IDENTIFICATION AND MANAGEMENT OF SEED BORNE FUNGI ASSOCIATED WITH SELECTED BRRI RICE VARIETIES

THESIS SUBMITTED FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY
IN
BOTANY

BY

TANIA SULTANA

LABORATORY OF MYCOLOGY AND PLANT PATHOLOGY

DEPARTMENT OF BOTANY

UNIVERSITY OF DHAKA

DHAKA-1000

January, 2021

Dhaka University Institutional Repository

IDENTIFICATION AND MANAGEMENT OF SEED BORNE FUNGI ASSOCIATED WITH SELECTED BRRI RICE VARIETIES

Thesis submitted for the degree of

DOCTOR OF PHILOSOPHY
IN
BOTANY

TANIA SULTANA
Reg. No. 86
Session: 2015 – 2016

LABORATORY OF MYCOLOGY AND PLANT PATHOLOGY

DEPARTMENT OF BOTANY

UNIVERSITY OF DHAKA

DHAKA-1000

January, 2021

Dhaka University Institutional Repository

CERTIFICATE

This is to certify that the research work embodying the results reported here in this thesis entitled ***"Identification and management of seed borne fungi associated with selected BRRI rice varieties"*** *by* ***TANIA SULTANA*** *has been carried out in the Laboratory of Mycology and Plant Pathology, Department of Botany, University of Dhaka under our supervision and guidance. It is further certified that the work presented here is original and suitable for submission in partial fulfillment for the Degree of Doctor of Philosophy in Botany.*

Co-Supervisor

Dr. Shamim Shamsi
Professor
Department of Botany
University of Dhaka.

Supervisor

Dr. Md. Abul Bashar
Professor
Department of Botany
University of Dhaka.

Dhaka University Institutional Repository

DEDICATION

This piece of work is dedicated to my Father Late Khalilur Rahman (may his soul rest in peace), Mother Sahanara Rahman, Mother-in-law Fazilatun-nesa, Son Roozbeh Taisir and my Reverent Teachers.

Dhaka University Institutional Repository

DECLARATION

I hereby declare that this dissertation is based on entirely my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of another degree or diploma at any other University. From this research work three papers are published in scientific journals.

Date: **Tania Sultana**

Dhaka University Institutional Repository

ACKNOWLEDGEMENTS

First of all, I would like to express the deepest appreciation to almighty Allah whose continuous blessings and kindly given me the wisdom, knowledge and ability to complete this dissertation.

I would like to express my deepest gratitude to my Supervisor **Prof. Dr. Md. Abul Bashar,** Department of Botany, University of Dhaka, for his full guidance, constructive criticism and continuous encouragement during the entire period of the research work as well as preparing the manuscripts.

I express my sincere appreciation and indebtedness to my Co-Supervisor **Prof. Dr. Shamim Shamsi,** Department of Botany, University of Dhaka for her continued overseeing and valuable advice. Enlightening discussion with her was always the drive to my progress.

I am cordially thankful to Dr. M. Shahadat Morshed, Professor, Department of Botany, University of Dhaka for his suggestion and endless inspiration throughout the period of my research work.

I am also very thankful to Sarowar Hosen, Assistant Professor, Department of Botany, University of Dhaka for his inspiration, valuable suggestion and constructive criticism during the study.

I am grateful to Professor Dr. Rakha Hari Sarker, Chairman, Department of Botany, University of Dhaka for providing all laboratory facilities during the tenure of research work.

I would like to express gratitude to all the members of Mycology and Plant Pathology Laboratory, Department of Botany, University of Dhaka for their continuous support and cordial help.

I express heartfelt gratitude to the Ministry of Science and Technology, Government of the People’s Republic of Bangladesh for providing financial assistance to this research work through Bangabandhu Science & Technology Fellowship Trust.

I feel proud to my beloved parents, brother, sisters and my husband for their support, encouragement and appreciation which inspired me to carry out this work.

The Authoress

Dhaka University Institutional Repository

CONTENTS

Dhaka University Institutional Repository

ABSTRACT

A total of twenty BRRI rice varieties i.e., BRRI dhan 56 to BRRI dhan 75 were collected from Bangladesh Rice Research Institute (BRRI) for seed quality analysis, detection and identification of fungi associated with seeds of rice varieties. Dry inspection indicated that the percentage of pure seeds ranged from 92 to 99%, spotted 0.05-0.80%, discolored 0.20-1.50%, inert matter 0.10-1.00% and weed seeds 0.05-0.30%. The highest germination was recorded in BRRI dhan 74 (94%) and the lowest in BRRI dhan 63 (78%). The highest mortality was recorded in BRRI dhan 65 (25.40%) and the lowest in BRRI dhan 74 (9.80%). Root length was highest in BRRI dhan 72 (5.37cm) and lowest in BRRI dhan 58 (2.20cm). Shoot length was highest in BRRI dhan 74 (8.90cm) and lowest in BRRI dhan 65 (4cm). BRRI dhan 74 showed the highest vigor index (1289.6) and lowest in BRRI dhan 65 (598.60) variety. The lowest average seed moisture was recorded in BRRI dhan 67 (9.80%) and highest in BRRI dhan 63 (11.83%).

25 fungal species were isolated from the selected rice varieties following Tissue planting method and Blotter method. The fungi were *Alternaria alternata, A. tenuissima, Aspergillus flavus, A. fumigatus, A. niger, A. ochraceus, A. terreus, Bipolaris multiformis, B. oryzae, B. sorokiniana, Chaetomium globosum, Curvularia lunata, Fusarium equiseti, F. fujikuroi, F. oxysporum, F. proliferatum, Microdochium fisheri, Nigrospora oryzae, Penicillium* sp., *Phanerochaete chrysosporium, Pestalotiopsis oxyanthi, Rhizopus stolonifer, Sarocladium oryzae, Syncephalastrum racemosum* and *Trichoderma viride.* Morphologically identified twenty-five fungi were selected for molecular identification. Out of the 25 fungal isolates, 13 were confirmed up to species level through ITS sequence based molecular analysis. Among the isolated fungi *Bipolaris multiformis, Microdochium fisheri* and *Pestalotiopsis oxyanthi* are the new record for Bangladesh.

In Tissue planting method *B. oryzae, M. fisheri, A. flavus, A. fumigatus* and *Penicillium* sp. were predominant in most of the rice varieties whereas *B. multiformis, P. chrysosporium* and *A. tenuissima* were recorded only in a few varieties of rice seeds. The highest fungal association was noticed in BRRI dhan 65 (75.46%) and lowest in BRRI dhan 73 (35.28%). In Blotter plate method *C. globosum, B. oryzae* and *A. niger* were predominant in most of the rice varieties whereas *A. terreus, M. fisheri* and *A. flavus* were

recorded only in a few varieties of rice seeds. The maximum fungal infection was observed in BRRI dhan 56 (35%) while minimum in BRRI dhan 65 (2.75%).

Correlation coefficient and regression analysis indicated that prevalence of fungi had significant effect on seed germination, pure seed, seedling mortality and moisture content. The present investigation suggested that out of 20 BRRI rice varieties, BRRI dhan 66, BRRI dhan 69 and BRRI dhan 74 showed better performances on the basis of percentage of pure seed, fungal association, seed germination and seedling mortality. Ten species of fungi were isolated from empty glume, flowering glume, embryo and endosperm. Six fungi *viz*., *B. oryzae, C. lunata, F. equiseti, F. fujikuroi, M. fisheri* and *N. oryzae* viz., showed positive results in pathogenicity test. These six fungi showed seed to seedling transmission nature in water agar test tube and earthen pot.

Ten fungicides i.e., Bavistin 50WP, Capvit 50WP, Dithane M-45, Greengel 72WP, Knowin 50 WP, Nativo75 WG, Ridomil Gold 68 WG, Score 250 EC, Thiovit 80 WG and Tilt 250 EC at 100, 200, 300, 400 and 500 ppm concentrations were tested against the six test pathogens following "poisoned food technique". Out of ten fungicides Bavistin showed the complete growth inhibition of *B. oryzae, C. lunata, F. equiseti, M. fisheri* and *N. oryzae* at all the tested concentrations. Tilt also completely inhibited the radial growth of *B. oryzae, C. lunata*, *F. fujikuroi, M. fisheri* and *N. oryzae* at all the concentrations. Knowin, Nativo, and Score were also found as most effective inhibitor of the test pathogens.

Out of ten leaf extracts namely *Adhatoda vasica* L., *Azadirachta indica* A. Juss., *Cassia alata* L., *Citrus limon* L., *Datura metel* L, *Heliotropium indicum* L, *Mangifera indica* L., *Moringa oleifera* Lam, *Psidium guajava* L. and *Vitex negundo* L were evaluated for their efficacy at 5, 10, 15 and 20% concentrations against the above mentioned six test pathogens. *Citrus lemon* completely inhibited the radial growth of *B. oryzae* and *M. fisheri* at all the concentrations. *Azadirachta indica* and *P. guajava* showed the complete growth inhibition of *C. lunata* and *M. fisheri*. *Azadirachta indica, C. alata* and *M. oleifera* showed highest radial growth inhibition of *F. equiseti*, *F. fujikuroi* and *N. oryzae* at 20% concentration. Moreover, *A. vasica, D. metel* and *V. negundo* also showed desired growth inhibition of the test pathogens.

Four antagonistic fungi were isolated from the rice field soil by serial dilution technique and were identified as *Aspergillus flavus* Link, *A. fumigatus* Fresenius, *A. niger* van

Tieghem and *Trichoderma viride* Pers. ex Gray. The soil fungi were evaluated for their antagonistic potentiality against *B. oryzae*, *C. lunata*, *F. equiseti*, *F. fujikuroi*, *M. fisheri* and *N. oryzae.* In dual culture colony interaction, out of four antagonistic fungi, *Trichoderma viride* showed highest growth inhibition of *B. oryzae* (61.67%), *C. lunata* (63.64%), *F. equiseti* (70.58%), *F. fujikuroi* (87.15%), *M. fisheri* (65.35%) and *N. oryzae* (63.64%).

Trichoderma viride showed the highest growth inhibition of *B. oryzae* (65.35%), *C. lunata* (54.37%), *F. equiseti* (63.90%), *F. fujikuroi* (57.12%), *M. fisheri* (62.40%) and *N. oryzae* (82.63%) owing to the effect of volatile metabolites. The maximum inhibition of radial growth of *C. lunata* (88.36%), *F. fujikuroi* (90.81%) and *M. fisheri* (68.40%) was observed owing to non-volatile metabolites of *T. viride* whereas *A. niger* showed the maximum inhibition of radial growth of *B. oryzae* (86.55%), *F. equiseti* (86.80%). Maximum inhibition of radial growth of *N. oryzae* (94.10%) was observed owing to non-volatile metabolites of *A. fumigatus.*

Evaluation of combined effects of fungicides, plant extracts and biocontrol agents were also performed against the six test pathogens. Out of twelve treatments T10 (Tilt + *A. indica* + *T. viride*), T3 (Bavistin + Tilt) and T7 (*T. viride*) showed highest germination percentage and seedling vigor index against *B. oryzae*, *C. lunata* and *F. fujikuroi*. On the other hand T3 (Bavistin + Tilt), T7 (*T. viride*) and T1 (Bavistin) also showed promising germination percentage and seedling vigor index against *F. equiseti*, *M. fisheri* and *N. oryzae*. These three test pathogens were completely controlled by the treatments used in the experiment.

.

Dhaka University Institutional Repository

LIST OF TABLES

Dhaka University Institutional Repository

Dhaka University Institutional Repository

LIST OF FIGURES

Dhaka University Institutional Repository

Dhaka University Institutional Repository

LIST OF PLATES

Dhaka University Institutional Repository

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important food crops mostly grown in tropical and sub-tropical climate. It is the staple food crop for more than half of the global population including Bangladesh. Rice provides 76% of calorie and 66% of total protein requirement of daily food intake (Bhuiyan *et al*. 2002). In Bangladesh, the total cultivable land covers about 75% (Ahmed *et al.* 2013). An area of 165 million hectares of land is cultivated with rice. About 744.4 million tones of rice production is now touching to the world (FAO 2014). In Bangladesh, rice production is increased more than three and a half during 1972 to 2019 (Fig.1). The majority of the rice produced comes from China, India, Indonesia, Bangladesh, Pakistan, Vietnam, Thailand, Myanmar, Philippines and Japan (Rao *et al.* 2010). The average world yield of rice is 4.4 tons/hectares and 2.14 tons /hectares is the average yield of rice in Bangladesh. Hence, the per hectare production of rice in Bangladesh is minimum in comparison to other countries (BBS 2012). In Bangladesh, more than 78 hybrid rice varieties are grown in the field (Bhandari *et al.* 2011).

Rice ranked first position by production during the year 2015-2016 among all the cereals in Bangladesh (BBS 2016). Thus, quality and healthy seed of high yielding rice varieties are very important to achieve this target. About 34.71 million metric tons is the total annual rice production in Bangladesh at the moment (BBS 2016) whereas 497.8 million tons is the world's total production (FAO 2016). Rice is the most important food for over two billion people in Asia and for hundreds of million in Africa and Latin America. In Asia about 90% rice is produced and consumed (Salim *et al.* 2003). The world's annual rice production must be increased from the present 560 to 750 million tons by 2020 to feed the ever-increasing population of these regions (Saranraj *et al.* 2013).

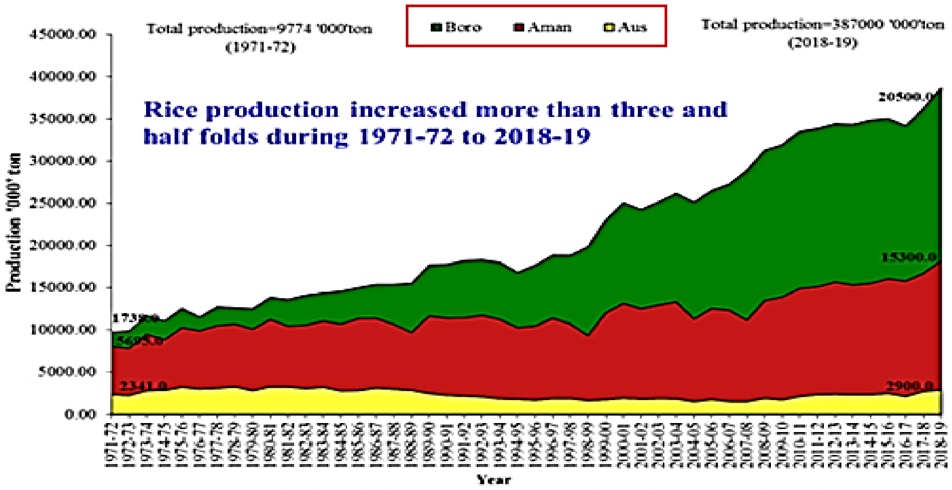


Fig. 1. Rice production during 1971-72 to 2018-19 in Bangladesh (Source: BRRI & BBS 2019).

Bangladesh is an agriculture-based country and its most of the people earn their livelihoods from farming and agriculture-related activities. At present total population of Bangladesh is 159.9 million. Two-third people of Bangladesh are engaged in livelihood activities related to rice. It provides nearly 43% of rural employment (BBS 2016).

For rice production and productivity lack of healthy rice seed is considered as one of the most important constraints in Bangladesh (Nazrul and Fakhrul 2010). Diseases affect rice production seriously throughout the growth period. Both productivity and grain quality is affected by diseases (Santos *et al.* 2009).

Rice suffers from more than 60 different diseases of which fungal disease is one of them (Fakir *et al.* 2002). In Bangladesh, approximately 2.5 million tons of rice, worth more than Tk. 12 thousand millions, is lost annually due to diseases caused by seed borne pathogens (Fakir *et al.* 2003). In Bangladesh, 43 diseases are known to occur on the rice crop of which 27 are seed borne and 14 are of major importance, 22 are caused by fungi (Fakir 2000).

Dhaka University Institutional Repository

Among the 22 seed-borne diseases of rice, Brown spot, Bakanae, Black kernel, Blast, Sheath blight, Sheath rot, Stem rot, Leaf scald and Grain spot are reported as destructive. They cause yield reduction, quality deterioration and germination failure (Mia and Mathur 1983, Shahjahan 1988, Khan *et al*. 1990, Bhutta and Hussain 1998, Gill *et al*. 1999, Wahid *et al.* 2001 and Haque *et al.* 2007).

Mew and Gonzales (2002) estimated that about 14-18% yield reduction was caused by these diseases worldwide. The infected seeds may fail to germinate, transmit disease from seed to seedling and from seedling to growing plants (Fakir *et al*. 2002). Seeds play an important role in the transmission of pathogens and development of plant diseases among the various modes of transmission of plant diseases. Seed-borne pathogens are externally or internally seed-borne, extra or intra embryonal or associated with the seeds (Neergaard 1979).

Most seed borne diseases caused by fungal pathogens are disastrous as they may decrease seed germination, cause seed discoloration, produce toxins that may be injurious to man and domestic animals. Several seed borne fungi associated with rice seeds have been isolated in many countries including Nigeria, Pakistan, Egypt, Bangladesh and Cameroon (Madbouly 2014, Suleiman 2013, Butt 2011, Ora 2011, Nguefack 2007).

For healthy and synchronous seedling good quality and viable seed is required. This is prerequisite for successful crop production, uniform crop growth, development and yield. Genetic and physical purity, high germination percentage and vigor, and free from seed-borne diseases and insects are the three major aspects of seed quality (Seshu and Dadlani 1989).

By the end of 2030, the country will need 50% more rice to meet the demand of growing human population (Khush and Brar 2002). So, the country has to face a challenge of producing an additional amount of food. The nutritional quality of rice appears to equal on surplus that of other cereals. Tropical and sub-tropical climate favors hybrid rice production. These are also favorable for its disease development. Pathogen free seed is the vital input in agriculture.

A total of five lac tons of seeds including the seeds of cereals and other crops per year is required of which only 18% seeds are produced by different seed organizations with care (Hossain and Dey 2011). Outside the supervision of Seed Certification Agency the rest 82% of the seeds remain uncertified with unknown quality (Rashid and Fakir 2000).

To identify the seed borne fungi of rice, conventional methods are used in Bangladesh and the methods rely on microscopic characteristics. The correct identification of a plant pathogenic fungus is important for the development of effective disease control management, quarantine purposes and as a basis for making decisions to protect agricultural crops as well as other natural resources from fungal pathogens (Rossman and Palm-Hernandez 2008).

Basic methods used to detect the organism mostly rely on microscopic, cultural and morphological approaches that require extensive time, labor and classical taxonomy knowledge (Nilsson *et al*. 2011). These approaches, although the cornerstone of fungal diagnostics, can lead to the unreliable results due to the problems in identification (Chalupová *et al.* 2014).

Due to the conventional method's limitations, molecular techniques came in use for the investigation of identification and classification problems. A high variety of molecular methods are increasingly becoming valuable tools in all aspects of fungal diagnostics. Rapid and accurate identification of fungal species based on DNA methodologies have allowed from a wide variety of samples (Mitchell and Zuccaro 2006). For the DNA sequence-based method, certain region of fungal genome is used during DNA sequencing and ITS (internal transcribed spacers) region is most common in this regard. The ITS region is commonly used as a conserved region during DNA sequencing and identified at the species level.

Proper seed treatment measures can substantially improve the quality of seed and significantly increase the yield. Chemicals are the main resources used to prevent and control the disease. However, the residue of the chemicals poses potential health hazard and environmental contamination (Alemu *et al*. 2014). Butt *et al.* (2011) reported the efficacy of different fungicides on the occurrence of fungi in stored rice grains.

Plant extracts can be successfully exploited as safer alternatives to conventional fungicides in modern agriculture (Kuepper 2003). They play an important role against fungi and have the potential to replace the synthetic fungicides (Tripathi and Shukla 2007). Mansur *et al*. (2013) obtained promising result with different plant extracts to reduce seed borne infection and increasing germination of rice.

The survey of literature indicates that no systematic approach has been made to study the various aspects of BRRI released rice varieties so as to find the possible methods of its control to save the crop from heavy losses. Therefore, the present investigation has been designed to identify the fungi associated with 20 BRRI rice varieties and to find out the possible control of important seed borne pathogens.

The aspects which have been studied in the present study is given below:

- Determination of seed health and quality status of the seeds of twenty BRRI rice varieties i.e., BRRI dhan 56 to BRRI dhan 75.
- Isolation, purification and preservation of the mycoflora associated with the selected BRRI rice seeds.
- Morphological and molecular identification of the fungi isolated from the selected BRRI rice seeds.

- Determination of pathogenic potentiality of the fungi associated with the selected BRRI rice seeds.
- Evaluation of seed to seedling transmission of pathogens associated with BRRI rice seeds.
- *In vitro* evaluation of fungitoxicity of extracts of selected higher plant parts against the test pathogens.
- Screening of locally available some fungicides against the selected test pathogens.
- Colony interaction between the test pathogens and some selected soil fungi and screening of antagonists.
- Effects of volatile and non-volatile culture filtrates of some selected soil fungi on the growth the test pathogens.
- Integrated approach to control the selected test pathogens.

REVIEW OF LITERATURE

2.1. Seed health and fungal association with rice seed

An experiment on the seed-borne fungi of rice was conducted in Nigeria by Esuruoso and Joaqui (1975) and the results revealed that *Drechslera oryzae*, *Pyricularia oryzae* and *Trichoconis padwickii* were seed-borne including some other fungi.

Shrestha *et al.* (1977) isolated *Alternaria, Cercospora, Curvularia, Drechslera, Epicoccum, Fusarium, Myrothecium, Nigrospora, Pyricularia, Phoma* and *Trichoconis* from rice seed.

Reddy and Khare (1978) noted four fungi in 42 rice seed samples collected from 4l districts in India. Of which, *Drechslera oryzae* and *Trichoconis padwickii* were associated with 18 samples. In individual sample the highest incidences of these fungi were 32 and 40%, respectively and both were internally as well as externally seed-borne.

Seed samples of l0 rice varieties were analyzed by Mendoza and Molina (1980) following blotter method of seed health test. They reported that *Drechslera oryzae*, *Trichoconis padwickii, Fusarium moniliforme, Curvularia oryzae, C. lunata* and *Aspergillus* spp were associated with the seeds. In Brazil, Caratelli and Saponaro (1983) isolated *Drechslera oryzae*, *Pyricularia oryzae* and *Trichoconis padwickii* from rice seed.

Shahjahan *et al*. (1988) reported thirteen organisms which were both externally and internally seed borne. Out of them *Drechslera oryzae, Fusarium* sp., *Chaetomium* sp., *Sarocladium oryzae* and *Trichoconis padwickii* were predominant. Ahmed *et al.* (1989) detected *Drechslera oryzae, Fusarium moniliforme, Pyricularia oryzae*, *Trichoconis padwickii* and *Curvularia lunata* in rice seed.

Mian and Fakir (1989) studied the occurrence of fungi associated with rough rice grains in the stored seeds of vars. Latishail and Nazirshail. The most predominant fungi in order of prevalence were *Helminthosporium oryzae, Curvularia lunata, Cladosporium cladosporioides, Aspergillus* spp and *Trichoconis padwickii* in Bangladesh, Fakir *et al.* (1990) detected *Dreschslera oryzae, Curvularia lunata, Fusarium* spp, *F. moniliforme, Phoma* sp. and *Trichoconis padwickii* in rice seed. Among these *F. moniliforme* was found to be the most prevalent.

Bokhary (1991) reported seed-borne fungi of rice in Saudi Arabia. Among the detected fungi *Curvularia, Alternaria, Aspergillus, Fusarium, Mucor* and *Penicillium* were the most frequent genera. A lower percentage of germination and higher percentage of fungal infection were observed in discolored grains than the normal grains.

Chai *et al.* (1991) isolated 21 fungal genera from 220 discolored rice samples collected from 17 provinces in China during 1987-88. *Alternaria, Curvularia, Fusarium* and *Penicillium* spp was the most frequent and widely distributed. Mishra and Dharam (1991) isolated 38 fungal species from discolored Padma grains and assumed that *Curvularia lunata* was probably the major cause of grain discoloration.

Islam *et al.* (1994) conducted seed health testing of 83 samples of rice collected from 15 districts of Bangladesh. The study revealed the association of seven fungal pathogens with rice grains. Incidence of these pathogens was found to vary with respect to location and source of collection. In general, infection was higher in farmers seed than those from government farms. Average incidence of *Drechslera oryzae* and *Alternaria padwickii* was much higher in the northern districts of the country compared to the south.

Mia *et al.* (1994) conducted a field experiment during 1988-1990 in four regions of Bangladesh i.e., Barishal, Comilla, Gazipur and Rajshahi over three rice growing seasons i.e., Aus, T. Aman and Boro and found that Aus season rice was most susceptible to grain spot disease. Incidence of grain spot varied with respect to variety and region.

Ten fungal species from seven genera (*Curvularia*, *Dreschslera*, *Trichothecium, Fusarium, Nigrospora*, *Aspergillus* and *Penicillium*) were isolated by Ali and Deka (1996) which were associated with grain discoloration of six rice cultivars.

Bicca *et al.* (1998) reported the occurrence of *Fusarium* spp, *Phoma* sp., *Helminthosporium* sp., *Rhynchosporium* sp., *Alternaria* sp., *Curvularia* sp., *Nigrospora oryzae.*, *Cladosporium* sp., *Aspergillus* spp, *Penicillium* spp and *Epicoccum* sp. in rice seeds.

Seeds of Mala and Pajam rice varieties were collected from 120 farmers (60 for Mala and 60 for Pajam), representing six villages under three unions of Mymensingh Sadar Thana for seed health test through standard blotter method by Fakir (1998). The test revealed that all the seed samples were infected by one or more fungal pathogens. The detected pathogens were *Curvularia lunata, Drechslera oryzae, Fusarium moniliforme, Fusarium* spp, *Phoma* spp and *Trichoconis padwickii*.

Khan *et al.* (1999) isolated *Fusarium moniliforme*, *F. semitectum*, *F. oxysporum, Alternaria alternata*, *A. padwickii*, *Curvularia oryzae*, *C. lunata*, *Drechslera oryzae*, *Pyricularia oryzae* and species of *Nigrospora*, *Phoma*, *Aspergillus* and *Penicillium* from 38 rice seed samples of 16 different varieties/lines. An experiment was conducted by Shamsi (1999) where 794 sheath rot affected rice samples from 317 varieties collected from all over Bangladesh. She established that *Sarocladium oryzae, Curvularia lunata*, *Drechslera oryzae* and *Nigrospora oryzae* causes sheath rot symptoms on various rice varieties.

Fakir *et al.* (2002) determined the quality of farmer's saved rice seeds of Rajshahi, Rangpur and Bogra district of Bangladesh before sowing. Five important pathogenic fungi *viz. Alternaria padwickii, Fusarium moniliforme, Bipolaris oryzae, Pyricularia* *oryzae* and *Sarocladium oryzae* were detected in rice seed samples and prevalence varied with respect to season and sites of seed collection.

Mew and Gonzales (2002) detected more than 100 fungal species on rice seeds. However, the detection frequency varied considerably. About 20 species of fungal pathogens were detected in rice seed.

Tripathi and Dubey (2004) reported that the most destructive seed-borne fungi of rice are *Bipolaris oryzae*, *Pyricularia oryzae*, *Sarocladium oryzae*, *Rhizoctonia solani*, *Sclerotium rolfsii*, *Fusarium* spp, *Curvularia oryzae* and *Nigrospora oryzae*.

Gopalakrishnan *et al*. (2010) recorded eight genera of fungi *viz. Alternaria*, *Aspergillus*, *Bipolaris*, *Chaetomium*, *Curvularia*, *Fusarium*, *Sarocladium* and *Trichoderma* with rice seed comprising twelve species. Among them, the most predominant one was *Bipolaris oryzae* followed by *Alternaria padwickii*.

Shamsi *et al.* (2010) found eight fungal species comprising 4 genera to be associated with three rice varieties (Kalijira, Kataribhog BR 34 and Jira dhan). The major disease causing fungi associated with rice seeds were *Aspergillus niger*, *Aspergillus* sp., *Curvularia* sp., *Cladosporium* sp., *Colletotrichum* sp., *Fusarium* sp., *Pyrenochaeta oryzae* and *Sarocladium oryzae*.

Butt *et al.* (2011) studied seed borne mycoflora of different stored grain of rice varieties. They reported that 27, 19, 17, 16 and 14% mycoflora was associated with the seeds of Basmati kernel, Basmati-385, Basmati- 370, Basmati-198 and KS-282, respectively. Four fungal species namely *Fusarium moniliforme*, *Alternaria* sp., *Helminthosporium* sp. and *Curvularia* sp. were isolated from different the rice varieties.

Habib *et al*. (2012) isolated 10 seed borne fungi from 15 varieties of rice collected from Rice Research Institute of Pakistan. *Curvularia* spp was the most predominant fungus which was followed by *Alternaria alternata*, *Aspergillus niger*, *Fusarium moniliforme*, *Rhizopus* spp, *A. flavus* and *Helminthosporium* spp.

Sharma and Kapoor (2016) isolated twelve genera of seed borne fungi *viz*., *Alternaria*, *Aspergillus*, *Bipolaris*, *Chaetomium*, *Curvularia*, *Epicoccum*, *Fusarium*, *Mucor*, *Penicillium*, *Phoma*, *Rhizopus* and *Rhizoctonia* comprising of sixteen species were found grown in Himachal Pradesh.

