	150		
		Total	**820**		
3	**Ghoraghat**	BARI Sarisha - 9	14	**1200**	**1.54**
		BARI Sarisha -14	524		
		BARI Sarisha -15	225		
		BINA Sarisha - 4	7		
		Sampad	35		
		Tori -7	5		
		Total	**810**		

Source: Department of Agricultural Extension, Khamarbari, Dhaka, Bangladesh, FY 2019-20.

4.1.11. Sources of seed, seed treating agents used in the surveyed areas

In the survey study, data was collected on sources of seed, seed treating agent and botanicals. It was observed that sources of seed were mainly home storage and BADC. Some farmers also collect the seed from local market and advance farmer. It was also observed that for seed treatments most of the farmer used Noin, Provax, Autostin and Rovral. Farmers were also use botanicals for seed treatment as a conventional practice. The informations are presented in Table 14.

Table 14. Source of seed, Seed treating agents used in the selected districts

Sl No	Districts	Source of seed	Seed treating agents
1	Jashore	Home storage	Provax
		BADC	Autostin
		Local Market	Rovral
		Advance Farmer	Botinical
2	Khulna	Home storage	Noin
		BADC	Provax
		Local Market	Autostin
		Advance Farmer	Rovral
			Botanical
3	Satkhira	Home storage	Noin
		BADC	Provax
			Autostin
4	Dhaka	Home storage	Provax
		BADC	Autostin
		Local Market	Rovral
			Botanical
5	Manikganj	Home storage	Noin
		BADC	Provax
		Local Market	Autostin
			Rovral
			Botanical
6	Tangail	Home storage	Provax
		BADC	Rovral
		Local Market	
		Advance Farmer	
7	Sirajganj	Home storage	Noin
		BADC	Provax
		Local Market	Autostin
		Advance Farmer	Botanical
8	Dinajpur	Home storage	Provax
		BADC	Autostin
			Rovral

4.1.12. Frequency of Mustard varieties cultivated in the selected districts

Different varieties of mustard were cultivated in the selected districts of Bangladesh. It was recorded that maximum cultivated area was occupied by BARI Sarisha -14 followed by Tori-7 and BARI Sarisha -15. It was aslo recorded that minimum area covered by Rye sarisha. The data are presented in the Table 15.

Table 15. Frequency percentage of Mustard varieties cultivated in the surveyed selected districts

Variety	Frequency	Percent (%)
Tori-7	11	9.17
BARI Sarisha-9	5	4.16
BARI Sarisha-14	77	64.17
BARI Sarisha-15	11	9.17
BINA Sarisha -4	8	6.67
BINA Sarisha -9	3	2.50
Rye	2	1.66
Others	3	2.50
	120	100

4.1.13. Sowing time of Mustard in the selected districts

Mustard sowing period was presented in figure 11. From the study, it was found that maximum mustard grower was sown in 16 – 30 November followed by the period of 1-15 November in the selected districts. On the other hand, the lowest sown period was 16 – 31 December.

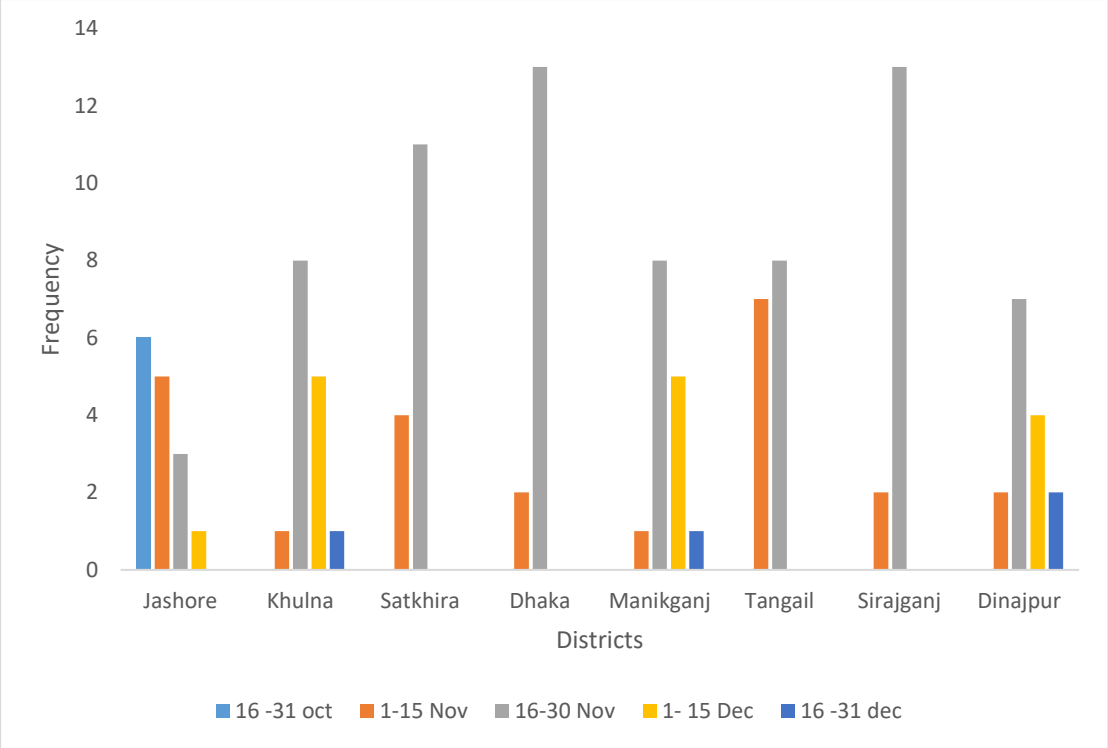


Figure 9. Sowing date of Mustard in the selected districts

4.1.14. District wise comparison of disease incidence for grey blight, white, rust, powdery mildew and sclerotinia stem rot

During the surveyed of the selected district of mustard growing areas, we found the incidence of four diseases like grey blight, white rust, powdery mildew and sclerotinia stem rot. Of these, the incidence of grey blight was higher in all districts. The highest disease incidence of grey blight was recorded in Dhaka and Sirajganj and the lowest disease incidence was observed in Satkhira. In case of white rust, the disease incidence was higher in the district of Khulna, Satkhira and manikganj and no disease incidence was found in Jashore, Dhaka, Sirajganj and Dinajpur. The highest disease incidence of powdery mildew was showed in Satkhira and Sirajganj in all selected districts and no disease incidence of powdery was found in Jashore, Dhaka, Tangail and Dinajpur. In case of Sclerotinia stem rot, the highest disease incidence was recorded in Tangail and no disease incidence was observed in Dhaka and Sirajganj (Figure 10).

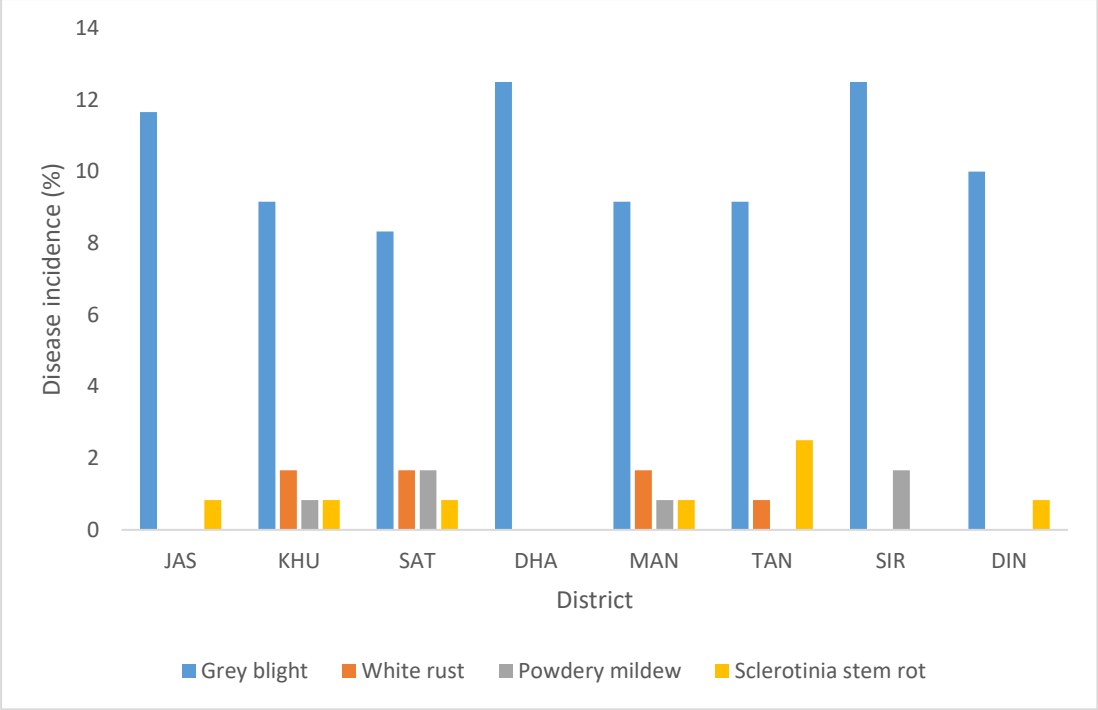


Figure 10. Disease incidence in selected districts

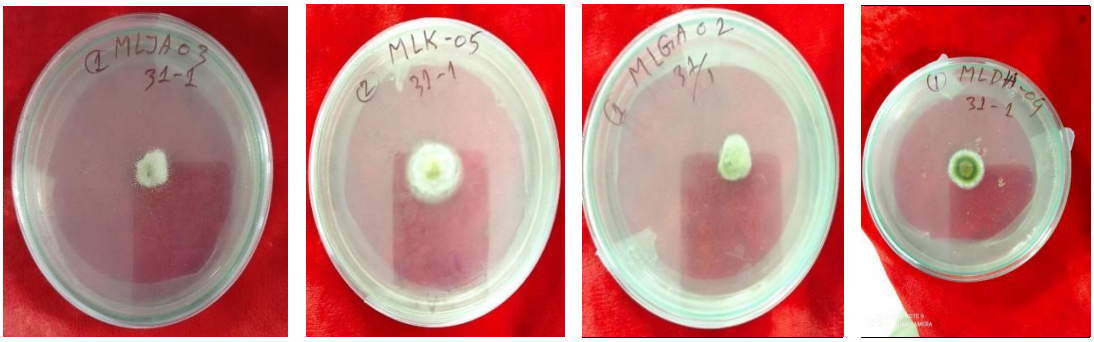
Experiment- 2: Identification and characterization of *Alternaria* isolates

4.2.1. Radial mycelial growth of 8 isolates of *Alternaria* spp. on PDA medium

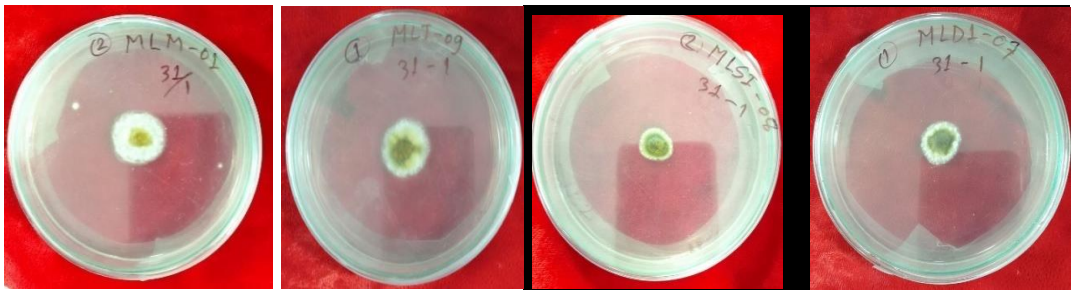
Among the different isolates, radial mycelial growth of *Alternaria* spp. varied significantly. The highest radial mycelial growth of *Alternaria* spp. was recorded in TAN (12.00 mm) followed by MAN (11.33 mm) and the lowest radial mycelial growth of *Alternaria* spp. was observed in JAS (6.50 mm) which was statistically similar to SAT (7.13 mm) after 3 days of inoculation. After 7 days of inoculation, the maximum radial mycelial growth of different isolates of *Alternaria* spp. was found in TAN (27.73 mm) and the minimum Radial mycelial growth of different isolates of *Alternaria* spp was observed in SAT which was 12.40 mm. After 14 days of inoculation, the highest radial mycelial growth of different isolates of *Alternaria* spp. was recorded in MAN (43 mm) followed by SIR (40.80 mm) whereas the lowest radial mycelial growth of different isolates of *Alternaria* spp was measured in SAT (18.13 mm).

Table 16. Radial mycelial growth of different isolates of *Alternaria* spp. at different days after incubation on PDA

Isolates	3 Days (mm)	7 Days (mm)	14 Days (mm)
JAS	6.50 $_{e}$	14.97 $_{d}$	35.43 $_{c}$
KHU	10.50 $_{b}$	20.27 $_{bc}$	31.90 $_{d}$
SAT	7.13$_{de}$	12.40 $_{d}$	18.13 $_{e}$
DHA	9.03 $_{c}$	25.50 $_{a}$	36.40 $_{c}$
MAN	11.33 $_{ab}$	24.33 $_{ab}$	43.00 $_{a}$
TAN	12.00 $_{a}$	27.73 $_{a}$	39.77 $_{b}$
SIR	8.07 $_{cd}$	16.93 $_{cd}$	40.80 $_{ab}$
DIN	8.17 $_{c}$	24.90 $_{a}$	34.23 $_{cd}$
CV (%)	6.37	12.45	4.25



1-JAS 2 - KHU 3 –SAT 4- DHA



5- MAN 6 - TAN 7 - SIR 8- DIN

Plate 9. Pure culture of *Alternaria* spp. on PDA after 3 days inoculation

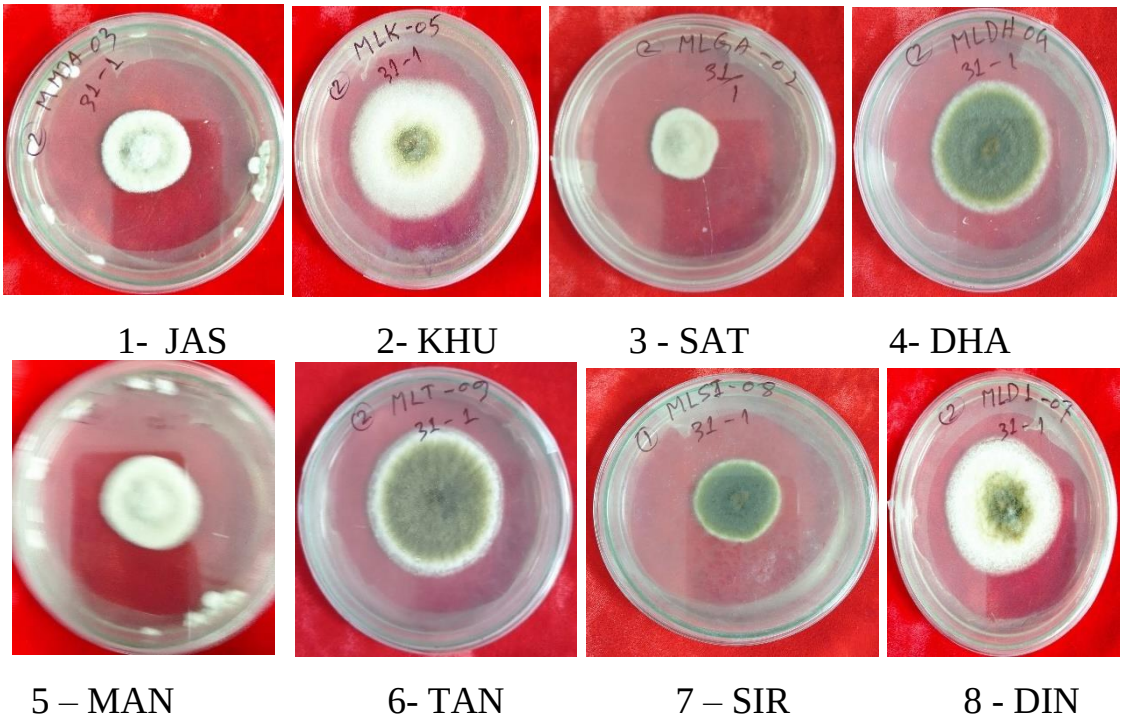


Plate 10. Pure culture of *Alternaria* spp. on PDA after 7 days inoculation

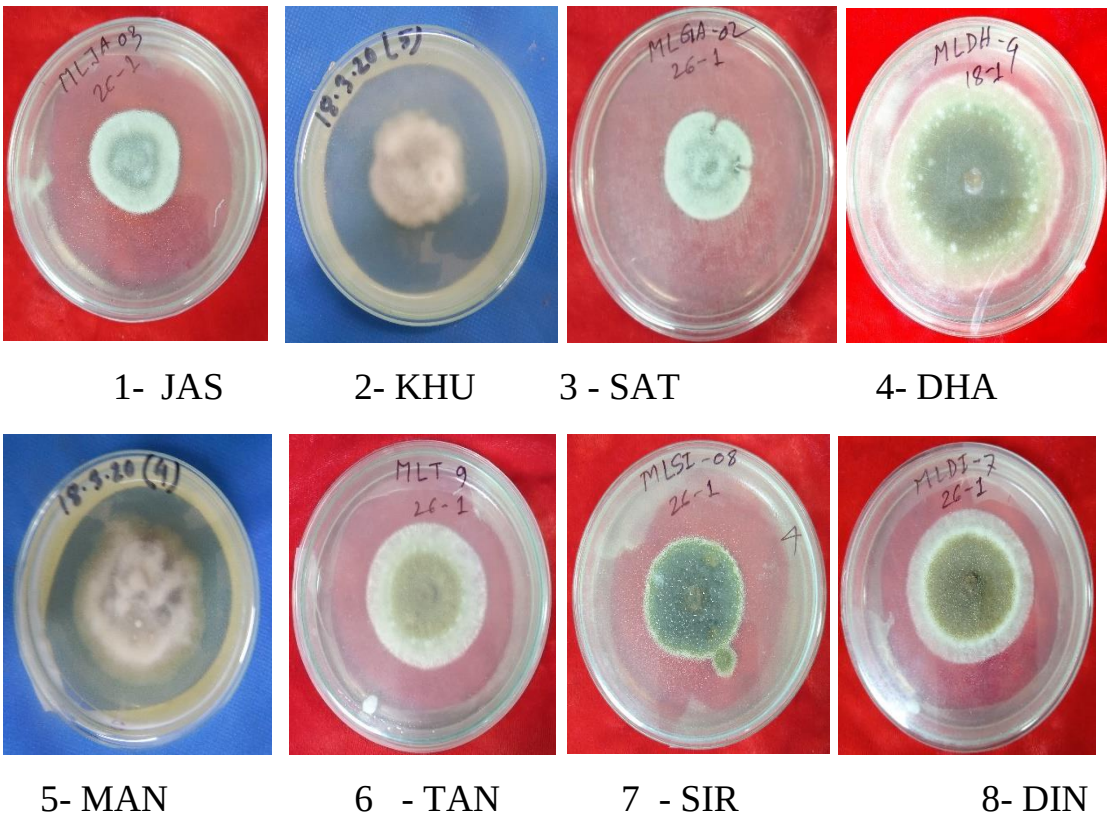
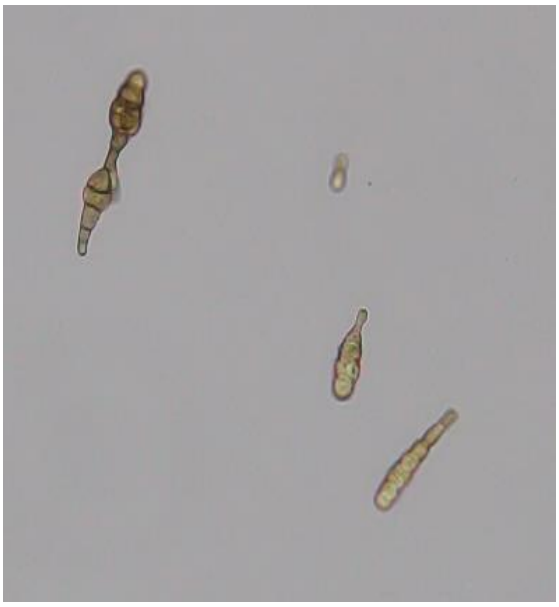
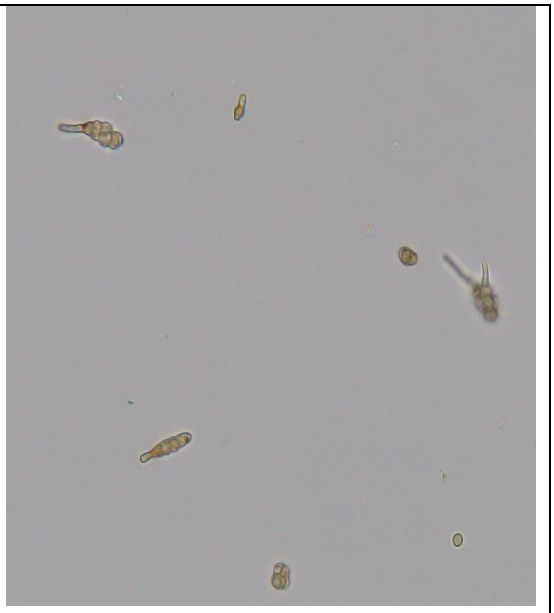