Patel and Solanki (2017) reported that seed borne fungi namely *Aspergillus*, *Curvularia*, *Chaetomium* and *Fusarium* was found to be increased during the storage period. Ten seed borne fungi *viz., Aspergillus candidus*, *A. flavus*, *A. nidulans*, *A. niger*, *Aspergillus* sp., *Chaetomium* sp. *Curvularia lunata*, *Curvularia* sp. *F. moniliforme* and *Fusarium* sp. were found with five rice varieties in South Gujarat, India.

2.2. Rice seed quality

The three major aspects of seed quality are genetic and physical purity, germination percentage and vigor and free from seed-borne diseases and insects (Seshu and Dadlani 1988).

Mian and Fakir (1989) studied the relationship between germinability and associated seed borne fungi of rice. They observed a positive correlation between increase in storage fungi and loss in germinability. They also found that the most predominant fungi in order of prevalence were *Helminthosporium oryzae, Curvularia lunata, Cladosporium cladosporioides, Aspergillus* spp and *Trichoconis padwickii*.

A total of 39 fungi belonging to 30 genera were studied by Misra *et al.* (1994) following blotter method of seed quality test. They found that the common species excepting *Pyricularia oryzae* and *Nakatia sigmoideum* were evenly distributed during dry season. During wet season distribution of *Drechslera* sp. and *Microdochium oryzae* was even.

They also observed that the infection of both apparently healthy and discolored seeds was highest with *Alternaria padwickii* followed by *Curvularia* sp.

Khare (1999) showed that varietal purity, germination percentage, moisture content, inert matter, weed seeds, objectionable weed seeds, other crop seeds and seed borne pathogens affected seed quality and seed certification.

An investigation was undertaken by Islam *et al.* (2000) with nine seed samples of rice cv. BR11 collected from farmer's storage and analyzed for *Bipolaris oryzae* incidence using storage blotter method. Incidence of *B. oryzae, Trichoconis padwickii, Curvularia lunata, Aspergillus* spp and *Penicillium* spp ranged from 0.0 to 64%, 16-48%. 12-21%, 0.0-19.5%, and 0.0-4%, respectively.

A detailed investigation to study the effect of different containers and additives on the quality of Boro rice seed was conducted by Fakir *et al*. (2003). Moisture content, germination, normal seedlings, abnormal seedlings, diseased seedlings and dead seeds ranged from 12.87 to 13.30%, 88.33 to 95.83%, 81.51 to 92.16%, 2.00 to 3.83%, 1.67 to 3.83% and 4.17 to 11.83%, respectively. They reported that these variations were owing to storage containers, storage periods and additives used. A total of 16 species of field fungi and l0 species of storage fungi was detected.

Rashid *et al.* (2007) worked with three T. aman rice cultivars collected from 2l selected farmers from Bangladesh and recorded 17 fungal species under 15 genera and one unidentified mycelium. Storage was found to be the most important factor that significantly affected the population of associated fungi.

Bodalka and Awadhiya (2009) reported that variety Kranti showed maximum discolored seeds (32.95%) followed by IR-36 (30.26%), Swarna (29.39%) and Mahamaya (29.46%). The least discoloration was observed in IR-64 variety (23.10%).

Mansur *et al.* (2013) isolated nine fungal species from the seeds of three varieties of rice namely BR6, Pajam and Joya from Parshuram upazila of Feni district. Apparently healthy seeds (61.50-78%), spotted seeds (6.15-12.90%), discolored seeds (4.80-14.25%), deformed seeds (2.00-7.25%), varietal mixtures (2.20-9.80%) and chaffy grains (0.95-6.50%) were found among the three rice varieties.

Bhuiyan *et al*. (2013) detected seven seed borne fungi from 40 rice seed samples collected from Narshingdi Sadar and Shibpur of Narshingdi district in Bangladesh. The seed samples were composed of apparently healthy seed, spotted seed, discolored seed, deformed seed, varietal mixture and chaffy grain.

2.3. Molecular identification of seed borne fungi of rice

Isolation of total genomic DNA from fungi suitable for polymerase chain reaction (PCR) amplification and other molecular applications was described by Amer *et al.* (2011). The main advantages of the method are: (1) does not require the use of liquid nitrogen for preparation of fungal DNA; (2) the mycelium is directly recovered from Petri dish cultures; (3) the quality and quantity of DNA obtained are suitable for molecular assays; (4) the technique is rapid and relatively easy to perform; (5) it can be applied to filamentous fungi from soil as well as from a fungi from other environmental sources and (6) it does not require the use of expensive and specialized equipment or hazardous reagents.

Sohaib *et al.* (2015) conducted an experiment to analyze occurrence of fungal species with contaminated rice grains in local markets. On the basis of phenotypic characters eight strains were isolated and further subjected to molecular analysis. The ITS regions by using ITS1 and ITS4 primers were amplified for each isolate. Phylogenetic analysis based on ITS regions revealed that all of these isolates belonged to genus *Aspergillus.* Four of these isolates were identified as *Aspergillus fumigatus* while remaining four strains were identified as *A. flavus*.

Nurulhidayah and Kalaivani (2015) reported that *Magnaporthe oryzae* is a plant-pathogenic fungus which causes rice blast. Five isolates of *Magnaporthe oryzae* were isolated from diseased leaf samples obtained from the field at Kompleks Latihan MADA, Kedah, Malaysia. Identification was done the basis of morphological and microscopic studies of the fungal spores and the lesions on the diseased leaves. Amplification of the internal transcribed spacer (ITS) was carried out with universal primers ITS1 and ITS4. The sequence of each isolate showed at least 99% nucleotide identity with the corresponding sequence in GenBank for *Magnaporthe oryzae*.

Magnaporthe oryzae was isolated from diseased leaf collected from MARDI Seberang Perai, Malaysia by Hasan *et al.* (2016). Molecular identification was performed by sequence analysis from internal transcribed spacer (ITS) region of nuclear ribosomal RNA genes. Phylogenetic affiliation of the isolated samples was analyzed by comparing the ITS sequences with those deposited in the GenBank database. The sequence of the isolate demonstrated at least 99% nucleotide identity with the corresponding sequence in GenBank for *Magnaporthe oryzae*.

Phylogenetic analysis was conducted using Internal Transcribed Spacer (ITS) region, and large subunit (LSU)-rDNA sequence data by Rana *et al.* (2017). The isolate was identified as *Microdochium fisheri* Hern. Restr. & Crous, as an endophyte of stem of greenhouse-grown *Oryza sativa* in UK.

The identification of *Cochliobolus carbonum* was done based on morpho-pathological characteristics and Internal Transcribed Spacer (ITS) region sequencing analysis by EL-Shafey *et al.* (2018). *Cochliobolus carbonum* were recorded as a novel pathogen causing seedling blight disease on rice. The molecular variation using ITS markers reflected a high level of genetic variation between the isolates. The ITS region sequencing of two isolates ECC-7 and ECC-9 was successfully analyzed, and alignment with 19 isolates of *Bipolaris zeicola* worldwide with 97% identify. Phylogenetic analysis of sequences resulted in a well resolved phylogeny. The data suggested that ITS region analysis was a potential tool for phylogenetic reconstruction of the new isolates and as was DNA barcode for identification of the fungal species. It confirmed that this organism was a seed-borne rice pathogen which causes seedling blight disease.

Molecular identification of fungal isolates via PCR utilizing Internal Transcribed Spacer (ITS) region universal primers was carried out Mohamed and Gomaa 2019. ITS region was amplified to confirm the species identification. DNA sequence of PCR products and analysis via BLAST and data of the GenBank showed that four isolates belonged to *F. graminearum*, four isolates belonged to *F. verticilliodies* and two isolates identified as *Bipolaris oryzae.* The phylogenetic tree revealed different levels of molecular variation among the fungal species compared to the international isolates deposited in the GenBank.

Ten pathogenic fungi of deuteromycetes were isolated from seven angiospermic hosts such as pointed gourd, tomato, rice, wheat, maize, chickpea and jute by Shamsi *et al.* (2019). Morphological characterization and molecular analysis were performed for accurate identification of the isolated pathogenic fungi. The sequence results obtained using the ITS1 and ITS4 primers were compared with NCBI GenBank and BOLD database using BLAST analysis.

.

2.4. Pathogenic seed borne rice fungi and seed to seedling transmission

Seed borne infection of rice by *Pyricularia oryzae* and its transmission to seedling were studied quantitatively with naturally infected seeds of three rice cultivars collected from three locations in Nepal by Manandhar *et al.* 1998. Transmission of *P. oryzae* from seeds to seedlings study was less. Seed transmission was found for light covering of the seeds with soil or for moist seedling without covering. Lower infection frequency was observed in seedlings raised in unsterilized soil than in seedlings raised in sterilized soil. Seedlings grown under low temperature (15 to 20ºC) did not develop blast lesions but when the same plants were transferred to high temperature (25 to 30ºC) blast lesions were detected.

An experiment on seed-borne transmission of fungi in discoloured seeds of hybrid rice was investigated by Vachspati *et al.* (2000). They detected many fungi like *Alternaria padwickii* and *Cochliobolus miyabeanus* from seed coat and endosperm.

Basak and Lee (2002) reported that six fungi namely, *Alternaria alternata*, *Aspergillus niger*, *Fusarium moniliforme, Fusarium* sp, *Penicillium* sp. and *Ustilago zeae* were associated with maize seeds. Prevalence of seed-borne fungi also varied. The highest percentages of seed borne fungi were recorded with *F. moniliforme* and the lowest in *Penicillium* sp. Transmission of all seed-borne pathogens from seed to seedlings were also detected by test tube seedling symptom test.

Hajano *et al.* (2011) reported rice blast caused by *Magnaporthe oryzae* is an infectious fungal disease. Seven fungi namely *Magnaporthe oryzae*, *Curvularia lunata, Helminthosporium oryzae*, *Fusarium moniliforme*, *Alternaria alternata*, *Nigrospora oryzae* and *Aspergillus niger* were isolated from seeds and affected leaves of five rice varieties. Pathogenicity test of *M. oryzae* conducted on apparently most susceptible variety IRRI-6 has confirmed the pathogenic nature of the fungus.

2.5. Fungicides to control seed borne rice pathogens

Farid *et al.* (2002) worked with Bavistin, Hinosan, Tilt 250 EC and Dithane M-45 against *Bipolaris oryzae.* Among the four fungicides Dithane was the best. Huynh and Ashok (2005) evaluated the efficacy of Vitavax, Thiram and Mancozeb which showed 80% germination. After six months of storage, they observed chemical residues on seeds.

Sagar and Hegde (2006) reported that Carbendazim was most effective in reducing the infection and recorded maximum seed germination and vigor index. Tricyclazole, Carboxin and Mancozeb were also effective to control seed mycoflora of rice.

Seed treatment with different fungicides exhibited insignificant effect on the occurrence of *F. moniliforme* and *Alternaria* sp. was reported by Butt *et al.* (2011). Antracal completely stopped the growth of *Helminthosporium* sp. and *Curvularia* sp. The other fungicides markedly suppressed the growth of *Helminthosporium* by 50%. Similarly, Topsin and Mencozeb suppressed the growth of *Curvularia* sp. by 50%.

Bhuiyan *et al*. (2013) used Vitavax and Bavistin as seed treating fungicides and Captan to manage seed-borne fungi. Vitavax eliminated all the seed-borne fungi and increased seed germination over the control whereas Bavistin reduced seed-borne infection and increased seed germination.

Selvaraj and Annamalai (2015) tested Carbendazim, Captaf, Mancozeb, Copper oxychloride, Ethanol and Methanol by poisoned food technique against *Sarocladium oryzae*. All the fungicides were proved effective significantly. Hossain *et al.* (2015) reported the efficacy of Bavistin, Sunphanate, Nativo and Carzeb which completely inhibited *Fusarium moniliforme in vitro.*

Ten fungicides namely, Bavistin, Salcox, Dithane, Indofil, Tall, Ridomil, Sulphur, Greengel, Hayvit and Capvit at 100, 200, 300, 400 and 500 ppm was evaluated by

Chowdhury *et al.* (2015) to control five pathogenic fungi in two rice varieties (BRRI 29 and Pajam). Tall was the best out of ten fungicides.

2.6. Plant extracts to control seed-borne rice pathogen

Miah *et al.* (1990) reported that extracts of garlic and neem were effective in controlling *Drechslera oryzae* in rice. Other workers also showed the presence of antifungal properties in garlic. Other plant extracts *viz.,* bishkatali, gagra, vatpata and bitter gourd were also found effective against the same pathogen. Several other workers also reported the antifungal activities of these plant species (Ashrafuzzaman and Khan 1992, Ashrafuzzaman and Hossain 1992).

Rahman *et al.* (1999) found that bishkatali, garlic, ginger and neem extracts were effective against seed-borne pathogens of wheat. However, garlic extract was found superior to other extracts followed by ginger and neem.

Four plant extracts *viz.*, biskatali, onion, garlic and neem were evaluated against *Bipolaris oryzae* by Farid *et al.* (2002). Lowest fungal infestations were recorded with leaf extract of *Paeonia tenuifolia* by Singh *et al.* (2004). Only six fungal species were isolated from seeds treated with leaf extract whereas seventeen fungal species were identified in control seeds.

Efficacy of different extracts of neem leaf on seed-borne fungi was observed by Mondall *et al.* (2009**)**. The growth of both the fungi was inhabited significantly ($p<0.01$) and controlled with the alcoholic and water extract at all the concentrations used. The alcoholic extracts of neem leaf were most effective in comparison to aqueous extract for retarding the growth of *Rhizopus* and *Aspergillus*.

Five different plant extracts *viz.*, garlic, allamanda, neem, chirata and bishkatali with two dilutions (1:1 & 1:2) for rice seed treatment was conducted by Ahmed *et al*. (2013).

Garlic extract was found best to reduce seed-borne infection and increased seed germination. Neem and chirata also increased seed germination.

Bhuiyan *et al.* (2013) reported that garlic extract was found best to reduce seed-borne infection and to increase seed germination. Neem, allamanda and bishkatali extracts also increased seed germination. Onion, kalijira, allamonda, garlic, neem, datura, turmeric, biskatali and shimul extracts were evaluated against seed borne pathogens of hybrid rice. Garlic was found superior to the other extracts followed by datura and allamanda (Faruq *et al*. 2014).

2.7. Bio agents to control seed borne rice fungi

Six isolates which showed promising efficiency as bio-control agents against rice seed borne pathogens were reported by Srinivas and Ramakrishnan (2005). Seed pelleting treatments with *Aspergillus terreus*, *A. flavus*, *Penicillium oxalicum*, *Sarocladium oryzae* and *Trichoderma viride* showed maximum reduction in the seed infection by *Heliminthosporium oryzae*. Control of brown spot disease was noticed in case of *A. terreus* and *A. flavus* treatment.

Paper towel method was used by Sivalingam *et al.* (2006) to study the infection on the seedling. Most of the seedlings raised from the infected seeds showed lesions on shoot, root or on the both. Bioagents treated seeds showed reduction in shoot infection. Combination of *T. harzianum* and *P. fluorescens* was most effective to reduce shoot infection and to enhance seed germination.

A total of 45 *Trichoderma* isolates were used by Khalili *et al.* (2012) against *Bipolaris oryzae.* They belonged to three species such as *Trichoderma harzianum*, *T. virens* and *T. atroviride*. *Trichoderma harzianum* and *T. virens* showed the highest disease control of the test pathogen in dual culture tests. They also showed increasing effects on the rice seedling growth.

Halgekar *et al.* (2014) showed that *Trichoderma viride* inhibited the mycelial growth of *Drechslera oryzae* which was followed by *Bacillus subtilis* and *Pseudomonas fluorescens*. Suppression of growth of *D. oryzae* was observed with *T. viride* and *T. harzianum* in dual culture. Inhibition of *Curvularia lunata* in rice was recorded with *Bacillus subtilis* (97.77%) followed by *T. viride* (96.44%) and *T. harzianum* (93.50%) in dual culture method.

Two species of *Trichoderma* was tested against *Sarocladium oryzae* by Selvaraj and Annamalai (2015). *Trichoderma harzianum* was found as the most effective to control *S. oryzae* which was followed by *T. viride*.

Seed-borne fungi cause enormous losses in rice production was reported by Khair and Subash (2018). Three species of *Trichoderma viz., T. viride, T. harzianum, T. hamatum* and three species of *Aspergillus viz., A. flavus, A. niger* and *A. terreus* were used against five important rice pathogenic fungi i.e., *Bipolaris oryzae, Curvularia lunata, Fusarium moniliformae, Sarocladium oryzae* and *Trichoconis padwickii* for the purpose of biological control. The highest radial growth inhibition was exhibited by *A. niger* and *T. harzianum* against *S. oryzae* (48.07%) and *T. padwickii* (65.40%), respectively. *Aspergillus terreus* produced distinct inhibition zone against all the five rice pathogens in dual culture. The use of culture filtrates of antagonists successfully reduced growth of rice pathogenic fungi. Maximum inhibition was recorded against *T. padwickii* by *A. niger* and *T. harzianum.*

2.8. Integrated control of seed-borne rice pathogen

Significant differences in germination percentage, root length, shoot length, seedling vigor and seedling mortality was observed by Waris *et al.* (2018). Susceptible variety Lalat was inoculated with *Helminthosporium oryzae, Fusarium fujikuroi* and *Curvularia lunata* and treated with different chemicals, biocontrol agents and plant extracts.

Carboxin + thiram resulted 100% germination of seed inoculated with *H. oryzae*. 10% garlic bulb extract increased germination of seed inoculated with *H. oryzae.* Carboxin + thiram (0.2%), carbendazim (0.1%) and garlic bulb (10%) extract recorded higher germination over *F. fujikuroi* inoculated untreated seed. Carboxin + thiram (0.1%) recorded nearly 100% reduction of seedling mortality but carbendazim (0.1%) possessed highest seedling vigor (3045.1). *Trichoderma viride* treated seeds exhibited significantly highest (96.67%) germination in comparison to other treatment in the seeds inoculated with *C. lunata*. Carboxin + thiram (0.1%), garlic bulb extract (10%) and garlic with datura and neem leaf in combination (10%) controlled the three pathogens to 100% *in vitro* condition.

MATERIALS AND METHODS

3.1. Collection of rice seed samples

Seeds of twenty BRRI released rice varieties *viz*., BRRI dhan56 to BRRI dhan75 were collected from Genetic Resources and Seed Division of Bangladesh Rice Research Institute (BRRI) Joydebpur, Gazipur. Samples were collected during the tenure of January 2016 to June 2018.

3.2. Preservation of rice seed samples

The samples were kept in brown paper bag, labeled properly and stored immediately in a dry safe place at room temperature 25±2°C in the Mycology and Plant Pathology Laboratory, Department of Botany, University of Dhaka, Bangladesh until used for further studies.

3.3. Seed quality analysis

3.3.1. Dry seed inspection

Seed quality analysis is an important observation for seed. The seeds were subjected to visual observation and examination under stereoscopic microscope. One hundred gm seeds of each sample were visually inspected to analyze the seed quality. The ratio of pure seeds, abnormal seeds, inert matter and weed seeds of twenty varieties of collected seed samples were determined. Seed contaminants and abnormal seeds were separated and recorded from each sample.

Purity status of rice seed: **a.** Seed contaminants: Inert matter
Weed seeds
b. Abnormal seeds: Discolored seeds
Spotted seeds

Per cent purity of seeds was determined with the following formula:

Per cent purity of seed $= \frac{\text{Weight of pure seed}}{\text{Total weight of seed}} \times 100$

3.3.2. Test of germination

According to the rules of ISTA (2001) 400 rice seeds were taken from the seed sample. 25 seeds were plated in each Petri dish. Whatman No.1 filter papers were soaked in distilled water and placed in 9 cm diameter Petri dish. Then 25 seeds were placed on the top of each filter paper. The lids of the Petri dishes were tightly fitted to avoid evaporation of water. The Petri dishes with rice seeds were placed in an incubator at 25±2°C. Seeds with plumule and radical after incubation were considered as sprouted seeds.

% Seed germination $= \frac{\text{Number of germinated seeds}}{\text{Total number of seeds tested}} \times 100$

3.3.3. Mortality test

Seedling mortality were determined after 10 days of incubation according to the following formula:

% Mortality $= \frac{\text{Number of dead seedling}}{\text{Total number of germinated seeds}} \times 100$

3.3.4. Seedling vigor test

The seedling vigor was determined by using the following formula of Baki and Andersen (1972) as shown below:

Vigor Index (VI) = Mean of root length + Mean of shoot length × Percentage of seed germination

3.3.5. Seed moisture content (%)

Moisture content was calculated according to Christensen and Lopez (1965).

3.4. Isolation, purification and identification of fungi associated with the seeds of BRRI rice varieties

Fungi associated with the seeds of twenty BRRI rice varieties were isolated separately by (a) Tissue planting method (CAB 1968) and (b) Blotter method (ISTA 1996)

For these methods of isolation approximately 400 seeds were used in each sample. The seeds were washed with sterile water and then surface sterilized by dipping in 10% chlorox solution for five minutes. The seeds were then washed with sterile water for three times. Finally, the seeds were placed on the sterilized filter paper inside the Petri plate to remove the excess surface water and kept in room temperature. The surface sterilized seeds thus prepared, were used for isolation purpose.

Tissue planting method: Surface sterilized seeds were placed on sterilized potato dextrose agar (PDA) medium in Petri plate in tissue planting method. A total of 400 seeds were kept in 20 sterilized Petri plates containing PDA medium which contained 15 ml of PDA with 1 drop of lactic acid. Petri plates with seeds were incubated at $25 \pm 2^{o}C$ for 7 days. After incubation, the fungi associated with the inoculum were recorded.

Blotter method: Blotter moist chamber were made by placing three layers of filter papers at the bottom of a 9 cm diameter Petri plate and sufficient water was added to soak the blotting papers. Then covered with the upper part of the Petri plate. The moist chambers were sterilized within an autoclave. A total of 400 seeds were transferred in 20 moist chambers. Surface sterilized seeds were inoculated in Petri plates and each Petri plate contained 20 seeds. The inoculated moist chambers were kept in an incubator at $25 \pm 2^{o}C$ for 7 days. The fungi associated with the seeds were recorded carefully after incubation.

Percentage frequency of occurrence of the fungal isolates was calculated by adopting the following formula of Spurr and Welty (1972) which is given below:

$$\% \text{ frequency} = \frac{\text{Total number of seed from which a fungal isolate was observed}}{\text{Total number of seeds}} \times 100$$

3.5. Isolation of fungi from different parts of rice seeds

The location of fungi in seed was studied by employing component plating technique (Shamsi *et al.* 1995). A total of twenty BRRI rice varieties (BRRI dhan 56 - BRRI dhan 75) was selected for the investigation of fungi associated with different parts of seed. Rice seed has four parts i.e., empty glume, flowering glume, embryo and endosperm. 100 seeds of all these varieties were taken. The seed parts were separated and then surface sterilized with 10% chlorox solution for five minutes. After five minutes, these parts were washed with distilled water for three times and placed in sterilized filter paper for soaking. Then the separated seed parts were placed in Petri plates containing sterilized Potato Dextrose Agar (PDA) medium. Each Petri plate contained 15ml of PDA medium with an additional 1 drop (0.03ml) of lactic acid which was used for checking the bacterial growth. Then the inoculated plates were incubated at 25 ± 2ºC for 7 days. The fungi isolated from seed parts were examined under compound microscope.

3.6. Morphological identification of seed borne fungi

Morphological studies of the fungal isolates were made in order to determine their identity. For microscopic observation, fungal structure like mycelia, spore bearing structures and spores were scrapped off from the surface with a scalpel or blade or picked up with a needle and was mounted in lacto phenol over a clean slide. In case of hyaline structures, a little amount of aniline blue (cotton blue) was added to the mounted fluid. A clean cover slip was placed over the material, excess fluid was removed by soaking with blotting paper and examined under microscope. The microscopic structural view of the fungi was taken by a digital camera. The diagram of microscopic structures was drawn with the aid of Camera Lucida.

Identification of the isolates were determined following standard literature (Thom and Raper 1945, Raper and Thom 1949, Gilman 1967, Booth 1971, Ellis 1971, 1976, Barnett and Hunter 1972, Sutton 1980 and Ellis and Ellis 1997).

3.7. Molecular identification of seed borne fungi

Molecular identification was done following Amer *et al*. (2011) with some modification.

3.7.1. DNA extraction

For DNA extraction, fungi were grown on PDA medium at 25±2 °C for 15 days. One gm fungal mycelia were taken in 1.5 ml Eppendorf tubes with a sterile spatula from the Petri plates. The mycelia were immediately grinded with a homogenizer machine with 400 µl sterile extraction buffers (200mM Tris- HCl, 250mM NaCl, 25mM EDTA, 0.5% SDS) in each Eppendorf. Then 6 µl of 20 mg/ml RNase was added in each Eppendorf. Stir with a vortex, so the mixture was homogenous. The tubes were transferred to 65ºC preheated water bath for 10 minutes. The samples were taken from the water bath and cooled down to room temperature.130 µl of 3 M sodium acetate was added and pH was adjusted to 5.2 in each sample. Samples were vortexed for 30s at maximum speed and incubated at -20º C for 10 minutes. The samples were centrifuged at 13,000 rpm for 15 minutes. The supernatants were transferred to fresh tubes, an equal volume of isopropanol was added to each sample, mixed well and samples were incubated at -20°C for 10 minutes. Samples were then centrifuged at 6000 rpm for 20 minutes. The supernatant was discarded and the pellet was washed with 700 µl of 70% ethanol twice. The DNA pellets were subsequently air dried in an oven at 40°C for at least 10 min. The resultant DNA pellet was then resuspended in 100 µl of 1 x TE (10 mM Tris-HCl, 1 mM EDTA) buffer (pH 8.0). The DNA was allowed to dissolve overnight at 4 ºC.

3.7.2. PCR amplification

Molecular identification of the isolates was performed using the internal transcribed spacer (ITS) region. PCR amplification was conducted using the ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers for the ITS gene. The PCR was carried out in 0.2 ml PCR tube with 25 reaction volume containing 2.00µl Template DNA, 12.5µl Master mix, 1.0µl Forward Primer, 1.0µl Reverse Primer and 8.5µl MilliQ H_2O. Reaction mixture was vortexed and centrifuged in a microcentrifuge. The PCR was initiated by an initial denaturation step at 94ºC for 5 minutes following 35 cycles of 94, 54 and 72ºC each for 30 sec, with a final extension step of 5 min at 72º C and ended with 4º C. The PCR amplified product were run 1% agarose gel. The gel was prepared using 1.0 g agarose powder containing ethidium bromide. Agarose gel electrophoresis was conducted in 1× TAE buffer at 90 Volts and 300 mA for 40 minutes. One molecular weight marker 1kb DNA ladder was electrophoresed alongside the ITS reactions. DNA bands were photographed by a Gel Documentation system (model: DI-HD, UK).

3.7.3. Sequencing analysis

PCR amplified products were purified by alcohol precipitation method (Islam and Mukherjee 2011) through automated sequencer in Centre for Advanced Research in Sciences (CARS), University of Dhaka. To identify the genus and species of the isolates, obtained sequences were compared with already available sequences in the National Center for Biotechnology Information (NCBI) using BLAST tool.

3.8. Pathogenicity test of isolated fungi

Seed inoculation technique described by Chowdhury *et al.* (2015) was used to test the pathogenicity of all isolated fungi. Four hundred seeds were selected from each variety of rice seed and soaked in distilled water in three beakers for 30 minutes separately and then surface sterilized with 10% chlorox for 5 minutes. Spore suspension of the test fungus at 10^4 /ml concentration was prepared in a 500 ml sterilized beaker. Two hundred seeds from each variety were placed in 250 ml beakers. Hundred ml of spore suspension with individual spore were added in seeds of each beaker and left undisturbed for 2 hours. Four hundred of each healthy and inoculated seeds of twenty rice varieties were selected and single seed was placed in sterilized 6inch cotton plugged test tubes containing 10 ml (2% agar) water agar medium. Healthy seeds served as control. Observation was made for 2 weeks at 3 days interval. Germination percentage of seeds, seed mortality, root and shoot length of seedlings were recorded on healthy and inoculated seeds of twenty rice varieties (Appendix I). The pathogens were re-isolated from the inoculated rice seeds to confirm Koch's postulates after 15 days of inoculation.

3.9. Transmission of pathogenic fungi from seed to seedling

Test tube-seedling symptom test: The test tube seedling symptom test developed by Khare *et al.* (1977) was used for this study. Test tube slants were prepared by pouring 6 ml of 2.0% water agar and sterilized in autoclaved for 10 minutes and 15 lbs. pressure at 121ºC. In all, rice varieties four i.e., BRRI dhan 57, 63, 65 and 73 having highest percentage of seed infection were employed in this experiment. The seeds were washed with sterile water and then surface sterilized by dipping in 10% chlorox solution for five minutes. One hundred seeds for each sample were used at the rate of one seed per test tube. The test tubes with the seeds were then incubated in the laboratory desk at room temperature (25 ± 2ºC). The mouth of the test tubes were properly plugged with cotton and the test tubes were placed on the wooden test incubation. The germinating seeds and seedlings in the test tube were examined for the presence of visible symptoms (seed rot, germination failure and infection or death of emerged seedlings) developed by the pathogens present in the seed (Appendix II). The symptoms produced on the germinating seeds and seedlings by the associated pathogen were confirmed by examining the seeds under stereo-binocular microscope.