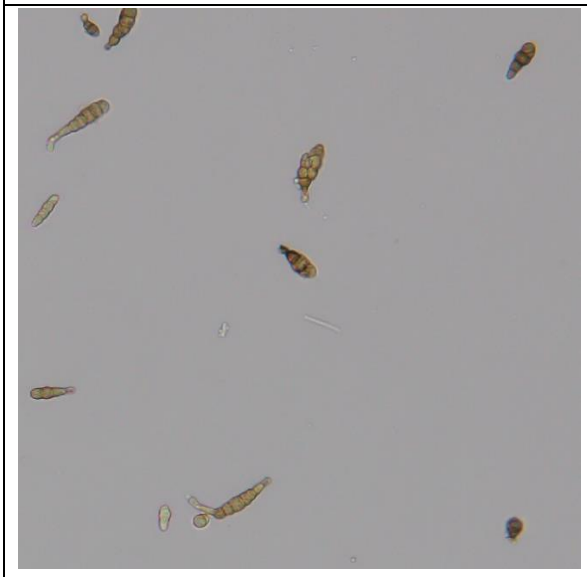
Plate 11. Pure culture of *Alternaria* spp. on PDA after 14 days inoculation



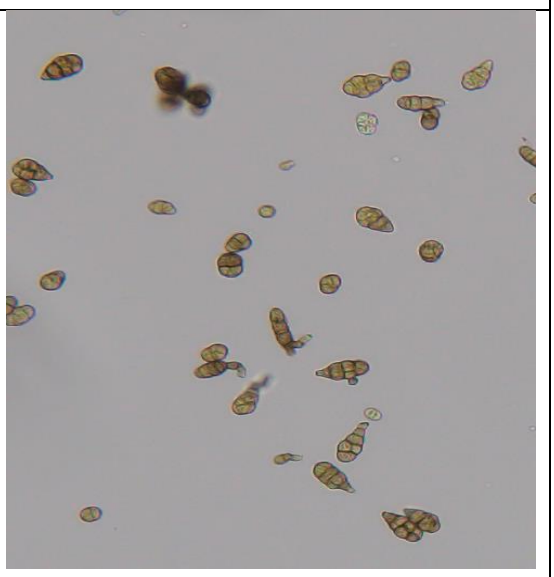
A – Conia of *Alternaria brassicae, Alternaria brassicicola* (JAS)



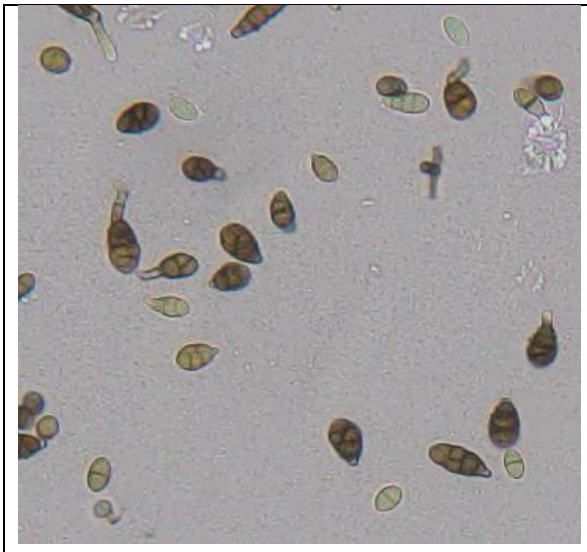
B – Conidia of *Alternaria alternata* (KHU)



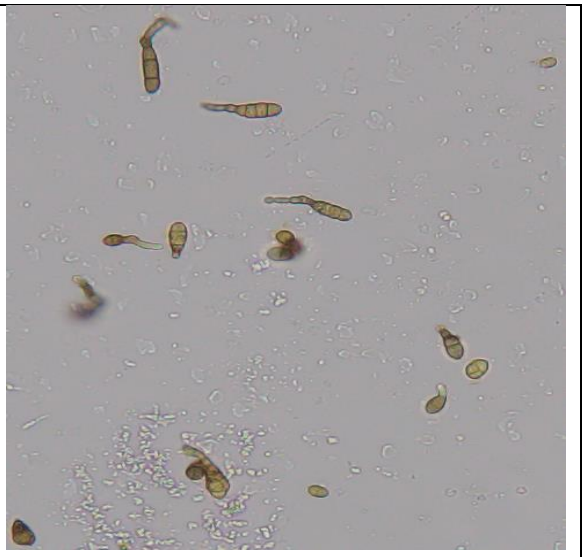
C – Conia of *Alternaria brassicicola* (SAT)



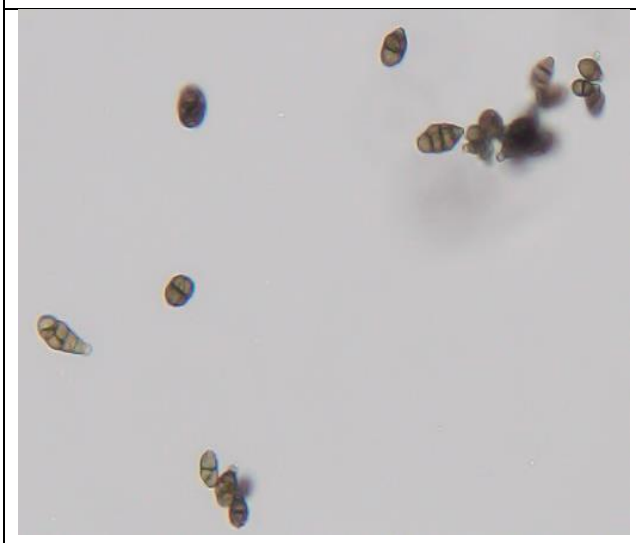
D– Conia of *Alternaria alternata* (DHA)



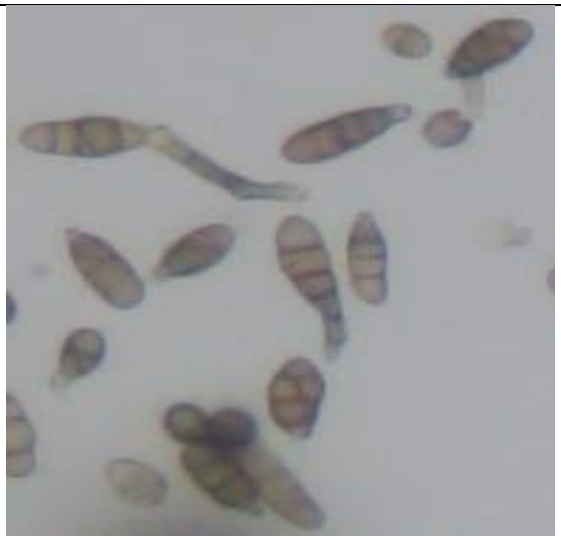
E– Conia of *Alternaria alternata* (MAN)



F - Conia of *Alternaria alternata, Alternaria brassicae* (TAN)



G - Conia of *Alternaria alternata* (SIR)



H – Conia of *Alternaria* spp (DIN)

Plate 12. Microscopic view of *Alternaria* spp. in the selected isolates viz., A- JAS, B- KHU, C- SAT, D – DHA, E- MAN, F – TAN, G – SIR and H – DIN (40X)

4.2.2. Colony characters of isolates of *Alternaria* spp. on PDA

Different variations were showed in colony characters of 8 isolates of *Alternaria* spp. regarding surface color, shape, texture, zonation and subsurface color (Table 17). From the results of the study of colony characters. It was observed that surface and subsurface color was black, white and greyish and black color. The texture was cottony and colony shape was circular in case of all isolates. In case of zonation, no zonation was found on surface of the culture but zonation was found in case of subsurface (Table 17).

Table 17. Colony characters of different isolates of *Alternaria* spp. on PDA media at 14 days

Isolates	Color		Texture	Colony Shape	Zonation	
	Surface	Subsurface			Surface	Subsurface
JAS	Black	Black center with white surrounding	Cottony	Circular	No Zonation	Zonation
KHU	Greyish	White	Cottony	Circular	No Zonation	Zonation
SAT	Greyish	White	Cottony	Circular	Zonation	No Zonation
DHA	Black	Black center with white surrounding	Cottony	Circular	No Zonation	Zonation
MAN	Greyish	White	Cottony	Circular	Zonation	No zonation
TAN	Greyish	Greyish center white surrounding	Cottony	Circular	No Zonation	Zonation
SIR	Black	Black center with white surrounding	Cottony	Circular	No Zonation	Zonation
DIN	Black	Black center with white surrounding	Cottony	Circular	No Zonation	Zonation

4.2.3. Morphological variation of conidia of different isolates of *Alternaria* spp.

4.2.3. Conidial characteristics of different isolates of *Alternaria* spp. on PDA media

Among different isolates of *Alternaria* spp. on PDA, variation was found in no of conidia, longitudinal septation and transverse septation of conidia. No of conidia varied from the range of 1.33 to 5.66. The highest no of conidia was recorded in TAN isolate (5.67) followed by JAS isolate (4.33) whereas the lowest no of conidia was found in KHU isolate (1.33) which was statistically similar to MAN isolate (2.33) and SIR isolate (2.33).

The maximum longitudinal septation of *Alternaria* spp. was observed in DIN isolate (6.67) followed by TAN isolate (6.33) and the longitudinal septation minimum transverse septation of *Alternaria* species was measured in SIR isolate that was 3.67 among the isolates.

Variation was obtained in transverse septation of *Alternaria* spp. in where it was varied from 1.00 to 2.33. Maximum transverse septation of Alternaria species was recorded in SAT isolate (2.33) whereas minimum transverse septation of Alternaria species was observed in SIR isolate (1.00) which was statistically similar to DIN (1.33). The data was presented in Table 18.

Table 18. Conidial characteristics of different isolates of *Alternaria* spp. on PDA media

Isolates	No of conidia	Longitudinal Septation	Transverse Septation
JAS	4.33 $_{b}$	6.00 $_{a}$	1.67$_{ab}$
KHU	1.33 $_{e}$	5.67$_{ab}$	1.67 $_{ab}$
SAT	2.67 $_{cd}$	6.00 $_{a}$	2.33 $_{a}$
DHA	3.67 $_{bc}$	5.33 $_{ab}$	1.67$_{ab}$
MAN	2.33 $_{de}$	5.33$_{ab}$	2.00 $_{ab}$
TAN	5.67$_{a}$	6.33$_{a}$	1.67$_{ab}$
SIR	2.33$_{de}$	3.67 $_{b}$	1.00 $_{b}$
DIN	2.67 $_{cd}$	6.67$_{a}$	1.33$_{ab}$
CV (%)	23.55	21.38	37.32

4.2.4. Size of conidia from different isolates of *Alternaria* spp.

From the study, it was observed that conidial length of *Alternaria* spp. varied from 12.30 to 25.09 µm. Among the isolates, the highest conidial length of *Alternaria* spp. was recorded in SAT isolate (25.09 um) which was statistically similar to TAN isolate (22.21um) whereas the lowest conidial length of *Alternaria* spp was found in SIR that was 12.30 um. There was no significant variation in all isolates in case of breath of the conidia.

Table. 19. Conidial Size of the *Alternaria* spp

Isolates	Conidial length (µm)[1]	Conidial breadth (µm)[1]
JAS	21.31 $_{ab}$	5.78 $_{a}$
KHU	20.59$_{ab}$	5.95$_{a}$
SAT	25.09$_{a}$	7.15$_{a}$
DHA	15.87$_{bc}$	7.02$_{a}$
MAN	17.07$_{bc}$	6.17$_{a}$
TAN	22.21$_{ab}$	5.57$_{a}$
SIR	12.30$_{c}$	6.33$_{a}$
DIN	16.82 $_{bc}$	6.30$_{a}$
CV (%)	21.46	19.53

4.2.5. Shape and size of *Alternaria* spp. on PDA media

Conidia color was varied from light to deep brown among the isolates of *Alternaria* spp (Plate 12). It was observed that light brown color was found in the isolates of Alternaria spp of JAS isolate and MAN isolate whereas brown color of conidia of *Alternaria* spp was showed in SAT isolate, KHU isolate and TAN isolate and deep brown of conidia of *Alternaria* spp was also showed in DHA isolate, SIR isolate and DIN isolate. From all collected isolates of Alternaria spp., muriform type conidia was found. All the isolates produced light brown to deep brown murifrom conidia with beak. Conidial septation was differed from the collected isolates of *Alternaria* spp. In case of the horizontal septation of each conidium was ranged from 3 -4 to 5 -7 and the vertical septation of each conidium was varied from 0-1 to 2-3. Of these, the highest horizontal septation of conidium of *Alternaria* species was recorded in JAS isolate (5 -7), TAN isolate (5 -7) and DIN isolate (5-7) whereas the lowest horizontal septation of conidium in

Alternaria species was observed in SIR isolate (3 – 4). The maximum vertical septation of each conidium was found in isolates of SAT isolate that was 2-3 and the minimum vertical septation was recorded in isolate of SIR isolate (0 -1).

Table 20. Conidial characteristics of *Alternaria* spp.

Isolates	Conidial type	Conidial color	Conidial septation (Range)	
			Horizontal	Vertical
JAS	Muriform	Light brown	5 -7	1-2
KHU	Muriform	Brown	5 - 6	1-2
SAT	Muriform	Brown	5-7	2-3
DHA	Muriform	Deep brown	4 - 7	1 -2
MAN	Muriform	Light brown	4-7	1-3
TAN	Muriform	Brown	5 -7	1-2
SIR	Muriform	Deep brown	3 - 4	0-1
DIN	Muriform	Deep brown	5-7	1-2

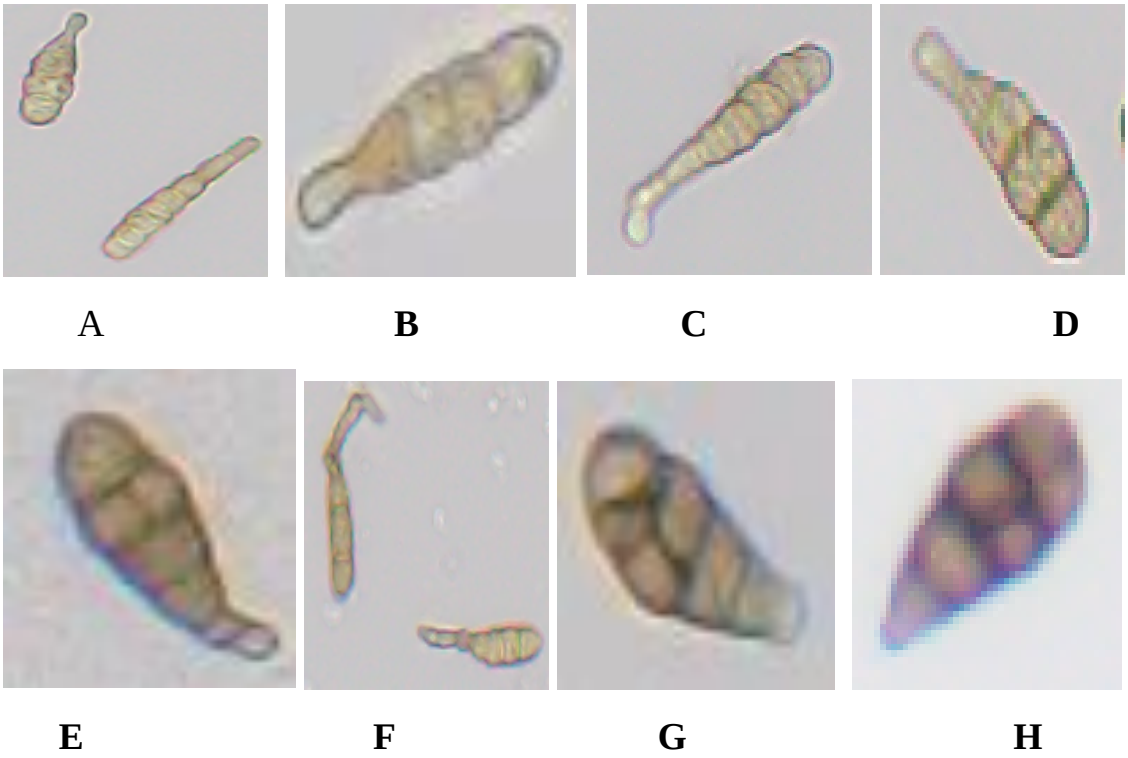


Plate 13: Condial characteristics of *Alternaria* spp. viz., A – Jashore, B- Khulna, C – Satkhira, D – Dhaka, E – Manikganj, F – Tangail, G – Sirajganj and H – Dinajpur (40X)

Experiment- 3: Pathogenicity Test

Pathogenicity

All the *Alternaria* isolates from mustard were found to be pathogenic in nature. Among the mustard isolates from different selected districts JAS isolate from Jashore was found to be highly pathogenic as the spots produced >1cm in diameter.

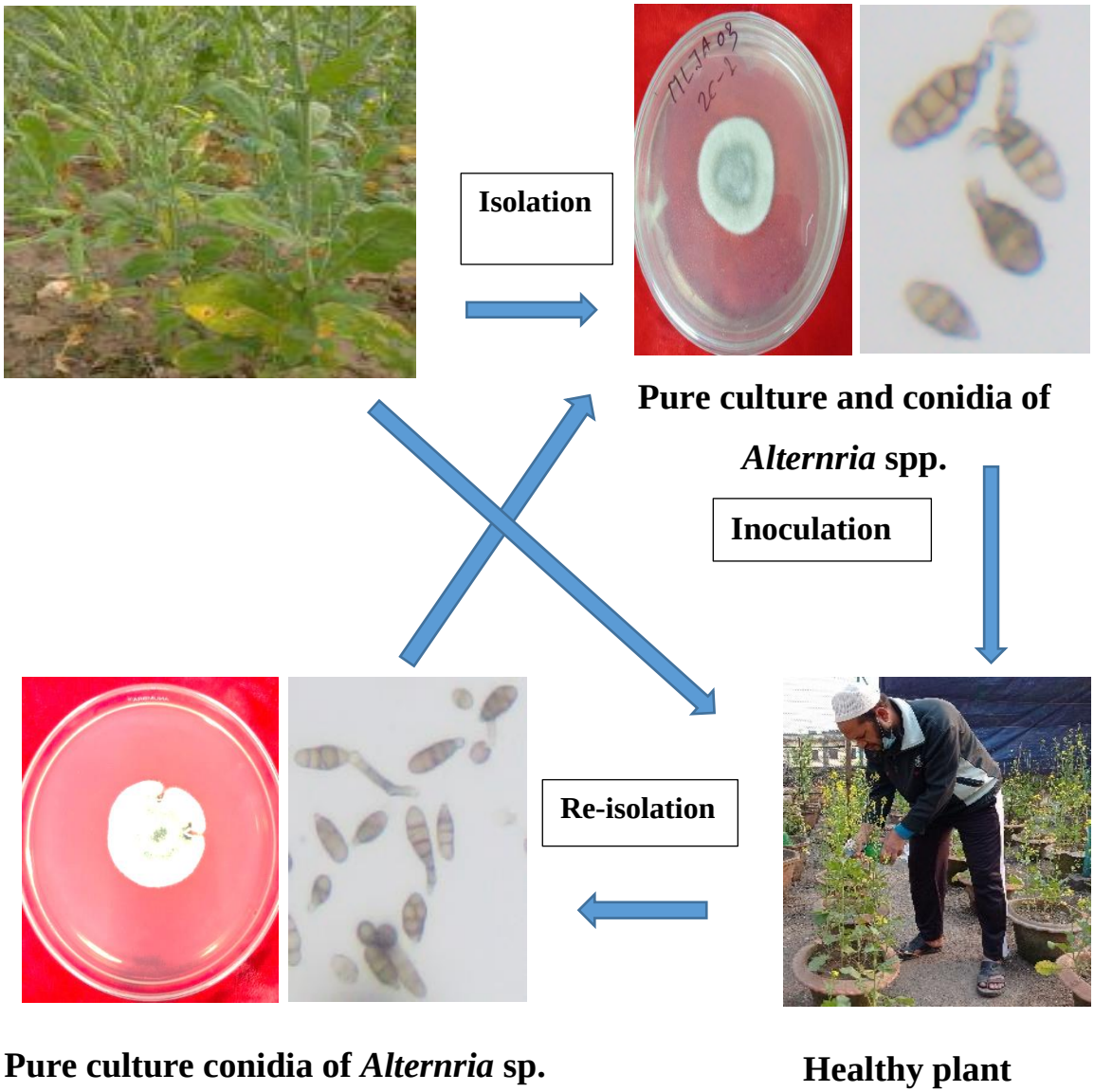


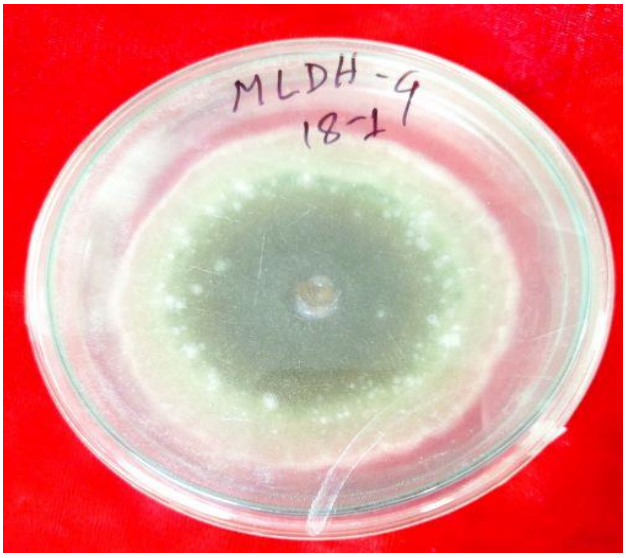
Plate 14. Establishment of pathogenicity test through Koch's postulates

Experiment- 4: Study on molecular variation among the selected *Alternaria* isolates

The experiment was conducted in the molecular laboratory to observe the molecular variation among the selected *Alternaria* isolates through molecular characterization. Searches for similarity of nucleotide sequences were performed using the BLASTn on the NCBI website (https:// www.ncbi.nlm.nih.gov) for matching with existing nucleotide sequences in the NCBI genbank data base to identify the fungal strain of *Alternaria*. Moreover, phylogenetic tree was developed with the nearest related *Alternaria* sp. to observe the relationship of the *Alternaria* isolates.

4.4.1. Isolates of *Alternaria* spp.

From the morphological and cultural study, it was observed that *Alternaria* isolates were produced brown colored, round shaped with cottony surface in the PDA medium. Brown growing mycelium margin were appeared in all the isolates. Surface color were olivacious green to black and circular shaped colonies were observed in all isolates on PDA medium. Colony texture were cottony to velvety. Surface color varied from light brown to black and pinkish. Zonation was found in some isolates and some did not produce on both surface and subsurface. All conidia were murifrom and light brown to deep brown in color (Plate 15).



Pure culture of *Alternaria*

Plate 15. Conidia of *Alternaria* sp. (Microscopic view) (40X)

4.4.2. PCR amplification

Total DNA was extracted from mycelia of the pure culture of Alternaria. After DNA extraction, fungal DNA was used as a template in PCR amplification using universal forward primer ITS4: TCC TCC GCT TAT TGA TAT GC and reverse primer ITS5: GGA AGT AAA AGT CGT AAC AAG G which designed to amplify fungal gene fragment of 700 bp. *Alternaria* fungal gene of 700 bp fragment was amplified and analyzed on 1.0% agarose gel along with 1 kb DNA ladder. In electrophoresis analysis, PCR product showed a single amplified fragment of ~ 700 bp as clear shown in Figure 11.

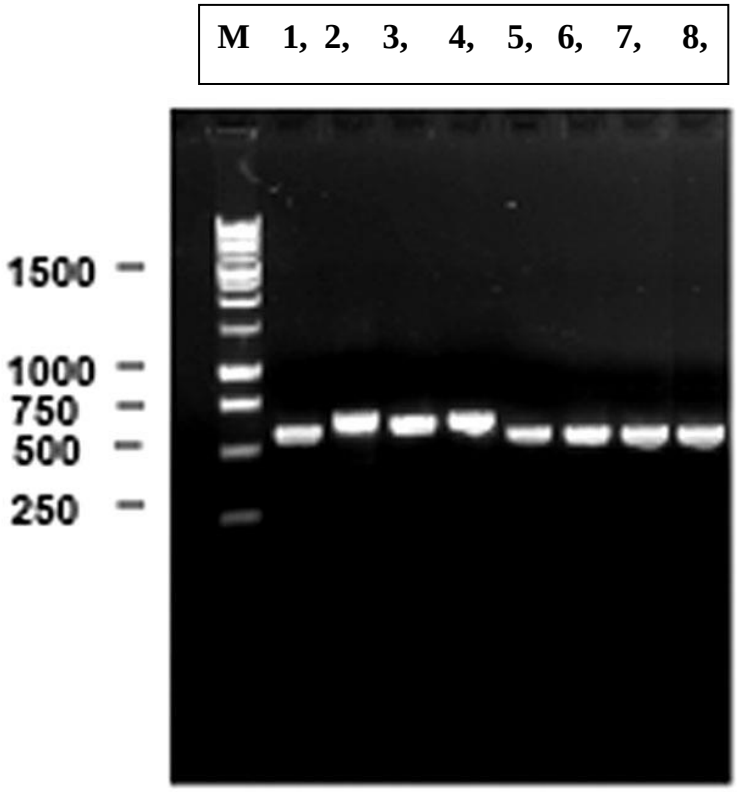


Figure 11. ITS profile of ITS4 and ITS5 primers generated from Fungus, Lane 1-8: DNA amplification at 700 bp, M: denotes 1 kb DNA ladder (Marker).

4.4.3. Sequencing and analysis

For sequencing, the PCR product was carried out in automated sequencer. The sequencing was conducted with reverse primer ITS5: GGA AGT AAA AGT CGT AAC AAG G. The sequences of eight isolates of *Alternaria* spp has been presented in Figure 12-19.

TAAATTGGTTCCTACCTGATCCGAGGTCAAAGTTGAAAAAAGGCTTA
ATGGATGCTAGACCTTTGCTGATAGAGAGTGCGACTTGTGCTGCGCT
CCGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAG
CAAAGCTAGAGACAAGACGCCCAACACCAAGCAAAGCTTGAGGGTA
CAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCGC
AATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACA
CTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAG
ATCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTG

CAATTACAAAAGGTTTATGTTTGTCCTAGTGGTGGGCGAACCCACCA
AGGAAACAAGAAGTACGCAAAAGACAAGGGTGAATAATTCAGCAAG
GCTGTAACCCCGAGAGATTCCAGCCCGCCTTCATATTTGTGTAATGA
TCCCTCCGCAGGTTCACCTACGGAGACCTTGTTACGACTTTTACTTCA

Figure 12. Nucleotide sequencing of JAS (Jashore) *Alternaria* isolate (FASTA Form)

TCAATTGGTTCCTACCTGATCCGAGGTCAAAGTTGAAAAAAGGCTTA
ATGGATGCTAGACCTTTGCTGATAGAGAGTGCGACTTGTGCTGCGCT
CCGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAG
CAAAGCTAGAGACAAGACGCCCAACACCAAGCAAAGCTTGAGGGTA
CAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCGC
AATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACA
CTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAG
ATCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTG
CAATTACAAAAGGTTTATGTTTGTCCTAGTGGTGGGCGAACCCACCA
AGGAAACAAGAAGTACGCAAAAGACAAGGGTGAATAATTCAGCAAG
GCTGTAACCCCGAGAGGTTCCAGCCCGCCTTCATATTTGTGTAATGA
TCCCTCCGCAGGTTCACCTACGGAGACCTTGTTACGACTTTTTACTTC
CA

Figure 13. Nucleotide sequencing of KHU (Khulna) *Alternaria* isolate (FASTA Form)

AATTGGTTCCTACCTGATCCGAGGTCAAAGTTGAAAAAAGGCTTAAT
GGATGCTAGACCTTTGCTGATAGAGAGTGCGACTTGTGCTGCGCTCC
GAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAGCA
AAGCTAGAGACAAGACGCCCAACACCAAGCAAAGCTTGAGGGTACA
AATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCGCAA
TGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTA
CTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATC
CGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGCAA
TTACAAAAGGTTTATGTTTGTCCTAGTGGTGGGCGAACCCACCAAGG
AAACAAGAAGTACGCAAAAGACAAGGGTGAATAATTCAGCAAGGCT

GTAACCCCGAGAGATTCCAGCCCGCCTTCATATTTGTGTAATGATCC
CTCCGCAGGTTCACCTACGGAGACCTTGTTACGACTTTTTACTTCCA

Figure 14. Nucleotide sequencing of SAT (Satkhira) *Alternaria* isolate (FASTA Form)

AANTGGTCCTACCTGATCCGAGGTCAAAGTTGAAAAAAGGCTTAATG
GATGCTAGACCTTTGCTGATAGAGAGTGCGACTTGTGCTGCGCTCCG
AAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAGCAA
AGCTAGAGACAAGACGCCCAACACCAAGCAAAGCTTGAGGGTACAA
ATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCGCAAT
GTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTA
CTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATC
CGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGCAA
TTACAAAAGGTTTATGTTTGTCCTAGTGGTGGGCGAACCCACCAAGG
AAACAAGAAGTACGCAAAAGACAAGGGTGAATAATTCAGCAAGGCT
GTAACCCCGAGAGGTTCCAGCCCGCCTTCATATTTGTGTAATGATCC
CTCCGCAGGTTCACCTACGGAGACCTTGTTACGACTTTTTACTTCCAG

Figure 15. Nucleotide sequencing of DHA (Dhaka) *Alternaria* isolate (FASTA Form)

ATTGGTTCCTAACCTGATCCGAGGTCAAAGTTGAAAAAAGGCTTAAT
GGGATGCTAGACCTTTGCTGATAGAGAGTGCGACTTGTGCTGCGCTC
CGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAGC
AAAGCTAGAGACAAGACGCCCAACACCAAGCAAAGCTTGAGGGTAC
AAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCGCA
ATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACT
ACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGA
TCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGC
AATTACAAAAGGTTTATGTTTGTCCTAGTGGTGGGCGAACCCACCAA
GGAAACAAGAAGTACGCAAAAGACAAGGGTGAATAATTCAGCAAGG
CTGTAACCCCGAGAGATTCCAGCCCGCCTTCATATTTGTGTAATGATC
CCTCCGCAGGTTCACCTACGGAGACCTTGTTACGACTTTTACTTCCAA

Figure 16. Nucleotide sequencing of MAN (Manikganj) *Alternaria* isolate (FASTA Form)

TAAATTGGTTCCTAACCTGATCCGAGGTCAAAGTTGAAAAAAAGGCT
TAATGGATGCTAGACCTTTGCTGATAGAGAGTGCGACTTGTGCTGCG
CTCCGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCC
AGCAAAGCTAGAGACAAGACGCCCAACACCAAGCAAAGCTTGAGGG
TACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGC
GCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCA
CACTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAG
AGATCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGAT
TGCAATTACAAAAGGTTTATGTTTGTCCTAGTGGTGGGCGAACCCAC
CAAGGAAACAAGAAGTACGCAAAAGACAAGGGTGAATAATTCAGCA
AGGCTGTAACCCCGAGAGGTTCCAGCCCGCCTTCATATTTGTGTAAT
GATCCCTCCGCAGGTTCACCTACGGAGACCTTGTTACGACTTTTTAAC
TTCCA

Figure 17. Nucleotide sequencing of TAN (Tangail) *Alternaria* isolate (FASTA Form)

TAAATTGGTTCCTAACCTGATCCGAGGTCAAAGTTGAAAAAAGGCTT
AATGGATGCTAGACCTTTGCTGATAGAGAGTGCGACTTGTGCTGCGC
TCCGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCA
GCAAAGCTAGAGACAAGACGCCCAACACCAAGCAAAGCTTGAGGGT
ACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCG
CAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCAC
ACTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGA
GATCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATT
GCAATTACAAAAGGTTTATGTTTGTCCTAGTGGTGGGCGAACCCACC
AAGGAAACAAGAAGTACGCAAAAGACAAGGGTGAATAATTCAGCAA
GGCTGTAACCCCGAGAGGTTCCAGCCCGCCTTCATATTTGTGTAATG
ATCCCTCCGCAGGTTCACCTACGGAGACCTTGTTACGACTTTTTACTT
CCAGA

Figure 18. Nucleotide sequencing of SIR (Sirajganj) *Alternaria* isolate (FASTA Form)

CAATTGGTTCCTACCTGATCCGAGGTCAAAGTTGAAAAAAGGCTTAA
TGGATGCTAGACCTTTGCTGATAGAGAGTGCGACTTGTGCTGCGCTC
CGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAGC
AAAGCTAGAGACAAGACGCCCAACACCAAGCAAAGCTTGAGGGTAC
AAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCGCA
ATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACT
ACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGA
TCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGC
AATTACAAAAGGTTTATGTTTGTCCTAGTGGTGGGCGAACCCACCAA
GGAAACAAGAAGTACGCAAAAGACAAGGGTGAATAATTCAGCAAGG
CTGTAACCCCGAGAGGTTCCAGCCCGCCTTCATATTTGTGTAATGATC
CCTCCGCAGGTTCACCTACGGAGACCTTGTTACGACTTTTTACTTCCA

Figure 19. Nucleotide sequencing of DIN (Dinajpur) *Alternaria* isolate (FASTA Form)

4.4.4. Similarity search for matching and multiple sequence alignment

Searches for similarity of nucleotide sequences were performed using the BLASTn on the NCBI website (https://www.ncbi.nlm.nih.gov) for matching with existing DNA sequences in the NCBI genbank to identify the fungal strain. The nucleotide sequences of the *Alternaria* isolates from the present study were showed 99% similarity with the existing nucleotide sequences of GenBank data base.

4.4.5. Development of phylogenetic tree

From the phylogenetic tree, it has been observed that *Alternaria* isolates from the present study are closely related to *Alternaria* sp. isolates globaly found that were data based in GenBank. It was also found that from the phylogenetic tree of *Alternaria* sp local isolates for grey blight disease infected mustard on the basis of nucleotide sequence showed close relationship. DHA, TAN, SIR, MAN and KHU isolates were closely related to *Alternaria alternata* and SAT and JAS related to *Alternria brsssicicola* (Figure 20).

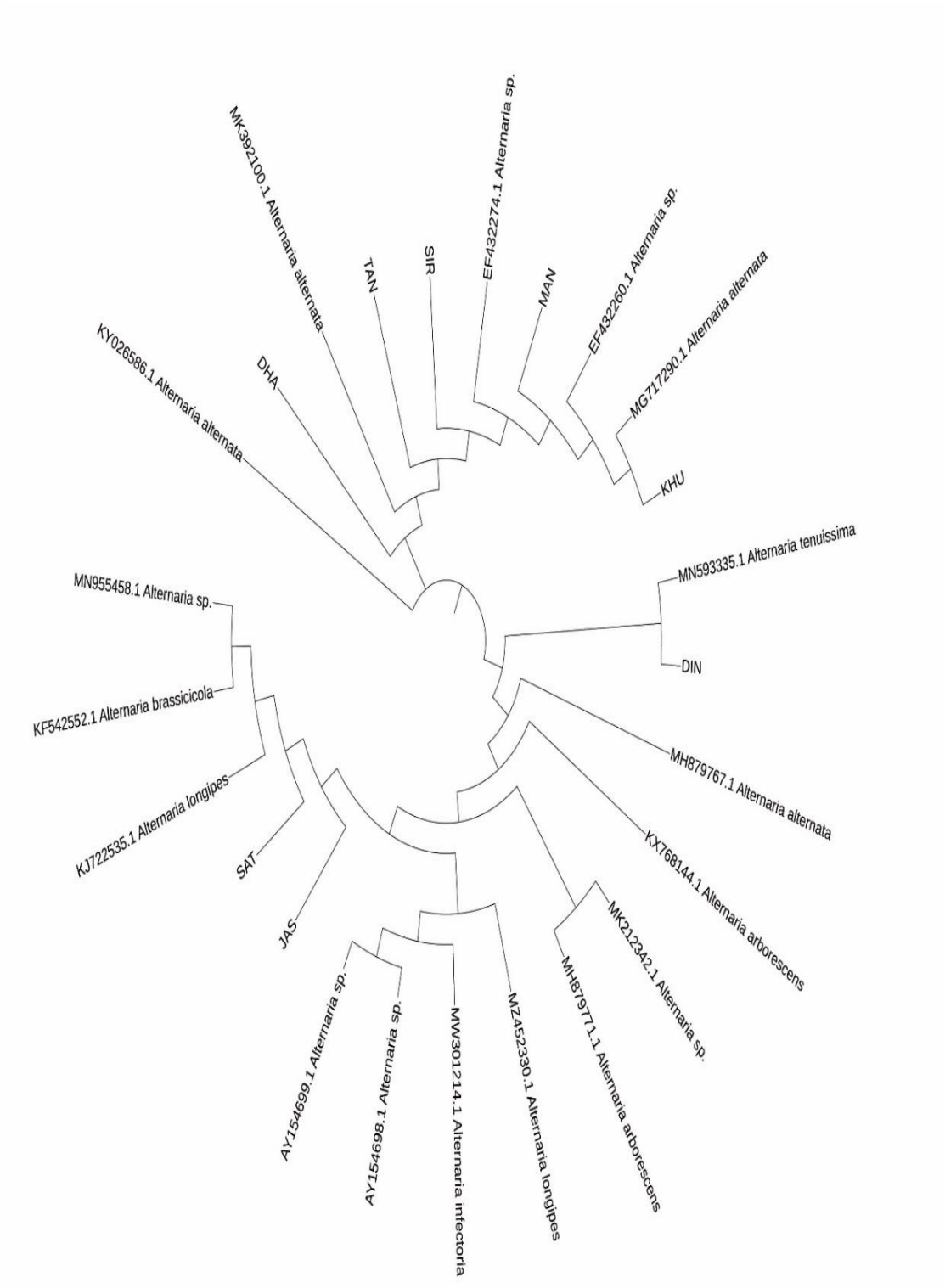


Figure 20. Phylogenetic tree generated by DNAMAN analysis system and Maximum Parsimoney method was selected to construct tree. All the globally found *Alternaria* isolates were very closely related to each other as shown in the tree