Pot culture test: According to Hansraj *et al*. (2013) discolored infected seeds were randomly selected and were grown in pots filled with sterilized soil. In case of control surface sterilized healthy seeds were inoculated with pure culture of pathogenic test fungi. Each pot was filled with sterilized soil and inoculated in upper 4 cm layer of the soil with culture grown in rice medium. Surface sterilized 100 rice seeds were sown per pot. The pots were kept in pot house and regularly watered. Appearance of seedling symptoms were recorded after 21 days of germination. For confirmation the organisms were examined under microscope.

3.10. Fungitoxicity of fungicides against the test pathogens

Ten fungicides with different active ingredients, *viz*., Bavistin 50 WP, Capvit 50 WP, Dithane M-45, Greengel 72 WP, Knowin 50WP, Nativo 75 WG, Ridomil Gold MZ 68 WG, Score 250 EC, Thiovit 80 WG and Tilt 250 EC were collected from the Krishi Upokoron Biponi Kendro, Khamarbari, Farmgate, Dhaka (Table 1). For each fungicide, a stock solution having the concentration of 10000 ppm was prepared. The calculated amount of stock solution of a fungicide was supplemented with sterilized PDA medium to get the final concentration of 100, 200, 300, 400 and 500 ppm (Appendix III). The concentrations of fungicides were expressed in terms of its active ingredients. Twenty ml of the supplemented medium of a particular concentration was poured in sterilized Petri plates and allowed to solidify. In control set, required amount of sterilized water instead of fungicide solution was added to PDA medium. Then the solidified medium was inoculated at the center of the Petri plate with a 5 mm mycelial agar disc cut from the margin of actively growing culture of the test pathogen. Three replications were maintained in each case. The inoculated plates were incubated at 25±2˚C. The radial growth of the colonies was measured after 5-7 days of incubation.

Table 1. Particulars of the fungicides used in the study.

Sl. No.	Fungicides	Active ingredient (s)	Manufacturer
1.	Bavistin 50 WP	50% Carbendazim (methyl Benimidazol-2-ylcarbamate)	BASF SE Germany.
2.	Capvit 50 WP	50% Copper oxychloride	Padma Agro Sprayers Company Ltd., Bangladesh.
3.	Dithane M-45	80% Mancozeb	Dow Agro Science, India.
4.	Greengel 72 WP	64% Mancozeb + 8% Metalaxyl	Green Bangla Agrovet Ltd.
5.	Knowin 50 WP	50% Carbendazim (methyl Benimidazol-2-ylcarbamate)	Sundat (S) Pte. Ltd., Singapore.
6.	Nativo 75 WG	50% Tebuconazole + 25% Trifloxystrobin	Bayer Crop Science Ltd.
7.	Ridomil Gold MZ 68 WG	4% Metalaxyl and 64% Mancozeb	Syngenta production France.
8.	Score 250 EC	250 g/L Difenoconazole	Syngenta (BD) Ltd.
9	Thiovit 80 WG	80% Sulpher	Syngenta (BD) Ltd.
10	Tilt 250 EC	25% Propiconazole	Syngenta crop production ag, Switzerland.

The per cent growth inhibition of each test pathogen was calculated by using the formula given below:

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

where, I = Per cent growth inhibition

C = Growth in control

T = Growth in treatment

3.11. *In vitro* effect of plant extracts on the radial growth of the test pathogens

A total of ten plant parts namely *Adhatoda vasica* L., *Azadirachta indica* A. Juss., *Cassia alata* L., *Citrus limon* L., *Datura metel* L., *Heliotropium indicum* L., *Mangifera indica* L., *Moringa oleifera* Lam., *Psidium guajava* L. and *Vitex negundo* were used for this experiment (Table 2). The plant parts were collected from the Botanical Garden of Curzon Hall Campus, University of Dhaka.

(a). Preparation of aqueous plant extracts

I. The desired parts of each plant were thoroughly washed in tap water, air dried and then used for fresh extract preparation.

II. Leaf extracts were prepared by crushing known weight of fresh materials with distilled water in ratio of 1:1 (w/v).

III. The pulverized mass of a plant part was squeezed through four folds of fine cloth and the extracts were centrifuged at 3000 rpm for 20 minutes to remove particulate matter. The supernatants were filtered through Whatman filter paper and the filtrate was collected in 250 ml Erlenmeyer flasks.

IV. In this method, the requisite amount of the filtrate of each plant extract was mixed with sterilized PDA medium to get 5, 10, 15 and 20% concentration.

(b). Inoculation of the test pathogens

I. The medium thus prepared was poured into sterilized Petri plates and was allowed to solidify. Each Petri plate was inoculated centrally with a 5 mm agar disc cut from the margin of actively growing culture of the test pathogens.

II. In control set, a Petri plate containing sterilized PDA medium with the requisite amount of distilled water instead of a plant extract was also inoculated with agar disc of the test pathogen in the same way as described above.

III. Three replications were maintained for both the experiments and control sets. The inoculated Petri plates were incubated at 25±2˚C. The radial growth of the colonies of the test pathogens was measured after 5 days of incubation.

Table 2. Particulars of angiospermic plants used in the present study.

Sl. No.	Plant species	Native name	Family	Used part
1.	*Adhatoda vasica* L.	Bashaok	Acanthaceae	Leaf
2.	*Azadirachta indica* A. Juss.	Neem	Meliaceae	Leaf
3.	*Cassia alata* L.	Dadmardan	Fabaceae	Leaf
4.	*Citrus limon* L.	Lebu	Rutaceae	Leaf
5.	*Datura metel* L.	Dhutura	Solanaceae	Leaf
6.	*Heliotropium indicum* L.	Hatishur	Boraginaceae	Leaf
7.	*Mangifera indica* L.	Aam	Anacardiaceae	Leaf
8.	*Moringa oleifera* Lam.	Sajne	Moringaceae	Leaf
9.	*Psidium guajava* L.	Payera	Myrtaceae	Leaf
10.	*Vitex negundo*	Nishinda	Verbenaceae	Leaf

(c) Calculation

The fungitoxicity of the plant parts extracts in terms of percentage inhibition of mycelial growth was calculated by using the following formula:

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

where, I = Per cent growth inhibition

C = Growth in control

T = Growth in treatment

3.12. Analysis of data

Computer package MSTAT- C was followed to analysis the data on different parameters and means were compared using Duncan's Multiple Range Test (DMRT). Inhibition percentage data of the radial growth of the pathogen in each replication were collected and evaluated by analysis of variance (ANOVA) by using STAR statistical program.

Interrelationships among storage mycoflora, seed germination, purity, seedling mortality and seed moisture of different varieties of rice seeds were done by MS EXCEL.

3.13. Evaluation of antagonistic potential of some soil fungi against the test pathogens

Some soil fungi were isolated from the rice field soil following serial dilution method (Krieg 1981). At first, 1 gm soil was added with 99 ml of distilled water in a conical flask, mixed it very well with a glass rod and marked as mother suspension. Then five test tubes each containing 9 ml sterilized distilled water were taken. 1ml of mother suspension was added into the 1st test tube and made it 10 ml. So, into the first test tube the mother suspension was diluted 10 times. After mixed it well, 1 ml of suspension from the 1st test tube was added into the 2nd test tube and made it 10 ml. So, into the 2nd test tube the mother suspension was diluted 100 times. This process was performed for rest of the test tubes and diluted the mother suspension 10, 100, 1000, 10000 and 100000 times. For each dilution, 1ml of suspension was poured into a sterilized Petri plate and then about 15 ml of sterilized melted PDA medium was added. The plate was moved gently on the Laminar air flow table to get a homogenous distribution of the suspension. Five replications were maintained for each dilution. All the Petri plates were incubated into 25±2^{o}C temperature. After 3 days, individual fungal colonies belonging to the genera *Aspergillus* and *Trichoderma* were sub-cultured on PDA slants randomly, from the culture plates and stored at 4ºC in an incubator for future studies. Identities of soil fungi were determined following the standard literature (Thom and Raper 1945, Raper and Thom 1949, Gilman 1967, Booth 1971, Ellis 1971, 1976, Barnett and Hunter 1972, Sutton 1980, Ellis and Ellis 1997).

Cultures were maintained by sub-culturing after four weeks intervals. From the isolated soil fungi, *Aspergillus flavus, A. fumigatus*, *A. niger* and *Trichoderma viride* were selected randomly to study colony interactions against the test pathogens.

3.14. Colony interactions

Colony interactions between the test pathogens and the selected soil fungi were studied in dual cultures on Potato dextrose agar medium. A Petri plate with 15 ml solidified PDA medium was inoculated with 5 mm mycelial agar disc of a pathogen and a soil fungus, 30 mm apart from each other. Three replications were maintained in each case. The inoculated plates were incubated at 25 ± 2˚C for 5 days. The colony growth of the pathogen was measured at the both sides, that is, towards and opposing each other from their central loci. The radial growth was measured after 5 days. Intermingled and inhibition zone was also measured during the same period.

Assessments of colony interaction between the test pathogens and soil fungi were done in terms of grades which were determined by the model of Skidmore and Dickinson (1976) (Appendix IV). The grades and types are as follows:

Grade 1 (Type A): Mutually intermingling growth where both the fungi grew into one another without any showing sign of interaction.

Grade 3 (Type Bi): Intermingling growth where the test fungus grew over the test pathogen either above or below or both resulting in suppression of growth of the test pathogen.

Grade 2 (Type Bii): Intermingled growth where the test pathogen grew over the test fungus resulting in reduction of growth of the test fungus.

Grade 4 (Type C): Slight inhibition where both the test pathogen and test fungus approached each other until almost in contact, leaving a narrow demarcation line (1-2 mm).

Grade 5 (Type D): Mutual inhibition of the test pathogen and the test fungus and the distance between the two is more than 2 mm.

The width of inhibition zone, intermingled zone and per cent inhibition of radial growth were the parameters used for the assessment of the colony interaction. The growth inhibition of the test fungi was according to the formula of Fokkema (1976).

$$\text{Per cent growth inhibition} = \frac{r1-r2}{r1} \times 100$$

where,

r_1 = denotes the radial growth of the pathogen towards the opposite side

r_2 = denotes the radial growth of the pathogen towards the antagonist.

The same method was followed for all possible combinations amongst the test pathogens and selected soil fungi.

3.15. Effect of volatile substances emanating from the cultures of the soil fungi on the radial growth of the test pathogens

The test pathogens and soil fungi selected for the present study were the same as in the experiment number 3.14. The method described by Dennis and Webster (1971 b) was followed for this study. The soil fungi were grown in 9 cm Petri plates on PDA medium for 5 days. After the inoculation at 25 ± 2˚C, the lid of each Petri plate was replaced by the same size bottom plate, containing 15 ml PDA medium, centrally inoculated with a test pathogen. Then Petri plates were covered by scotch tape so that no volatile substances can be moved from the inside of the Petri plates (Appendix V). Control was also prepared in the same way but the test pathogen at the bottom. Three replications were maintained in each test pathogen. These sets were incubated at 25 ± 2^{o}C. Colony diameters of the test pathogen, in all the sets, were measured and the per cent inhibition in the colony diameter of the test pathogen was calculated after 7th day of incubation.

The formula of per cent growth inhibition is given below:

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

where, I = Per cent growth inhibition

C = Growth in control

T = Growth in treatment

3.16. Effect of culture filtrates (non-volatile metabolites) of the soil fungi on the radial growth of the test pathogens

The test pathogens selected for the present study were the same as in the experiment number 3.15. Three equal size blocks each of individual fungus, cut from the actively growing margins of 5 days old cultures, were inoculated separately into the 250 ml conical flasks each containing 100 ml sterilized Potato dextrose broth medium. After 10 days of incubation at 25±2˚C, the culture of a soil fungus was filtered first through a Whatman filter paper and then centrifuged at 3000 rpm for 20 minutes.

5, 10, 15 and 20 ml culture filtrates of each soil fungus were added in 95, 90, 85 and 80 ml sterilized PDA medium separately. The conical flask containing the PDA medium and culture filtrates was moved in different directions gently on the laminar air flow table to get the homogenous distribution of the supplemented medium. Each Petri plate contained 15 ml of PDA medium and metabolites with an addition of 1 drop (ca 0.03) of lactic acid which was used to check the bacterial growth. Each Petri plate was inoculated centrally with a 5 mm agar disc, cut from the margin of actively growing culture of a test pathogen. In the control, Petri plate containing PDA medium without culture filtrates were inoculated with a test pathogen as described above. In control set, equal amount of sterilized water was added with the PDA medium instead of culture filtrate. Three replications were maintained for each treatment. All the Petri plates were incubated at 25±2˚C. The radial growth of the colonies was measured after 5 days of incubation.

The per cent inhibition of each test pathogen was calculated with the formula given below:

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

where, I = Per cent growth inhibition

C = Growth in control

T = Growth in treatment

3.17. Integrated approach to control the test pathogens

Integrated approach was done according to Waris *et al.* (2018) with some modification. The experiment was conducted in the earthen pot in Botanical Garden, Department of Botany, University of Dhaka. Best performed two fungicides, two leaf extracts and one antagonistic fungus were tested in the pot to control the test pathogens. The seeds were surface sterilized with 10% chlorox solution for five minutes (Appendix VI). Then the seeds were washed 4-5 times in sterile distilled water. Spore suspensions were prepared from 10 days old cultures, using sterile distilled water. Each spore suspension contained 10^7-10^8 cfu /ml spores. Then the seeds were inoculated with equal volume of spore suspension of each test fungus separately and left for 2 hour in sterilized Petri dishes. The inoculated seeds were then treated with the various combination of fungicides, plant extracts and biocontrol agents enlisted in the Table 3. The fungicides were mixed with correct amount of water for their respective dose. Spore suspension of antagonistic fungus was made in sterile distil water, plant extracts were also prepared as mentioned earlier and treated to the pre-inoculated seeds. The seeds were sown in $12^4 \times 8^4$ sized pots containing sterile soil.

Experimental design was CRD and RBD, having three replications. The observations were recorded after 14 days of showing. Final data were recorded after 21 days. The data were recorded as germination percentage of seeds, seedling mortality, root length, shoot length and seedling vigor index.

Table. 3. Components of different treatments with their dose.

Treatments	Components of treatments	Dose
T1	Bavistin	100 ppm
T2	Tilt	100 ppm
T3	Bavistin+Tilt	100 ppm conc
T4	*Azadirachta indica*	10% conc
T5	*Citrus lemon*	10% conc
T6	*A. indica+ C. lemon*	10% conc.
T7	*Trichoderma viride*	10% conc
T8	Bavistin+ *A. indica+ T. viride*	10% conc.
T9	Bavistin+ *C. lemon + T. viride*	10% conc.
T10	Tilt + *A. indica+ T. viride*	10% conc.
T11	Tilt + *C. medica + T. viride*	10% conc.
T12	Inoculated but not treated (positive control)	
T13	Uninoculated healthy seed (negative control)	

RESULTS AND DISCUSSION

Rice is the important staple food of the majority of the world population. The yield of rice is affected by many biotic and abiotic stresses out of which diseases occupy a major role. Most of the diseases of rice are seed-borne in nature. Different varieties harbor various levels of seed-borne mycoflora. The experiments were conducted for the seed quality analysis, detection of different fungal association, their isolation, purification, morphological and molecular identification, pathogenicity test, seed to seedling transmission and suitable management practices. The detail results are described below.

4.1. Seed quality analysis

For seed quality analysis the percentage of pure seeds is presented in Table 4. According to Seed Certification Agency the accepted range of pure seed of rice is 96 to 99% in Bangladesh. Dry inspection indicated that the percentage of pure seeds ranged from 92 to 99%. The highest percentage of pure seed was found in the variety of BRRI dhan 74 (99%) and lowest in BRRI dhan 62 (92%) (Table 4, Fig. 2).

4.1.1. Seed contaminants

Seed contaminants and its frequency of occurrence in twenty BRRI rice varieties are shown in Table 4. Two types of contaminants i.e., inert matter and weed seed were found. Among the twenty BRRI rice varieties, the highest percentage of inert matter was observed in the variety of BRRI dhan 61 (2.00%) while the lowest in BRRI dhan 68 (0.20%). The highest percentage of weed seeds was found in the variety of BRRI dhan 63 (1.00%) whereas the lowest in BRRI dhan 73 (0.10%). BRRI dhan 70 was free of inert matter and weed seeds. (Table 4).

4.1.2. Abnormal seeds

Abnormal seeds and its frequency of occurrence in twenty BRRI rice varieties are presented in Table 4. Two types of abnormal seeds were observed namely spotted and discolored. The highest percentage of spotted seed was recorded in the variety of BRRI dhan 62 (3.00%) and lowest in BRRI dhan 66 (0.10%). Spotted seed was not observed in BRRI dhan 71, BRRI dhan 72 and BRRI dhan 74. The highest percentage of discolored seeds were found in the variety of BRRI dhan 60 (2.50%) and lowest in BRRI dhan 74 (0.60%). No discolored seed was recorded in BRRI dhan 64 and BRRI dhan 68. (Table 4).

Fakir *et al.* (2002) recorded 91.20 to 98.89% pure seed, 14.43 to 24.44% discolored seed and 33.72 to 37.71% spotted seed in the rice samples collected from Rajshahi, Rangpur and Bogra regions of Bangladesh. Approximately similar results were reported by Naher *et al.* (2016) in three rice varieties i.e., BR 11, BRRI dhan 30 and BRRI dhan 33. The highest percentage (83.35) of pure seed was in BRRI dhan 30. They also reported the lowest percentage of spotted seed (2.75) and discolored seed (2.16) in sample of BRRI dhan 30 and BR11, respectively.

Uddin (2005) studied the farmer seeds of Begum Ganj Upazilla in Noakhali and recorded spotted seed (27.84 to 44.77%), discolored seed (3.93 to 8.94%) and inert matter (0.50 to 0.34%). Islam *et al*. (2007) reported maximum pure seed (99.01%) in seed samples of trained farmers and minimum (96.19%) in untrained farmers.

Table 4. Purity status of rice seeds collected from BRRI, Gazipur.

Rice varieties	Pure seed (% weight)	Abnormal seeds (% weight)		Inert matter (% weight)	Weed seeds (% weight)
		Spotted	Discolored		
BRRI dhan 56	98	0.20	1.30	0.30	0.20
BRRI dhan 57	96	1.00	1.90	0.90	0.20
BRRI dhan 58	98	0.30	1.30	0.40	-
BRRI dhan 59	97	0.50	1.50	1.00	-
BRRI dhan 60	96	1.00	2.50	-	0.50
BRRI dhan 61	95	1.30	1.00	2.00	0.70
BRRI dhan 62	92	3.00	2.30	1.80	0.90
BRRI dhan 63	94	0.90	2.20	1.90	1.00
BRRI dhan 64	97	0.90	-	1.50	0.60
BRRI dhan 65	96	1.20	1.80	1.00	-
BRRI dhan 66	98	0.10	1.00	0.50	0.40
BRRI dhan 67	94	2.50	1.50	1.80	0.20
BRRI dhan 68	98	1.00	-	0.20	0.80
BRRI dhan 69	97	0.70	1.00	1.00	0.30
BRRI dhan 70	98	1.20	0.80	-	-
BRRI dhan 71	98	-	1.70	-	0.30
BRRI dhan 72	96	-	2.00	1.50	0.50
BRRI dhan 73	95	2.00	1.90	1.00	0.10
BRRI dhan 74	99	-	0.60	0.40	-
BRRI dhan 75	98	0.80	0.70	0.30	0.20

-represents absence of respective parameters

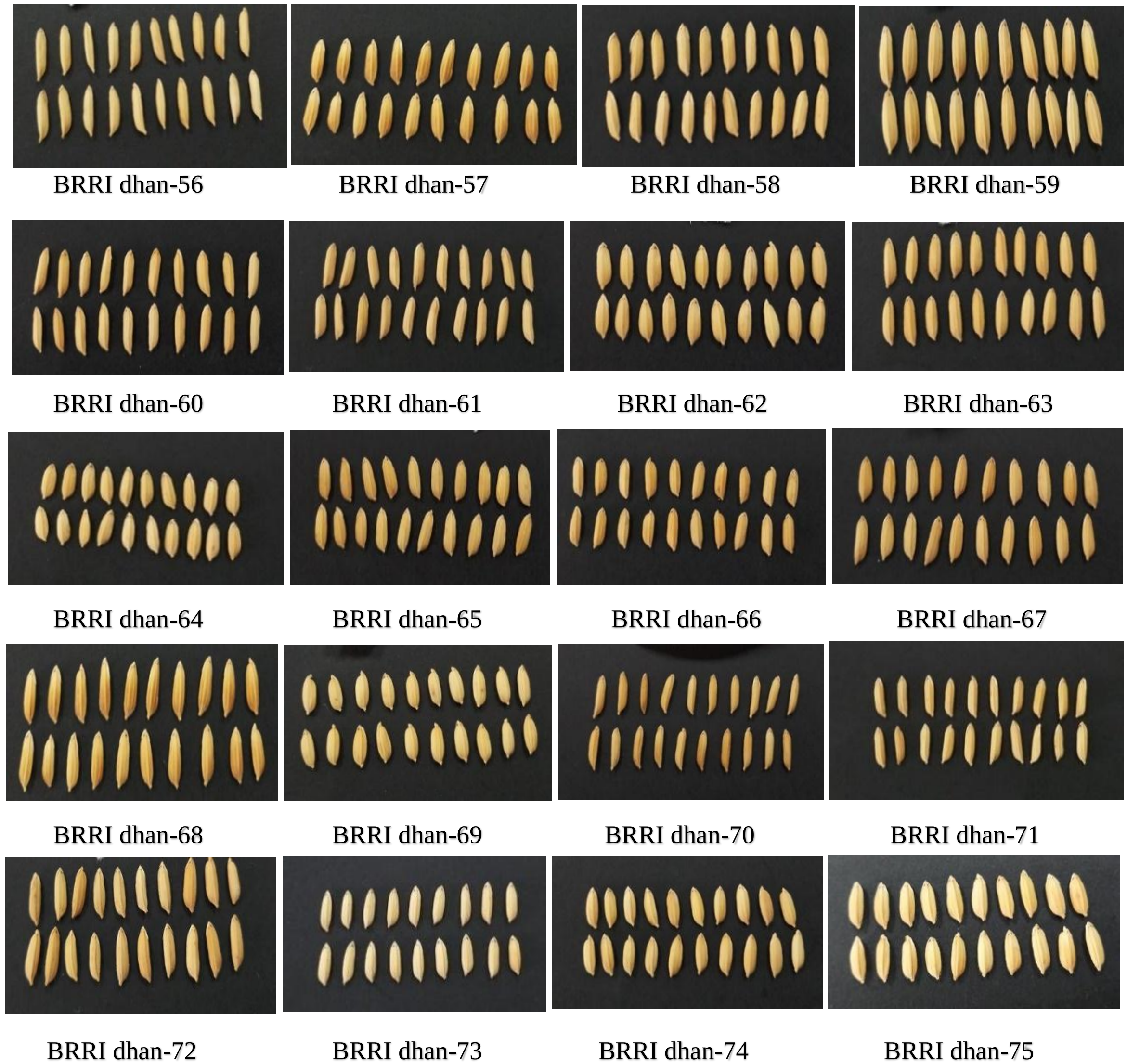


Fig. 2. Different varieties of BRRI rice seeds collected from BRRI, Joydebpur.

4.1.3. Determination of germination

The present study revealed that BRRI rice varieties showed different percentage of germination at different periods of time. The germination percentage of seed ranged from 78 to 94%. Among the rice varieties, the germination percentage was highest (94%) in BRRI dhan 74 and lowest (78%) in BRRI dhan 63 (Table 5, Plate 1). This is probably due to the fungal infection or high moisture content or poor storage facilities and handling. The prevalence of seed-borne infection is also responsible for lower germination (Fakir

1998, Islam *et al.* 2003). The standard germination percentage is between 92-98% (Anonymous 1990).

4.1.4. Mortality percentage of seedling of rice varieties

Mortality percentage of seedling ranged between 9.8% to 25.4%. The highest mortality percentage value of rice seedling was recorded in BRRI dhan 65 (25.40%) and lowest in BRRI dhan 74 (9.80%) (Table 5).

4.1.5. Root and shoot length of rice seedlings in different varieties

The length of root was highest in BRRI dhan 72 (5.37 cm) and lowest in BRRI dhan 58 (2.20 cm), whereas shoot length was highest in BRRI dhan 74 (8.90 cm) and lowest in BRRI dhan 65 (4.00 cm) (Table 5).

4.1.6. Vigor index value

The present study revealed that, BRRI dhan 74 showed the highest (1289.6) vigor index and lowest (598.60) in BRRI dhan 65 (Table 5).

4.1.7. Percentage of seed moisture content of rice varieties

Seed moisture of the BRRI rice varieties were measured at three different time intervals i.e., 2, 6 and 10 months from the initial seed storage time at room temperature (28 ± 2ºC) within conical flask. The lowest average seed moisture (9.80%) was recorded in BRRI dhan 67 and the highest (11.83%) in BRRI dhan 63 (Table 5).

Table 5. Per cent germination, seedling mortality, seedling growth, vigor index and moisture of rice seeds after seven days of incubation.

Rice variety	Germination (%)	Mortality (%)	Seedling growth		Vigor index	Average moisture (%)
			Root length (cm)	Shoot length (cm)		
BRRI dhan 56	85	12.55	4.26	5.50	829.6	11.06
BRRI dhan 57	80	21.80	4.33	5.37	776	11.56
BRRI dhan 58	90	15.10	2.20	5.40	684	11.00
BRRI dhan 59	86	10.99	4.92	7.58	1075	10.30
BRRI dhan 60	80	20.28	3.25	7.30	844	11.24
BRRI dhan 61	81	13.45	3.33	6.66	809.19	11.43
BRRI dhan 62	85	20.50	4.00	7.82	1004.7	10.43
BRRI dhan 63	78	15.00	3.30	6.62	773.76	11.83
BRRI dhan 64	82	12.55	2.33	6.60	732.26	11.20
BRRI dhan 65	82	25.40	3.30	4.00	598.60	11.80
BRRI dhan 66	86	18.82	5.00	5.90	937.4	10.83
BRRI dhan 67	92	14.50	2.49	5.50	771.88	9.80
BRRI dhan 68	90	12.70	2.21	5.00	648.9	10.66
BRRI dhan 69	92	16.66	3.27	5.60	816.04	10.63
BRRI dhan 70	85	14.99	4.39	7.22	986.85	11.20
BRRI dhan 71	80	11.10	5.22	5.80	881.6	10.90
BRRI dhan 72	90	22.25	5.37	8.20	1221.3	11.10
BRRI dhan 73	92	12.20	5.19	6.97	1118.7	10.03
BRRI dhan 74	94	9.80	4.82	8.90	1289.6	11.20
BRRI dhan 75	88	15.35	4.48	8.0	1098.2	11.40

A good number of workers reported the effect of seed cleaning and washing on germination and seedling disease of rice (Hasan *et al.* 2001). The varietal purity, germination percentage, moisture content, inert matter, weed seeds, objectionable weed seeds and seed borne pathogens affect seed quality and seed certification (Khare 1999). Lower germination rate of seeds in the present study might be due to the prevalence of seed-borne infection is in agreement with the findings of Fakir 1998 and Islam *et al*. 2003.

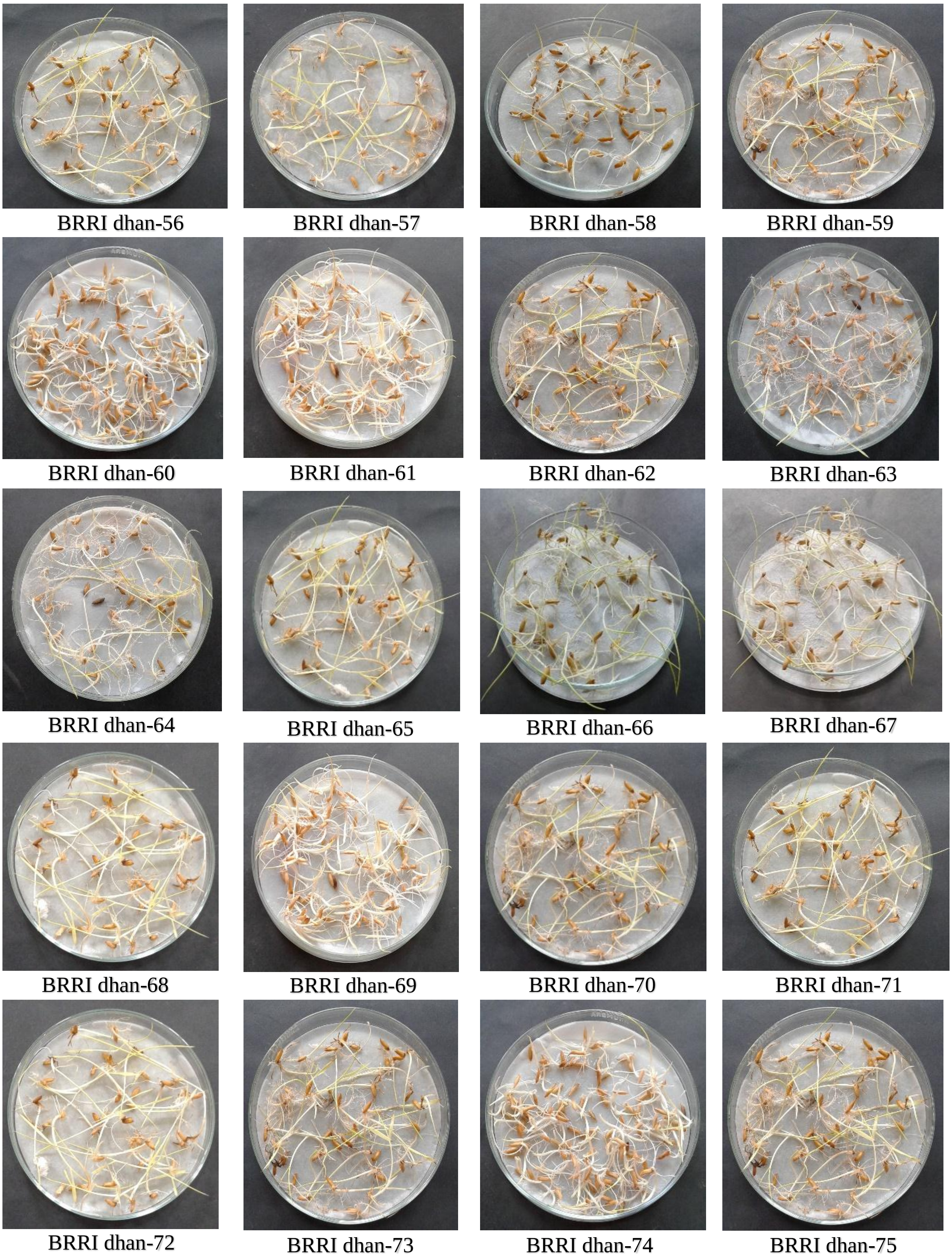


Plate 1. Germination of rice seeds of BRRI dhan 56 to BRRI dhan 75.