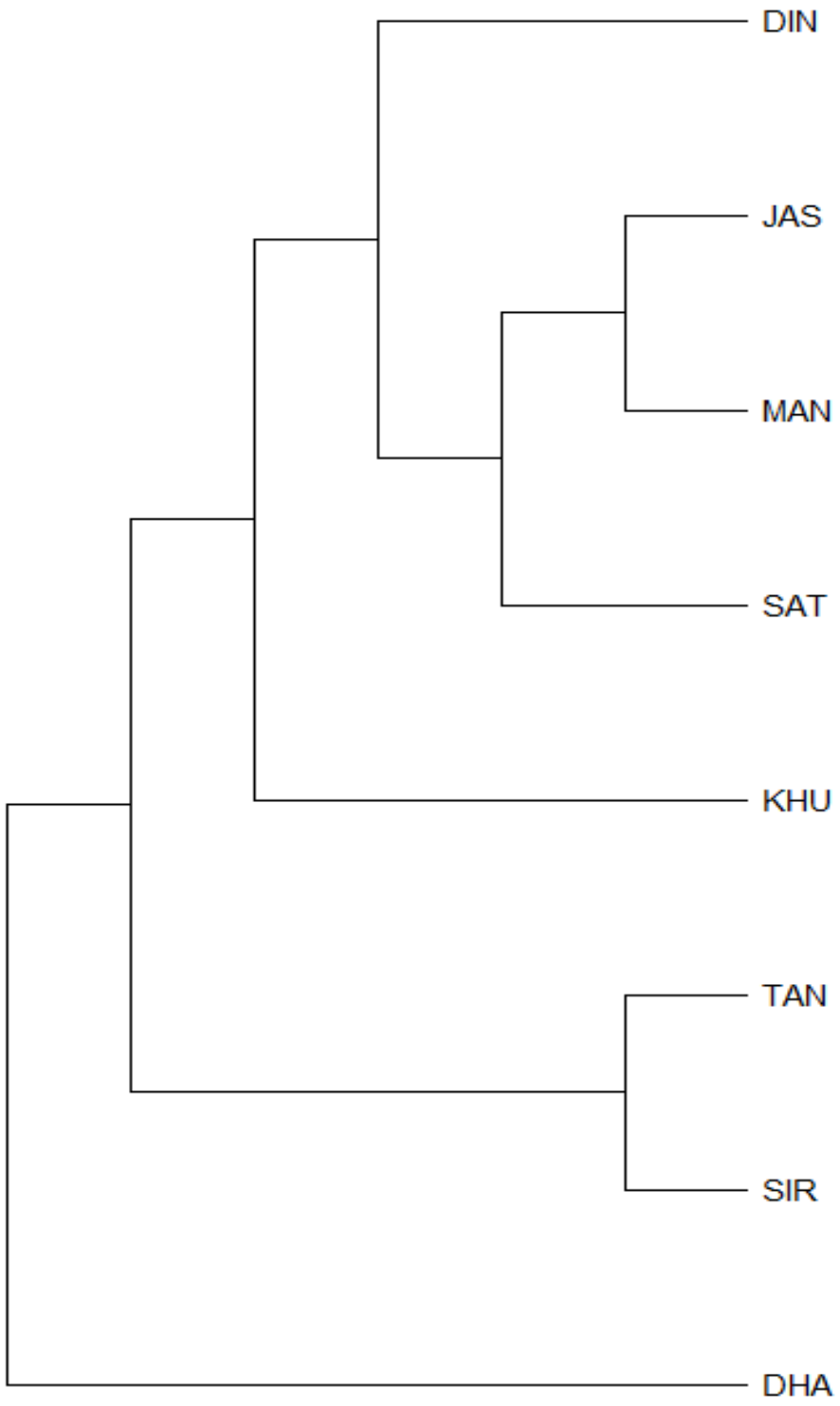


Figure 21. Phylogenetic tree of *Alternaria* sp isolates for grey blight disease infected mustard on the basis of nucleotide sequence and generated from 1000 bootsrap trees. The scale bar is in fixed nucleotide substations per sequence position

Experiment- 5: Evaluation the efficacy of selected fungicides, botanicals, bio- agent and their combined effect against the *Alternaria* (*in -vitro*)

4.5.1. Effect of selected treatments in the radical growth of *Alternaria* on PDA media *in-vitro* condition at 3, 7 and 14 DAI

The data recorded on the effect of selected treatments in the radical growth of *Alternaria* spp. on PDA medium *in- vitro* condition and ranged from 0.00-6.83%, 0.00 - 23.67% and 0.00 - 35.67% at 3, 7 and 14 DAI respectively (Table 21). It was found that the treatments varied significantly from each and other. Among the selected treatments, there was no radial mycelial growth of *Alternaria* spp was recorded in T_4 (Rovral 50 WP), T_6 (Dithane M-45) and T_{13} (Lantana extract+ Dithana M-45) at 3, 7, and 14 DAI and the highest radial mycelial growth of Alternaria species was found in T_1 (control) at all observations. Among the chemical treatments, the lowest radial mycelial growth of *Alternaria* spp was observed in T_4 (Rovral 50 WP) and T_6 (Dithane M-45) at all observations. The highest radial mycelial growth of *Alternaria* spp was recorded in T_5 (Amistar Top 325 EC) at all observations. Among the botanical treatments, the lowest radial mycelial growth of *Alternaria* spp was recorded in T_8 (Lantana leaf extract, 1.90mm, 8.50 mm and 10.50 mm) at all observations and the highest radial mycelial growth of *Alternaria* spp was found in T_9 (Alamanda leaf extract, 5.83mm, 15.67 mm and 27.67mm at all observation. Among the combined treatments, there was no radial mycelial growth of *Alternaria* spp was found in T_{13} (Lantana leaf extract+ Dithane M -45) and the highest radial mycelial growth of *Alternaria* spp was recorded in T_{12} (Datura leaf extract + Amistar Top, 5.0 mm, 9.16mm and 22.83 mm) at all observations. From the study, it was revealed that the highest radial growth inhibition (100%) of Alternria species was recorded in T_{13} (Lantana leaf extract+ Dithane M -45), T_4 ((Rovral 50 WP) and T_6 (Dithane M-45). The lowest radial growth inhibition (22.42%) of Alternria species was found in T_8 (Allamanda leaf extract,) over the control (T_1) treatment.

Table 21. Effect of selected treatments in the radical growth of *Alternaria* spp. on PDA medium in vitro condition at 3, 7 and 14 DAI

Treatment	3 Days (mm)	7 Days (mm)	14 Days (mm)	Growth inhibition (%)
T_1 (Control)	6.83 ^{a}	23.67 ^{a}	35.67 ^{a}	---
T_2 (*Trichoderma harzianum* suspension)	3.47 ^{d}	18.90 ^{c}	18.90 ^{d}	47.01
T_3 (Autostin 50 WDG)	1.00 ^{ef}	2.90^{i}	5.90 ^{g}	83.46
T_4 (Rovral 50 WP)	0.00 ^{f}	0.00 ^{j}	0.00 ^{h}	100
T_5 (Amistar Top 325 SC)	3.50 ^{d}	19.67 ^{b}	19.67 ^{d}	44.86
T_6 (Dithane M-45)	0.00 ^{f}	0.00 ^{j}	0.00 ^{h}	100
T_7 (Neem leaf extract)	3.63 ^{e}	12.50 ^{g}	19.00 ^{f}	46.73
T_8 (Allamanda leaf extract)	5.83 ^{ab}	15.67^{d}	27.67 ^{b}	22.42
T_9 (Lantana leaf extract)	1.90 ^{cd}	8.50 ^{f}	10.50 ^{d}	70.56
T_{10} (Datura leaf extract)	5.67 ^{ab}	13.50 ^{e}	23.67 ^{c}	33.64
T_{11} (Neem leaf extract+ Rovral)	1.00 ^{h}	4.00 ^{h}	15.50 ^{e}	56.55
T_{12} (Datura leaf extract + Amistar Top)	5.00 ^{bc}	9.16 ^{g}	22.83 ^{c}	36
T_{13} (Lantana leaf extract + Dithane M- 45)	00.00 ^{f}	00.00 ^{j}	00.00 ^{h}	100
T_{14} (Allamanda leaf extract + Autostin)	4.10 ^{cd}	8.83 ^{g}	19.67 ^{d}	53.27
CV (%)	16.00	2.42	4.63	------

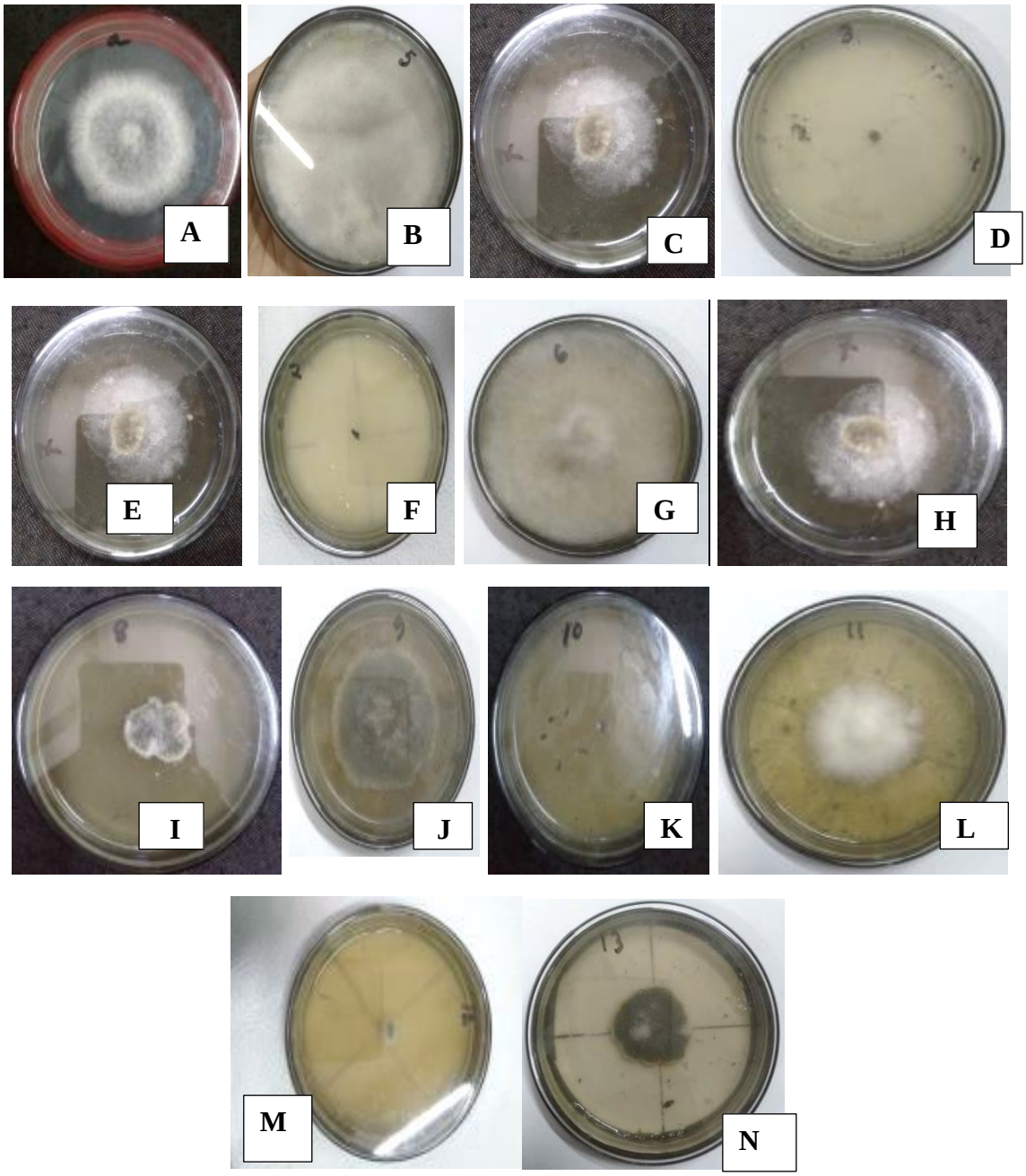


Plate 16. Showing radial growth inhabition of *Alternaria* isolates by applying selected treatments A- Control, B- *Trichoderma harzianum* suspension, C – Autostin 50 WDG, D - Rovral 50 WP, E - Amistar Top 325 SC, F - Allamanda leaf extract, G - Lantana leaf extract, H – Datura leaf extract, I – Neem leaf extract+ Rovral, J – Datura leaf extract + Amistar Top, M - Lantana leaf extract + Dithane M- 4 and N- Allamanda leaf extract + Autostin

Experiment - 6: Integrated management of grey blight of mustard through selected fungicides, botanicals, bio - agent and their combined effect

4.6.1. Effect of different treatments on disease incidence (%) at 50, 60 and 70 DAS

The effect of selected treatments on percent disease incidence for grey blight of mustard was recorded and the disease incidence ranged from 43.0–59.02%, 45.16–69.35% and 55.91–79.20% at 50, 60 and 70 DAS, respectively (Table 22)**.** From the study, it was found that among treatments, the lowest disease incidence was recorded in T_5 (Dithane M-45) and the highest in T_1 (control) at all observation. Among the chemical treatments, the lowest disease incidence was found in T_5 (Dithane M-45) followed by T_3(Autostin) where the highest disease incidence was recorded in T_4 (Rovral). Among the botanical treatments, the lowest disease incidence was found in T_9 (Lantana leaf extract) followed by T_{10} (Datura leaf extract) while the highest disease incidence was recorded in T_7 (Neem leaf extract). Among the combined treatments, the highest disease incidence was recorded in T_{11} (Neem + Rovral), where the lowest disease incidence was found in T_{13} (Lantana+ Dithane M-45). From these results it was also revealed that the T_2 treatment (*Trichoderma harzianum* suspension) was showed the moderate disease incidence and gave best performance for controlling grey blight disease of mustard.

Table 22. Disease incidence (%) under different treatments at 50, 60 and 70 DAS

Treatments	Disease incidence (%)		
	50 DAS	60 DAS	70 DAS
T_1 (Control)	59.02 ^{a}	69.35 ^{a}	79.20 ^{a}
T_2 (*Trichoderma harzianum* suspension)	47.29 ^{cd}	52.69 $^{b-d}$	63.63 $^{b-d}$
T_3 (Autostin 50 WDG)	47.31 ^{cd}	52.60 $^{b-d}$	56.63 ^{d}
T_4 (Rovral 50 WP)	51.34 $^{a-d}$	56.45 $^{a-d}$	67.92 $^{a-d}$
T_5 (Dithane M-45)	43.00 ^{d}	45.16 ^{d}	55.91 ^{d}
T_6 (Amistar Top 325 SC)	49.20^{b-d}	53.76 $^{b-d}$	61.47 $^{b-d}$
T_7 (Neem leaf extract)	56.83 ^{ab}	56.99 $^{a-d}$	73.48 ^{ab}

T_8 (Allamanda leaf extract)	54.32^{abc}	67.20 ^{ab}	72.40 $^{a-c}$
T_9 (Lantana leaf extract)	46.81 ^{cd}	51.06 ^{cd}	65.05 $^{b-d}$
T_{10} (Datura leaf extract)	50.91^{a-d}	54.30 $^{b-d}$	67.64 $^{a-d}$
T_{11} (Neem leaf extract+ Rovral)	57.55 ^{ab}	60.21 $^{a-c}$	68.18 $^{a-d}$
T_{12} (Datura leaf extract + Amistar Top)	51.54^{a-d}	56.99 a-d	67.70 $^{a-d}$
T_{13} (Lantana leaf extract + Dithane M- 45)	49.41^{b-d}	55.73 $^{a-d}$	59.14 ^{cd}
T_{14} (Allamanda leaf extract + Autostin)	54.84^{a-c}	61.82 $^{a-c}$	67.02 $^{a-d}$
CV (%)	10.64	15.66	12.41

4.6.2. Effect of different treatments on disease severity (%) at 50, 60 and 70 DAS

The data recorded on the effect of selected treatments on percent disease severity for grey blight of mustard, it ranged from 11.89-16.84%, 18.49-21.77% and 22.09-37.54% at 50, 60 and 70 DAS, respectively (Table 23). It was observed that the treatments varied significantly from each and other. Among the selected treatments, the lowest disease severity was found in T_{13} (Lantana + Dithane M-45) at 50 DAS but less disease severity was found in T_2 (18.49%, 26.08%, *Trichoderma harzianum* suspension) at 60 and 70 DAS and the highest disease severity was recorded in T_1 (control) at all observations. Among the chemical treatments, the lowest disease severity was recorded in T_5 (Dithane M-45) at all observations. The highest disease severity was recorded in T_4 (15.26% Rovral) at 50 DAS, but at 60 DAS, the highest disease severity was found in T_3 (20.92%, Autostin) and at 70 DAS it was higher in T_6 (29.27%, Amistar top). Among the botanical treatments, the lowest disease severity was recorded in T_9 (Lantana leaf extract, 12.57%, 19.44% and 28.89%,) at all observations and the highest disease severity was found in T_8 (Allamanda leaf extract, 12.99%, 20.21%) at 50, 60 DAS. But at 70 DAS, the highest disease severity was found in T_7 (Neem leaf extract, 34.37%). Among the combined treatments, the lowest disease severity was found in T_{13} (Lantana + Dithane M-45) at all observations and the highest disease severity was recorded in T_{11} (14.42%, 32.01%) at 50 and 70 DAS, but it was found higher in T_{12} (Datura + Amistar top, 20.74%) at 60 DAS.

Table 23. Disease severity (%) under different treatments at 50, 60 and 70 DAS

Treatments	Disease severity (%)		
	50 DAS	60 DAS	70 DAS
T_1 (Control)	16.84 [a]	21.77 [a]	37.54 [a]
T_2 (*Trichoderma harzianum* suspension)	12.72 [cd]	18.49 [d]	26.08 [b-d]
T_3 (Autostin 50 WDG)	13.82 [b-d]	20.92 [ab]	23.83 [cd]
T_4 (Rovral 50 WP)	15.26 [ab]	20.20 [a-d]	26.14 [b-d]
T_5 (Dithane M-45)	12.77 [cd]	19.17 [b-d]	22.09 [d]
T_6 (Amistar Top 325 SC)	13.30 [b-d]	20.29 [a-d]	29.27 [a-d]
T_7 (Neem leaf extract)	12.63 [cd]	19.50 [b-d]	34.37 [ab]
T_8 (Allamanda leaf extract)	12.99 [cd]	20.21 [a-d]	32.84 [a-c]
T_9 (Lantana leaf extract)	12.57 [cd]	19.44 [b-d]	28.89 [a-d]
T_{10} (Datura leaf extract)	12.73 [cd]	20.05 [a-d]	29.44 [a-d]
T_{11} (Neem leaf extract+ Rovral)	14.42 [bc]	20.10 [a-d]	32.01 [a-c]
T_{12} (Datura leaf extract + Amistar Top)	13.75 [b-d]	20.74 [a-c]	30.67 [a-d]
T_{13} (Lantana leaf extract + Dithane M- 45)	11.89 [d]	18.66 [cd]	26.32 [b-d]
T_{14} (Allamanda leaf extract + Autostin)	12.97 [cd]	19.73 [a-d]	27.17 [b-d]
CV (%)	9.55	6.44	19.79

4.6.3. Effect of different treatments on disease severity index (%) at 50, 60 and 70 DAS

The effect of selected treatments on percent disease severity index for grey blight of mustard was estimated, it ranged from 18.40-43.45%, 27.87-49.35%, 32.16 - 67.38% at 50, 60 and 70 DAS, respectively (Table 24). Among the set treatments, minimum disease severity index was recorded in T_2 (*Trichoderma harzianum* suspension, 18.40%) followed by T_5 (Dithane M-45, 19.19%) at 50 DAS, but at 60 and 70 DAS, the lowest disease severity index was found in T_5 (Dithane M-45) and maximum was recorded in control plot (T_1) at all observations. Among the chemical treatments, the lowest disease severity index was recorded in T_5 (Dithane M-45) at all observations and the highest disease was found in T_3 (28.78%, Autostin) at 50 DAS while the highest in T_4 (41.60% and 47.74%) at 60 and 70 DAS, respectively. In case of botanical treatments, the lowest disease severity index was found in T_9 (Lantana leaf extract) at all observations and the

highest disease severity index was recorded in T_8 (Allamanda leaf extract) at 50 DAS but at 60 and 70 DAS, disease severity index was found higher in T_7 (34.23%, Neem leaf extract) and T_{10} (48.45%, Datura leaf extract). Among the combined treatments, the lowest disease severity index was found in T_{13} (Lantana+ Dithane M-45) at all observations. The highest disease severity index was recorded in T_{12} (Datura + Amister top) at 50 DAS, but at 60 and 70 DAS, it was found higher in T_{11} (Neem+ Rovral, 35.38% and 49.10% respectively).