4.2. Isolation of seed borne fungi associated with rice seeds

A total of 25 species of fungi were isolated from twenty varieties of rice seeds (Plates 2-4). The fungi were *Alternaria alternata, A. tenuissima, Aspergillus flavus, A. fumigatus, A. niger, A. ochraceus, A. terreus, Bipolaris multiformis, B. oryzae, B. sorokiniana, Chaetomium globosum, Curvularia lunata, Fusarium equiseti, F. fujikuroi, F. oxysporum, F. proliferatum, Microdochium fisheri, Nigrospora oryzae, Penicillium* sp.*, Pestalotiopsis oxyanthi, Phanerochaete chrysosporium, Rhizopus stolonifer, Sarocladium oryzae, Syncephalastrum racemosum* and *Trichoderma viride.* Among the isolated fungi *Bipolaris multiformis, Microdochium fisheri* and *Pestalotiopsis oxyanthi* are the new record for Bangladesh (Siddique *et al.* 2007, Shamsi *et. al* 2018 and Helal *et. al* 2018).

4.2.1. Per cent incidence of fungal association with BRRI rice seeds by Tissue planting method after harvest

Results of the present investigation revealed that the rice seeds are quite frequently infected by fungi. In the present study, a total of 20 fungal species were isolated from the selected BRRI rice varieties following Tissue planting method after harvesting (Table 6). The highest frequency percentage of *Microdochium fisheri* was noticed in BRRI dhan 71, *B. oryzae* and *A. fumigatus* in BRRI dhan 63, *A. ochraceus* and *P. oxyanthi* in BRRI dhan 66, *A. terreus* and *A. niger* in BRRI dhan 59, *Penicillium* sp. on BRRI dhan 73, *N. oryzae, A. flavus, A. tenuissima* and *B. sorokiniana* in BRRI dhan 72, *S. racemosum* in BRRI dhan 69, *R. stolonifer* in BRRI dhan 74, *F. oxysporum* and *C. lunata* in BRRI dhan 58, *F. equiseti* in BRRI dhan 65, *F. proliferatum* in BRRI dhan 71, *A. alternata* in BRRI dhan 57, and *S. oryzae* in BRRI dhan 60 variety. Among these fungi *M. fisheri, B. oryzae, Aspergillus* spp and *Penicillium* sp. were predominant in most of the rice varieties (Table 6).

More than eight species of fungi were found to be associated with BRRI dhan 60, BRRI dhan 61, BRRI dhan 62, BRRI dhan 63, BRRI dhan 71 and BRRI dhan 72 varieties (Table 6). Out of twenty fungal species *M. fisheri* showed highest mean per cent incidence (10.9) whereas *A. tenuissima* showed the lowest per cent incidence (0.63) in BRRI rice seeds. The maximum total fungal association (98.94%) was recorded in BRRI dhan 63 whereas the minimum association (30%) in BRRI dhan 67.

Ora *et al.* (2011) found ten seed borne pathogens *viz., Alternaria tenuissima, Aspergillus flavus, A. niger, Bipolaris oryzae, Curvularia lunata, Fusarium moniliforme, Penicillium* sp., *Nigrospora oryzae, Rhizopus stolonifer* and *Xanthomonas* spp. associated with rice seeds. The highest incidence of *Xanthomonas* spp was noticed on Tinpata whereas *B. oryzae* on Aloron, *F. moniliforme* on ACI-1, *R. stolonifer* on Tia, *A. tenuissima* on Hira-1, *C. lunata* on Aloron, *Penicillium* sp. and *A. flavus* on BRRI hybrid dhan-1 and *A. niger* on Taj-1. *Nigrospora* sp. was recorded only on Hira-1. Of all the pathogens *Xanthomonas* spp, *B. oryzae, Aspergillus* sp., *F. moniliforme* and *R. stolonifer* were predominant.

Table 6. Per cent incidence of fungal association with BRRI rice seeds by Tissue planting method after harvest.

SL. No.	Name of fungi	Per cent association of fungi with BRRI rice seeds																				Mean (%)
		BRRI dhan 56	BRRI dhan 57	BRRI dhan 58	BRRI dhan 59	BRRI dhan 60	BRRI dhan 61	BRRI dhan 62	BRRI dhan 63	BRRI dhan 64	BRRI dhan 65	BRRI dhan 66	BRRI dhan 67	BRRI dhan 68	BRRI dhan 69	BRRI dhan 70	BRRI dhan 71	BRRI dhan 72	BRRI dhan 73	BRRI dhan 74	BRRI dhan 75	
1	***Alternaria alternata***	-	7.7	-	-	-	4	-	-	-	-	-	-	-	-	-	3.34	3.78	-	-	-	0.94
2	***A. tenuissima***	-	-	5.27	-	-	-	-	-	-	-	-	-	-	-	-	-	7.5	-	-	-	0.63
3	***Aspergillus flavus***	6.66	9.61	2.63	-	-	7.85	9.09	3.12	6.45	-	-	3.23	7.4	8.82	-	-	11.43	-	11.33	-	4.38
4	***A. fumigatus***	-	5.77	-	-	7.14	3.93	12.1	20.84	-	-	-	-	5.72	5.88	4.33	-	-	6.98	-	6.25	3.94
5	***A. niger***	-	-	-	13.34	-	-	6.06	-	-	7.5	6.9	-	-	-	6.52	6.67	11.33	-	-	-	2.91
6	***A. ochraceus***	4.45	3.85	2.64	6.67	9.52	7.85	-	12.5	-	5	20.68	-	-	-	-	-	-	9.3	5.72	-	4.4
7	***A. terreus***	-	-	-	20	4.76	-	-	-	-	-	-	-	-	2.94	-	-	-	-	-	-	1.38
8	***Bipolaris oryzae***	17.78	7.69	10.5	16.66	9.52	23.52	24.2	25	22.58	17.5	6.9	12.19	-	-	-	-	-	-	-	-	9.7
9	***B. sorokiniana***	-	-	5.27	-	-	-	-	-	-	7.5	-	-	-	-	-	3.34	7.55	-	-	-	1.18
10	***Curvularia lunata***	-	1.92	7.9	-	-	-	-	2.08	3.23	-	-	-	-	-	6.52	-	-	4.66	-	-	1.31
11	***Fusarium equiseti***	-	-	-	6.67	-	-	-	-	-	10	-	-	-	-	8.7	-	-	-	-	-	1.26
12	***F. oxysporum***	4.45	-	13.1	-	4.76	5.88	-	4.17	-	2.5	-	-	-	5.88	-	-	-	-	8.58	-	2.46
13	***F. proliferatum***	-	1.93	-	-	7.15	-	-	-	-	-	-	4.87	-	-	3.34	10	3.78	-	-	-	1.55
14	***Microdochium fisheri***	-	15.38	-	-	23.8	9.8	18.1	22.91	8.06	-	-	-	22.22	-	8.7	41.67	18.86	5.37	5.72	18.75	10.9
15	***Nigrospora oryzae***	-	-	-	-	-	-	-	3.12	-	-	-	-	-	-	-	10	15.09	4.66	-	-	1.64
16	***Penicillium* sp.**	2.22	7.7	-	-	11.9	9.8	9.09	5.2	-	-	6.9	9.75	7.5	-	13.04	-	-	18.6	-	12.5	5.71
17	***Pestalotiopsis oxyanthi***	-	-	-	-	-	-	-	-	-	5	13.8	-	-	-	10.86	-	-	4.66	-	-	1.71
18	***Rhizopus stolonifer***	6.67	-	-	-	-	-	-	-	-	-	-	-	-	5.88	-	-	5.66	-	11.43	-	1.48
19	***Sarocladium oryzae***	4.45	3.85	-	-	9.52	-	-	-	6.45	5	6.9	-	-	-	-	-	-	-	-	-	1.8
20	***Syncephalastrum racemosum***	-	-	5.27	-	-	-	-	-	-	-	-	-	-	14.7	-	-	-	-	-	-	0.99
Total association (%)		46.6	65.95	52.6	63.34	88.08	72.63	78.7	98.94	46.77	60	62.08	30	42.74	44.1	62.01	75.02	84.98	54.23	42.48	37.5	

-represents no growth of respective fungi

4.2.2. Per cent incidence of fungal association with BRRI rice seeds by Tissue planting method after six months of storage

A total of 16 fungal species were isolated from the selected BRRI rice seeds after six month of storage (Table 7). The highest frequency percentage of *A. flavus* was noticed in BRRI dhan 65; *A. fumigatus* in BRRI dhan 72, *A. niger* in BRRI dhan 71, *A. ochraceus* in BRRI dhan 67, *A. terreus* in BRRI dhan 57, *B. oryzae* in BRRI dhan 61, *F. fujikuroi* and *M. fisheri* in BRRI dhan74, *F. oxysporum* in BRRI dhan 58 and *Penicillium* sp. in BRRI dhan 70. On the other hand *A. alternata,* was only found in BRRI dhan 56, 58 and 73, *C. lunata* in BRRI dhan 59, 74 and 75 varieties. *Rhizopus stolonifer* in BRRI dhan 60, 63, 66 and *S. racemosum* in BRRI dhan 56, 57, 58 rice varieties. *Nigrospora. oryzae* and *Trichoderma viride* was found in BRRI dhan 67, 68 and 61, 62 rice varieties, respectively. The present result revealed that with the increase of storage period the frequency of *A. flavus, A. fumigatus, M. fisheri* and *Penicillium* sp. was also increased.

Among the 16 fungal species, *A. fumigatus* showed highest mean per cent incidence (8.70%) whereas *A. alternata* showed lowest mean per cent incidence (0.65%) on BRRI rice seeds. The maximum fungal association (84.60%) was found in BRRI dhan 57 and minimum (25%) in BRRI dhan 73.

Habib *et al.* (2012) collected fifteen varieties of rice from Rice Research Institute, Kala Shah Kaku and detected seven fungi with seeds of different varieties. *Helminthosporium* spp was the predominant fungus in all the samples tested with a range from 22.23-31.95% and the range of other fungi i.e., *Alternaria alternata, Aspergillus niger, Rhizopus* spp, *Fusarium moniliforme, A. flavus* and *Curvularia* spp were 16.66-20.83%, 16.65-20.85%, 13.83-16.65%, 12.5-18.05%, 9.73-19.43% and 6.95-11.11%, respectively.

Table 7. Per cent incidence of fungal association with BRRI rice seeds by Tissue planting method after six months of storage.

SL. No.	Name of fungi	Per cent association of fungi with BRRI rice seeds																				Mean (%)
		BRRI dhan 56	BRRI dhan 57	BRRI dhan 58	BRRI dhan 59	BRRI dhan 60	BRRI dhan 61	BRRI dhan 62	BRRI dhan 63	BRRI dhan 64	BRRI dhan 65	BRRI dhan 66	BRRI dhan 67	BRRI dhan 68	BRRI dhan 69	BRRI dhan 70	BRRI dhan 71	BRRI dhan 72	BRRI dhan 73	BRRI dhan 74	BRRI dhan 75	
1	***Alternaria alternata***	3.58	-	4.48	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5.00	-	-	0.65
2	***Aspergillus flavus***	10.71	8.58	5.98	15.60	-	-	-	7.41	6.45	44.40	-	-	-	7.14	15.30	4.00	13.34	-	12.19	7.70	7.94
3	***A. fumigatus***	7.15	11.43	11.90	-	13.64	8.34	16.60	-	-	3.70	12.50	-	4.87	14.29	7.70	8.00	20.00	-	14.64	19.24	8.70
4	***A. niger***	-	-	-	6.25	-	4.16	-	-	9.68	-	-	-	14.60	-	11.54	16.00	3.84	-	-	-	3.30
5	***A. ochraceus***	-	7.52	8.96	-	-	-	-	-	-	-	-	13.34	-	-	-	-	-	-	-	11.53	2.06
6	***A. terreus***	14.29	17.15	8.96	-	-	16.60	3.30	7.40	-	-	-	-	-	-	-	-	-	-	-	-	3.38
7	***Bipolaris oryzae***	14.29	14.28	14.90	-	-	25.0	-	14.82	19.36	7.40	18.75	-	-	-	-	-	-	-	7.32	3.84	6.97
8	***Curvularia lunata***	-	-	-	9.09	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9.75	7.70	1.32
9	***Fusarium fujikuroi***	-	-	-	12.50	4.50	-	-	-	-	-	-	-	-	-	-	-	3.34	10.00	14.64	-	2.24
10	***F. oxysporum***	3.58	-	5.98	-	-	-	-	-	-	-	-	-	-	-	-	4.00	3.34	-	-	-	0.84
11	***Microdochium fisheri***	-	-	-	-	-	-	-	7.41	6.45	11.10	-	-	-	-	-	-	13.34	10.00	24.40	23.07	4.78
12	***Nigrospora oryzae***	-	-	-	-	-	-	-	-	-	-	-	13.33	7.42	-	-	-	-	-	-	-	1.03
13	***Penicillium* sp.**	-	17.15	2.98	-	-	-	-	3.70	-	-	-	-	4.87	10.72	23.07	-	6.67	-	-	6.70	3.79
14	***Rhizopus stolonifer***	-	-	-	-	4.54	-	-	14.81	-	-	6.25	-	-	-	-	-	-	-	-	-	1.28
15	***Syncephalastrum racemosum***	3.58	8.58	4.48	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.83
16	***Trichoderma viride***	-	-	-	-	-	8.34	6.67	-	-	-	-	-	-	-	-	-	-	-	-	-	0.75
Total association (%)		57.40	84.60	68.60	34.30	31.80	62.50	26.60	55.50	41.90	66.60	37.50	26.60	31.80	32.10	57.60	32.00	63.80	25.00	82.90	79.70	

-represents no growth of respective fungi

4.2.3. Per cent incidence of fungal association with BRRI rice seeds by Tissue planting method after ten months of storage

The prevalence of fungi with the seeds of rice after ten months of storage is presented in Table 8. The frequency percentage of *Aspergillus* spp, *B. oryzae, M. fisheri* and *Penicillium* sp. gradually increased with the increase of storage period.

The highest frequency percentage of *Penicillium* sp. and *A. alternata* was noticed in BRRI dhan 70, *A. fumigatus* and *M. fisheri* in BRRI dhan 64, *A. flavus* in BRRI dhan 62, *B. oryzae, B. sorokiniana* and *S. racemosum* in BRRI dhan 65, *A. niger* in BRRI dhan 71, *A. terreus* and *B. multiformis* in BRRI dhan 60, *C. lunata* and *F. equiseti* in BRRI dhan 66, *S. oryzae* in BRRI dhan 74, *F. fujikuroi* and *P. chrysosporium* in BRRI dhan 73, *N. oryzae* and *R. stolonifer* in BRRI dhan 68 variety. Among the 18 fungi *Penicillium* sp. showed highest mean per cent incidence (9.63) which was followed by *A. flavus* (6.63), *B. oryzae* (6.33), *A. fumigatus* (6.04) and *M. fisheri* (4.64). *Syncephalastrum racemosum* showed lowest mean per cent incidence (0.21). The maximum association (92.5%) were recorded in BRRI dhan 65 whereas the minimum (19.3%) in BRRI dhan 72.

Similarly, Archana and Prakash (2013) reported sixteen genera of fungi *viz*., *Acremonium, Alternaria, Aspergillus, Bipolaris, Chaetomium, Cladosporium, Curvularia, Exserohilum, Fusarium, Microdochium, Nigrospora, Phoma, Pyricularia, Rhizoctonia, Rhizopus* and *Verticillium* comprising twenty-seven species were found to be associated with the rice seed samples. Among them, the most predominant was *Bipolaris oryzae* which was associated with 82.08% seed samples, followed by *Alternaria padwickii* (63.36%), *Curvularia lunata* (46.08%), *Pyricularia oryzae* (44.64%), *A. alternata* (34.56%), *Fusarium moniliforme* (27.36%) and *C. pallescens* (21.6%). *Aspergillus flavus* and *C. oryzae* had an incidence of 15.84%.

Table 8. Per cent incidence of fungal association with BRRI rice seeds by Tissue planting method after ten months of storage.

Sl. No.	Name of fungi	Per cent association of fungi with BRRI rice seeds																				Mean (%)
		BRRI Dhan 56	BRRI Dhan 57	BRRI Dhan 58	BRRI Dhan 59	BRRI Dhan 60	BRRI Dhan 61	BRRI Dhan 62	BRRI Dhan 63	BRRI Dhan 64	BRRI Dhan 65	BRRI Dhan 66	BRRI Dhan 67	BRRI Dhan 68	BRRI Dhan 69	BRRI Dhan 70	BRRI Dhan 71	BRRI Dhan 72	BRRI Dhan 73	BRRI Dhan 74	BRRI Dhan 75	
1	***Alternaria alternata***	-	-	-	-	-	-	-	-	-	2.5	-	-	-	2.22	2.7	-	2.3	-	-	-	0.48
2	***Aspergillus flavus***	12.2	2.5	10	8	8.7	8.7	16.7	-	15.3	16.3	6.7	8.7	12	4.6	-	-	-	2.3	-	-	6.63
3	***A. fumigatus***	-	6.7	-	-	-	16.3	1.7	-	18.8	18.7	-	2.3	8.5	10	10	8.6	6.5	-	4.5	8.3	6.04
4	***A. niger***	4.8	6.7	6.7	5.7	-	-	4.6	4.7	-	4.3	3	-	-	3.3	8.7	10.7	-	5.6	-	-	3.44
5	***A. terreus***	-	-	4.3	3.33	8.7	-	-	4	-	-	4.7	2.3	2.3	-	-	-	-	-	2.3	-	1.59
6	***Bipolaris multiformis***	-	-	-	-	4	-	-	-	-	-	1.3	2	-	-	-	-	-	-	-	-	0.36
7	***B. oryzae***	4.3	10	8.6	-	8.6	10	4.3	10.3	-	16	10.6	12.4	10.3	-	-	-	8.5	-	12.7	-	6.33
8	***B. sorokiniana***	-	-	-	-	-	-	-	-	-	7.7	2.2	3.7	2.7	-	-	-	-	2.3	-	-	0.93
9	***Curvularia lunata***	-	-	2	5.6	-	-	-	4.6	-	7.7	8.3	-	3.3	-	-	-	-	4.3	-	-	1.79
10	***Fusarium equiseti***	-	-	-	-	-	-	-	-	4.3	2.7	5.6	4	2.3	-	-	5.6	-	-	2.7	-	1.36
11	***Fusarium fujikuroi***	-	-	-	-	4.3	-	-	-	-	-	-	-	-	-	-	-	-	5.6	3	-	0.64
12	***Microdochium fisheri***	10.3	8.3	-	6.3	10	-	-	8.3	12	-	-	-	-	8.6	10	8.5	-	-	-	10.5	4.64
13	***Nigrospora oryzae***	-	-	-	-	-	-	-	1.6	-	-	-	2.3	2.7	-	-	-	-	-	-	-	0.33
14	***Penicillium* sp.**	10.66	10.9	5.6	12	15.7	13	14	9.7	12.7	10	-	10.3	16	12.7	30.7	8.7	-	-	-	-	9.63
15	***Phanerochaete chrysosporium***	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.3	2	2.8	-	-	0.35
16	***Rhizopus stolonifer***	-	-	-	-	-	-	-	2	-	2	-	2	2	-	-	-	-	1.3	-	-	0.46
17	***Sarocladium oryzae***	-	-	-	-	2	-	-	-	-	2.3	-	-	-	-	-	-	-	2.6	8.3	2.3	0.87
18	***Syncephalastrum racemosum***	-	-	-	-	-	-	-	-	-	2.3	-	2	-	-	-	-	-	-	-	-	0.21
Total association (%)		42.2	45.1	37.2	40.9	62	48	41.3	45.2	63.1	92.5	42.4	52	62.1	42	63	44	19.3	26.8	33.5	21.1	

-represents no growth of respective fungi

4.2.4. Average per cent incidence of fungal association with BRRI rice seeds in three replications by Tissue planting method

Average per cent incidence of fungi associated with twenty varieties of rice seeds in three replications is presented in Table 9 and Figs 3-4. *Aspergillus flavus* was found in all the varieties examined. Highest mean average frequency percentage of total association was observed in *Bipolaris oryzae* (152.2%) and lowest in *B. multiformis* (2.42%). These were the most predominant fungi in terms of prevalence. The eight predominant fungi were *Aspergillus flavus, A. fumigatus, A. niger, A. ochraceus, A. terreus, B. oryzae, Microdochium fisheri* and *Penicillium* sp. varied in prevalence with respect to variety and time duration whereas *Bipolaris multiformis*, *Phanerochaete chrysosporium, Alternaria tenuissima, Trichoderma viride, Fusarium proliferatum*, *Pestalotiopsis oxyanthi, Bipolaris sorokiniana* and *Syncephalastrum racemosum* were recorded only with a few varieties of rice seeds. The highest average fungal association was found in BRRI dhan 65 (75.46%) followed by BRRI dhan 63, BRRI dhan 57 and the lowest was in BRRI dhan 73 (35.28%).

Table 9. Average per cent incidence of fungal association with BRRI rice seeds of three replications by Tissue planting method.

SL No.	Name of fungi	Per cent association of fungi with BRRI rice seeds																				Total association (%)
		BRRI dhan 56	BRRI dhan 57	BRRI dhan 58	BRRI dhan 59	BRRI dhan 60	BRRI dhan 61	BRRI dhan 62	BRRI dhan 63	BRRI dhan 64	BRRI dhan 65	BRRI dhan 66	BRRI dhan 67	BRRI dhan 68	BRRI dhan 69	BRRI dhan 70	BRRI dhan 71	BRRI dhan 72	BRRI dhan 73	BRRI dhan 74	BRRI dhan 75	
1	***Alternaria alternata***	1.2	2.56	1.49	-	-	1.33	-	-	-	0.83	-	-	-	0.74	0.9	1.11	2.02	1.66	-	-	13.84
2	***A. tenuissima***	-	-	1.75	-	-	-	-	-	-	-	-	-	-	-	-	-	2.5	-	-	-	4.25
3	***Aspergillus flavus***	9.85	6.89	6.2	7.86	2.9	5.51	8.59	3.51	9.4	20.23	2.23	3.97	6.46	6.85	5.1	1.33	8.25	0.76	7.84	2.56	126.29
4	***A. fumigatus***	2.38	7.96	3.96	-	6.92	9.52	10.13	6.94	6.26	7.46	4.16	0.76	6.39	10.05	7.34	5.53	8.83	2.32	6.38	11.26	124.55
5	***A. niger***	1.6	2.23	2.23	8.43	-	1.38	3.55	1.56	3.22	8.93	3.3	-	4.86	1.1	8.92	11.12	5.05	1.86	-	-	69.34
6	***A. ochraceus***	1.48	3.79	3.86	2.22	3.17	2.61	-	4.16	-	1.66	6.89	4.44	-	-	-	-	-	3.1	1.9	3.84	43.12
7	***A. terreus***	4.76	5.71	4.42	7.77	4.48	5.53	1.1	3.8	-	-	1.56	0.76	0.76	0.98	-	-	-	-	0.76	-	42.39
8	***Bipolaris multiformis***	-	-	-	-	1.33	-	-	-	-	-	0.43	0.66	-	-	-	-	-	-	-	-	2.42
9	***B. oryzae***	12.12	10.65	11.33	5.55	6.04	19.5	9.5	16.7	13.98	13.63	12.08	8.19	3.43	-	-	-	2.83	-	6.67	-	152.2
10	***B. sorokiniana***	-	-	1.75	-	-	-	-	-	-	2.56	0.73	1.23	0.9	-	-	1.11	2.51	0.76	-	-	11.55
11	***Curvularia lunata***	-	0.64	3.3	1.86	3.03	-	-	2.22	1.07	2.56	2.76	-	1.1	-	2.17	-	-	2.98	3.25	-	26.94
12	***Fusarium equiseti***	-	-	-	2.22	-	-	-	-	1.43	4.23	1.86	1.33	0.76	-	2.9	1.86	-	-	0.9	-	17.49
13	***F. fujikuroi***	-	-	-	4.16	2.93	-	-	-	-	-	-	-	-	-	-	-	1.11	5.2	5.88	-	19.28
14	***F. oxysporum***	2.67	-	6.36	-	1.58	1.96	-	1.39	-	0.83	-	-	-	1.96	-	1.33	1.11	-	2.86	17.44	39.49
15	***F. proliferatum***	-	0.65	-	-	2.38	-	-	-	-	-	-	1.62	-	-	1.11	3.33	1.26	-	-	-	10.35
16	***Microdochium fisheri***	3.43	7.89	-	2.1	11.26	3.26	6.03	12.87	8.83	3.7	-	-	7.4	2.86	6.23	16.72	10.73	5.12	10.04	6.4	124.87
17	***Nigrospora oryzae***	-	-	-	-	-	-	-	1.57	-	-	-	5.21	3.37	-	-	3.33	5.03	1.55	-	-	20.06
18	***Penicillium* sp.**	4.29	11.91	2.86	4	9.2	7.6	7.69	6.2	4.23	3.33	2.3	6.68	9.45	7.8	22.27	2.9	2.22	6.2	-	-	121.22
19	***Pestalotiopsis oxyanthi***	-	-	-	-	-	-	-	-	-	1.66	4.6	-	-	-	3.62	-	-	1.55	-	-	11.43
20	***Phanerochaete chrysosporium***	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.76	0.66	0.93	-	0.76	3.11
21	***Rhizopus stolonifer***	2.22	-	-	-	1.51	-	-	5.6	-	0.66	2.08	0.66	0.66	1.96	-	-	1.88	0.43	3.81	-	21.45
22	***Sarocladium oryzae***	1.48	1.28	-	-	3.84	-	-	-	2.15	2.43	2.3	-	-	-	-	-	-	0.86	2.76	-	17.1
23	***Syncephalastrum racemosum***	1.2	2.86	3.25	-	-	-	-	-	-	0.76	-	0.66	-	4.9	-	-	-	-	-	-	13.63
24	***Trichoderma viride***	-	-	-	-	-	2.78	2.22	-	-	-	-	-	-	-	-	-	-	-	-	-	5.0
Total association (%)		48.68	65.02	52.76	46.17	60.57	60.98	48.81	66.52	50.57	75.46	47.28	36.17	45.54	39.2	60.56	50.43	55.99	35.28	53.05	42.26	

-represents no growth of respective fungi

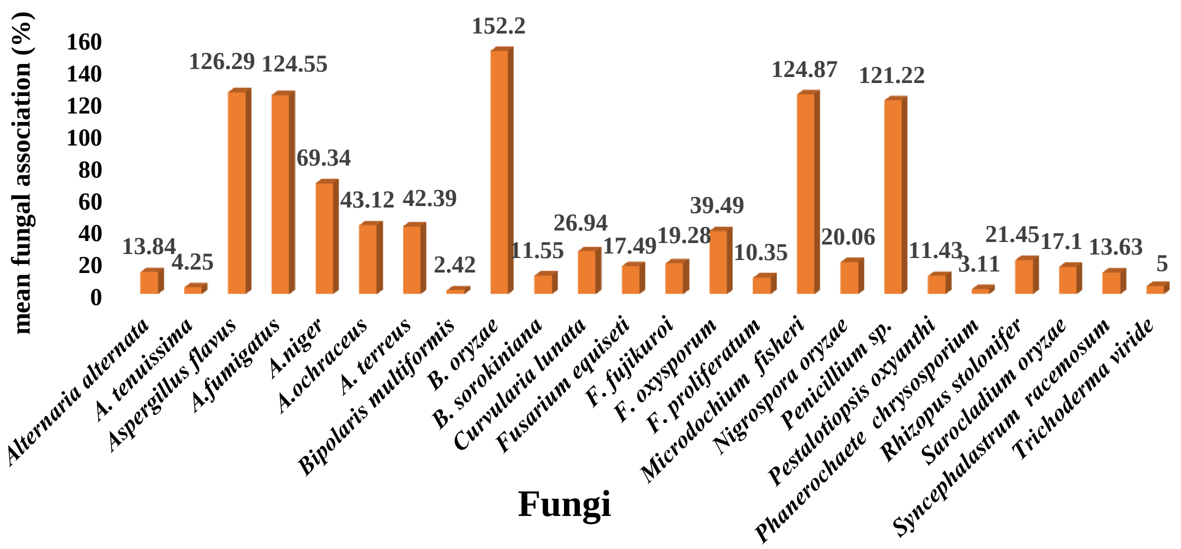


Fig. 3. Average per cent association of fungi of three replications by Tissue planting method.