Table 24. Disease severity index (%) under different treatments at 50, 60 and 70 DAS

Treatments	Disease severity index (%)		
	50 DAS	60 DAS	70 DAS
T_1 (Control)	43.45 ^{a}	49.35 ^{a}	67.38 ^{a}
T_2 (*Trichoderma harzianum* suspension)	18.40 ^{d}	30.38 ^{bc}	46.50 $^{b-d}$
T_3 (Autostin 50 WDG)	28.78 ^{bc}	36.30 ^{bc}	44.58 $^{b-d}$
T_4 (Rovral 50 WP)	26.63 $^{b-d}$	41.60 ^{ab}	47.74 ^{bc}
T_5 (Dithane M-45)	19.19 ^{cd}	27.87 ^{c}	32.16 ^{e}
T_6 (Amistar Top 325 SC)	24.20 $^{b-d}$	32.40 ^{bc}	39.23 $^{c-e}$
T_7 (Neem leaf extract)	29.09 ^{bc}	34.23 ^{bc}	45.49 $^{b-d}$
T_8 (Allamanda leaf extract)	29.90 ^{b}	30.50 ^{bc}	44.74 $^{b-d}$
T_9 (Lantana leaf extract)	27.84 $^{b-d}$	28.71 ^{bc}	37.96 ^{de}
T_{10} (Datura leaf extract)	28.08 $^{b-d}$	32.76 ^{bc}	48.45 ^{bc}
T_{11} (Neem leaf extract+ Rovral)	30.16 ^{b}	35.38 ^{bc}	49.10 ^{b}
T_{12} (Datura leaf extract + Amistar Top)	32.81 ^{b}	33.85 ^{bc}	41.22 $^{b-e}$
T_{13} (Lantana leaf extract + Dithane M- 45)	25.59 $^{b-d}$	28.80 ^{bc}	38.20 ^{de}
T_{14} (Allamanda leaf extract + Autostin)	28.98 ^{bc}	34.01 ^{bc}	45.20 $^{b-d}$
CV (%)	21.94	22.66	12.65

4.6.4. Effect of different treatments on pod infection (%) at 55, 65 and 75 DAS

The effect of selected treatments on percent pod infection was recorded and ranged from 0.65-2,97%, 1.73-7.25% and 6.72-34.63% at 55, 65 and 75 DAS (Table 25), respectively. From the study, it was recorded that among set treatments, the lowest pod infection was found in T_5 (Dithane M-45) where the

highest was recorded in T_1 (Control) at all observations. Among the chemical treatments, Dithane M-45 (T_5) was gave the best result, which was statistically similar with T_6 (Amistar top) and T_4 (Rovral). Among the botanical treatments, the lowest pod infection was found in T_9 (Lantana leaf extract) at 55 and 75 DAS whereas it was recorded the lowest in T_8 (Allamanda leaf extract) at 65 DAS. The highest pod infection found in T_8 (Allamanda leaf extract) at 55 and 75 DAS whereas it was found higher in T_9 (Lantana leaf extract) at 65 DAS. Among the combined treatments, the highest pod infection was recorded in T_{11}(Neem + Rovral) at 55 and 65 DAS but at 75 DAS, it was found higher in T_{11} (Neem leaf extract + Rovral). From these results it was also revealed that the T_2 treatment (*Trichoderma harzianum* suspension) was gave the moderate result in terms of pod infection (%) and showed the best performance for controlling grey blight disease of mustard.

Table 25. Pod infection (%) under different treatments at 55, 65 and 75 DAS

Treatments	Pod infection (%)		
	55 DAS	65 DAS	75 DAS
T_1 (Control)	2.97 ^{a}	7.25 ^{a}	34.63 ^{a}
T_2 (*Trichoderma harzianum* suspension)	1.31 ^{bc}	2.92 ^{bc}	19.68 ^{bc}
T_3 (Autostin 50 WDG)	1.14 ^{bc}	2.75 ^{bc}	17.21bc
T_4 (Rovral 50 WP)	1.02 ^{bc}	2.30 ^{bc}	12.78 ^{cd}
T_5 (Dithane M-45)	0.65 ^{c}	1.73 ^{c}	6.72 ^{d}
T_6 (Amistar Top 325 SC)	0.88 ^{bc}	1.78 ^{c}	9.56 ^{cd}
T_7 (Neem leaf extract)	0.94 ^{bc}	3.23 ^{bc}	16.85 $^{b-d}$
T_8 (Allamanda leaf extract)	1.73 ^{abc}	2.79 ^{bc}	23.05 ^{b}
T_9 (Lantana leaf extract)	1.18 ^{bc}	4.59 ^{b}	13.79 $^{b-d}$
T_{10} (Datura leaf extract)	1.57 ^{bc}	3.50 ^{bc}	18.55 ^{bc}
T_{11} (Neem leaf extract+ Rovral)	1.40 ^{bc}	2.53 ^{bc}	19.54 ^{bc}
T_{12} (Datura leaf extract + Amistar Top)	1.23 ^{bc}	2.75 ^{bc}	13.82 $^{b-d}$
T_{13} (Lantana leaf extract + Dithane M- 45)	1.21 ^{bc}	2.39 ^{bc}	12.30 ^{cd}
T_{14} (Allamanda leaf extract + Autostin)	1.96 ^{ab}	3.60 ^{bc}	17.41 ^{bc}
CV (%)	56.25	46.19	36.08

4.6.5. Effect of different treatments on yield and yield contributing parameters in mustard against grey blight disease

The effect on grain yield of mustard under different treatments were summarized and significant variation was found in fresh and dry condition (Table 26). In case of fresh weight, among the treatments. The highest grain yield (1357.3 gm/plot) was recorded in T_{13} (Lantana + Dithane M-45) followed by T_5 (Dithane M-45, 1347.7 gm/plot) and T_9 (Lantana leaf extract, 1318.9 gm/plot), whereas the lowest yield was found in T_1 (Control, 998.4 gm/plot). In dry condition, maximum grain yield was obtained in combined treatment T_{13} (Lantana + Dithane M-45, 1136.3 gm/plot), while minimum grain yield was found in T_1 (control, 811.7 gm/plot).

Table 26. Grain yield of Mustard under different treatments

Treatments	Fresh wt.(gm/ plot)	Dry wt. (gm/plot)	1000-seed weight (gm)	Wt. (MT/ha)
T_1 (Control)	998.4 ^{d}	811.7 ^{e}	3.15 ^{e}	1.34 ^{f}
T_2 (*Trichoderma harzianum* suspension)	1152.5 $^{a-d}$	990.1 $^{a-e}$	4.55 bc	1.65 $^{a-e}$
T_3 (Autostin 50 WDG)	1035.5 ^{cd}	905.1 ^{de}	4.15 ^{d}	1.52 ^{ef}
T_4 (Rovral 50 WP)	1115.7 $^{b-d}$	978.4 $^{b-e}$	4.41 cd	1.71 $^{a-e}$
T_5 (Dithane M-45)	1347.7 ^{a}	1114.7 ^{ab}	5.03 ^{a}	1.89 ^{a}
T_6 (Amistar Top 325 SC)	1214.1 $^{a-c}$	1089.1 $^{a-c}$	4.93 ^{a}	1.81 ^{ab}
T_7 (Neem leaf extract)	1028.0 ^{cd}	910.4 $^{c-e}$	4.09 ^{d}	1.56 $^{c-f}$
T_8 (Allamanda leaf extract)	1300.0 ^{ab}	1122.3 ^{ab}	4.92 ^{a}	1.78 $^{a-d}$
T_9 (Lantana leaf extract)	1318.9 ^{ab}	1172.4 ^{a}	5.16 ^{a}	1.84 ^{ab}
T_{10} (Datura leaf extract)	1029.6 ^{cd}	969.1 $^{b-e}$	4.55 bc	1.62 $^{b-e}$
T_{11} (Neem leaf extract+ Rovral)	1254.1 ^{ab}	1072.8 $^{a-d}$	4.90 ^{a}	1.79 $^{a-c}$
T_{12} (Datura leaf extract + Amistar Top)	1113.9 $^{b-d}$	996.3 $^{a-d}$	4.83 ab	1.54 $^{d-f}$
T_{13} (Lantana leaf extract + Dithane M-45)	1357.3 ^{a}	1136.3 ^{ab}	5.00 ^{a}	1.86 ^{ab}
T_{14} (Allamanda leaf extract + Autostin)	1181.6 $^{a-d}$	910.9 $^{c-e}$	4.26 cd	1.5 ^{ef}
CV (%)	10.70	10.78	4.38	4.97

4.6.6. Relationship between grain yield with percent diseases incidence, severity, severity index and pod infection

Different treatments that were used in the present study regarding grain yield (ton/ha) and disease incidence (%), disease severity (%), disease severity index (%) and pod infection (%). In case of relationship between grain yield (MT/ha) and disease incidence (%), it was revealed that grain yield was decreased with increased of disease incidence. The highest grain yield (1.89 MT /ha) was found in T_5 (Dithane M - 45) where the disease incidence was recorded the lowest (55.91%) at 70 DAS. On the other hand, the lowest grain yield (1.34 MT/ha) was found while the disease incidence was recorded the highest (79.20%) in control treatment (T_1) at 70 DAS as depicted in the Figure 36. In case of relationship between grain yield (MT/ha) and disease severity (%), it was revealed that grain yield was decreased with increased of disease severity. The highest grain yield (1.89 MT /ha) was found in T_5 (Dithane M-45) where the disease severity was recorded the lowest (22.09%) at 70 DAS. On the other hand, the lowest grain yield (1.34 MT/ha) was found while the disease severity was recorded the highest (37.54%) in control treatment (T_1) at 70 DAS as depicted in the Figure 37. In case of relationship between grain yield (MT/ha) and disease severity index (%), it was revealed that grain yield was decreased with increased of disease severity index. The highest grain yield (1.89 MtT /ha) was found in T_5 (Dithane M-45) where the disease severity index was recorded the lowest (32.16%) at 70 DAS. On the other hand, the lowest grain yield (1.34 MT/ha) was found while the disease severity index was recorded the highest (67.38%) in control treatment (T_1) at 70 DAS as depicted in the Figure 38. In case of relationship between grain yield (MT/ha) and pod infection (%), it was revealed that grain yield and seed quality was decreased with increased of pod infection. The highest grain yield (1.89 MT /ha) was found in T_5 (Dithane M-45) where the pod infection was recorded the lowest (6.72%) at 75 DAS. On the other hand, the lowest grain yield (1.34 MT/ha) was found while the pod infection was recorded the highest (34.63%) in control treatment (T_1) at 75 DAS as depicted in the Figure 22-25.

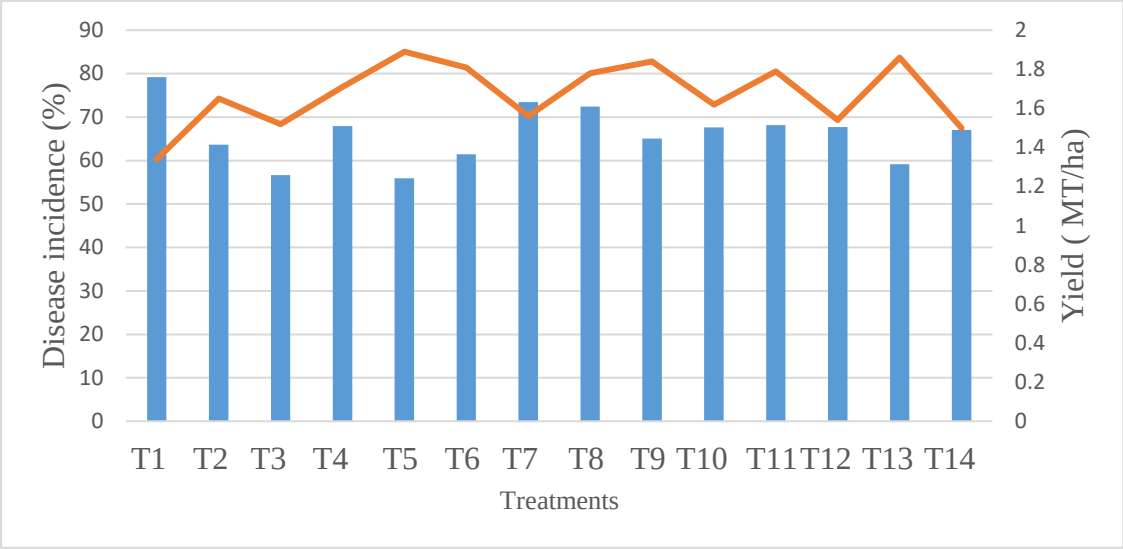


Figure 22. Relationship between disease incidence (%) at 70 DAS and yield (MT/ha)

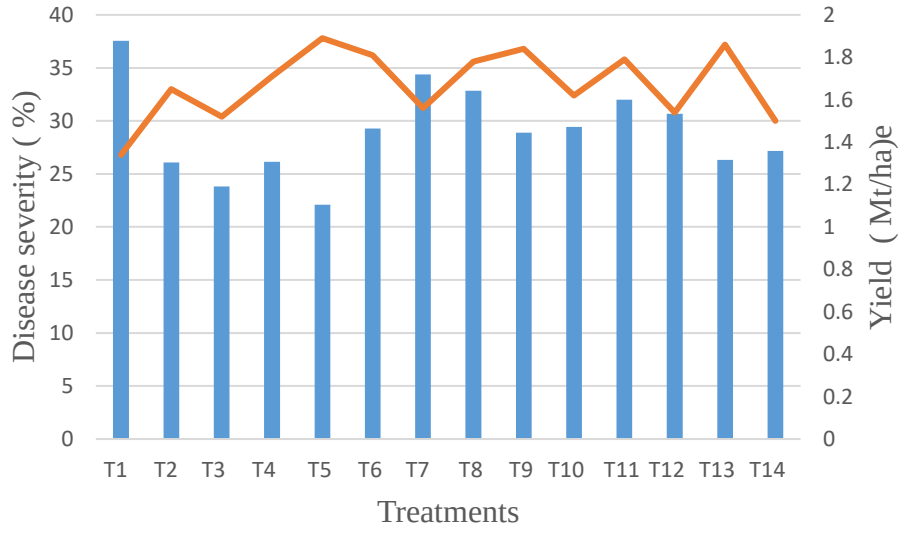


Figure 23. Relationship between disease severity (%) at 70 DAS and yield (MT/ha)

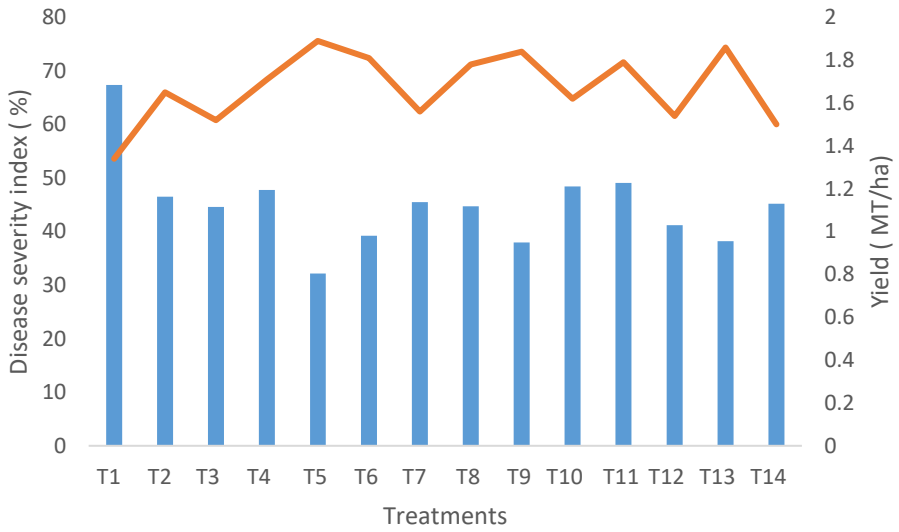


Figure 24. Relationship between disease severity index (%) at 70 DAS and yield (MT/ha)

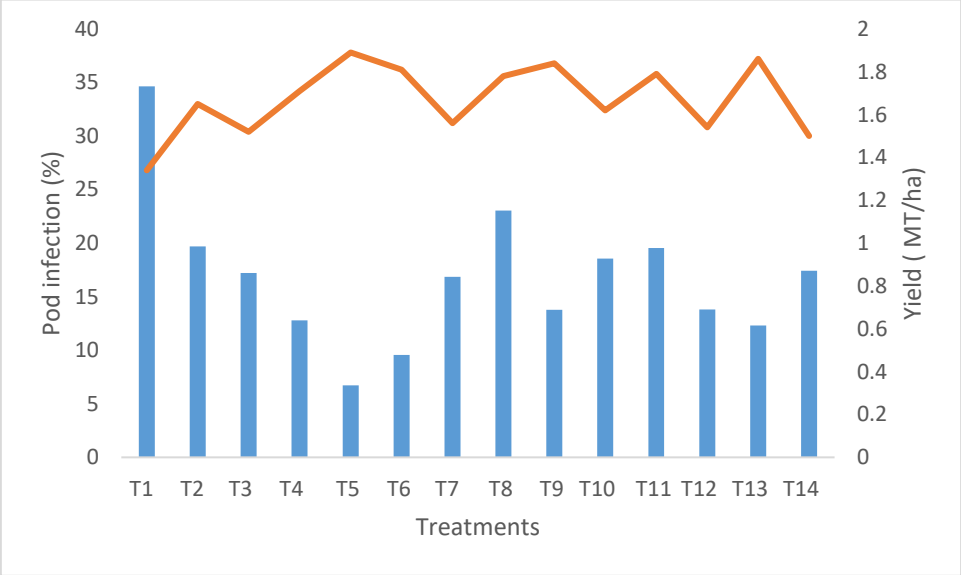


Figure 25. Relationship between Pod infections (%) at 75 DAS and yield (MT/ha)

DISCUSSION

Experiment: 1. Symptomatology of recorded Mustard diseases

A survey on major fungal diseases of mustard was done in selected districts of Bangladesh. From the survey study, it was observed that some major diseases were commonly found in all surveyed areas and disease was identified on the basis of visible characteristics symptoms. The identified diseases were Alternaria or grey blight, white rust, powdery mildew and sclerotinia stem rot. Among the diseases grey blight disease caused by Alternaria was found more prevalent. Bakr *et al.* (2009) conducted a survey study where 14 mustard diseases were recorded (fungus-9, virus-2, bacteria-1, nematode-1 and parasitic plant-1) in Bangladesh. Meena *et al.* (2010) studied that more than thirty diseases are known to occur on Brassica crops in India. These include Alternaria blight, white rust, downy mildew, powdery mildew etc. Kharbanda and Tewari (1996) reported that a number of diseases affect mustard in the field in which the most important are Alternaria blight (*Alternaria brassicae*), powdery mildew (*Erysiphe cruciferarum*) and white mold or sclerotinia rot (*Sclerotinia sclerotiorum*). Sclerotinia sclerotiorum is the most devastating disease of rapeseed-mustard in Bangladesh. Sclerotinia is present in all rapeseed and mustard growing regions and is considered economically damaging disease.

Characteristics symptoms of Alternaria or Grey blight were found in the lower leaves as small circular brown necrotic spots which gradually increase in size. In severe cases many concentric spots coalesce to cover large patches showing blighted symptoms. Circular to linear dark brown spots were also observed on stems and pods. Infected pods produce small discolored and shriveled seeds. Karthikeyan *et al.* (2021) and Valkonen and Koponen (1990) described that symptoms of this disease include presence of irregular, often circular brown to dark brown colour leaf spots on the leaves with concentric lines inside the spots. Gray blight causes blight of leaf, pod and stem as well as seed abnormalities (Meah *et al.*,1988).

During the field observation it was noticed for the typical symptoms of white rust appears white or creamy yellow pustules of various shape and size appears on the surface of the leaves, mainly on the lower surface resulting the leaves become thick, fleshy, enrolled and their size becomes reduced and young stems and inflorescence are infected to produces deformities like swelling and distortion of the floral parts. This observation agreed with the observation of Sangeetha. and Siddaramaiah (2007), they described that the first symptom of white rust appeared as small, white raised pustules on leaves.

In the surveyed areas, it was observed that the symptoms of powdery mildew where grayish white irregular necrotic patches developed on the lower surface of the leaves. Mark L. Gleason (2013) reported that symptoms appeared as circular to irregular white colonies, which subsequently showed abundant hyphal growth on both leaf surfaces. Severely infected plants were unmarketable due to leaf discoloration, and most were not harvested.

Sclerotinia stem rot also identified from survey areas. The symptoms were water soaked spots near to crown region develop in stems which later covered with cottony white mycelium. Sclerotinia stem rot, also referred to as white mold, is caused by the fungal pathogen *Sclerotinia sclerotiorum.* The fungus survives from year to year as hard dark structures called sclerotia. Sclerotia are variously shaped bodies of tightly packed white mycelium covered with a dark, melanized protective coat. These spores are released during favorable weather conditions and can collapsible to other fields in air currents. Bolton (2006) described that *Sclerotinia sclerotiorum* is a necrotrophic pathogen with a wide host range, causing symptoms that vary from host to host. The sign of white mycelium is one of the most important characteristics of *S. sclerotiorum* infection.