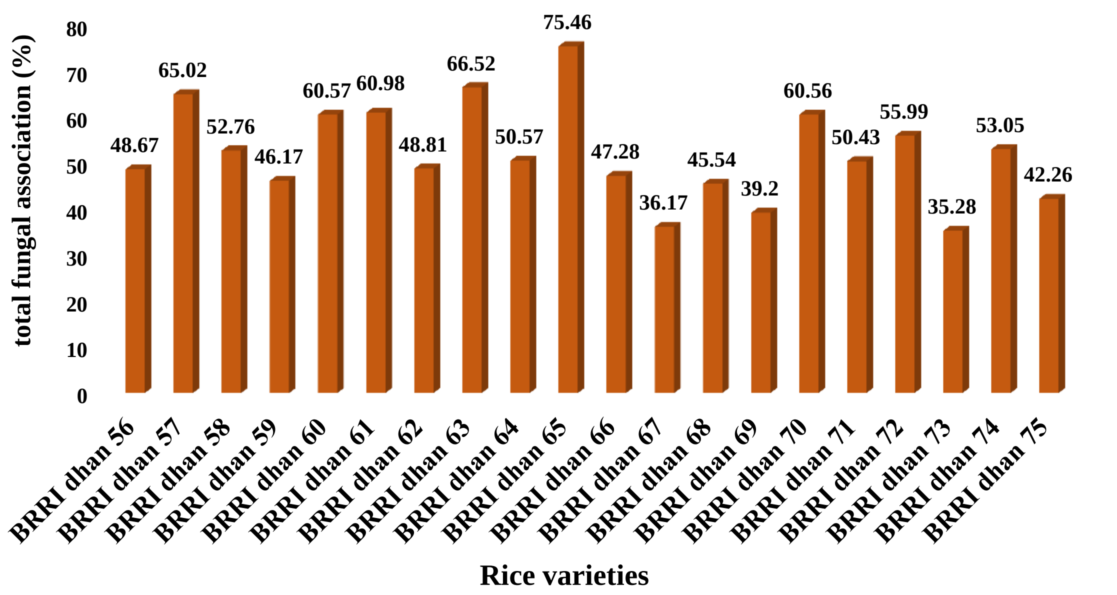
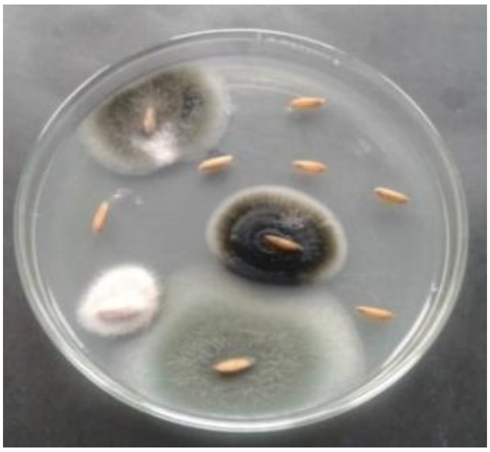
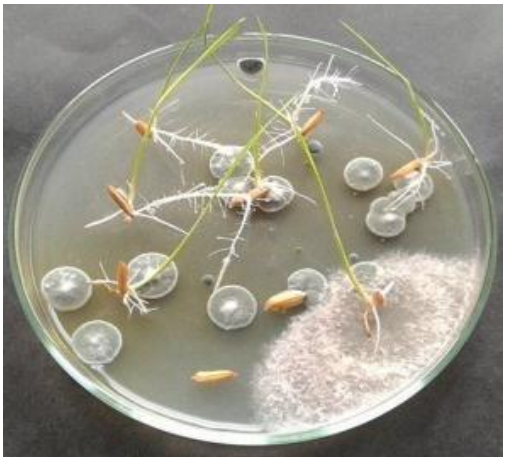


Fig. 4. Average per cent total association of fungi with BRRI rice varieties in three replications by Tissue planting method.

BRRI dhan-56 BRRI dhan-57



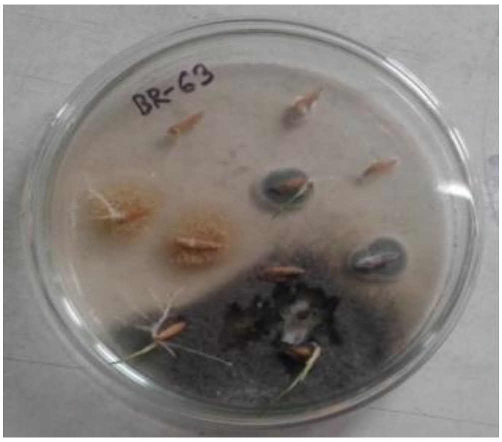
BRRI dhan-58 BRRI dhan-59



BRRI dhan-60 BRRI dhan-61

Plate 2. Fungi associated with the seeds of BRRI dhan 56 to BRRI dhan 61.

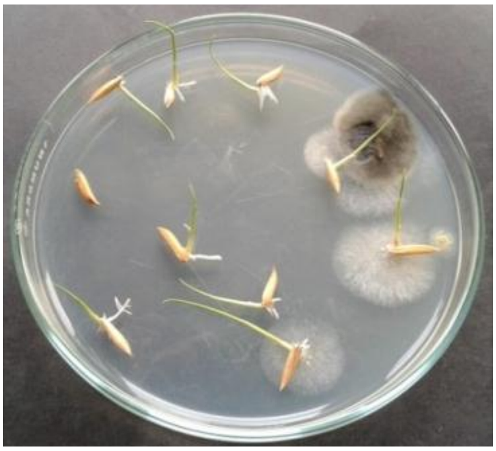
BRRI dhan-62



BRRI dhan-63

BRRI dhan-64

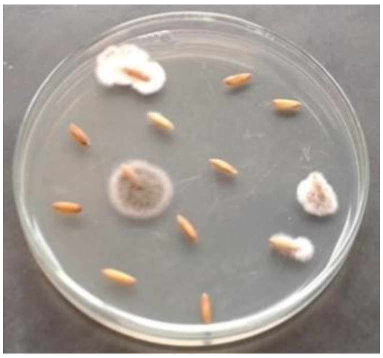
BRRI dhan-65



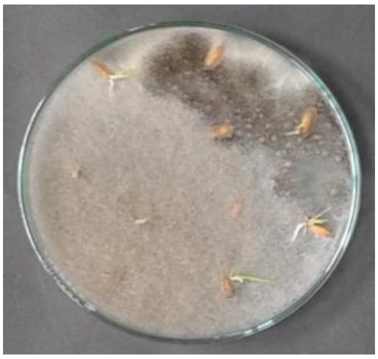
BRRI dhan-66

BRRI dhan-67

Plate 3. Fungi associated with the seeds of BRRI dhan 62 to BRRI dhan 67.



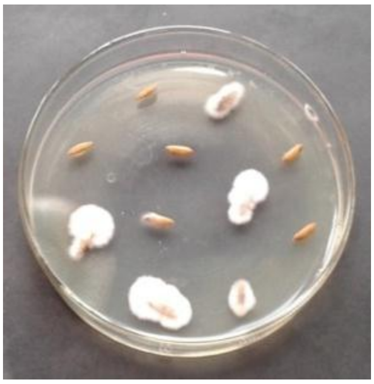
BRRI dhan-68



BRRI dhan-69

BRRI dhan-70

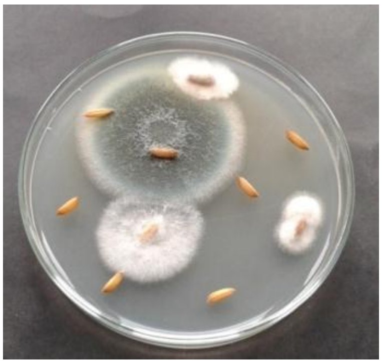
BRRI dhan-71



BRRI dhan-72

BRRI dhan-73

BRRI dhan-74



BRRI dhan-75

Plate 4. Fungi associated with the seeds of BRRI dhan 68 to BRRI dhan 75.

4.2.5. Per cent incidence of fungal association with BRRI rice seeds by Blotter method

In the present study a total of 13 fungi were isolated from different varieties of rice seeds by blotter method presented in Table 10, Figs 5-6 and Plate 5. The highest frequency percentage of *S. oryzae* was noticed in BRRI dhan 63; *B. oryzae* in BRRI dhan 64, *C. globosum* in BRRI dhan 56, *A. niger* in BRRI dhan 68, *C. lunata* in BRRI dhan 61, *Penicillium* sp. in BRRI dhan 59 and *A. terreus* in BRRI dhan 72 varieties. In blotter method, *Chaetomium globosum, Bipolaris oryzae* and *Aspergillus niger* were predominant in most of the rice varieties. *Microdochium fisheri* and *Sarocladium oryzae* were recorded only with a few varieties of rice seeds. The maximum mean fungal association was noticed in *C. globosum* (2.16%) and minimum in *A. terreus* (0.41%) (Fig. 5). The highest total association of fungi was found in BRRI dhan 56 (35%) which was followed by BRRI dhan 64, BRRI dhan 68 and the lowest in BRRI dhan 65 (2.75%) (Table 10, Fig. 6).

The results are in agreement with the findings of Naher *et al.* (2016) who detected six fungal species *viz., Alternaria padwickii, Aspergillus* spp, *Bipolaris oryzae, Curvularia lunata, Fusarium moniliforme* and *F. oxysporum* from the 3 rice varieties such as BR11, BRRI dhan 30 and BRRI dhan 33 by blotter method. Similarly, Ora *et al.* (2011) found 12 seed borne fungi *viz., Alternaria tenuissima, Aspergillus* spp, *Bipolaris oryzae, Chaetomium globosum, Curvularia lunata, Fusarium moniliforme, Penicillium* sp., *Phoma* sp., *Nigrospora oryzae, Rhizopus stolonifer, Tilletia barclyana* and *Xanthomonas oryzae* by blotter method. Of all the microbes *R. stolonifer, Aspergillus* spp, *B. oryzae* and *F. moniliforme* were predominant.

Higher infestation was recorded in agar plate method as compared to blotter method in the present study. Seed borne mycoflora of rice showed variation in their composition depending on variety and detection of seed samples collected.

Table 10. Per cent incidence of fungal association with BRRI rice seeds by blotter method.

Sl. No.	Name of fungi	Per cent association of fungi with BRRI rice seeds																				Mean (%)
		BRRI Dhan 56	BRRI Dhan 57	BRRI Dhan 58	BRRI Dhan 59	BRRI Dhan 60	BRRI Dhan 61	BRRI Dhan 62	BRRI Dhan 63	BRRI Dhan 64	BRRI Dhan 65	BRRI Dhan 66	BRRI Dhan 67	BRRI Dhan 68	BRRI Dhan 69	BRRI Dhan 70	BRRI Dhan 71	BRRI Dhan 72	BRRI Dhan 73	BRRI Dhan 74	BRRI Dhan 75	
1	***Aspergillus flavus***	1.25	-	-	1	-	1.25	0.5	0.75	5.25	0.5	-	-	2.5	0.25	-	-	-	-	-	-	0.66
2	***A. fumigatus***	7	-	0.75	-	-	2.25	1.5	-	3.25	-	-	-	5	-	-	1	-	-	1.75	-	1.12
3	***A. niger***	5	-	8.5	-	-	1.5	-	-	1.25	-	2.2	-	8.75	-	2.25	-	-	4.5	-	-	1.69
4	***A. terreus***	0.75	-	-	-	-	-	1	0.75	0.5	-	1	-	0.5	-	-	-	2.25	1.5	-	-	0.41
5	***Bipolaris multiformis***	5	-	-	2.5	0.75	-	2	-		-	-	-	3.2	-	1.5	-	-	-	-	-	0.86
6	***Bipolaris oryzae***	-	-	3.75	2.25	5.2	-	-	-	12.5	-	-	8.5	-	1.5	2	-	1.25	-	-	3.75	2.03
7	***Chaetomium globosum***	9.25	7.5	-	-	4.5	1	5.2	1	1.25	-	2.25	-	3.5	-	-	-	-	2.25	1	4.5	2.16
8	***Curvularia lunata***	-	-	-	4	-	5.75	2.2	-	1.25	-	-	-	-	3.5	-	1	-	-	-		0.88
9	***Fusarium fujikuroi***	4.75	1.5	1	2	1.7	-	-	-	2	1	-	1.75	3.7	2.25	1.5	4.5	-	-	1	2	1.53
10	***Microdochium fisheri***	-	3.5	-	0.75	-	-	-	0.5	-	1.25	3.5	-	-	-	-	-	-	-	-	-	0.47
11	***Penicillium* sp.**	2	-	-	4.25	-	-	1	1.25	-	-	-	2	1.5	1.25	-	-	-	-	-	-	0.66
12	***Sarocladium oryzae***	-	-	-	-	4.54	-	-	14.81	-	-	6.25	-	-	-	-	-	-	-	-	-	1.28
13	***Syncephalastrum racemosum***	-	-	1.25	-	-	2.75	-	-	2.75	-	1.5	-	-	2	-	2.25	-	-	-	-	0.62
Total association (%)		35	12.5	15.2	16.7	16.6	14.5	13.4	19	32.2	2.75	16.7	12.2	28.6	10.7	7.25	8.75	3.5	8.25	3.75	10.2	

-represents no growth of respective fungi

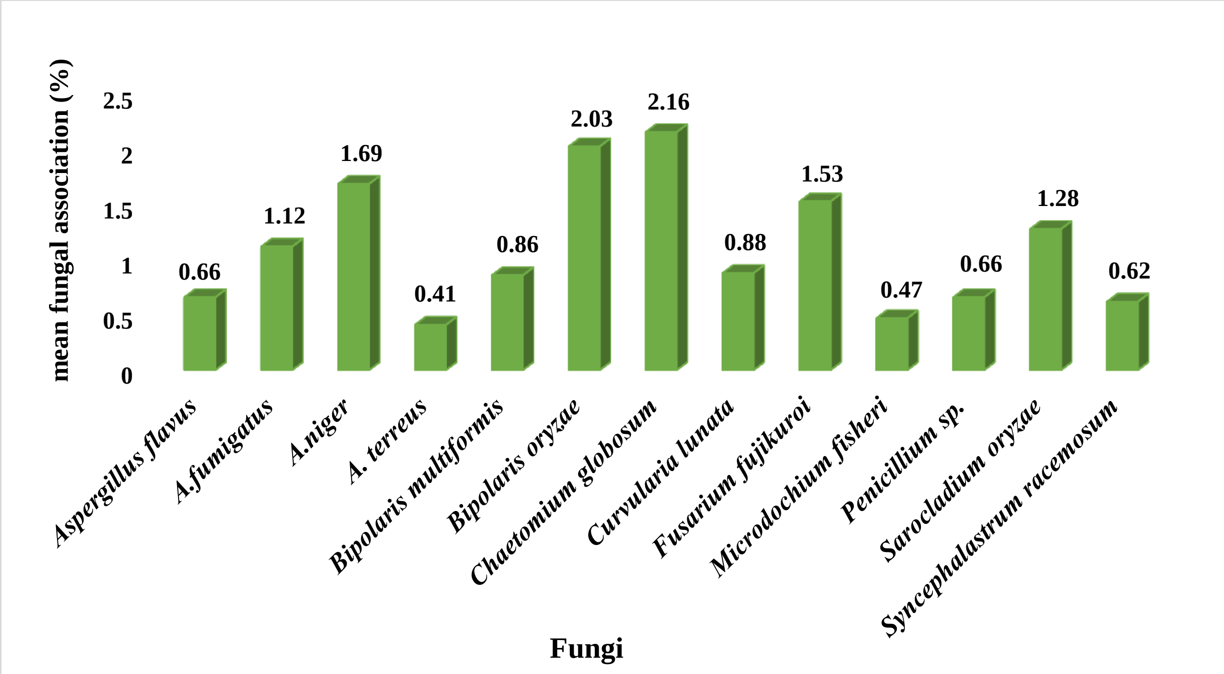


Fig. 5. Per cent mean association of fungi with BRRI rice seeds by Blotter method.

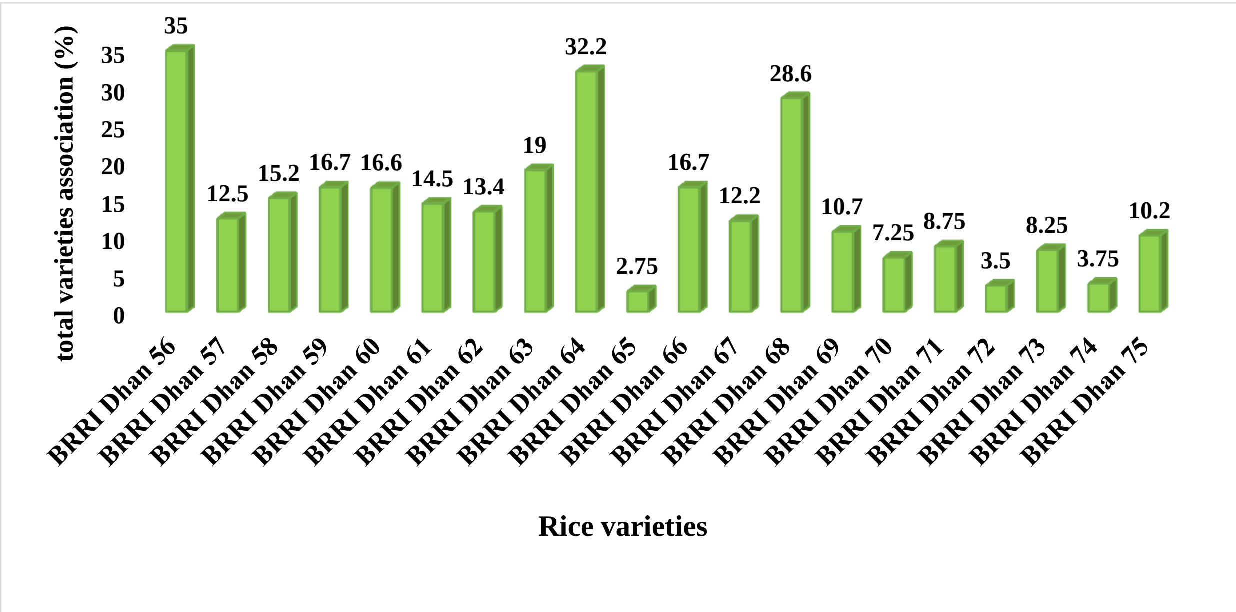


Fig. 6. Per cent total association of fungi with BRRI rice varieties by Blotter method.

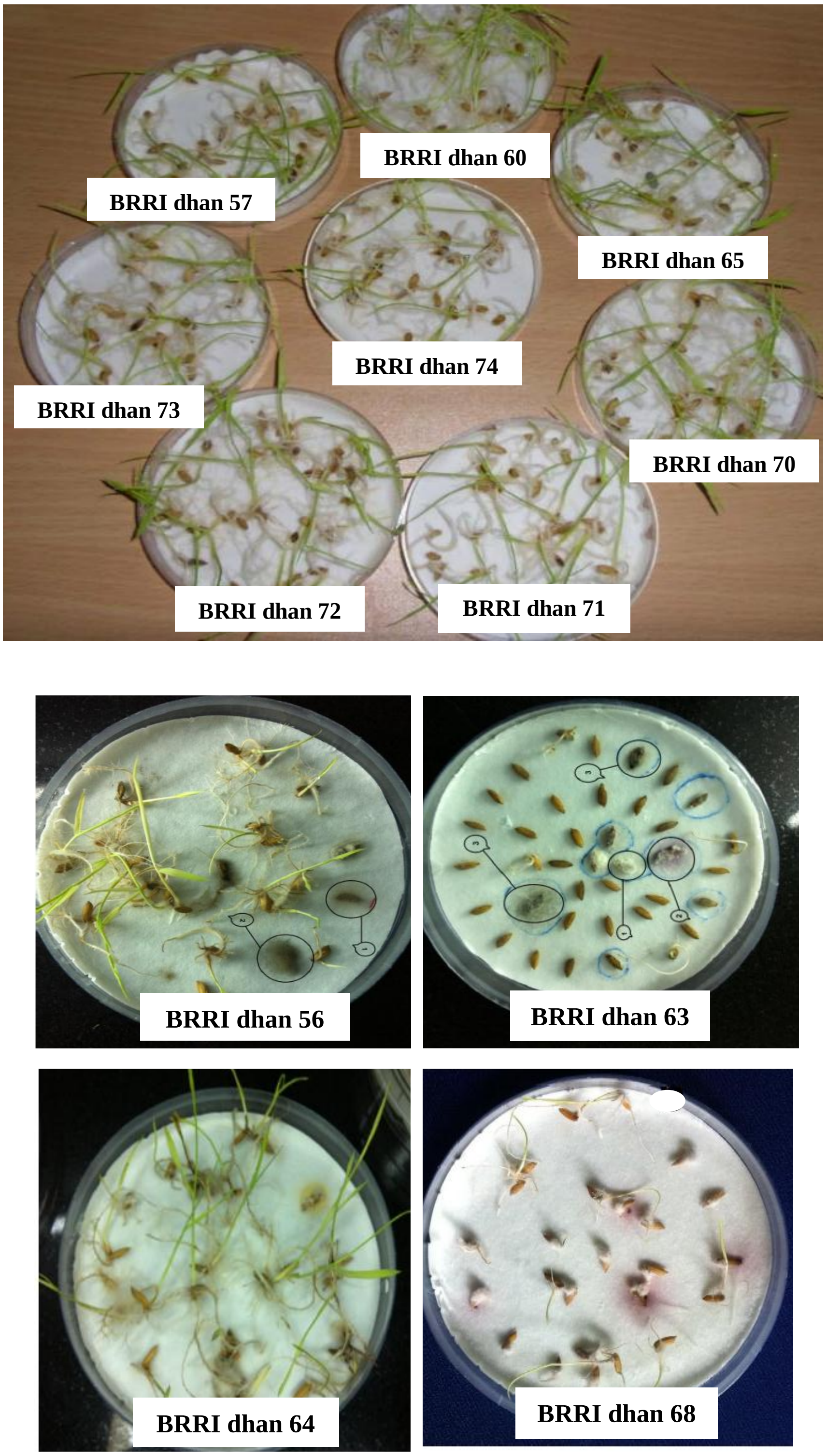


Plate 5. Fungi associated with the seeds of BRRI rice varieties by Blotter method.

4.3. Fungal association with different parts of seeds of selected BRRI rice varieties

For histopathological study associated seed borne fungi were isolated from four parts of BRRI rice seeds such as empty glume, flowering glume, embryo and endosperm. Ten species of fungi were found to be associated with different parts of randomly selected rice seeds. The fungi were *Aspergillus flavus, A. fumigatus, A. niger*, *Curvularia lunata, Fusarium proliferatum, Nigrospora oryzae, Penicillium* sp., *Pestalotiopsis oxyanthi, Rhizopus stolonifer* and *Trichoderma viride*. Among them *A. flavus, A. fumigatus, A. niger* and *T. viride* were found in most of the parts of rice seeds as well as maximum BRRI rice varieties whereas *P. oxyanthi* was rarely found in seed parts and BRRI rice varieties (Table 11, Plate 6). Out of ten isolated fungi two *viz., C. lunata* and *N. oryzae* were found to be pathogenic to BRRI rice varieties.

Empty glume of BRRI dhan 57 and BRRI dhan 75 was completely affected by *A. flavus* whereas *P. oxyanthi* was only observed in the empty glume of BRRI dhan 65. On the other hand, flowering glume, embryo and endosperm were totally affected by *Fusarium proliferatum* in BRRI dhan 65.

The results of present investigation is in agreement with the findings of Shamsi *et al.* (1995) who reported presence of five fungal species associated with different seed parts of sheath rot infected BR 14 and purbachi. The associated fungi were *A. alternata, C. lunata, F. pallidoroseum*, *N. oryzae* and *S. oryzae*. *Fusarium pallidoroseum* was exclusively isolated from purbachi seeds and other fungi were isolated from both the rice varieties. The variation in number and fungal species might be owing to the differences of rice varieties.

Table 11. Fungal incidence with different parts of seeds of selected BRRI rice varieties.

SL No	Name of Fungi	Fungal incidence with different parts of BRRI rice seeds																																							
		BRRI Dhan 56				BRRI Dhan 57				BRRI Dhan 58				BRRI Dhan 59				BRRI Dhan 60				BRRI Dhan 61				BRRI Dhan 62				BRRI Dhan 63				BRRI Dhan 64				BRRI Dhan 65			
		EG	FG	EM	EN	**EG**	FG	EM	EN	**EG**	FG	EM	EN	**EG**	FG	EM	EN	**EG**	FG	EM	EN	**EG**	FG	EM	EN	**EG**	FG	EM	EN	**EG**	FG	EM	EN	**EG**	FG	EM	EN	**EG**	FG	EM	EN
1	***Aspergillus flavus***	-	-	-	20	100	-	86	75	20	20	-	-	80	15	20	-	-	25	-	25	60	-	20	34	-	19	-	17	-	-	-	-	72	-	84	78	-	-	-	-
2	***A. fumigatus***	-	40	43	-	-	-	-	-	30	-	-	25	-	-	-	-	-	-	-	-	-	13	20	34	50	10	-	34	15	-	20	29	-	-	-	-	-	-	-	-
3	***A. niger***	-	-	57	20	-	25	-	-	-	25	-	50	-	-	20	-	34	-	-	25	-	13	-	34	50	55	-	50	72	72	40	72	15	25	-	-	-	-	-	-
4	***Curvularia lunata***	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	25	-	20	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	***Fusarium proliferatum***	-	-	-	-	-	-	-	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	100	100
6	***Nigrospora oryzae***	-	-	-	-	-	-	-	13	-	-	-	-	-	15	-	-	34	-	-	-	-	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	***Penicillium* sp.**	-	-	-	40	-	-	-	-	20	-	-	-	-	15	-	-	34	50	-	25	20	-	-	-	-	-	-	-	-	-	-	-	-	-	17	23	-	-	-	-
8	***Pestalotiopsis oxyanthi***	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	-	-	-
9	***Rhizopus stolonifer***	-	-	-	-	-	50	-	-	-	13	33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15	-	-	15	75	-	-	-	-	-	-
10	***Trichoderma viride***	-	60	-	20	-	25	15	-	30	63	67	25	20	58	60	-	-	-	-	-	-	63	60	-	-	19	-	-	15	15	40	-	-	-	-	-	-	-	-	-

Table 11 (cont.).

SL No	Name of Fungi	Fungal incidence with different parts of BRRI rice seeds																																							
		BRRI Dhan 66				BRRI Dhan 67				BRRI Dhan 68				BRRI Dhan 69				BRRI Dhan 70				BRRI Dhan 71				BRRI Dhan 72				BRRI Dhan 73				BRRI Dhan 74				BRRI Dhan 75			
		EG	FG	EM	EN	**EG**	FG	EM	EN	**EG**	FG	EM	EN	**EG**	FG	EM	EN	**EG**	FG	EM	EN	**EG**	FG	EM	EN	**EG**	FG	EM	EN	**EG**	FG	EM	EN	**EG**	FG	EM	EN	**EG**	FG	EM	EN
1	*Aspergillus flavus*	-	13	67	43	67	50	72	72	75	13	34	20	67	67	60	60	20	80	25	58	25	38	50	-	40	50	-	34	86	50	71	25	20	12	40	-	100	33	-	50
2	*A. fumigatus*	-	-	-	-	-	-	-	15	-	-	-	-	-	-	-	-	20	20	-	15	-	-	-	20	-	33	50	-	-	34	-	-	20	-	-	-	-	67	67	-
3	*A. niger*	50	50	-	43	34	50	-	15	-	50	34	40	-	34	17	20	20	-	50	28	25	25	16	20	60	-	-	50	-	-	-	25	-	25	-	25	-	-	-	-
4	*Curvularia lunata*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20	-	-	-	-	-	-	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	25
5	*Fusarium proliferatum*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	33	-
6	*Nigrospora oryzae*	-	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20	-	-	-	-	-	-	-	-	20	-	-	-	-	-	-	-
7	*Penicillium* sp.	50	25	33	-	-	-	-	-	25	13	34	40	-	-	-	-	20	-	25	-	-	-	-	-	-	-	50	17	14	-	28	-	20	25	20	50	-	-	-	25
8	*Pestalotiopsis oxyanthi*	-	-	-	-	-	-	-	-	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	50	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	*Rhizopus stolonifer*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	*Trichoderma viride*	-	-	-	15	-	-	29	-	-	25	-	-	34	-	17	-	-	-	-	-	50	38	17	20	-	17	-	-	-	17	-	50	20	38	40	25	-	-	-	-

EG=Empty glume, FG=Flowering glume, EM=Embryo and EN=Endosperm.