Incidence of major diseases of Mustard

From the survey study, the incidence of four diseases like grey blight, white rust, powdery mildew and sclerotinia stem rot were recorded, it was so high in all districts. The highest disease incidence of grey blight was recorded in Dhaka and Sirajganj and the lowest disease incidence was observed in Satkhira. In case of

white rust, the disease incidence was higher in the district of Khulna, Satkhira and Manikganj and no disease incidence was found in Jashore, Dhaka, Sirajganj and Dinajpur. The highest disease incidence of powdery mildew was showed in Satkhira and Sirajganj in all selected districts and no disease incidence of powdery mildew was found in Jashore, Dhaka, Tangail and Dinajpur. In case of sclerotinia stem rot, the highest disease incidence was recorded in Tangail and no disease incidence was observed in Dhaka and Sirajganj.

It was noticed that sources of seed were mainly home storage and Govt. agency (BADC), and for seed treatments most of the farmer used chemical fungicides. Farmers were also use botanicals for seed treatment as a conventional practice. From the frequency study, it was found that maximum cultivated area was occupied by HYV variety BARI Sarisha -14 followed by BARI Sarisha -15 and local variety Tori-7, and minimum area covered by Rye sarisha. From the survey study, it was also found that maximum mustard grower was sown seeds during 16 – 30 November.

Experiment-2. Identification and characterization of *Alternaria* isolates cultural and morphological characters of *Alternria* isolates on PDA

Three species of *Alternaria* were identified from grey blight infected mustard leaves those were *A. brassicae, A. brassicola and A. alternata*. Among the different isolates, radial mycelial growth of *Alternaria* spp. was varied significantly at different days after inoculation. Radial mycelial growth was measured at 3, 7 and 14 days after inoculation. In respect of cultural characteristics, all isolates of *Alternaria* spp. showed variation in mycelial growth, colony color, shape, textures, subsurface color, zonation conidia production and sporulation time. Almost same observation was reported by Rufaida Monowara *et al.* (2017) where they studied with 10 *Alternaria* isolates, and reported that all 10 isolates showed significant variations in respect of their cultural and morphological characteristics on different artificial culture media. The results are also similar with Yadav *et al.* (2016), they were identified

morphological and cultural characters of *A. brassicae* isolates from four different locations, colonies of all the isolates were circular in shape. Deep *et al.* (2014) observed thirty-two isolates of *A brassicicola* for colony color and radial growth. Colony colour of *A. brassicicola* varied from olive green to dark olivacious black on PDA. The colour of colonies varies between light brown to black on PDA. Diversity in colony colour, growth, its margin and topography were noticed among the isolates of *A. brassicae* by different workers (Sharma *et al.*, 2013, Pramila *et al.*, 2014, Singh *et al.*, 2015, Singh *et al.*, 2016, and Singh and Singh, 2018).

Morphological variation of conidia of different isolates of *Alternaria* spp.

Among different isolates of *Alternaria* spp. morphological variation was found in number of conidia, longitudinal septation and transverse septation of conidia. Number of conidia varied from the range of 1.33 to 5.66. The highest number of conidia was recorded in TAN isolate (5.67) followed by JAS isolate (4.33) whereas the lowest number of conidia was found in KHU isolate (1.33) which was statistically similar to MAN isolate (2.33) and SIR isolate (2.33). The maximum longitudinal septation of *Alternaria* spp. was observed in DIN isolate (6.67) followed by TAN isolate (6.33) and the longitudinal septation minimum transverse septation of *Alternaria* species was measured in SIR isolate that was 3.67 among the isolates. Variation was also obtained in transverse septation of *Alternaria* spp. in where it was varied from 1.00 to 2.33. Maximum transverse septation of *Alternaria* spp. was recorded in SAT isolate (2.33) whereas minimum transverse septation of *Alternaria* spp. was observed in SIR isolate (1.00) which was statistically similar to DIN_{Ab} (1.33). These results are in agreements with earlier workers (Sharma *et al*., 2013; Pramila *et al*., 2014; Singh *et al.*, 2016; Kumar and Singh 2018, Singh and Singh, 2018), who observed morphological variability in different geographical isolates within Alternaria isolates. Khan *et al.* (2007a) observed that conidial length varied from 112.0 µm, to 185.6 µm. The maximum conidial length (185.6 µm), breadth (17.6 µm), length of conidial beak varied from 42.0-116 µm and number of septa (horizontal, 9.7 and vertical 2.4)

were recorded. Khuble *et al.* (2011) reported average conidial length ranged from 21.00 µm to 298.00 µm. The average minimum conidial length was observed in isolate ABC (55.23 µm) and maximum in ABD (152.17 µm). The beak length of conidia ranged from 12.00 µm to 144.00 µm however, average was minimum in ABH (35.50 µm) and maximum in ABL (88.40).

Size of conidia from different isolates of *Alternaria* spp.

From the present study, it was observed that conidial length of *Alternaria* spp. varied significantly. Among the isolates, the highest conidial length of *Alternaria* spp. was recorded in SAT isolate (25.09 µm) which was statistically similar to TAN isolate (22.21 µm) whereas the lowest conidial length of *Alternaria* spp. was found in SIR $_{Ab}$ isolate that was 12.30 µm. Variation of conidial breath of *Alternaria* spp. was observed that was 5.57 to 7.15 µm but no statistically significant data was found among the isolates. Monowara *et al.* (2017) reported that variations were observed in accordance with length, breadth and beck on different isolates of *Alternaria* spp. on PDA media. Ramjegathesh and Ebenezar (2012) collected ten isolates of *A. alternata* the length and width of conidia were varied from 30.99 -42.47 µm and 11.90-17.37 µm, respectively.

Conidial color of different *Alternaria* spp. on PDA

Conidia color was varied from light to deep brown among the isolates of *Alternaria* spp. It was observed that light brown color was found in the isolates of *Alternaria* spp. of JAS isolate and MAN isolate whereas brown color of conidia of *Alternaria* spp. was showed in SAT isolate, KHU isolate and TAN isolate and deep brown of conidia of *Alternaria* spp. was also showed in DHA isolate, SIR isolate and DIN isolate. From all collected isolates of *Alternaria* spp. muriform type conidia was found. All the isolates produced light brown to deep brown murifrom conidia with beak. Conidial septation was differed from the collected isolates of *Alternaria* spp. This finding was supported by Kumar *et al.* (2014) and Giri (2013). They were also found murifrom conidia which were brownish black. Some researcher worked with *A. brassicae* and found murifrom, obclavate conidia with brownish black.

Experiment - 3. Pathogenicity Test

All the *Alternaria* isolates were found to be pathogenic in nature that was demonstrated by pathogenicity test. Among the eight (8) isolates from different selected districts, JAS isolate from Jashore district was found to be highly pathogenic as the spots produced >1cm in diameter. These findings are similar to Akhter *et al*. (2012), eight mustard varieties (SAU-1, BINA-6, TORI-7, BARI-9, BARI-6, SOFOL, AGRANI and SS-75) were evaluated for their reaction against Alternaria blight (*Alternaria brassicae*) under natural condition at the experimental field of Sher-e-Bangla Agricultural University, Dhaka during winter season from November 2007 to February 2008. According to Prasad *et al*. (2008), who found that increasing inoculum concentration significantly increased the infection of *A. helianthi* in sunflower, whereas Doullah and Okazaki (2015) used the concentration of conidial suspension at 5×10^4 conidia/ml for inoculation of *B. rapa* leaf with *A. brassicicola*.

Experiment-4: Molecular Variation among the Selected *Alternaria* isolates

Pathogenic and molecular variability of *Alternaria* isolates

Due to the systematic and taxonomic usefulness the ITS region has been used in classifying fungi, as it is suitable size for PCR amplification, restriction analysis and sequencing procedures, and ITS regions are variable among species as well (Jung *et al.,* 2002). In the present study 8 *Alternaria* isolates were amplified and analyzed. *Alternaria* fungal gene was amplified using transcribed spacer region primers; forward primer ITS4: TCCTCCGCTTATTGATATGC and reverse primer ITS5: GGAAGTAAAAGTCGTAACAAGG and found the PCR products of amplicons of ~ 700bp. Analysis of ITS revealed that all *Alternaria* isolates were 99% similar to the *Alternaria alternata* in NCBI database.. Sharma *et al.* (2013) also characterized 32 Indian isolates using ITS primers between 550- 600 bp. Kumar *et al.* (2014) also performed DNA fingerprinting using ISSR primers to study genetic diversity among 32 isolates of *A. brassicae* isolates. Molecular relationships amongst *Alternaria* spp. based on nucleotide sequence and host

specific toxins (Kusaba and Tsuge, 1994, 1995) or with other related fungi have been analyzed (Pryor and Gilbertson, 2002; Chou and Wu., 2002). DNA/nucleotide sequences among *Alternaria* spp. pathogenic to crucifers has been reported from one isolate each of *A. brassicae, A. brassicicola, A. raphani* and *A. alternata* (Jasalavich *et al.,* 1995). BLAST analysis of the internal transcribed spacer region of all thirty two *A. brassicae* isolates in this study showed high similarity among the isolates with *Alternaria* isolates of the NCBI database. Results of the study is supported by the one previous study, the genetic distance between the isolates of *Alternaria isolates* ranged from 0.00 -0.04 suggesting that they are closely related (Bock *et al.,* 2002).

The internal transcribed spacer sequences were very variable in both base composition and length. The nucleotide sequences were highly conserved, but enough variability was present to distinguish isolate clearly. Phylogenetic analysis of the sequence data sets by both parsimony and maximum likelihood methods clearly separated genera and species isolates. All the *Alternaria isolates* were closely related. Jasalavich *et al.* (1995) found that *A. brassicicola* is actually more closely related to *A. raphani* than to *A. brassicae* based on the DNA sequences data. *A. alternata, A. brassicae, A. brassicicola* and *A. raphani* formed clade of very closely related sister taxa. The nucleotide sequences resolved two subclades of species within *Alternaria* at a level of confidence of 99%, based on five informative sites contained within the 700 bp at the 3'-end of the sequences alignment. Complete resolution of the species of *Alternaria* was achieved with the ITS sequence data which were much variable and contained more phylogenetically informative sites. The study indicates that *A. brassicae* and *A. brassicicola* encompasses less genetic variability. Chou and Wu (2002) studied the phylogenetic analysis of internal transcribed spacer regions (ITS 1and ITS 2) of different fungi and they got positioned filament beaked *Alternaria* as a monophyletic group discrete from the other members of genus *Alternaria*. Filament beaked *Alternaria* spp. formed a well-supported group. The second group consisted of small spored *A. brassicicola* and the large spored

A. brassicae. This study also indicates less genetic diversity in *Alternaria* species causing Alternaria leaf blight on mustard.

Experiment-5. Evaluation of the Efficacy of selected Fungicides, Botanicals, Bio- agent and their combined effect against *Alternaria (In-vitro)*

Effect of selected treatments in reduction of the radial mycelial growth of *Alternaria* isolates on PDA medium at different DAI (*in-vitro*)

In the present study (*in-vitro*), the efficacy of some selected fungicides, botanicals, biological-agent and their combined effect were evaluated before conducting the management experiment in the field conditions. The data was recorded at different DAI in the radical mycelial growth of *Alternaria* isolates on PDA medium. It was found that the treatments varied significantly from each and other. Among the chemical treatments, the lowest radial mycelial growth was observed in T_4 (Rovral 50 WP) and T_6 (Dithane M-45) treatments and the highest radial mycelial growth was recorded in T_5 (Amistar Top 325 EC) treatment at all observations. Among the botanical treatments, the lowest radial mycelial growth was recorded in T_9 (Lantana leaf extract) treatment and the highest radial mycelial growth was found in T_8 (Allamanda leaf extract) treatment at all observation. Among the combined treatments, the highest radial mycelial growth was recorded in combined treatment, Datura leaf extract + Amistar Top (T_{12}), and there was no radial mycelial growth was found in combined treatment in (T_{13}) (Lantana leaf extract+ Dithana M – 45) upto last observation. The selected bio-agent (*Trichoderma harzianum* suspension) was also showed significant results in reduction of mycelial growth of *Alternaria* isolates in *in-vitro* condition. These results support to Meena *et al*. (2004) evaluated bio-control agent (*Trichoderma viride*) and fungicides (Mancozeb and Carbendazim) for controlling Alternaria blight in Indian mustard. Fungicide Mancozeb and Carbendazim caused 100% reduction in mycelia growth, while the bio-control agent significantly reduced disease severity. Mamta *et al.* (2016) evaluated of biocontrol agents in controlling blight disease of mustard. In dual culture test *Trichoderma harzianum* caused maximum inhibition of radial growth of *Alternaria brassicae*. Khan *et al*. (2007), Singh *et*

al. (2008) tested efficacy of different fungicides (Apron 35 SD, Ridomil MZ 72 WP and Carbendazim 50%) and bioagent (*Trichoderma harzianum* and *Pseudomonas flurescence*) alone and in combination against Alternaria blight and found seed treatment with *Trichoderma harzianum* @ 10g/kg seed + 3 foliar sprays of same bioagent @ 10% was most effective in minimizing the disease intensity. Neeraj *et al.* (2020) was conducted an experiment to evaluate 7 (seven) chemical fungicides under *in-vitro* condition against *Alternaria brassicae* by poison food technique at 0.05%, 0.1% and 0.2% concentration and it was found that in case of 0.1% and 0.2% concentration of fungicides the minimum growth was recorded.

Experiment- 6: Integrated management of grey blight of mustard through selected fungicides, botanicals, bio-agent and their combined effect

Effect of selected treatments on disease incidence (%)

The effect of selected treatments on percent disease incidence for grey blight of mustard was recorded**.** From the study, it was found that among the chemical treatments, the lowest disease incidence was found in T_5 (Dithane M-45) treatment followed by T_3 (Autostin) treatment where the highest disease incidence was recorded in T_4 (Rovral). Among the botanical treatments, the lowest disease incidence was found in T_9 (Lantana leaf extract) treatment followed by T_{10} (Datura leaf extract) treatment while the highest disease incidence was recorded in T_7 (Neem leaf extract) treatment. Among the combined treatments, the highest disease incidence was recorded in T_{11} (Neem + Rovral) treatment, where the lowest disease incidence was found in T_{13} (Lantana+ Dithane M-45) treatment. From these results it was also revealed that the T_2 treatment (*Trichoderma harzianum* suspension) was showed the moderate disease incidence and gave best performance for controlling grey blight disease of mustard. Prasad *et al.* (2003) conducted an experiment to determine the losses due to Alternaria blight under protected and unprotected conditions. The protected plots were sprayed with

0.25% mancozeb starting from 40 days after sowing and 3 subsequent sprays at 15-day intervals. The disease appeared 45 days after sowing. Treatment with mancozeb reduced disease incidence in all the genotypes. The lowest disease intensity was recorded in the protected plots compared to the unprotected plots in the two subsequent years. Singh and Singh (2006) conducted a field experiment to develop spray schedule(s) for the management of grey blight caused by *Alternaria brassicae and A. brassicicola* applying three consecutive sprays of Mancozeb 75 WP (0.2%) at fortnightly intervals, beginning at the disease initiation resulted in the lowest leaf blight incidence and pod blight intensity.

Effect of selected treatments on disease severity (%)

The data recorded on the effect of selected treatments on percent disease severity for grey blight of mustard and it was observed that the treatments varied significantly from each and other. Among the chemical treatments, the lowest disease severity was recorded in T_5 (Dithane M-45) treatment at all observation and the highest disease severity was found in T_3 (20.92%, Autostin) treatment, but at 70 DAS it was higher in T_6 (29.27%, Amistar top) treatment. Among the botanical treatments, the lowest disease severity was recorded in T_9 (Lantana leaf extract) treatment at all observations and the highest disease severity was found in T_8 (Allamanda leaf extract) treatment, but at 70 DAS, the highest disease severity was found in T_7 (Neem leaf extract) treatment. Among the combined treatments, the lowest disease severity was found in T_{13} (Lantana + Dithane M-45) treatment at all observations and the highest disease severity was recorded in T_{11} (Neem leaf extract+ Rovral) at 50 DAS and 70 DAS, but it was found higher in T_{12} (Datura + Amistar top) treatment at 60 DAS. These results are agreed with previous research reports. Chattopadhyay and Bagchi (1994) reported that the lowest severity and the highest yields were obtained following four foliar sprays with Mancozeb (0.2%) at intervals of 15 days, starting from 30 days after sowing. Three sprays at 45, 60 and 75 days after sowing gave the highest benefit ratios. Prasad (2006) was carried an experiment to evaluate the efficacy of different spraying combinations of three fungicides (ridomil [metalaxyl], carbendazim and

mancozeb) and five plant extracts (*Datura stramonium, Eucalyptus globosus, Azadirachta indica, Allium sativum and Allium cepa*) against *Alternaria* blight (*Alternaria brassicae*) of Indian mustard cv. Varuna. Comparative analysis of various spraying schedules revealed that first spray of carbendazim (0.1%) + mancozeb (0.2%) followed by two sprays of mancozeb (0.2%) at early sowing (20 October) was the best combination in reducing the disease severity on leaves (18.7%) and pods (10.4%) and in increasing yield (1295.8 kg/ha), 1000-seed weight (5.12 g) and oil content (42.6%).

Effect of selected treatments on disease severity index (%)

The effect of selected treatments on percent disease severity index for grey blight of mustard was estimated. It was estimated that among the set treatments, minimum disease severity index was recorded in T_2 (*Trichoderma harzianum* suspension) treatment followed by T_5 (Dithane M-45) treatment at 50 DAS, but at 60 and 70 DAS, the lowest disease severity index was found in T_5 (Dithane M-45) treatment. Among the chemical treatments, the lowest disease severity index was recorded in T_5 (Dithane M-45) treatment at all observations and the highest disease was found in T_3 (Autostin) treatment at 50 DAS while the highest in T_4 (Rovral 50 WP) treatment at 60 and 70 DAS, respectively. In case of botanical treatments, the lowest disease severity index was recorded in T_9 (Lantana leaf extract) treatment at all observations and the highest disease severity index was recorded in T_8 (Allamanda leaf extract) treatment at 50 DAS but at 60 and 70 DAS, the disease severity index was found higher in T_7 (Neem leaf extract) treatment and T_{10} (Datura leaf extract) treatment. Among the combined treatments, the lowest disease severity index was found in T_{13} (Lantana+ Dithane M-45) treatment at all observations. The highest disease severity index was recorded in T_{12} (Datura + Amister top) treatment at 50 DAS, but at 60 and 70 DAS, it was found higher in T_{11} (Neem+ Rovral) treatment. Results are agreed with some reported results regarding the disease severity index (%)/ leaf area diseased (% LAD) of mustard. Alam (2007) reported that the effect of different treatments on leaf area diseased (% LAD) was found to be significant at different days after sowing (DAS) in response to the application of different chemicals

fungicides and botanicals. Percent leaf area diseases (LAD) of mustard (SAU Sarisha-1) increased gradually with the advancement of crop age. At 85 DAS, the highest percent leaf area diseased was found at control treatment and the lowest leaf area diseased was recorded from the Rovral treated plots followed by Allamanda, Dithane M-45, Bavistin, Ridomil MZ-72 and Garlic treated plots.

Effect of selected treatments on pod infection (%)

The effect of selected treatments on percent pod infection was recorded. From the study, it was recorded that among set treatments, the lowest pod infection was found in T_5 (Dithane M-45) treatment where the highest was recorded in T_1 control treatment at all observations. Among the chemical treatments, Dithane M-45 (T_5) gave the best result, which was statistically similar with T_6 (Amistar top) and T_4 (Rovral) treatments. Among the botanical treatments, the lowest pod infection was found in T_9 (Lantana leaf extract) treatment at 55 and 75 DAS whereas it was recorded the lowest in T_8 (Allamanda leaf extract) treatment at 65 DAS. The highest pod infection found in T_8 (Allamanda leaf extract) treatment at 55 and 75 DAS whereas it was found higher in T_9 (Lantana leaf extract) treatment at 65 DAS. Among the combined treatments, the highest pod infection was recorded in T_{11} (Neem + Rovral) treatment at all observation. From these results it was also revealed that the T_2 treatment (*Trichoderma harzianum* suspension) was gave the moderate result in terms of pod infection (%) and showed the best performance for controlling grey blight disease of mustard. Almost similar observation was reported by Godika *et al.* (2001), when they conducted a field experiment to evaluate the efficacy of different chemical fungicides viz. Mancozeb, Ridomil MZ (mancozeb+metalaxyl), Captan, Rovral (iprodione), Bayletan [triadimefon] and Copper oxychloride, against Alternaria blight (*Alternaria brassicae*) and observed that all the fungicides significantly controlled the disease, but their efficacy varied.