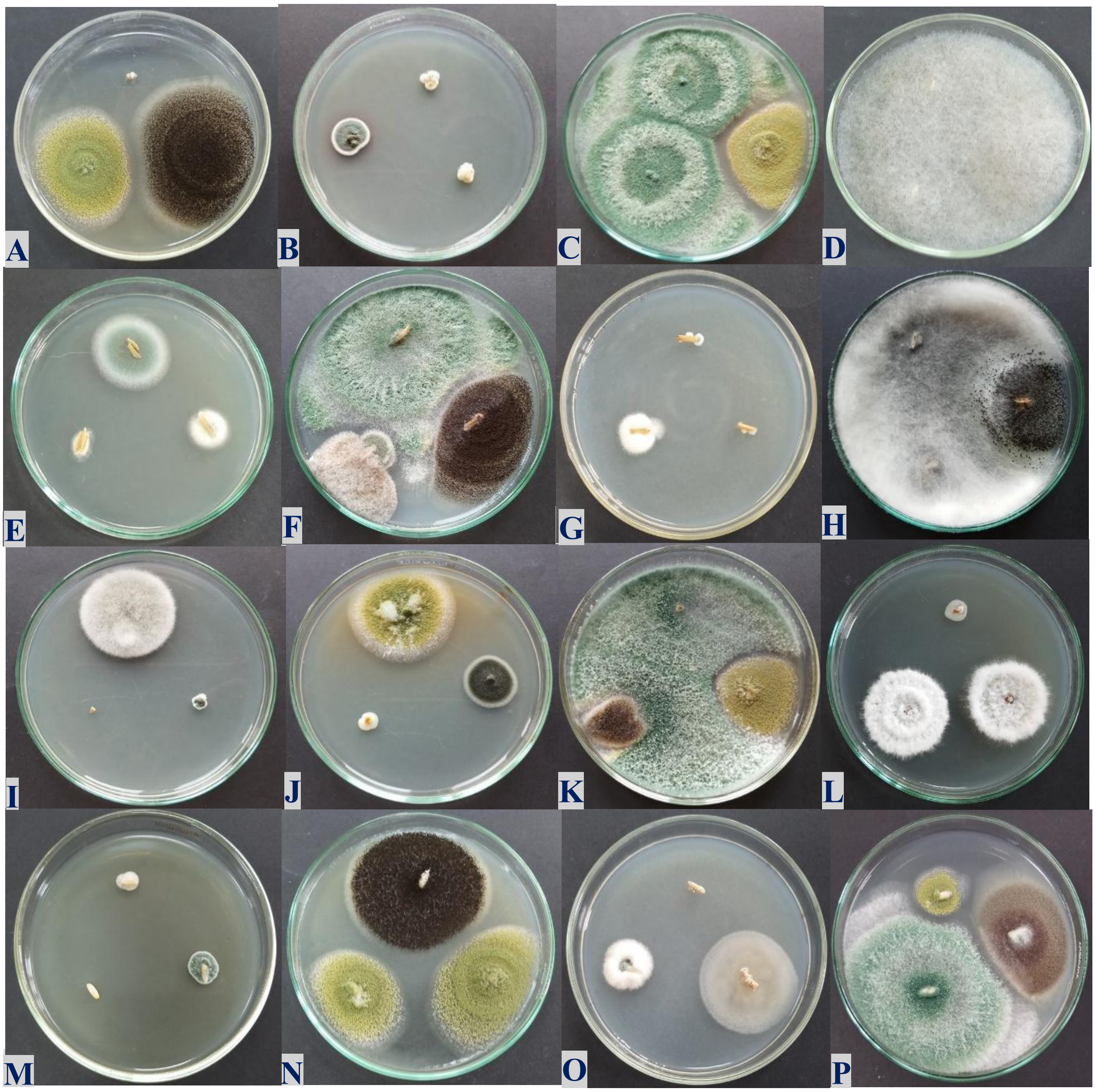


Plate 6. Fungal association with different parts of seed of selected BRRI rice varieties. Empty glume (A-D), Flowering glume (E-H), Embryo (I-L) and Endosperm (M-P).

4.4. Interrelationship between the quality factors through correlation and regression analysis

In this study, some interrelationship between the quality factors through correlation and regression analysis has been estimated which is very much important in controlling seed quality. Significant relationship has been estimated in all the cases.

In case of Tissue planting method Fig. 7A. shows the relationship between percentage of germination rate and frequency percentage of fungi and negative correlation between the two variables. Here regression line gives a downward sloping curve which means that germination of seeds decreased when the percentage of fungi increased or the germination of seed increased when the percentage of fungi decreased. In the present study, the correlation co-efficient value between percentage of fungi and percentage of germination was -0.742 (Mamun *et al.* 2016).

Fig. 7B. shows the relationship between seedling mortality and frequency percentage of fungi and positive correlation between the two variables. Here regression line gives an upward sloping curve which means that both the variable change in the same direction i.e. the mortality of seeds increased when the percentage of fungi increased. The correlation co-efficient value between percentage of fungi and seedling mortality was +0.451 (Mamun *et al.* 2016).

Fig. 7C. shows the relationship between purity of seeds and frequency percentage of fungi and negative correlation between the two variables. In this case the regression line gives a downward sloping curve which indicates that the occurrence of fungi decreased when purity of seed increased and vice versa. The correlation co-efficient value between percentage of fungi and purity of seeds was -0.570.

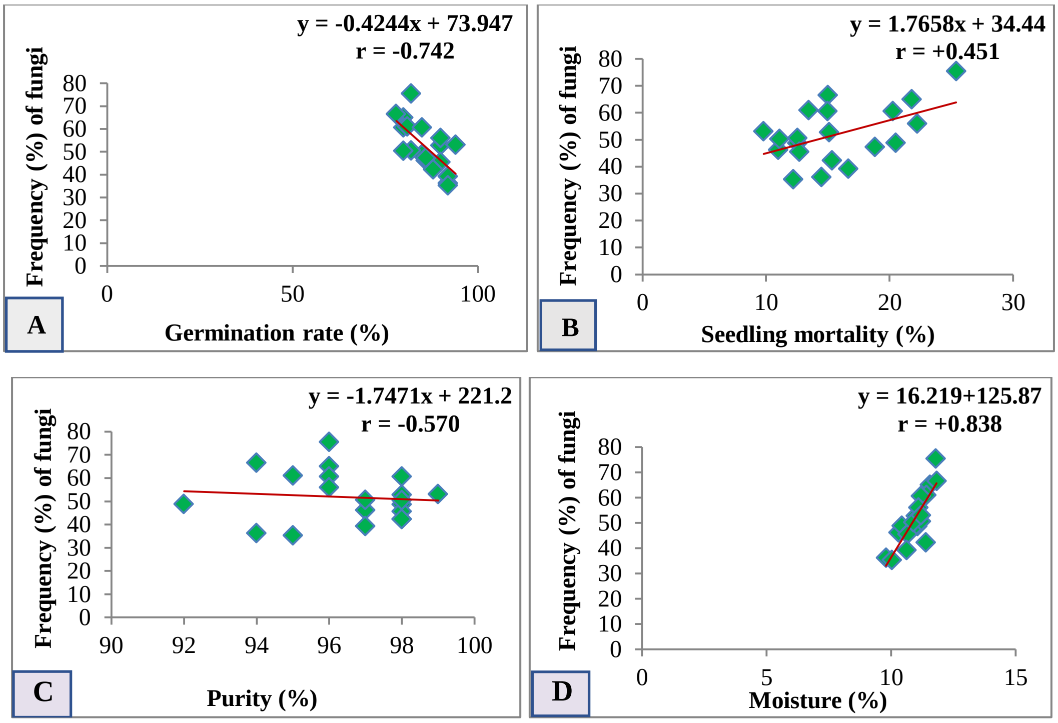


Fig 7. Correlation co-efficient and regression analysis between germination rate (%) and frequency (%) of fungi (A), seedling mortality (%) and frequency (%) of fungi (B), purity (%) and frequency (%) of fungi (C) and moisture content (%) and frequency (%) of fungi (D).

The relationship between seed moisture and frequency percentage of fungi shows positive correlation between the two variables (Fig.7D). Here regression line gives upward slopping curve, which means that both the variable change in the same direction i.e., increase or decrease of one variable increase or decrease of other variable. The correlation co-efficient value between percentage of fungi and seed moisture was +0.838. Fig. 8A. shows the relationship between seed germination and purity percentage of seed and positive correlation between the two variables. Here regression line gives an upward sloping curve which means that both the variable change in the same direction i.e., the germination increased when the purity of seed increased or the germination decreased when the purity of seed decreased. The correlation co-efficient value between purity and germination of seed was +0.556. (Khatun and Shamsi 2016).

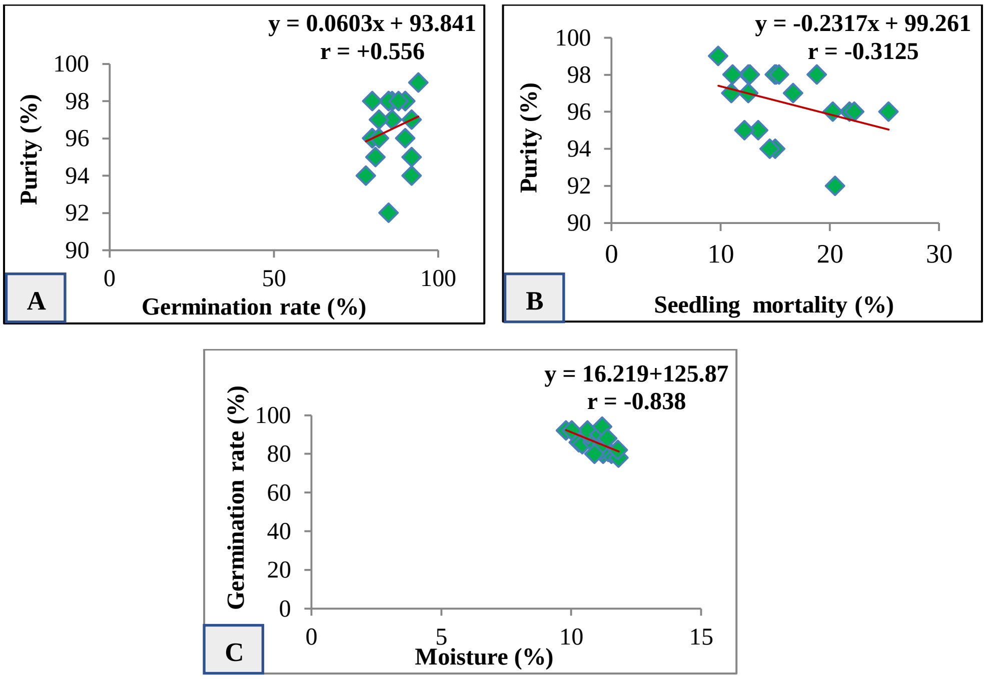


Fig 8. Correlation co-efficient and regression analysis between germination rate (%) and purity (%) of fungi (A), seedling mortality (%) and purity (%) of fungi (B) and moisture content (%) and germination rate (%) of fungi (C).

The relationship between seedling mortality and purity of seed shows negative correlation between the two variables (Fig.8B). Here regression line has given a downward sloping curve which indicates that the increased of one variable decreased of other variable i.e., when purity of seeds increases then seedling mortality decreased or when purity of seeds decreased then seedling mortality increased. The correlation co-efficient value between seedling mortality and purity was -0.3125.

Fig.8C. shows the relationship between seed moisture and germination of seeds and negative correlation between the two variables. In this case the regression line gives a downward sloping curve which means that the increase of one variable decreased of other variable i.e., when moisture increases then germination of seed decreased or when seed moisture decreases then germination of seed increased. The correlation co-efficient value between germination rate and seed moisture was -0.838.

4.5. Morphological identification of seed borne fungi of BRRI rice varieties

Alternaria alternata (Fr.) Keissler, Beih. Bot., Zbl. **29**: 434 (1972) (Fig. 9A).

Colonies usually black or olivaceous black, sometimes grey. Conidiophores golden brown, smooth, up to 28-99 μm long and 3-5 μm thick with one or several conidial scars. Conidia formed in long, often branched chains, obclavate with a short conidial or cylindrical beak, sometimes up to but not more than one third the length of the conidium, pale to mid golden brown, smooth or verruculose, overall length 22.5-52.2 μm, 4.5-16.3 μm thick in the broadest part; beak pale 2.5-5 μm thick.

Alternaria tenuissima (Kunze ex Pers.) Wiltshire, Trans. Br. Mycol. Soc. **18**:157 (1933) (Fig. 9B).

Colony dark blackish brown to black. Conidiophores solitary up to 30 x 3-6 μm, simple or branched, straight or flexuous, more or less cylindrical, septate, pale or mid pale brown. Conidia solitary, straight or curved, obclavate, conidium ellipsoidal, tapering gradually to the beak, usually shorter, pale to mid clear golden brown, usually smooth, 22.2-70.3×10.2-25.4 μm, body generally with 4-7 transverse and several longitudinal or oblique septa, beak shorter than or the same length as the body, cylindrical, 3-5 μm thick.

Aspergillus flavus Link, Magazin der Gesellschaft Naturforschenden Freunde Berlin (Fig.9C).

Colony on PDA was grayish powdery and fast growing. Conidial heads were yellow to green, became brownish in edge. Conidiophores were less than 1 mm length and 10-

20 µm diameter, vesicle was globose to subglobose. Conidia were globose, minutely accumulate and measured 2.5-3.5 µm.

Aspergillus fumigatus Fresenius. Beitragezur Mykologie 3:**81** (1863) (Fig.9D).

Colonies greenish, mycelia well developed, septate. Cells are multinucleate. Conidiophores are long, often with a foot cell, straight or flexuous, swollen at the apex into a spherical vesicle. Conidia catenulate, dry, usually globose, echinulate and smooth. Colonies of the fungus produced thousands of minute pale green conidia 2-3 µm diameter.

Aspergillus niger van Tieghem Ann. Sci. Nat. Bot. Ser. 5, **8**: 240 (1867) (Fig.9E).

Colonies effuse, black. Mycelium well developed, septate, profusely branched and hyaline. Cells are multinucleate. Conidiophores are very long, often with a foot cell, straight or flexuous, swollen at the apex in to a spherical vesicle. Surface of vesicle covered by closely packed more or less clavate branches. Conidia catenulate, dry, usually globose, echinulate, dark brown in color.

Aspergillus ochraceus K. Wilh., Beitrage zur Kenntnis der Pilzgattung**: 66** (1877) (Fig.9F).

Colonies yellow to yellow-orange, ochraceus or buff, powdery to granular. Conidial heads radiate, later splitting into several columns. Conidiophores brownish, 1-1.5 µm long, rough walled. Vesicles globose; phialides biseriate covering almost the entire surface of the vesicle. Conidia spherical to sub spherical, 2.5-3.5 µm in diameter, smooth walled to finely roughened. Pink to vinaceous-purple coloured, irregular shaped sclerotia (up to 1 mm diam.) may be formed in some isolates.

Aspergillus terreus Thom. Amer. J. Bot. **5**(2): 85 (1918) (Fig.9G).

Colonies moderately fast rapidly growing flat, velvety to slightly granular, or powdery, occasionally floccose with thin irregular margins, cinnamon-buff to brown, rarely orange-brown, consisting of a dense felt of conidiophores with reverse yellow to pale brown. An isolate with deep orange colonies with lemon yellow diffusible pigment has been described. Conidial heads pale-brown, long, densely columnar, characteristically appearing fan-shaped. Conidiophores short, 100-250 mm long, flexuous, smooth walled with dome-shaped vesicle, 10-20 mm diameter. Phialides biseriate on upper two third of the vesicle. Conidia hyaline, smooth-walled, spherical to broadly elliptical, 1.5-2.5 mm diameter.

Bipolaris multiformis (Jooste) Alcorn, Mycotaxon **17**:68 (1983) (Fig.9H).

Colonies effuse, grey, dark blackish brown or black. Conidiophores solitary flexuous or geniculate, septate, pale to mid brown. Conidia straight, ellipsoidal, oblong or cylindrical, rounded at the ends, pale to mid brown. The main axis 14-23×1.5-2.0 µm. The terminal branches are tapering towards the apex. Conidia hyaline, smooth, aseptate, cylindrical, 2 - 14 × 1.5 - 1.8 µm.

Bipolaris oryzae (Breda de Haan) Shoemaker **37**(5): 883 (1959) (Fig.9I).

Colonies on PDA was slowly growing, dark to slightly black becoming cottony towards the margin, zonated and black on the reverse side. Conidiophores were short and long. Conidia were dark brown to olivaceous brown, obclavate, fusiform, 5-11 pseudo septa and measured 13.37-125.68 ×10.52-18.65 µm.

Bipolaris sorokiniana (Sacc.) Shoemaker Canadian J. Bot. **37**(5): 883 (1959) (Fig.9J).

Colonies olivaceous brown to very dark becoming generally lighter towards the periphery, margin mostly smooth. Conidiophores brown, short, erect, in most cases single, bearing 1-6 conidia. Conidia ellipsoid, dark brown, mostly straight or slightly curved, broadest in the middle, ends rounded, scar clear within the basal cell. Terminal portion of the end cells sub hyaline, 6-9 pseudoseptate, 48.0-88.6 ×17.2-25.8 µm.

Chaetomium globosum Kunze ex Fr., Systema Mycologicum **3**:255 (Fig.9K).

Colony is punctiform, greyish, numerous on substrate. Hyphae brown septate, profusely branched. Perithecia dark brown with long hairy wavy appendages. Ascospores lemon shaped, 5.2-6 × 2.8-4 µm.

Curvularia lunata (Wakker) Boedijin, Mycol. Pap. 106: 2-43 (1966) (Fig.9L).

Colonies effuse, brown, grey or black, hairy, cottony or velvety. Conidiophores solitary, mostly unbranched, straight, mostly flexuous geniculate, mid brown, septate up to 250 µm. Conidia mostly 3-septate, dark brown, mostly curved, smooth, 25.2-14.4 × 7.2-13.5 µm.

Fusarium equiseti E.J Butler & Hafiz Khan) W. Gams, (1971) (Fig.10A).

Colony white and slightly dark towards the periphery of the Petri dish. Mycelia were hyaline, conidiophores were single, and conidia were hyaline 3-4 septa, measuring 68.6-165.5 × 10.8-16.9 µm.

Fusarium fujikuroi Gibberellafujikuroi (Sawada) Wollenw., (1931) (Fig.10B).

Colony white, floccus to slightly felt. Conidia were hyaline, fusiform, ovate or clavate; one or two celled, measured 26.7-73.6 × 8.1-17.0 µm. Mycelium sparse to densely floccose or felted. Conidiophores hyaline, 0-2 septate.

Fusarium oxysporum Schlecht, Flora berol. **2**: 139, (1824) (Fig.10C).

Mycelium delicate white in color in culture plate. Microconidia borne on simple phialides arising laterally on the hyphae. Microconidia generally abundant, variable, oval-ellipsoid, cylindrical, straight, 5-12 × 2.2-3.5 µm. Macroconidia thin walled, generally 3-5 septate, fusoid-subulate and pointed at both ends; 3 septate 27-46 × 3-5 µm, 5 septate 35-60 × 3-5 µm.

Fusarium proliferatum (Matsush.) Nirenberg ex Gerlach& Nirenberg (1976) (Fig.10D).

Colony white to light pinkish, floccose aerial mycelium on PDA. Pigments produced on PDA varied from white, light yellowish-brown to reddish-brown, light pink, light to deep purple brown with or without concentric rings and light violet or deep violet with concentric rings. Macroconidia were hyaline, delicate, slightly sickle-shaped or almost straight, 3-5 septate and produced in sporodochia. The size of macroconidia averaged 17.39-38.1×1.9-3.1 µm. Microconidia were hyaline,1-2 celled, fusiform to oval. The microconidia were agglutinated in short to long chains and or in false heads.

Microdochium fisheri Hern. -Restr. &Crous, Persoonia **36**:68, (2016) (Fig.10E).

Colonies were flat, margin entire, slightly raised to umbonate centre, white with reverse greyish orange. Mycelium was superficial and immersed. Hyphae smooth-walled, septate, branched, hyaline. Conidia solitary, simple, smooth-walled, 1 septate (rarely 2 septate), fusiform, subpyriform to clavate, hyaline, 4.8-12×1.6-3.6 µm apex rounded, base tapering towards a subtruncate and unthickened hilum. Conidia sometimes form a floret appearance on conidiogenous cells. Conidiogenous cells mainly terminal, mono and polyblastic, denticulate, straight or curved, cylindrical to slightly inflated in the median region, 7-31.5 ×1.5-3 µm, hyaline, smooth. Conidiophores micronematous, arising as lateral, branches from superficial mycelium, smooth-walled, simple to branched, hyaline 12.5-90×1.4-3 µm.

Nigrospora oryzae (Berkeley & Broome) Petch, J. Indian bot. Soc. **24** (1924) (Fig.10F).

Colonies at first white with small shining black conidia easily visible under a low power dissecting microscope, later brown or black when sporulation is abundant. Conidia solitary, with a violent discharge mechanism, acrogenous, simple, spherical. Conidiophores micronematous or semi macronematous, branched, flexuous,

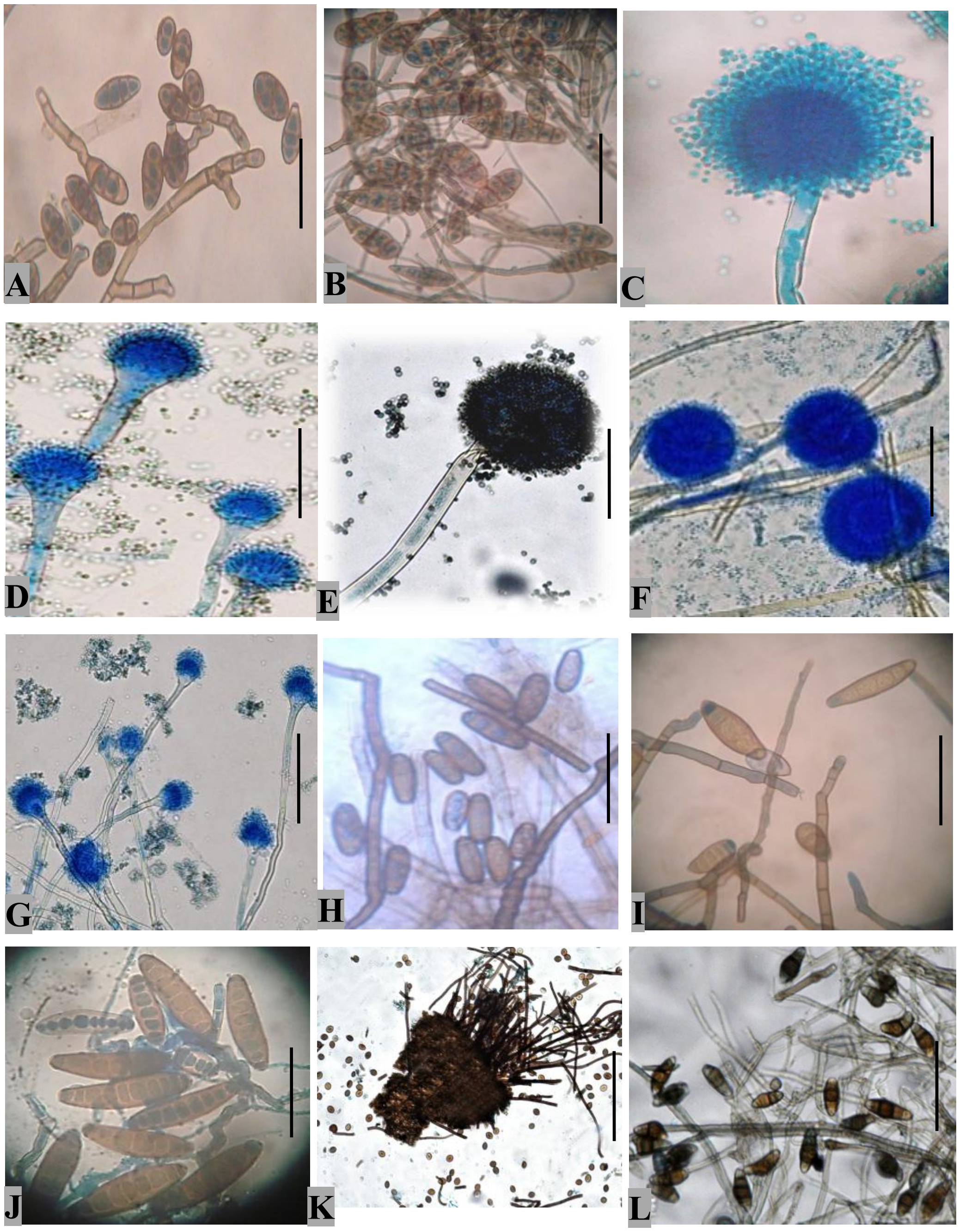


Fig. 9. Conidiophores with conidia of **A**. *Alternaria alternata,* **B**. *A. tenuissima,* **C.** *Aspergillus flavus,* **D**. *A. fumigatus,* **E**. *A. niger,* **F**. *A. ochraceus,* **G**. *A. terreus,* **H**. *Bipolaris multiformis,* **I**. *B. oryzae*, **J**. *B. sorokiniana*, **K**. Perithecia with dark brown city and ascospores of *Chaetomium globosum* and **L**. Conidiophores with conidia of *Curvularia lunata* (Bar = 50 µm).

colorless to brown, smooth. Conidiogenous cells 6-9 µm diameter.

Penicillium Link. (Fr.) Sacc. Bur. Anim. Ind., Bul. **118**:31-33 (1910) (Fig.10G).

Colony on PDA was velvety with areal mycelium and very fast growing and ashy colour. The reverse colour of the plate was yellow to brownish. Conidiophores were smooth, vesiculate, containing phialides, conidia were globose, 3-3.5 µm diameter. Conidia hyaline or brightly colored in mass, one celled, mostly globes or ovoid, produced basipetally.

Pestalotiopsis oxyanthi (Thum.) Steyaert, Bulletin du Jardin Botanique de I'Etat a Bruxelles **19** (3): 329 (1949) (Fig.10H).

Colonies white, cottony, reverse white. Hyphae septate, branched, hyaline. Acervuli black, small, shining. Conidiophores septate, branched, dark brown, cylindrical or lageniform, formed from the upper cells of the pseudoparenchymata. Conidia fusiform, straight or slightly curved, mostly 3 euseptate: basal cells hyaline, truncate, with an endogenous, cellular appendage: apical cell conic, hyaline, with 2 or more apical, simple or branched, spathulate or espathulate appendages: mediam cells brown, sometimes versicoloured, thicker-walled, smooth, 14-23×5-7.5 µm.

Phanerochaete chrysosporium Burds., Mycotaxon 1(2): 124 (1974) (Fig.10I).

Mycelium white to gray, abundant in culture; conidia gray or tan in mass,1 celled, short cylindric to rounded, catenulate, formed acropetally; conidiophores branched, its cell differing little from the older conidia.

Rhizopus stolonifer (Ehrenb.:Fr.) Vuillemin. Toney Bot.Clup. **69**:592-616. (1902) (Fig.10J).

Colony on PDA was initially white, cottony, mycelium hyaline, aseptate, rhizoids well developed at nodes, sporangiophores arised in clusters, irate, aseptate, light brown, 629.5-1002.5 ×5.5-11.7 µm. Spores were round to ovule, hyaline or grayish brown, one celled smooth, 3.8 to 6.4 µm in diameter. Columella present. Sporangium produces non-motile, brownish sporangiospores, 4-6 µm in diameter.

Sarocladium oryzae (Sawada) W. Gams & D. Hawksworth (1976) (Fig.10K).

Colony appears white, compact or cottony, reverse yellowish pink. The fungus produces whitish, sparsely branched, septate mycelia. Conidiophores arising from mycelia slightly thickened from hyphae, branched once or twice, each times with 3-4 branches in a whorl. The main axis 14-23 x 1.5-2.0 µm. The terminal branches are tapering towards the apex. Conidia hyaline, smooth, aseptate, cylindrical 2-0-14 x 1.5-1.8 µm. Chlamydospores absent.

Syncephalastrum racemosum Cohn ex J. Schrot. Kryptogamen-Flora von Schlesien 3-**1**(2): 217 (1886) (Fig.10L).

Colonies transparent, fluffy, grow very rapidly and fill the Petri plate on PDA medium in 48 hours. Mycellium grow rapidly, abundantly branched. Sporangiophores are frequently branched and rather short. Conidiophores erect, branched tips enlarged bearing a head of rod-shaped merosporangia (4-6×9-60 µm) each producing a row of nearly spherical spores, resembling a chain of conidia. Each merosporangium contains a single row of 3-18 merosporangiospores.

Trichoderma viride Pers. Neues Magazin fur die Botanik **1**:92 (1794) (Fig.10M).

Colony effuse, light green. Conidiophores hyaline, much branched, bearing phialides single or in groups. Conidia hyaline, powdery mass, 1 celled, ovoid, borne in small terminal clusters 3.5-5 µm, usually easily recognized by its rapid growth and green patches or cushions of conidia. It is used in the commercial production of enzyme cellulase.

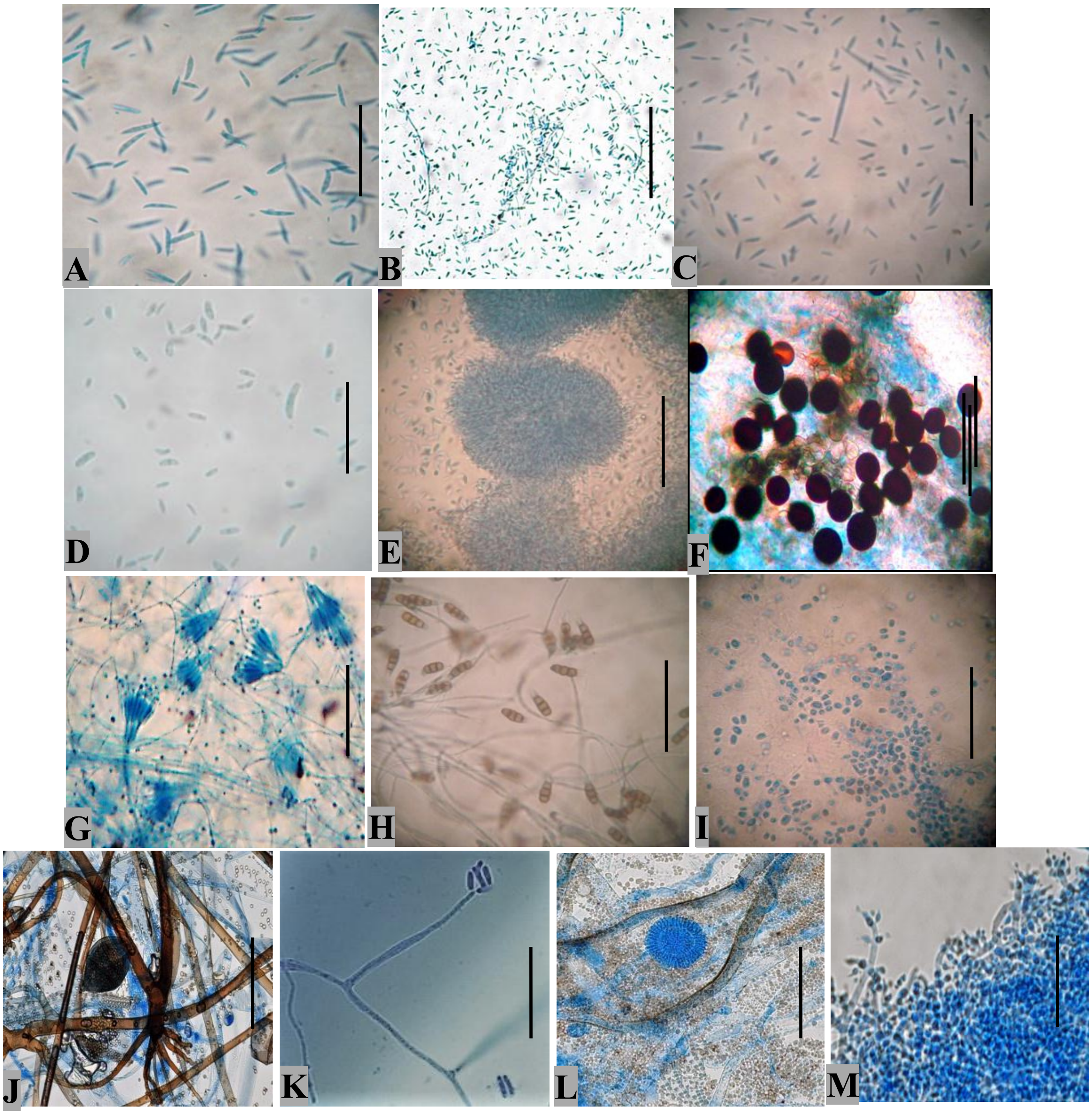


Fig. 10. Macro and microconidia of **A**. *Fusarium equiseti,* **B**. *F. fujikuroi,* **C**. *F. oxysporum,* **D.** *F. proliferatum,* **E.** Sporodochia with conidia of *Microdochium fisheri*, **F.** Conidia of *Nigrospora oryzae,* **G.** Conidiophores with conidia of *Penicillium* sp., **H.** *Pestalotiopsis oxyanthi,* **I.** Conidia of *Phanerochaete chrysosporium,* **J.** Sporangium with sporangiophores of *Rhizopus stolonifer,* **K.** Conidiophores with conidia of *Sarocladium oryzae,* **L.** Conidiophores with merosporangia of *Syncephalastrum racemosum* and **M**. Conidiophores with conidia of *Trichoderma viride.* (Bar = 50 µm).

4.6. Molecular identification of seed borne fungi of BRRI rice varieties

Morphologically identified twenty-five fungi were selected for molecular identification. Among the 25 isolates, some isolates were unable to identify up to species level based on the morphological features only. Therefore, molecular characterization of the fungal isolates was conducted for proper identification using ITS sequence analysis. Out of the 25 fungal isolates, 13 were confirmed up to species level through ITS sequence based molecular analysis (Table 12).

Genomic DNA was isolated successfully from the thirteen isolates. PCR was conducted using ITS1 (Forward) and ITS4 (Reverse) primers and ~600 bp DNA band was amplified (Fig. 11). Sequence analysis of the amplified DNA through BLAST search in GenBank was conducted and found 85 to 99% similarity with partial sequence of 18S ribosomal RNA gene; complete sequence of internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, and partial sequence 28S ribosomal RNA gene of different isolates. (Table 12). ITS1 and ITS4 primers depicted isolate species identities more than 90% sequence similarity except the isolate number 3 and 11 which showed 88 and 85% sequence similarity, respectively.

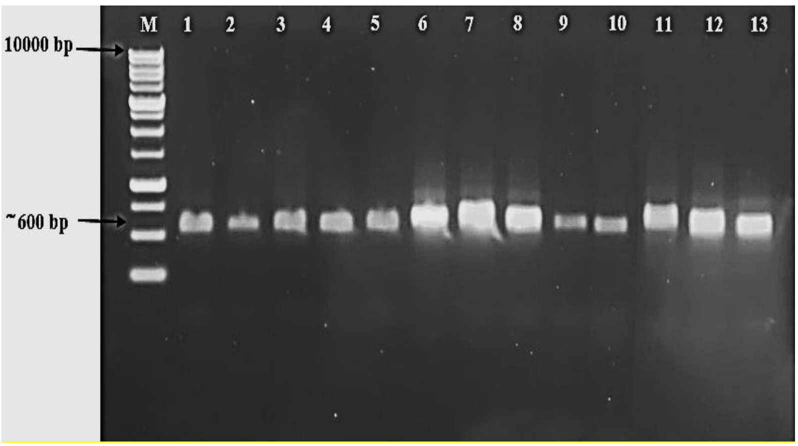


Fig.11. Gel electrophoresis of the PCR product of 13 fungal isolates performed by ITS1 (F) and ITS4 (R) primers and showing ~600 bp amplification.

Results obtained from the BLAST analysis showed that morphologically identified *Fusarium* sp.1 99% nucleotide identities with *Fusarium equiseti* isolate PAK54; 98% nucleotide identities with *Fusarium fujikuroi* isolate AFI SGDB1DT; 96% nucleotide identities with *Microdochium fisheri* strain CBS 242.91; 95% nucleotide identities with *Alternaria alternata* isolate ZB11060984 and *Alternaria tenuissima* strain M9; 93% nucleotide identities with *Bipolaris oryzae* strain L3-2, *Bipolaris sorokiniana* strain JN-1 *Curvularia lunata* isolate CU 563 and *Fusarium proliferatum* isolate ND3; 92% nucleotide identities with *Bipolaris multiformis* isolate CBS480.74; 91% nucleotide identities with *Pestalotiopsis oxyanthi* strain FR3-CGR7; 88% nucleotide identities with *Fusarium oxysporum* strain MD-24 and 85% nucleotide identities with *Phanerochaete chrysosporium* voucher GK 02 (Table 12).

From the comparison between morphological and molecular identification, it was clear that out of 13 fungal isolates, morphological identification of two fungal isolates did not match with molecular identification (Table 12). On the other hand, in case of *Fusarium* sp. it was difficult to identify up to species label by morphological identifications. Besides, two unidentified fungi were detected by the analysis of nucleotide sequences. *Bipolaris sorokiniana* identified as *B. multiformis* and *B. spicifera* identified as *B. sorokiniana.*

Table 12. Identification of fungal isolates using ITS sequence comparison with data from GenBank through BLAST search.

Isolates No.	Morphologically identified fungi	ITS sequence of test fungi matches with						
		Accession No.	Description	Max score	Total score	Query coverage (%)	E-value	Identity (%)
1	***Alternaria alternata***	KX783412.1	*Alternaria alternata* isolate ZB11060984	845	845	62	0.0	95
2	***A. tenuissima***	JX523613.1	*Alternaria tenuissima* strain M9	854	854	64	0.0	95
3	***Bipolaris sorokiniana***	KJ909771.1	*Bipolaris multiformis* isolate CBS480.74	732	732	58	0.0	92
4	***B. oryzae***	KP638341.1	*Bipolaris oryzae* strain L3-2	747	747	67	0.0	93
5	***B. spicifera***	KT310049.1	*Bipolaris sorokiniana* strain JN-1	732	732	66	0.0	93
6	***Curvularia lunata***	KU221491.1	*Curvularia lunata* isolate CU 563	747	747	67	0.0	93
7	***Fusarium* sp.1**	KY523100.1	*Fusarium equiseti* isolate PAK54	909	909	97	0.0	99
8	***Fusarium* sp.2**	KP998524.1	*Fusarium fujikuroi* isolate AFI SGDB1DT	905	905	85	0.0	98
9	***Fusarium* sp.3**	JQ886424.1	*Fusarium oxysporum* strain MD-24	601	601	97	9e-168	88
10	***Fusarium* sp.4**	MG735754.1	*Fusarium proliferatum* isolate ND3	582	582	79	3e-162	93
11	***Pestalotiopsis* sp.**	KP900246.1	*Pestalotiopsis oxyanthi* strain FR3-CGR7	695	695	80	0.0	91
12	**Unidentified fungus 1**	KP859015.1	*Microdochium fisheri* strain CBS 242.91	832	832	98	0.0	96
13	**Unidentified fungus 2**	KP998195.1	*Phanerochaete chrysosporium* voucher GK 02	673	1106	82	0.0	85

The present investigation suggested that molecular technique was more accurate and rapid means of fungal identification. ITS-based molecular methods might be an important complement to conventional mycological detection by culture, which is becoming increasingly important in clinical mycology as well as plant pathology.

4.7. Pathogenicity test of the fungi associated with seeds of BRRI rice varieties

A total of twenty five species of fungi *viz., Alternaria alternata, A. tenuissima, Aspergillus flavus, A. fumigatus, A. niger, A. ochraceus, A. terreus, Bipolaris multiformis, B. oryzae, B. sorokiniana, Chaetomium globosum, Curvularia lunata, Fusarium equiseti, F. fujikuroi, F. oxysporum, F. proliferatum, Microdochium fisheri, Nigrospora oryzae, Penicillium* sp.*, Pestalotiopsis oxyanthi, Phanerochaete chrysosporium, Rhizopus stolonifer, Sarocladium oryzae, Syncephalastrum racemosum* and *Trichoderma viride* were isolated and identified from the seeds of twenty BRRI rice varieties. All the isolated fungi were selected for pathogenicity test.

4.7.1. Pathogenic potentiality of the isolated fungi

Pathogenicity of test fungi were done following seed inoculation technique described by Chowdhury *et al.* (2015). Comparatively fresh seeds of BRRI rice varieties (BRRI dhan 57, 66, 67, 70 and 74) were used for this experiment. Healthy seeds without inoculation with fungus served as control and put inside test tube containing PDA medium and was kept at room temperature. Besides, other seeds were inoculated with individual fungus and kept inside the test tube with PDA medium and kept at room temperature. Twenty five isolated fungi were used separately in this experiment. Three rice seeds were within each test tube. After 21 days of incubation disease symptoms were recorded. Control set did not show any sign or symptoms of disease whereas fungi such as *Bipolaris oryzae, Curvularia lunata, Fusarium equiseti, F. fujikuroi, Microdochium fisheri* and *Nigrospora oryzae* showed disease symptoms and they were treated as pathogenic seed borne fungi (Figs 12-14). The fungi associated with the infected seedlings were isolated by tissue culture method. The individual colony of each fungus showed identical colonies of inoculated fungus (Figs 12-14).

The growth of fungus from the infected seedling satisfied Koch’s postulates. So, after pathogenicity test *Bipolaris oryzae*, *Curvularia lunata*, *Fusarium equiseti*, *F. fujikuroi*, *Microdochium fisheri* and *Nigrospora oryzae* were recognized as seed borne pathogens of BRRI rice varieties.

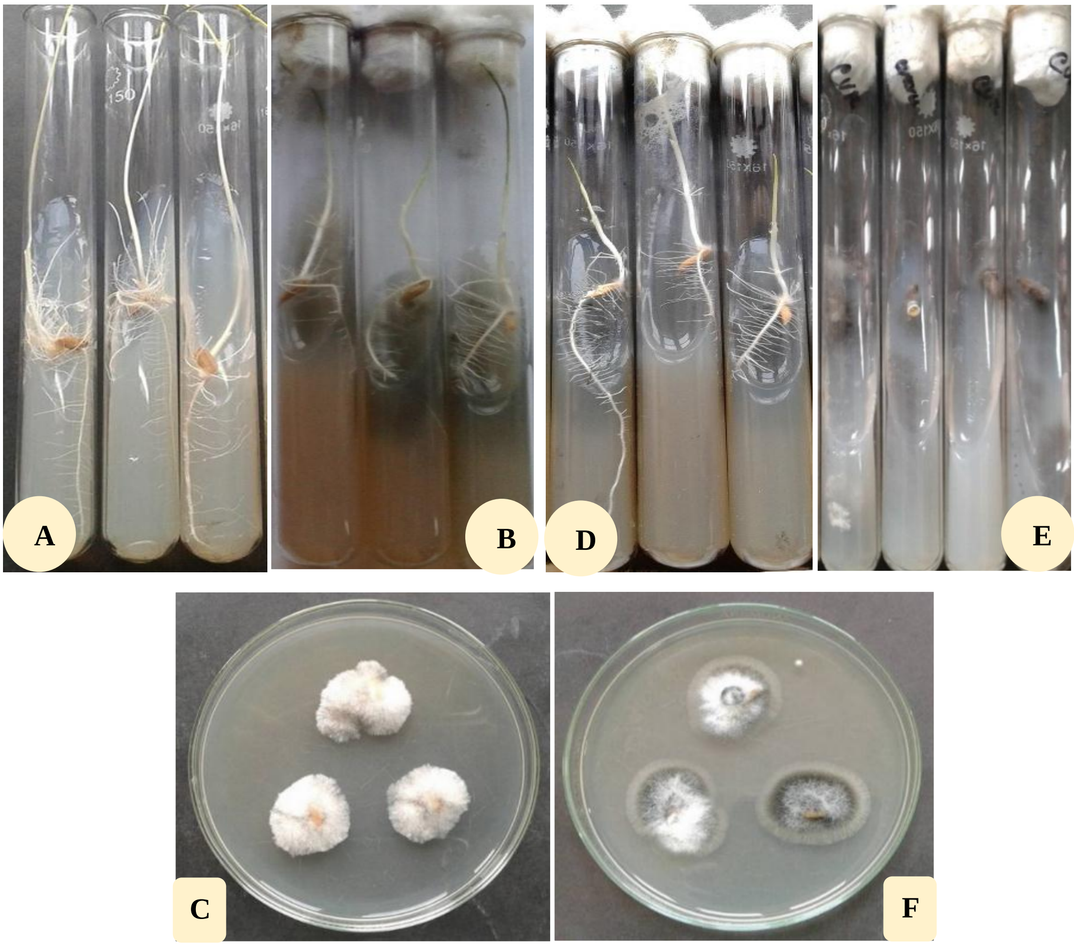


Fig. 12. Pathogenicity test of *Bipolaris oryzae* and *Curvularia lunata.*

A & D: Control healthy seedlings,
B & E: Infected seedlings and
C & F: Re-isolated fungal colonies of *Bipolaris oryzae* and *Curvularia lunata*, respectively.

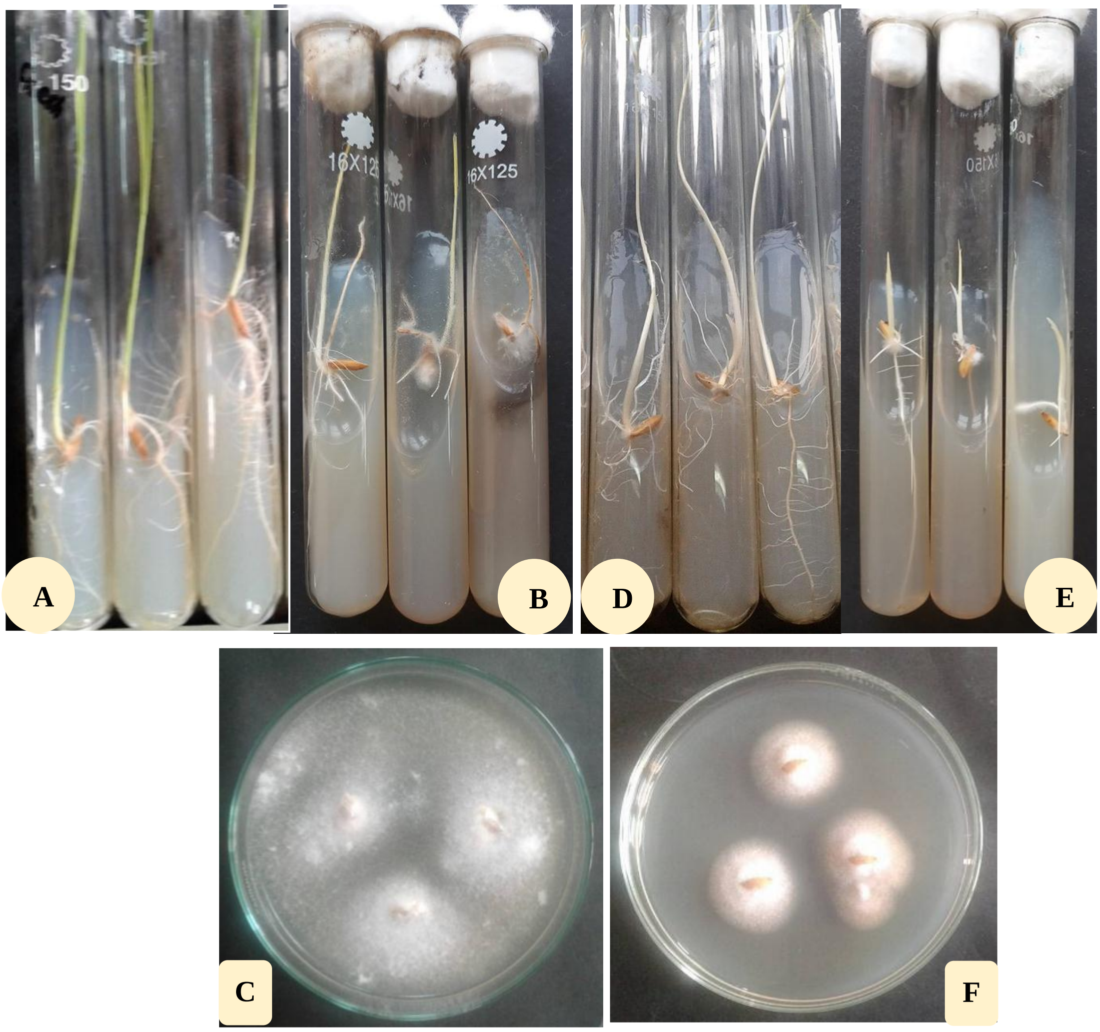


Fig. 13. Pathogenicity test of *Fusarium equiseti* and *Fusarium fujikuroi*.

A & D: Control healthy seedlings,
B & E: Infected seedlings and
C & F: Re-isolated fungal colonies of *Fusarium equiseti* and *Fusarium fujikuroi*, respectively.

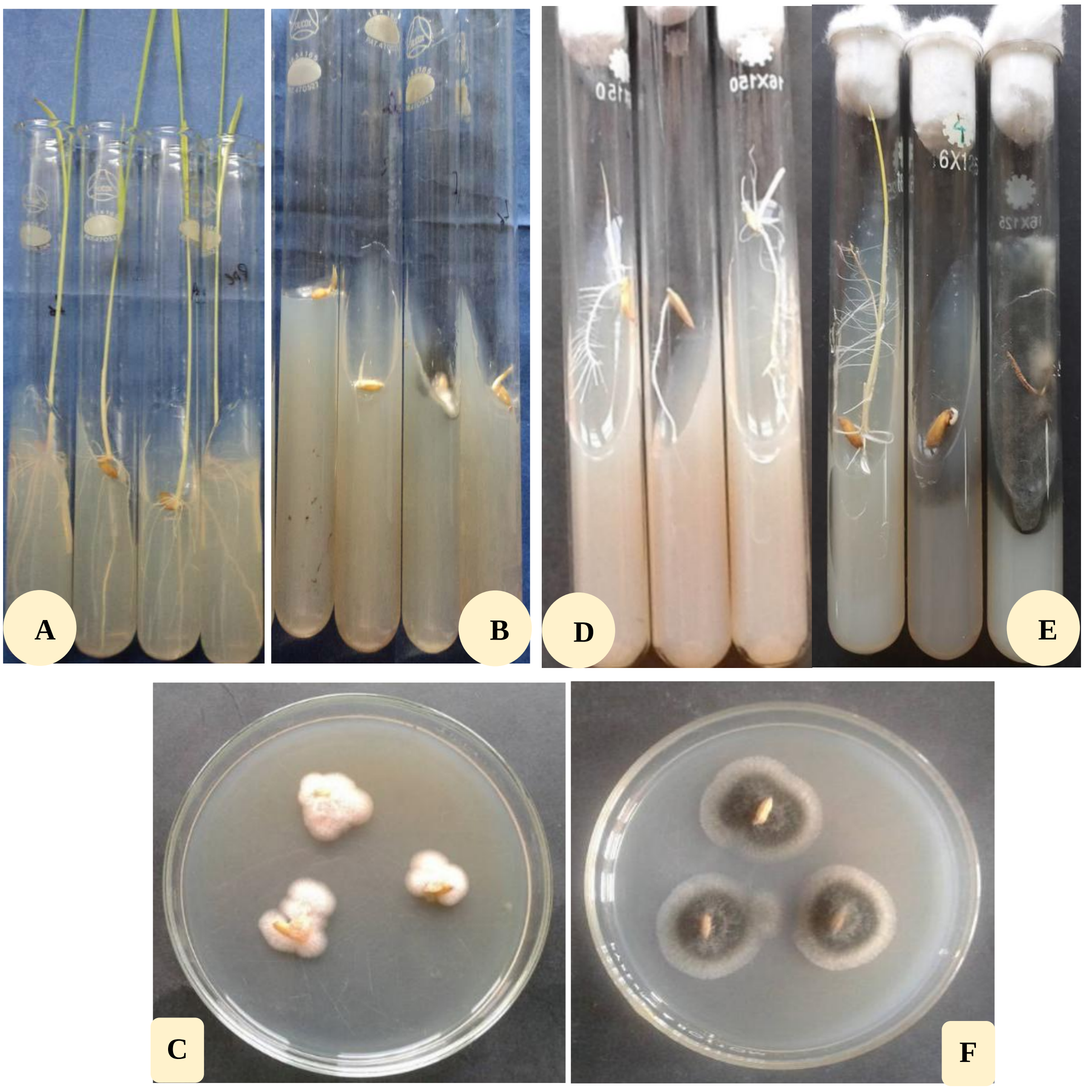


Fig. 14. Pathogenicity test of *Microdochium fisheri* and *Nigrospora oryzae*.

A & D: Control healthy seedlings,
B & E: Infected seedlings and
C & F: Re-isolated fungal colonies of *Microdochium fisheri* and *Nigrospora oryzae* respectively.

4.7.2. Effects of pathogenic fungi on the rice seeds

The effects of six test fungi on the seedlings of BRRI rice varieties are presented in Table 13 and Fig.15. All the tested fungi reduced the length of roots and shoots of rice seedlings. In control seedlings, the average shoot length was 82.33 mm whereas the highest shoot length (52.35 mm) was recorded in *Bipolaris oryzae* inoculated seedlings and lowest shoot length (31.91mm) was recorded on *Curvularia lunata* inoculated seedlings. In control, the average root length was 42.83 mm whereas the highest root length 37 mm was observed in *Fusarium equiseti* inoculated seedlings and lowest root length 20.52 was shown by *Bipolaris oryzae* inoculated seedlings (Table 13 and Fig. 15). Seedlings of control set showed 90% germination whereas the highest germination percentage was 77.67 in *Bipolaris oryzae* inoculated seedlings and the lowest germination percentage was 38% in *Nigrospora oryzae* inoculated seedlings. Control seedlings showed 25.67% seedling mortality whereas the highest mortality percentage was 48 in *Curvularia lunata* inoculated seedlings and the lowest mortality was 27.67% in *Fusarium fujikuroi* inoculated seedlings (Table 13 and Fig. 15).

Table 13. Effects of pathogenic fungi on germination, mortality, root and shoot length of rice seedlings.

Treatments	Germination percentage	Mortality percentage	Average root length (mm)	Average shoot length (mm)
Control	**90^{a}**	**25.67^{d}**	**42.83^{a}**	**82.33^{a}**
Bipolaris oryzae	**77.67^{b}**	**40.83^{ab}**	**20.52^{f}**	**52.35^{b}**
Curvularia lunata	**60.00^{d}**	**48^{a}**	**30^{c}**	**31.91^{e}**
Fusarium equiseti	**73.67^{b}**	**36.33^{bc}**	**37^{b}**	**$45b^{cd}$**
Fusarium fujikuroi	**67.67^{c}**	**27.67^{d}**	**27^{cd}**	**39^{de}**
Microdochium fisheri	**52^{e}**	**42.33^{ab}**	**21.53^{ef}**	**48.54^{bc}**
Nigrospora oryzae	**38^{f}**	**35^{c}**	**24.46^{de}**	**42^{cd}**
CV%	**2.99**	**6.75**	**4.36**	**5.49**

Mean followed by the same letter (s) within a column did not differ significantly and dissimilar letter (s) within a column differ significantly at 5% level by DMRT.

Seed borne pathogens cause enormous losses of crops. Seed borne disease causes seed rot, germination failure and seedling mortality and then reduce rice production. The infected seeds fail to germinate, transmit disease from seed to seedling and from seedling to growing plants. Most pathogens causing abnormal seedling of rice are seed borne (Guerrero *et al.* 1972). Seed borne pathogens also affect seed quality **(**Khare 1999). The highest lethal seed infection caused by *Fusarium moniliforme*, *Trichoconis padwickii* and *Curvularia* spp was observed by Islam *et al.* (2000). The present findings are also in agreement with results of the above mentioned workers.

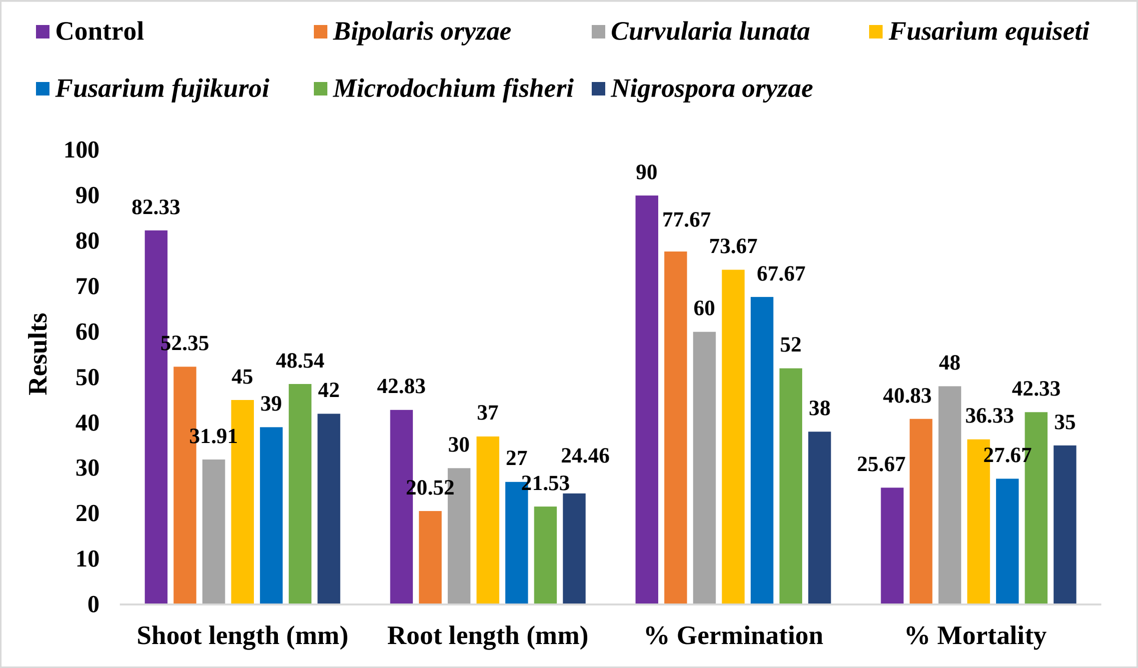


Fig. 15. Effects of pathogenic fungi on germination, mortality, root and shoot length of rice seedlings.

4.8. Transmission of fungal pathogens from seed to seedling

Out of twenty five isolated fungi, six test pathogens *viz., Bipolaris oryzae, Curvularia lunata, Fusarium equiseti, F. fujikuroi, Microdochium fisheri* and *Nigrospora oryzae* were selected for seed to seedling symptoms test in water agar test tubes (Fig. 16). These fungi were tested for their pathogenic effects on rice seeds and seedlings. All the seedlings showed disease symptoms except control and the pathogens were re-isolated from the infected seedlings.