Effect of selected treatments on yield and yield contributing parameters in Mustard against grey blight

The effect on grain yield of mustard under set treatments were summarized and significant variation was found in fresh and dry condition. In case of fresh weight, among the treatments. The highest grain yield was found in T_{13} (Lantana + Dithane M-45) treatment followed by T_5 (Dithane M-45) and T_9 (Lantana leaf extract) treatments whereas the lowest yield was recorded from in control treatment. In dry condition, maximum grain yield was obtained in combined T_{13} (Lantana + Dithane M -45) treatment, while minimum grain yield was recorded from control treatment. Results are agreed with report by Singh and Singh (2006), they reported that the highest, seed yield and 1000-seed weight were found in two consecutive sprays with Mancozeb followed by a third spraying with Ridomil MZ 72. Among the treatments, the highest yield was recorded in T_5 (Dithane M-45) and the lowest yield was found in control treatment. Results from this study are also almost similar with report by Godika *et al.* (2001), they reported that the highest yield of mustard was found in Rovral followed by Mancozeb (Dithane M-45) and Ridomil MZ. Shrestha *et al.* (2005) also reported that Mancozeb (Dithane M-45) and Iprodione (Rovral) had effectively reduced grey blight disease in the sprayed plots and increased seed yield. 1000-seed weight was significantly affected by grey blight disease of mustard under different treatments. Among the treatments, Lantana Leaf extract was gave the best results for 1000-seed weight, which was significantly different from all other treatments. The lowest 1000- seed weight was found in control treatment.

Relationship between grain yield with percent diseases incidence, severity, severity index and pod infection

Arranged treatments that were used in the present study regarding grain yield (ton/ha) and disease incidence (%), disease severity (%), disease severity index (%) and pod infection (%). In case of relationship between grain yield (Mton/ha) and disease incidence (%), it was revealed that grain yield was decreased with increased of disease incidence. Results of relationship study are found to be

similar with some previous reports. In a field trial, Howlider *et al.* (1991) used 5 fungicides (Dithane M-45, Thiovit, Delan, Topsin M and Cupravit) at 3 doses in controlling Alternaria/Grey blight of mustard. Five sprays were applied with first spray at 40 days' growth stage maintaining an interval of 8 days. Dithane M-45 was proved the best with reduction of 73 % in leaf spot severity and siliqua spotting corresponding to obtain an increase of 30% seed yield. Chattopadhyay and Bagchi (1994) also reported that the severity of leaf blight of mustard, caused by *Alternaria brassicae*, was negatively correlated with seed yield.

CHAPTER - V

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

In the present study, in total six (6) experiments were conducted sequentially. The survey study was conducted in the selected eight districts; Jashore, Khulna, Satkhira, Dhaka, Manikganj, Tangail, Sirajganj and Dinajpur in Bangladesh. For conducting this study, three (3) upazila were selected from each of the district. Four diseases namely, grey blight, white rust, powdery mildew and sclerotinia stem rot of mustard were found in all selected survey areas. Of these, grey blight disease of mustard was found commonly with higher disease intensity. It was also observed that most of the farmers were cultivated high yielding mustard varieties like BARI Sarisha-14 which vulnerable to grey blight disease.

Samples were collected from different survey areas and causal agent isolated at Molecular Biology and Plant Virology Laboratory, under Department of Plant Pathology, SAU. The causal agent was identified as *Alternaria brassicae*, *Alternaria brassicicola and Alternaria alternata*. The radial mycelial growth was recorded at 3, 7 and 14 DAI. After 14 days of inoculation, the highest radial mycelial growth of different isolates of *Alternaria* species was recorded in MAN (43 mm) isolate followed by SIR (40.80 mm) isolate whereas the lowest radial mycelial growth was measured in SAT (18.13 mm) isolate. Different variations were also showed in colony characters of different isolates of *Alternaria* spp. regarding surface color, shape, texture, zonation and subsurface color. It was observed that surface and subsurface color was black, white and greyish and black color. The texture was cottony and colony shape was circular in case of all isolates. In case of zonation, no zonation was found on surface of the culture but zonation was found in case of subsurface. No of conidia varied from the range of 1.33 to 5.66. The highest number (5.67) of conidia was recorded in TAN isolate whereas the lowest number (1.33) of conidia was found in KHU isolate. Variation was found in transverse septation which was varied from 1.00 to 2.33.

The pathogenicity was determined based on parameter related to grey blight disease development after inoculation. From the pathogenicity test, it was found that all the *Alternaria* isolates from mustard were found to be pathogenic in

nature. Among the mustard isolates eight isolates, JAS isolate was found to be highly pathogenic as the spots produced >1cm in diameter.

The experiment- 4 was conducted in the molecular laboratory to observe the molecular variation among the selected *Alternaria* isolates through molecular characterization. Searches for similarity of nucleotide sequences were performed using the BLASTn on the NCBI website (https:// www.ncbi.nlm.nih.gov) for matching with existing nucleotide sequences in the NCBI genbank data base to identify the fungal strain of *Alternaria.* For conduction this experiment, total DNA was extracted from mycelia of the pure culture of *Alternaria*. After DNA extraction, fungal DNA was used as a template in PCR amplification using universal forward primer ITS4: TCC TCC GCT TAT TGA TAT GC and reverse primer ITS5: GGA AGT AAA AGT CGT AAC AAG G which designed to amplify fungal gene fragment of 700 bp. *Alternaria* fungal gene of 700 bp fragment was amplified and analyzed on 1.0% agarose gel along with 1 kb DNA ladder. In electrophoresis analysis, PCR product showed a single amplified fragment of ~ 700 bp. For sequencing, the PCR product was carried out in automated sequencer. The sequencing was conducted with reverse primer ITS5: GGA AGT AAA AGT CGT AAC AAG G. The nucleotide sequences of the *Alternaria* isolates from the present study were showed 99% similarity with the existing nucleotide sequences of GenBank data base. From the phylogenetic tree, it has been observed that *Alternaria* isolates from the present study are closely related to *Alternaria* sp. isolates globaly found that were data based in GenBank. It was also found that from the phylogenetic tree of *Alternaria* sp local isolates for grey blight disease infected mustard on the basis of nucleotide sequence showed close relationship.

In case of *in-vitro* management study, the data recorded on the effect of selected treatments in the radial mycelial growth of *Alternaria* on PDA medium and radial mycelial growth was ranged from 0.00-6.83%, 0.00 - 23.67% and 0.00 - 35.67% at 3, 7 and 14 DAI, respectively. It was found that the treatments varied significantly from each and other. Among the selected treatments, there was no radial mycelial growth of Alternaria species was recorded in T_4 (Rovral 50 WP),

T_6 (Dithane M-45) and T_{11} (Neem leaf extract+ Rovral) at 3, 7, and 14 DAI and the highest radial mycelial growth of *Alternaria* spp. was found in T_1 (control) at all observations. Among the chemical treatments, the lowest radial mycelial growth of *Alternaria* spp. was observed in T_4 (Rovral 50 WP) and T_6 (Dithane M-45) at all observations. The highest radial mycelial growth of *Alternaria* spp. was recorded in T_5 (Amistar Top 325 EC) at all observations. Among the botanical treatments, the lowest radial mycelial growth of *Alternaria* spp. was recorded in T_7 (Neem leaf extract, 1.90mm, 8.50 mm and 10.50 mm) at all observations and the highest radial mycelial growth of *Alternaria* spp. was found in T_8 (Allamanda leaf extract, 5.83mm, 15.67 mm and 27.67mm at all observation. Among the combined treatments, there was no radial mycelial growth of *Alternaria* spp. was found in T_{11} (Neem leaf extract+ Rovral) and the highest radial mycelial growth of *Alternaria* spp. was recorded in T_{12} (Datura leaf extract + Amistar Top, 5.0 mm, 9.16mm and 22.83 mm) at all observations.

In- vivo experiment was also laid in the Randomized Complete Block Design (RCBD) with three replications. Multiple treatments i.e. 14 treatments were used in the experiment namely T_1 = Control (No spray applying) , T_2 = *Trichoderma harzianum* suspension @0.2%, T_3 = Autostin 50 WDG @0.3%, T_4 = Rovral 50 WP @0.2%, T_5 = Dithane M-45 @ 0.3%, T_6 = Amistar Top 325 SC @0.1%, T_7 = Neem leaf extract (10%), T_8 = Allamanda leaf extract (10%), T_9 = Lantana leaf extract @10%, T_{10} = Datura leaf extract @10%, T_{11} = Neem leaf extract+ Rovral, T_{12} = Datura leaf extract + Amistar Top, T_{13} (Lantana leaf extract + Dithane M- 45), T_4 = Rovral 50 WP for controlling the grey blight disease of Mustard. From the study, it was revealed that application of biological agent, chemical, plant extract and their combind effect significantly influenced all most all of the parameters. The effect of selected treatments on percent disease incidence for grey blight of mustard was varied from 55.91–79.20% at 70 DAS. The lowest disease incidence was observed in T_5 (Dithane M-45) and the highest in T_1 (control) at all observation. From the study, T_2 treatment (*Trichoderma harzianum* suspension) was showed the moderate disease incidence and gave best performance for controlling grey blight disease of mustard. In case of percent disease severity for

grey blight of mustard ranged from 22.09-37.54% at 70 DAS. The highest disease severity was recorded in T_1 (control) at all observations and the lowest was found in T_5 (Dithane M-45) at all observations. The effect of selected treatments on percent disease severity index for grey blight of mustard was ranged from 32.16 -67.38% at 50, 60 and 70 DAS. Among the set treatments, highest disease severity index was found in T_1(Control) at 70 DAS whereas the lowest disease severity index was observed in T_5 (Dithane M-45). Percent of pod infection was ranged from 0.65-2,97%, 1.73-7.25% and 6.72-34.63% at 55, 65 and 75 DAS respectively on the effect of set treatments. From the study, it was recorded that among the treatments, the lowest pod infection was found in T_5 (6.72%, Dithane M-45) where the highest was recorded in T_1 (34.63%, Control) at all observations. Remarkable varaiation was found on the effect on grain yield of mustard under different treatments. The maximum grain yield was recorded in T_5 (Dithane M-45, 1.89 MT/ha) and the minimum grain yield was observed in T_1 (control, 1.34 MT/ha).

Considering the findings of the present study, the following conclusions were drawn:

1. Grey blight of Mustard caused by *Alternaria brassicae, Alternaria brassicicola and Alternria alternata* is widely distributed in the growing regions of Bangladesh and the disease is remarkably influenced by environmental factors that favours development of the disease.
2. Variabilities exist among the isolates of *Alternaria brassicae*, *Alternaria brassicicola and Alternria alternata* associated with grey blight of mustard in Bangladesh. The existence of physiological races of the pathogen in Bangladesh might be the reasons of diversified severity of the disease in different growing regions in the country.
3. Results of a pathogenicity test of eight isolates of *Alternaria* found to be pathogenic causing grey blight disease of mustard and the isolates were sharply varied in terms of degree of pathogenicity.
4. *Alternaria* isolates from the present study are closely related to *Alternaria* spp. isolates globally found that were data based in GenBank. It was also found that

from the phylogenetic tree of *Alternaria* spp., local isolates for grey blight disease infected mustard on the basis of nucleotide sequence showed close relationship.

5. Based on the *in-vitro* study to evaluate the selected chemical fungicides and botanicals, Rovral 50 WP, Dithane M-45, Neem leaf extract+ Rovral and Lantana leaf extract + Dithane M-45 were found the most effective against *Alternaria* pathogen.
6. Based on results of the *in-vivo* study to evaluate the selected treatments, it may be concluded that among the botanical treatments, lantana leaf extract gave better results and among the chemical fungicides, Dithane M-45 performed the best. In combined effect of the treatments, Lantana leaf extract + Dithane M-45 also gave the satisfactory results to manage the grey blight disease of mustard. Treatments gave the best result regarding yield and yield attributes. From the findings on different parameters studied, the treatment Lantana leaf extract, Dithane M-45 and their combined treatment can be used in further field trials and then may be recommended for management of grey blight mustard. Above all, more research should be conducted on molecular characterization of *Alernaria* isolates associated with grey blight disease of mustard and its integrated management.

CHAPTER – VI

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APPENDICES

Appendix - I. Questionnaire of the study

Title of the Project

Molecular Characterization of *Alternaria* Isolates Associated with Grey Blight Disease of Mustard and Its Integrated Management

'' Structural Questionnaire''

1. Information from DAE personnel at upazilla level:

Name:……
Designation:……
Address:……

Cell No:

E-mail No:

2.0. Information from growers/farmers:

2.1 Name:…… …

2.2 Gender: Male-1 Female-2

2.3 Age: (Code:1 =20 or less,2=21to30,3=31 to 40,4=41to50, 5=51 or abo

2.4 Educational background:(Code: 1= 0 to4, 2=5 to 7,3=8 to SSC,4=HSC,5= Graduate or above

2.5 Area cultivated: …… Bigha

2.6 Previous crops history of selected area (Mustard cultivated or not?) Yes No

2.7 Communication with others organization: DAE/BADC/ NGO/Bank/Others

3.0. Information about Mustard seed:

3.1. Source of seeds:

a) Home storage ☐

b) BADC ☐

c) Local market: ☐

d) Advance farmer ☐

f) Company seeds ☐

g) NGO's supply ☐

3.2. If source of seed from company, then please be specific ----------

3.3. Information about seed treatments:

a) Hot water treatment ☐

b) Use of botanicals ☐

c) Use of fungicides ☐

d) Other ☐

3.4. Name of the fungicides used for seed treatment:

a) Noin ☐

b) Provex ☐

c) Autostin ☐

d) Rovral ☐

e) Other ☐

4.0. Information about Mustard varieties:

4.1 Name of the Mustard varieties used:

a) Tori -7 ☐

b)BARI sarisha- 9 ☐

c) BARI sarisha- 11 ☐

d) BARI sarisha- 14 ☐

e) BARI sarisha- 15

f) BARI sarisha- 16

g) BARI sarisha -17

h) BINA sarisha -3

i) BINA sarisha -4

j) BINA sarisha –5

k) BINA sarisha –6

l) BINA sarisha –7

m) BINA sarisha –8

n) BINA sarisha –9

o) Rye sarisha- 5

p) Others

4.2. Mostly cultivated varieties:

a) Local

b) Improve varieties/ HYV

4.3. Date of sowing:

5.1. Major disease of mustard symptoms that appear at investigated area:

a) Grey Blight

b) White Rust

c) Downy Mildew

d) Powdery Mildew

e) Bacterial Blight

f) *Sclerotinia* Stem Rot

g) Mosaic Virus

5.2. Incidence of Grey Blight on the basis of typical morphological symptoms:

a) Severe / Epidemic form

b) Moderate

c) Low infection

d) No-infection

5.3. Incidence of White Rust

a) Severe / Epidemic form

b) Moderate

c) Low infection

d) No-infection

5.4. Incidence of Powdery Mildew

a) Severe / Epidemic form

b) Moderate

c) Low infection

d) No-infection

5.5. Incidence of Downy Mildew

a) Severe / Epidemic form

b) Moderate

c) Low infection

d) No-infection

5.6. Incidence of Bacterial Blight

a) Severe / Epidemic form

b) Moderate

c) Low infection

d) No-infection

5.7. Incidence of *Sclerotinia* Stem Rot

a) Severe / Epidemic form

b) Moderate

c) Low infection

d) No-infection

5.8. Incidence of Mosaic Virus

a) Severe / Epidemic form

b) Moderate

c) Low infection

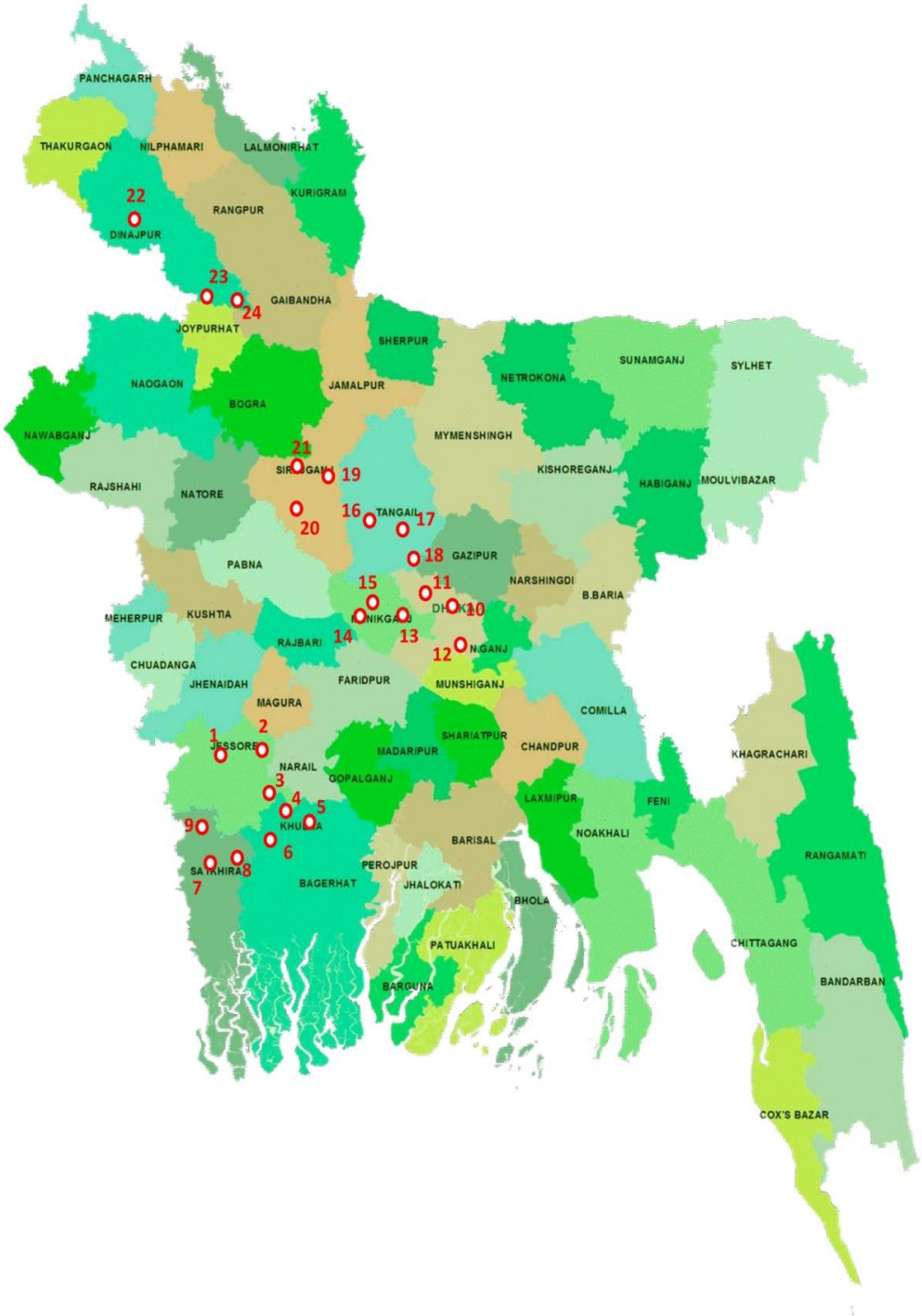
d) No-infection

Signature of Farmer/Grower

Signature of Researcher

Signature of Research Coordinator

Appendix - II. Geographical representation of sampling area

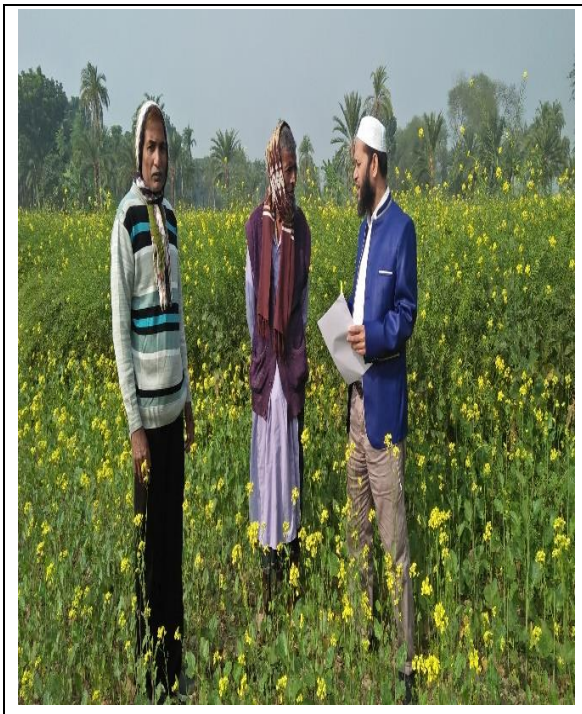


1 - Jashore Sadar, 2-Jhikargacha, 3-Abhynagar, 4- Khulna Phultala, 5-Digholia, 6-Dumuria, 7- Satkhira Sadar, 8-Tala, 9-Kalaroa, 10-Savar, 11-Dhamrai, 12-Keraniganj, 13- Manikganj Sadar, 14-Shivalaya, 15-Ghoir, 16- Tangail Sadar, 17-Basail, 18-Mirzapur, 19- Sirajganj Sadar, 20-Ullapara, 21-Raiganj, 22- Dinajpur Sadar, 23-Hakimpur and 24-Ghoraghat

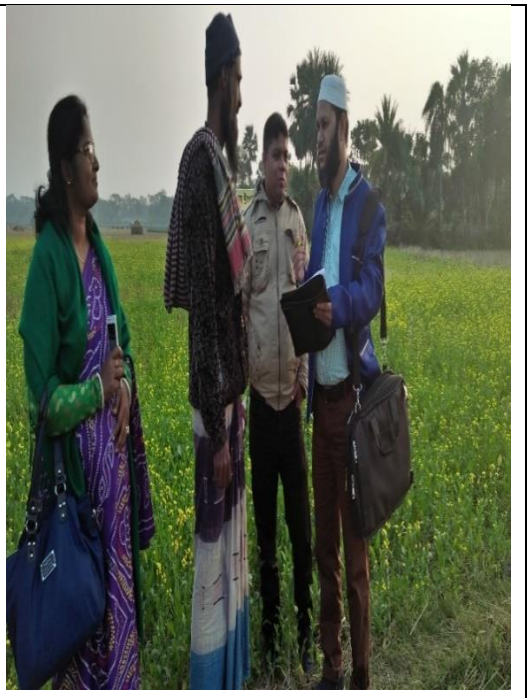
Appendix - III. Non – random sampling process

Appendix - IV. Random data sampling from mustard field to collect fungus infected sample for further analysis

Appendix - V. Data collected from different selected districts



A - Jashore



B - Khulna

C - Satkhira

D - Dhaka

E - Manikganj

F - Tangail

G - Sirajganj

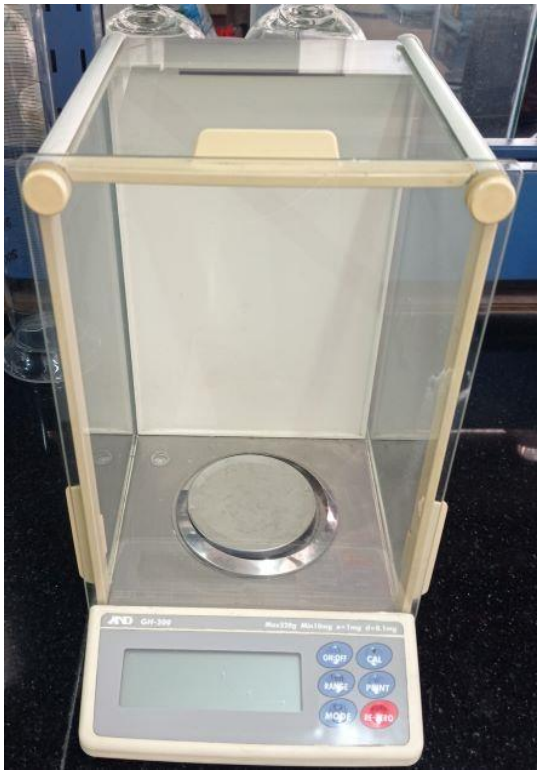
H - Dinajpur

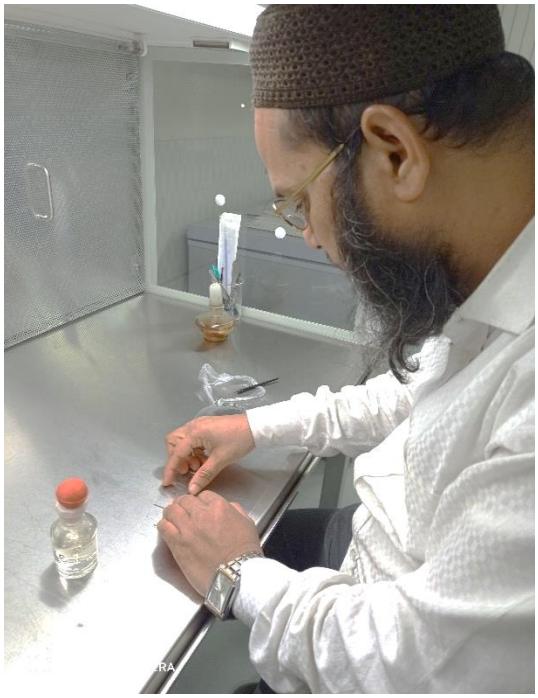
Appendix - VI. Collected diseased sampling in the laboratory

lll

Appendix - VII. Working in the laboratory

Appendix - VIII. Instrument used for the *in –vitro* experiment





Appendix- IX: ANOVA table of the laboratory experiment

01: Radial mycelial growth (mm) at 3 days

Source of variance	Degree of freeedom	Sume of squares	Mean of squares	F value	Probability
Replication	2	0.3733	0.1867	35.65	0.0000
Factor	7	83.7850	11.9693		
Error	14	4.7000	0.3357		
Total	23	88.8583			
Coefficient of Variation: 6.37%					

02: Radial mycelial growth (mm) at 7 days

Source of variance	Degree of freeedom	Sume of squares	Mean of squares	F value	Probability
Replication	2	14.006	7.0029	13.89	0.0000
Factor	7	657.686	93.9552		
Error	14	94.668	6.7620		
Total	23	766.360			
Coefficient of Variation: 12.45%					

03: Radial mycelial growth (mm) at 14 days

Source of variance	Degree of freeedom	Sume of squares	Mean of squares	F value	Probability
Replication	2	3.77	1.885	81.02	0.0000
Factor	7	1251.53	178.790		
Error	14	30.90	2.20		
Total	23	1286.20			
Coefficient of Variation: 4.25%					

04: Number of conidia

Source of variance	Degree of freeedom	Sume of squares	Mean of squares	F value	Probability
Replication	2	1.7500	0.87500	10.36	0.0001
Factor	7	39.2917	5.61310		
Error	14	7.5833	0.54167		
Total	23	48.6250			
Coefficient of Variation: 23.55%					

05. Longitudinal Septation of the conodia

Source of variance	Degree of freeedom	Sume of squares	Mean of squares	F value	Probability
Replication	2	1.7500	0.87500	1.74	0.1787
Factor	7	17.6250	2.51786		
Error	14	20.5.96	2.0250		
Total	23	39.6250			
Coefficient of Variation: 21.38%					

06. Transverse Septation of the conidia

Source of variance	Degree of freeedom	Sume of squares	Mean of squares	F value	Probability
Replication	2	0.58333	0.29167	1.23	0.3496
Factor	7	3.33333	0.47619		
Error	14	5.41667	0.38690		
Total	23	9.33333			
Coefficient of Variation: 37.32%					

07. Conidial size (µm)[1] in length

Source of variance	Degree of freeedom	Sume of squares	Mean of squares	F value	Probability
Replication	2	1.1816	43.6926	3.08	0.0348
Factor	7	355.094	50.7278		
Error	14	230.571	16.4693		
Total	23	588.028			
Coefficient of Variation: 21.46%					

08: Radial mycelial growth (mm) at 3 days after applying selected treatments

Source of variance	Degree of freeedom	Sume of squares	Mean of squares	F value	Probability
Factor	13	216.108	16.6237	72.49	0.0000
Error	28	6.421	0.2293		
Total	41	222.528			
Coefficient of Variation: 16.00%					

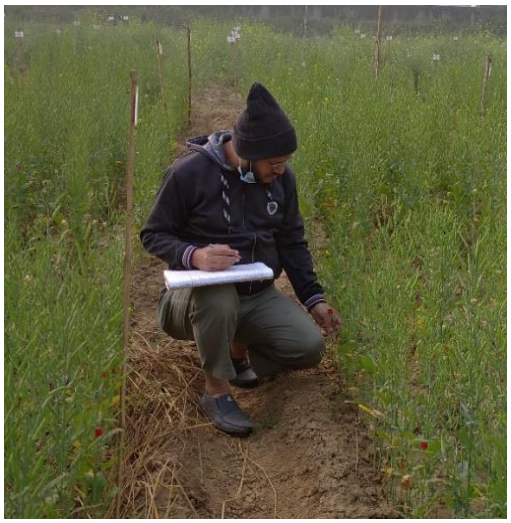
09: Radial mycelial growth (mm) at 7 days after applying selected treatments

Source of variance	Degree of freeedom	Sume of squares	Mean of squares	F value	Probability
Factor	13	2401.35	184.719	3214.11	0.0000
Error	28	1.61	0.057		
Total	41	2402.96			
Coefficient of Variation: 2.44%					

10: Radial mycelial growth (mm) at 14 days after applying selected treatments

Source of variance	Degree of freeedom	Sume of squares	Mean of squares	F value	Probability
Factor	13	4513.29	347.176	120.86	0.0000
Error	28	80.43	2.872		
Total	41	4593.72			
Coefficient of Variation: 10.99%					

Appendix-X: Pictorial view of the field experiment (*in-vivo*)



Appendix-XI: Particulars of the Agro-ecological Zone of the Experimental site

Agro-ecological region: Madhupur Tract (AEZ-28).

Land Type: Medium high land.

General soil type: Non-Calcareous Dark gray floodplain soil

Soil series: Tejgaon

Topography: Up land

Location: SAU Farm, Dhaka.

Field level: Above flood level.

Drainage: Fairly good.

Firmness (consistency): Compact to friable when dry.

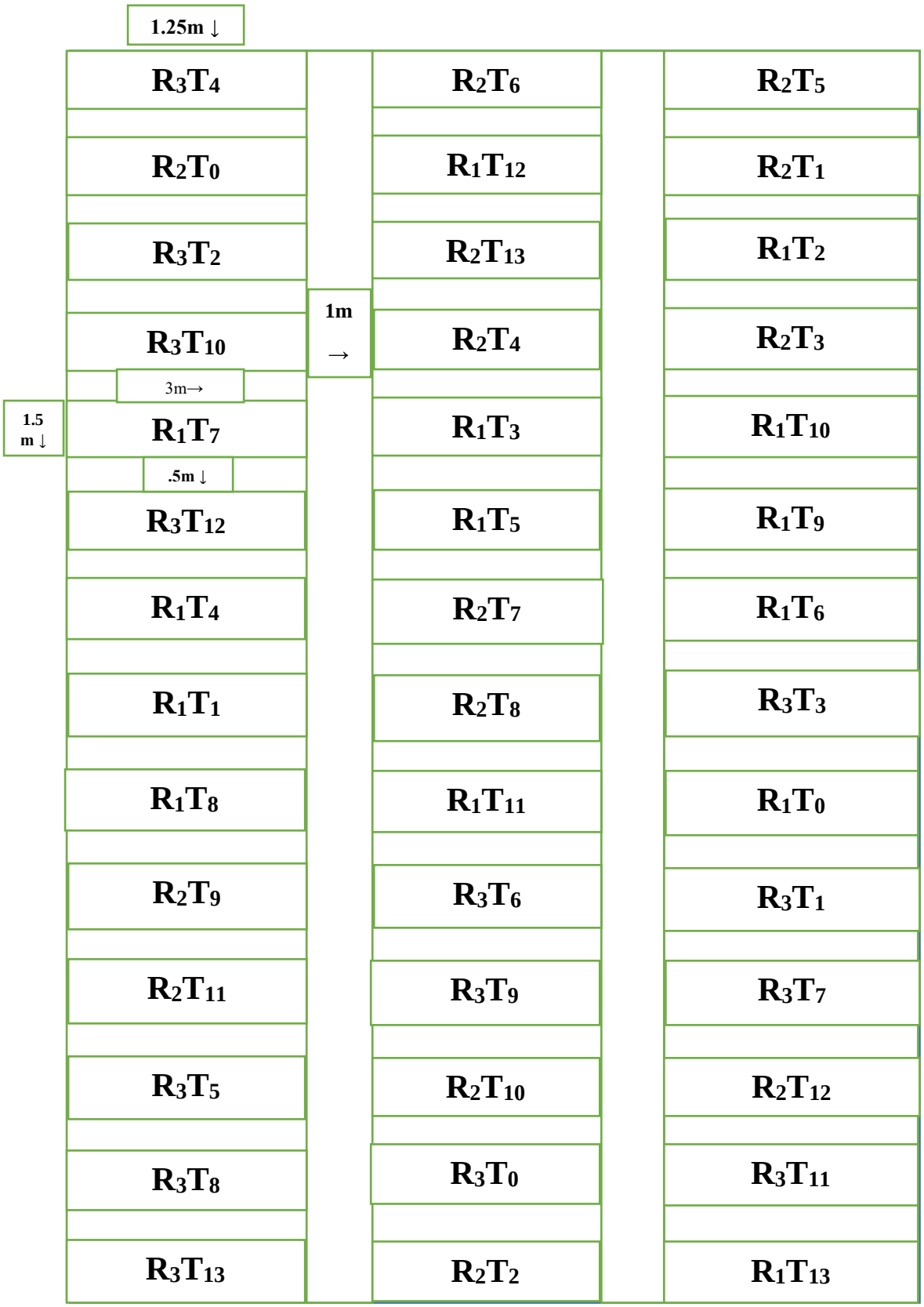
Appendix-XII: Monthly mean weather

Monthly mean of daily maximum, minimum and average temperature, relative humidity, total rainfall and sunshine hours during November/2021 to February/2022

Year	Month	Air temperature (0 c)			Relative humidity (%)	Rain fall (mm)	Sun shine (hr)
		Maximum	Minimum	Mean			
2021	November	31.5	18.2	24.1	68	40	o.7
	December	25.5	15	21	72	0	1.2
2022	January	24	15.8	19.8	70.6	1.56	1.0
	February	29.2	16.4	22.2	52.5	0	1.5

Source: Bangladesh Meteorological Department (Climate division) Agargoan, Sher-e-Bangla Nagar, Dhaka-121

Appendices -XIII. Lay out of the field experiment



Appendix-XIV: ANOVA table of the field experiment

01: Percent of disease incidence at 50 DAS

Source of variance	Degree of freeedom	Sume of squares	Mean of squares	F value	Probability
Replication	2	87.39	43.6926	2.16	0.045
Factor	13	840.02	64.6171		
Error	26	777.23	29.8935		
Total	41	1704.64			
Coefficient of Variation: 10.64%					

02: Percent of disease incidence at 60 DAS

Source of variance	Degree of freeedom	Sume of squares	Mean of squares	F value	Probability
Replication	2	105.18	52.591	1.53	0.1734
Factor	13	1567.46	120.574		
Error	26	2052.96	78.96		
Total	41	3725.60			
Coefficient of Variation: 15.66%					

03: Percent of disease incidence at 70 DAS

Source of variance	Degree of freeedom	Sume of squares	Mean of squares	F value	Probability
Replication	2	49.78	24.892	1.89	0.0816
Factor	13	1649.55	126.889		
Error	26	1748.39	67.246		
Total	41	3447.73			
Coefficient of Variation: 12.41%					

04: Percent of disease severity at 50 DAS

Source of variance	Degree of freeedom	Sume of squares	Mean of squares	F value	Probability
Replication	2	26.61	13.3050	3.03	0.079
Factor	13	65.191	5.0147		
Error	26	43.097	1.6576		
Total	41	134.898			
Coefficient of Variation: 9.55%					

05: Percent of disease severity at 60 DAS

Source of variance	Degree of freeedom	Sume of squares	Mean of squares	F value	Probability
Replication	2	14.8237	7.41185	1.41	0.2215
Factor	13	30.2322	2.32555		
Error	26	42.9678	1.65261		
Total	41	88.0237			
Coefficient of Variation: 6.44%					

06: Percent of disease severity at 70 DAS

Source of variance	Degree of freeedom	Sume of squares	Mean of squares	F value	Probability
Replication	2	250.77	125.383	1.61	0.1466
Factor	13	691.24	53.172		
Error	26	859.71	33.066		
Total	41	1801.71			
Coefficient of Variation: 19.79%					

07: Percent of severity index at 50 DAS

Source of variance	Degree of freeedom	Sume of squares	Mean of squares	F value	Probability
Replication	2	26.39	13.194	3.42	0.0037
Factor	13	1520.69	116.976		
Error	26	890.45	34.248		
Total	41	2437.52			
Coefficient of Variation: 21.94%					

08: Percent of severity index at 60 DAS

Source of variance	Degree of freeedom	Sume of squares	Mean of squares	F value	Probability
Replication	2	28.71	14.353	1.63	0.1390
Factor	13	1368.03	105.233		
Error	26	1674.93	64.42		
Total	41	3071.66			
Coefficient of Variation: 22.66%					

09: Percent of severity index at 70 DAS

Source of variance	Degree of freeedom	Sume of squares	Mean of squares	F value	Probability
Replication	2	91.81	45.903	6.07	0.0000
Factor	13	2542.39	195.569		
Error	26	837.68	32.218		
Total	41	3471.88			
Coefficient of Variation: 12.65%					

10: Percent of pod infection at 55 DAS

Source of variance	Degree of freeedom	Sume of squares	Mean of squares	F value	Probability
Replication	2	1.2145	0.60725	1.65	0.1337
Factor	13	12.8237	0.98644		
Error	26	15.5256	0.59714		
Total	41	29.5638			
Coefficient of Variation: 56.25%					

11: Percent of pod infection at 65 DAS

Source of variance	Degree of freeedom	Sume of squares	Mean of squares	F value	Probability
Replication	2	7.719	3.85961	2.75	0.0137
Factor	13	75.780	5.82919		
Error	26	55.159	2.12151		
Total	41	138.658			
Coefficient of Variation: 46.19%					

12: Percent of pod infection at 75 DAS

Source of variance	Degree of freeedom	Sume of squares	Mean of squares	F value	Probability
Replication	2	166.86	83.428	3.65	0.0024
Factor	13	1753.76	134.905		
Error	26	961.60	36.985		
Total	41	2882.22			
Coefficient of Variation: 36.08%					

13: 1000 – Seed weight (gm/plot)

Source of variance	Degree of freeedom	Sume of squares	Mean of squares	F value	Probability
Replication	2	5646.36	2823.18	2.17	0.0446
Factor	13	928.72	71.44		
Error	26	854.51	32.87		
Total	41	7429.59			
Coefficient of Variation: 4.38%					

14: Grain fresh weight (gm /plot)

Source of variance	Degree of freeedom	Sume of squares	Mean of squares	F value	Probability
Replication	2	0.05258	0.02629	1.04	0.4464
Factor	13	2.33203	0.17939		
Error	26	4.48389	0.17246		
Total	41	6.86850			
Coefficient of Variation: 10.70%					

15: Grain dry weight (gm/plot)

Source of variance	Degree of freeedom	Sume of squares	Mean of squares	F value	Probability
Replication	2	0.0465	0.02325	9.74	0.0000
Factor	13	18.1952	1.39963		
Error	26	3.7373	0.14374		
Total	41	21.9790			
Coefficient of Variation: 6.84%					

16: Grain weight (Mton/ha)

Source of variance	Degree of freeedom	Sume of squares	Mean of squares	F value	Probability
Replication	2	0.00325	0.001623	1.32	0.2636
Factor	13	0.09257	0.007121		
Error	26	0.14025	0.005394		
Total	41	0.23607			
Coefficient of Variation: 4.97%					