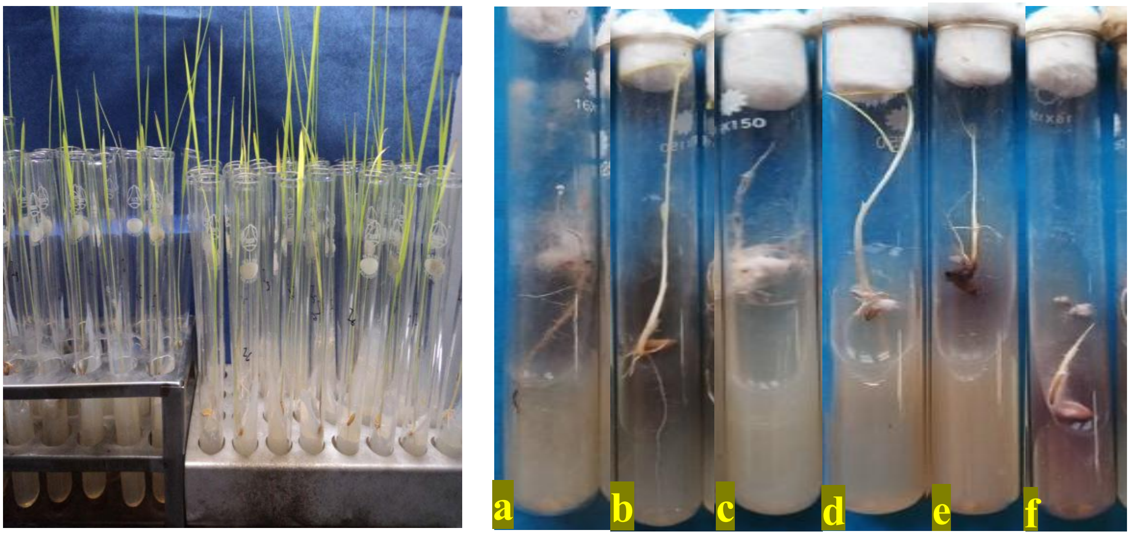
After 21 days of incubation, all the seedlings showed characteristic symptoms excepts control set. Inoculated pathogens were re-isolated from the infected seedlings. All the pathogenic fungi showed seed transmission nature that means pathogenic fungi transferred from seeds to seedlings. In control set, 90.33% seed germination was found whereas the highest germination was 85.35% in *Bipolaris oryzae* inoculated seeds and the lowest germination was 50% in *Curvularia lunata* inoculated seeds. Healthy seedlings (control) showed 20% mortality whereas the highest mortality was 37.67% in *Fusarium equiseti* inoculated seedlings and the lowest mortality was 19% in *Microdochium fisheri* inoculated seedlings (Table 14).

Results presented in Table 14 revealed that, yellowing of leaf followed by blight symptom were observed in seedlings after 21 days of inoculation. The re-isolation of the pathogens was made from infected leaves of seedlings raised from inoculated seeds, which yielded the fungus identical with the original *Bipolaris oryzae. Curvularia lunata* showed seedling rot symptoms, *Fusarium equiseti* and *F. fujikuroi* showed lanky tillers and stunted growth symptoms, *Microdochium fisheri* showed seedling blight symptom and *Nigrospora oryzae* showed seedling rot symptoms. Re-isolation was done in PDA medium. The re-isolated fungi were identical with the original culture.

Table 14. Seed to seedling transmission nature of test pathogens in test tube.

Test pathogen	Seed germination (%)	Seedling mortality (%)	Symptoms on seedling
Bipolaris oryzae	85.35^{a}	30.00^{bc}	Yellowing of leaves, blight
Curvularia lunata	50.00^{e}	27.67^{bc}	Seedling rot
Fusarium equiseti	72.33^{c}	37.67^{a}	Stem rot, stunting, wilting
Fusarium fujikuroi	73.33^{bc}	22.33^{cd}	Lanky tillers
Microdochium fisheri	55.67^{de}	19.00^{d}	Seedling blight
Nigrospora oryzae	62.33^{d}	34.33^{ab}	Seedling rot
Control	90.33^{a}	20.00^{d}	No symptoms
CV%	4.97	10.07	

Mean followed by the same letter (s) within a column did not differ significantly and dissimilar letter (s) within a column differ significantly at 5% level by DMRT.



A B

Fig. 16. Transmission of test pathogens from seeds to seedlings determined by test-tubes seedling symptom test.

A. Healthy seedling without fungal infection, **B.** Seedling infection caused by **(a)** *B. oryzae,* **(b)** *C. lunata,* **(c)** *F. equiseti,* **(d)** *F. fujikuroi,* **(e)** *M. fisheri* and **(f)** *N. oryzae.*

The results on transmission of test pathogens from seed to seedlings in pot experiment are presented in Table 15. The results revealed that six test pathogens found to be transmitted from seed to seedling and had different types of symptoms on seedlings (Fig.17).

The pathogen *Curvularia lunata* showed the highest percentage of seed to seedling transmission i.e., 18.58%. It was followed by *Fusarium fujikuroi* (17.21%), *F. equiseti* (13.14%), *Bipolaris oryzae* (12.92%), *Nigrospora oryzae* (12.26%) and *Microdochium fisheri* (11.91%). Healthy seed (control) did not show any symptoms on seedlings (Table 15).

It was also observed that the symptoms produced in agar test tube method were similar to the symptoms produced in pot culture condition. Hence, it was proved that the six pathogenic seed borne fungi showed seed transmissible in nature.

Table 15. Transmission of test pathogens from seed to seedlings in pot experiment.

Test pathogens	Seed germination (%)	No. of seedlings exhibiting symptoms	Seed to seedling transmission of disease (%)	Symptoms on seedling
Bipolaris oryzae	36.33[c]	5.00[ab]	12.92[bc]	Yellowing of leaves, blight
Curvularia lunata	47.67[b]	8.00[a]	18.58[a]	Seedling rot
Fusarium equiseti	35.50[c]	5.25[ab]	13.14[bc]	Stem rot, stunting, wilting
Fusarium fujikuroi	31.0[cd]	5.00[ab]	17.21[ab]	Lanky tillers
Microdochium fisheri	14.67[e]	3.33[bc]	11.91[c]	Seedling blight
Nigrospora oryzae	28.00[d]	7.33[a]	12.26[c]	Seedling rot
Control	71.00[a]	-	-	No symptoms
CV%	5.91	28.10	13.46	

Mean followed by the same letter (s) within a column did not differ significantly and dissimilar letter (s) within a column differ significantly at 5% level by DMRT.

Fig. 17. Symptoms on rice seedlings in pot experiment after treatment with test pathogens. **A**. Healthy seedling (Control), **B.** *Bipolaris oryzae,* **C.** *Curvularia lunata,* **D.** *Fusarium equiseti,* **E.** *F. fujikuroi,* **F**. *Microdochium fisheri* and **G.** *Nigrospora oryzae.*

4.9. Toxicity of fungicides against the test pathogens

Amongst the ten fungicides used in the present investigation, Bavistin 50WP, Dithane M-45, Knowin 50WP and Score 250 EC was systemic fungicide while Capvit 50WP, Greengel 72 WP, Thiovit 80 WG and Tilt 250 EC were protectant fungicides. Nativo 75 WG and Ridomil Gold 68 WG were both systemic as well as protectant fungicides.

The details of these fungicides given in Table 1. The results with regard to their effect on the radial growth inhibition of *Bipolaris oryzae, Curvularia lunata, Fusarium equiseti, F. fujikuroi, Microdochium fisheri* and *Nigrospora oryzae* owing to 100, 200, 300, 400 and 500 ppm concentrations are presented in Tables 16-21, Figs 18-23 and Plates 7-8. All the fungicides inhibited the radial growth of the six test pathogens. The extent of growth inhibition, however, varied amongst the test pathogens (Figs 18-23).

4.9.1. Toxicity of fungicides against *Bipolaris oryzae*

Amongst the ten fungicides complete inhibition of radial growth of *Bipolaris oryzae* was observed with Bavistin and Tilt at all the treated concentrations. Knowin showed complete growth inhibition of the fungus at 300, 400 and 500 ppm concentrations whereas Nativo showed complete inhibition at 400 and 500 ppm concentrations. Dithane, Greengel and Score showed complete inhibition at 500 ppm concentrations. Capvit, Ridomil and Thiovit showed 82.33, 84.47 and 52.04% inhibition of radial growth of *B. oryzae*, respectively at 500 ppm concentration (Table 16, Fig. 18 and Plates 7-8).

Capvit, Dithane, Greengel, Knowin, Nativo, Ridomil and Score showed 59.89, 60.0, 63.62, 85.53, 62.84, 43.97 and 80.06% radial growth inhibition of the fungus at 200ppm concentration, respectively. They also showed 54.16, 56.83, 44.31, 84.48, 56.08, 31.23 and 75.03% radial growth inhibition of the fungus at 100 ppm concentrations, respectively. The lowest inhibition was shown by Thiovit at all tested concentrations (Table 16, Fig.18 and Plates 7-8).

The toxicity of these fungicides against *Bipolaris oryzae* at 100 ppm concentration in descending order was Bavistin = Tilt > Knowin > Score > Dithane > Nativo > Capvit > Greengel > Ridomil > Thiovit (Table 16).

Different scientists studied with wide range of fungicides and found many of them effective for brown spot of rice disease fungus *Drechslera oryzae* (Poudel *et al.* 2019, Chowdhury and Shamsi 2016, Gupta *et al*. 2013, Shamima *et al*. 2013, Geetha and Sivaprakasam 1993). The results of the present investigation are in agreement with the result of Chowdhury and Shamsi (2016) where they reported complete inhibition of *Drechslera oryzae* at all the treated concentration with Bavistin. Geetha and Sivaprakasam (1993) also reported that Bavistin was effective against *Bipolaris oryzae*, the causal agent of brown leaf-spot of rice. Similarly, Poudel *et al.* (2019) found Tilt as the most effective fungicides to reduce the disease severity against rice brown leaf spot disease caused by *B. oryzae.*

Table 16. Toxicity of fungicides against *Bipolaris oryzae* at different concentrations.

Name of fungicides	% inhibition of radial growth at different concentrations (ppm)				
	100	**200**	**300**	**400**	**500**
Bavistin	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Capvit	54.16^{d}	59.89^{d}	66.46^{d}	74.52^{c}	82.33^{b}
Dithane	56.83^{d}	60.0^{d}	66.60^{d}	72.0^{c}	100^{a}
Greengel	44.31^{e}	63.62^{d}	70.42^{d}	90.00^{b}	100^{a}
Knowin	84.48^{b}	85.53^{b}	100^{a}	100^{a}	100^{a}
Nativo	56.08^{d}	62.84^{d}	70.00^{d}	100^{a}	100^{a}
Ridomil	31.23^{f}	43.97^{e}	57.01^{e}	75.26^{c}	84.47^{b}
Score	75.03^{c}	80.06^{c}	82.0^{c}	86.34^{b}	100^{a}
Thiovit	18.82^{g}	23.0^{f}	24.89^{f}	30.65^{d}	52.04^{c}
Tilt	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
CV%	1.70	2.33	2.12	1.58	1.23

Mean followed by the same letter (s) within a column did not differ significantly and dissimilar letter (s) within a column differ significantly at 5% level by DMRT.

Remarks of efficiency gradient of *Bipolaris oryzae*: Bavistin = Tilt > Knowin > Score > Dithane > Nativo > Capvit > Greengel > Ridomil > Thiovit.

The present findings are also in confirmation with the results reported by Azher *et al.* (2013) where they found Mancozeb as the excellent chemical control agent of brown spot of rice caused by *D. oryzae*. Farid *et al.* (2002) reported that Bavistin, Dithane, Hinosan and Tilt were effective against *Bipolaris oryzae*. Dithane was the best with 100% reduction of the prevalence of the pathogen and inhibited the mycelial growth at 0.3% of the seed weight as seed treatments and 500 ppm as mycelial growth inhibition test which was followed by Tilt, Hinosan and Bavistin. All the test fungicides were effective against *Bipolaris oryzae* at higher concentration. These findings fully supported the results obtained in the present investigation.

Jha *et al.* (2004) reported Bavistin as the best inhibiting fungicide followed by Kavach and Emissan against *Drechslera oryzae* in their studies. Kumar *et al.* (1997) found Mancozeb as the best control agent followed by Thiram against *D. oryzae*. Sisterna and Ronco (1994) reported Dithane as the best fungicide against *B. oryzae*. Dithane significantly reduced rice seed borne infection of *B. oryzae* (Rao and Ranganathaiah 1988). Misra and Singh (1972) reported Dithane as the suitable fungicide followed by Tilt, Hinosan and Bavistin at 500 ppm concentration showed complete growth inhibition of *Bipolaris oryzae*.

4.9.2. Toxicity of fungicides against *Curvularia lunata*

Out of ten fungicides, the complete inhibition of growth of *Curvularia lunata* was observed with Bavistin, Knowin and Tilt at all the treated concentrations. Ridomil and Score showed complete growth inhibition at 400 and 500 ppm concentrations whereas Capvit, Greengel and Thiovit showed 61.88, 82.00 and 65.00% growth inhibition at 500 ppm concentration, respectively. Dithane and Nativo showed complete inhibition at 500 ppm concentration. Capvit, Dithane, Greengel, Nativo and Thiovit showed 53.08, 82.33, 72.0, 84.09 and 50.0% radial growth inhibition at 400 ppm concentration, respectively (Table 17, Fig. 19 and Plates 7-8).

Capvit, Dithane, Greengel, Nativo, Ridomil, Score and Thiovit showed 44.08, 72.99, 62.64, 74.81, 60.70, 65.15 and 44.47% growth inhibition at 300 ppm and 42.20, 64.66, 62.64, 70.66, 55.08, 64.74 and 43.33% radial growth inhibition at 200 ppm concentration, respectively. They also showed 12.17, 50.00, 52.00, 63.33, 47.00, 54.75 and 33.67% radial growth inhibition at 100 ppm concentrations, respectively (Table 17, Fig. 19 and Plates 7-8). The lowest activity was shown by Capvit at 100 ppm concentration. The toxicity of these fungicides against *Curvularia lunata* at 100 ppm concentration in descending order was Bavistin = Knowin = Tilt > Nativo > Score > Greengel > Dithane > Ridomil > Thiovit > Capvit (Table 17).

Table 17. Toxicity of fungicides against *Curvularia lunata* at different concentrations.

Name of fungicides	% inhibition of radial growth at different concentrations (ppm)				
	100	**200**	**300**	**400**	**500**
Bavistin	100[a]	100[a]	100[a]	100[a]	100[a]
Capvit	12.17[f]	42.20[d]	44.08[e]	53.08[d]	61.88[d]
Dithane	50.00[cd]	64.66[bc]	72.99[b]	82.33[b]	100[a]
Greengel	52.00[cd]	62.64[c]	63.00[c]	72.0[c]	82.00[b]
Knowin	100[a]	100[a]	100[a]	100[a]	100[a]
Nativo	63.33[b]	70.66[b]	74.81[b]	84.09[b]	100[a]
Ridomil	47.00[d]	55.08[d]	60.70[c]	100[a]	100[a]
Score	54.75[c]	64.74[bc]	65.15[c]	100[a]	100[a]
Thiovit	33.67[e]	43.33[e]	44.47[d]	50.00[d]	65.00[c]
Tilt	100[a]	100[a]	100[a]	100[a]	100[a]
CV%	2.84	2.97	2.52	2.19	1.06

Mean followed by the same letter (s) within a column did not differ significantly and dissimilar letter (s) within a column differ significantly at 5% level by DMRT.

Remarks of efficiency gradient of *Curvularia lunata*: Bavistin = Knowin = Tilt > Nativo > Score > Greengel > Dithane > Ridomil > Thiovit > Capvit.

Khatun and Shamsi (2016) reported that Bavistin showed complete radial growth inhibition of *Curvularia lunata* at 500 ppm concentration which is similar to the present investigation where Bavistin showed 100% growth inhibition of the fungus at all the tested concentrations. Nahar and Shamsi (2020) worked on five fungicides *viz*., Acrobat, Autostin, Capvit, Nativo and Thiovit where Nativo showed complete growth inhibition of *Curvularia lunata* at all the treated concentrations which is also in agreement of the existing research work. Rao *et al.* (2018) reported that Bavistin and Nativo proved to be effective in inhibiting the mycelial growth of *C. lunata* which is also similar to the present work.

In contrast to the present study Al-Ameen *et al.* (2017) observed complete inhibition of radial growth of *Curvularia lunata* with Dithane and Tilt at 500 ppm concentration and 90% inhibition with Greengel at the same concentration. Besides, Mamun *et al.* (2016) reported that Dithane and Tilt completely inhibited the radial growth of *C. lunata* at 100, 200, 400 and 500 ppm concentrations. But Dithane only showed complete growth inhibition of *C. lunata* at 500 ppm concentration in the present research work.

4.9.3. Toxicity of fungicides against *Fusarium equiseti*

The complete inhibition of growth of *Fusarium equiseti* was observed with Bavistin and Knowin at all the tested concentrations. Nativo and Tilt showed complete growth inhibition at 500 ppm concentration whereas Capvit, Dithane, Greengel, Ridomil, Score and Thiovit showed 80, 60, 76.50, 64.41, 61.50 and 65.50% at the same concentration, respectively (Table 18, Fig. 20 and Plates 7-8).

Tilt also showed complete growth inhibition of *Fusarium equiseti* at 400 ppm concentration whereas Capvit, Dithane, Greengel, Nativo, Ridomil, Score and Thiovit showed 73.72, 40.17, 66.49, 83.67, 61.28, 44.37 and 56.12% growth inhibition, at the same concentration respectively (Table 18, Fig. 20 and Plates 7-8). The toxicity of these fungicides against *Fusarium equiseti* at 100 ppm in descending order was Bavistin = Knowin > Tilt > Greengel > Nativo > Capvit > Score > Dithane > Thiovit > Ridomil (Table 18).

Table 18. Toxicity of fungicides against *Fusarium equseti* at different concentrations.

Name of fungicides	% inhibition of radial growth at different concentrations (ppm)				
	100	**200**	**300**	**400**	**500**
Bavistin	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Capvit	24.23^{de}	42.48^{d}	62.33^{c}	73.72^{c}	80.00^{b}
Dithane	19.02^{fg}	24.23^{f}	32.09^{g}	40.17^{h}	60.00^{f}
Greengel	40.59^{c}	51.84^{c}	62.11^{c}	66.49^{d}	76.50^{c}
Knowin	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Nativo	27.49^{d}	41.66^{d}	46.90^{e}	83.67^{b}	100^{a}
Ridomil	11.52^{h}	42.61^{d}	51.83^{d}	61.28^{e}	64.41^{de}
Score	20.30^{ef}	32.63^{e}	42.00^{f}	44.37^{g}	61.50^{ef}
Thiovit	14.61^{gh}	22.00^{f}	42.00^{f}	56.12^{f}	65.50^{d}
Tilt	63.67^{b}	71.00^{b}	74.30^{b}	100^{a}	100^{a}
CV%	3.83	2.88	2.60	1.87	1.35

Mean followed by the same letter (s) within a column did not differ significantly and dissimilar letter (s) within a column differ significantly at 5% level by DMRT.

Remarks of efficiency gradient of *Fusarium equseti*: Bavistin = Knowin > Tilt > Greengel > Nativo > Capvit > Score > Dithane > Thiovit > Ridomil.

Radial growth inhibition of *Fusarium* spp with Bavistin, Dithane, Contaf, Cupravit and Benlate was reported previously by several scientists (Fravel 2005, Iqbal *et al.* 2010, Chowdhury *et al.* 2015, Mamun *et al.* 2016). Rathod and Pawar (2013) reported that the combination of Dithane and Cupravit at 0.4% significantly reduced the mycelial growth of *Fusarium* spp. Seed treatment with Bavistin, Sunphanate, Nativo and Carzeb completely inhibited the growth of *Fusarium moniliforme in vitro* condition at their low (2.5 gm/L) concentration (Hossain *et al*. 2015).

Chakraborty *et al.* (2009) found Bavistin at 0.5% happened to be the most efficient one against *Fusarium solani* under *in vitro* condition. Ridomil was very effective against *Fusarium oxysporum* (Fravel *et al.* 2005).

It is clear that the fungicides *viz*., Bavistin and Knowin used in the present investigation showed promising results against *Fusarium* spp. whereas Capvit, Dithane, Greengel and Ridomil did not show promising results as compared to other research works. The same fungicides also showed different effects on the different species of the same fungi in the present investigation. This variation might be due to the selection of different species of the same fungi. Singh and Singh (1970) observed that reaction of *Fusarium* spp to fungicides varies from species to species and sometimes even from isolate to isolate of the same species.

4.9.4. Toxicity of fungicides against *Fusarium fujikuroi*

The complete inhibition of growth of *Fusarium fujikuroi* was observed with Score and Tilt at all the tested concentrations. Bavistin, Dithane, Knowin and Nativo showed complete growth inhibition of the fungus at 500 ppm concentration whereas Capvit, Greengel, Ridomil and Thiovit showed 68, 86.67, 78.36 and 77.67% growth inhibition at the same concentrations, respectively (Table 19, Fig. 21 and Plates 7-8). At 400 ppm concentration, Bavistin and Knowin showed 100% growth inhibition which was followed by Thiovit (66.51%), Greengel (64.33%), Dithane (62.00%), Capvit (58.00%), Ridomil (56.00%) and Nativo (43.67%).

Bavistin showed complete inhibition of *F. fujikuroi* at 300 ppm concentration which was followed by Knowin (76.66%), Thiovit (55.43%), Capvit (48.00%), Ridomil (47.67%), Dithane (42.33%), Greengel (36.63%) and Nativo (32.88%) (Table 19, Fig. 21 and Plates 7-8).

The toxicity of these fungicides against *Fusarium fujikuroi* at 100 ppm in descending order was Score = Tilt > Knowin > Bavistin > Thiovit > Dithane > Greengel > Ridomil > Capvit > Nativo (Table 19).

The results of the present work is in agreement with the findings of Bashar (1992) where Bavistin checked the growth of *F. oxysporum f.* sp. *ciceri* completely at 100 ppm concentration. Bashar also noted that Dithane failed to check the growth of the pathogen completely even at 3,000 ppm concentration.

Table 19. Toxicity of fungicides against *Fusarium fujikuroi* at different concentrations.

Name of fungicides	% inhibition of radial growth at different concentrations (ppm)				
	100	**200**	**300**	**400**	**500**
Bavistin	34.11^{c}	52.00^{b}	100^{a}	100^{a}	100^{a}
Capvit	15.33^{f}	27.33^{d}	48.00^{d}	58.00^{c}	68.00^{d}
Dithane	26.33^{de}	33.67^{c}	42.33^{e}	62.00^{bc}	100^{a}
Greengel	22.47^{e}	26.00^{de}	36.63^{f}	64.33^{b}	86.67^{b}
Knowin	42.00^{b}	51.00^{b}	76.66^{b}	100^{a}	100^{a}
Nativo	15.27^{f}	22.38^{e}	32.88^{f}	43.67^{d}	100^{a}
Ridomil	16.33^{f}	23.67^{de}	47.67^{d}	56.00^{c}	78.36^{c}
Score	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Thiovit	27.67^{d}	36.00^{c}	55.43^{c}	66.51^{b}	77.67^{c}
Tilt	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
CV%	3.89	3.50	2.43	2.83	1.38

Mean followed by the same letter (s) within a column did not differ significantly and dissimilar letter (s) within a column differ significantly at 5% level by DMRT.

Remarks of efficiency gradient of *Fusarium fujikuroi*: Score = Tilt > Bavistin > Thiovit > Dithane > Greengel > Ridomil > Capvit > Nativo.

In contrast to the present study, Muthomi *et al.* (2007) reported that Capvit completely inhibited the growth of *Fusarium graminearum* in *in vitro* condition. But in the present investigation Capvit did not show complete growth inhibition of *Fusarium fujikuroi* even at 500 ppm concentration. This variation might be due to the selection of different species of the fungi.

Various works have been done by several scientists in Japan on the resistance phenomena of *Fusarium fujikuroi* against Benzimidazoles and Triflumizole (Ogawa 1988, Hamamura *et al.* 1989, Ishii and Takeda 1989, Ogawa and Takeda 1990, Omatsu *et al.* 1990). The pathogen could tolerate as high as 1000 ppm of the Benzimidazoles (Yasuda 1986).

4.9.5. Toxicity of fungicides against *Microdochium fisheri*

The radial growth of *Microdochium fisheri* was completely inhibited by Bavistin, Knowin and Tilt at all the tested concentrations. Capvit and Greengel showed complete growth inhibition at 500 ppm concentration. At 500 ppm concentration Dithane showed 53.40%, Nativo 54.69%, Ridomil 64.27%, Score 62.23% and Thiovit showed 63.27% growth inhibition of the pathogen. (Table 20, Fig. 22 and Plates 7-8).

Lowest activity was shown by Ridomil at 100 ppm and that was 16.77%. The toxicity of these fungicides against *Microdochium fisheri* at 100 ppm concentration in descending order was Bavistin = Knowin = Tilt > Score > Dithane > Greengel > Nativo > Capvit > Thiovit > Ridomil (Table 20).

Table 20. Toxicity of fungicides against *Microdochium fisheri* at different concentrations.

Name of fungicides	% inhibition of radial growth at different concentrations (ppm)				
	100	200	300	400	500
Bavistin	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Capvit	22.01^{d}	35.62^{c}	36.21^{f}	53.90^{c}	100^{a}
Dithane	29.71^{bc}	36.73^{c}	42.90^{d}	47.12^{d}	53.40^{c}
Greengel	28.64^{c}	34.45^{c}	44.09^{cd}	53.71^{c}	100^{a}
Knowin	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Nativo	25.33^{cd}	36.05^{c}	37.76^{ef}	45.80^{d}	54.69^{c}
Ridomil	16.77^{e}	28.18^{d}	40.39^{de}	59.32^{b}	64.27^{b}
Score	34.05^{b}	41.15^{b}	47.02^{bc}	53.43^{c}	62.23^{b}
Thiovit	22.42^{d}	26.73^{d}	50.30^{b}	52.67^{c}	63.27^{b}
Tilt	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
CV%	3.17	2.79	2.25	2.26	2.56

Mean followed by the same letter (s) within a column did not differ significantly and dissimilar letter (s) within a column differ significantly at 5% level by DMRT.

Remarks of efficiency gradient of *Microdochium fisheri*: Bavistin = Knowin = Tilt > Score > Dithane > Greengel > Nativo > Capvit > Thiovit > Ridomil.

4.9.6. Toxicity of fungicides against *Nigrospora oryzae*

Amongst ten fungicides the complete inhibition of the radial growth of *Nigrospora oryzae* was observed with Bavistin and Tilt at all the tested concentrations. The complete inhibition of radial growth of *Nigrospora oryzae* was also observed with Knowin, Nativo and Score at 300, 400 and 500 ppm concentrations. Dithane and Ridomil also showed complete inhibition at 400 and 500 ppm concentrations. Greengel showed complete inhibition at 500 ppm concentration whereas Capvit showed 65.77% and Thiovit 72.56% growth inhibition at the same concentration, respectively. Capvit, Greengel and Thiovit showed 62.93, 82.33, and 50.63% radial growth inhibition at 400 ppm concentration, respectively (Table 21, Fig. 23 and Plates 7-8). The toxicity of these fungicides against *Nigrospora oryzae* at 100 ppm in descending order was Bavistin = Tilt > Knowin > Score > Dithane > Ridomil > Nativo > Greengel > Capvit > Thiovit (Table 21).

Niaz *et al.* (2008) worked with four fungicides, Neem seed powder and Sodium hypochlorite where Ridomyl was found effective against seed borne mycoflora *Nigrospora* sp. of maize followed by Neem seed powder and Sodium hypochlorite. Ridomil also showed promising effect against *Nigrospora oryzae* in the present investigation.

Table 21. Toxicity of fungicides against *Nigrospora oryzae* at different concentrations.

Name of fungicides	% inhibition of radial growth at different concentrations (ppm)				
	100	200	300	400	500
Bavistin	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Capvit	27.97^{f}	42.40^{f}	54.00^{e}	62.93^{c}	65.77^{b}
Dithane	63.67^{c}	73.67^{c}	82.50^{b}	100^{a}	100^{a}
Greengel	42.98^{e}	53.00^{e}	64.00^{d}	82.33^{b}	100^{a}
Knowin	74.84^{b}	83.18^{b}	100^{a}	100^{a}	100^{a}
Nativo	48.87^{d}	67.24^{d}	100^{a}	100^{a}	100^{a}
Ridomil	53.07^{d}	65.64^{d}	77.05^{c}	100^{a}	100^{a}
Score	66.83^{c}	74.62^{c}	100^{a}	100^{a}	100^{a}
Thiovit	19.23^{g}	32.55^{g}	42.23^{f}	50.63^{d}	72.56^{b}
Tilt	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
CV%	3.05	2.36	6.04	1.50	1.37

Mean followed by the same letter (s) within a column did not differ significantly and dissimilar letter (s) within a column differ significantly at 5% level by DMRT.

Remarks of efficiency gradient of *Nigrospora oryzae*: Bavistin = Tilt > Knowin > Score > Dithane > Ridomil > Nativo > Greengel > Capvit >Thiovit.

In this study, out of ten fungicides Bavistin showed the complete growth inhibition of *B. oryzae*, *C. lunata*, *F. equiseti*, *M. fisheri* and *N. oryzae* at all the tested concentrations. Tilt showed the complete growth inhibition of *B. oryzae*, *C. lunata*, *F. fujikuroi*, *M. fisheri* and *N. oryzae.* Nativo, Score and Knowin were also found as the most effective inhibitor of the test pathogens.

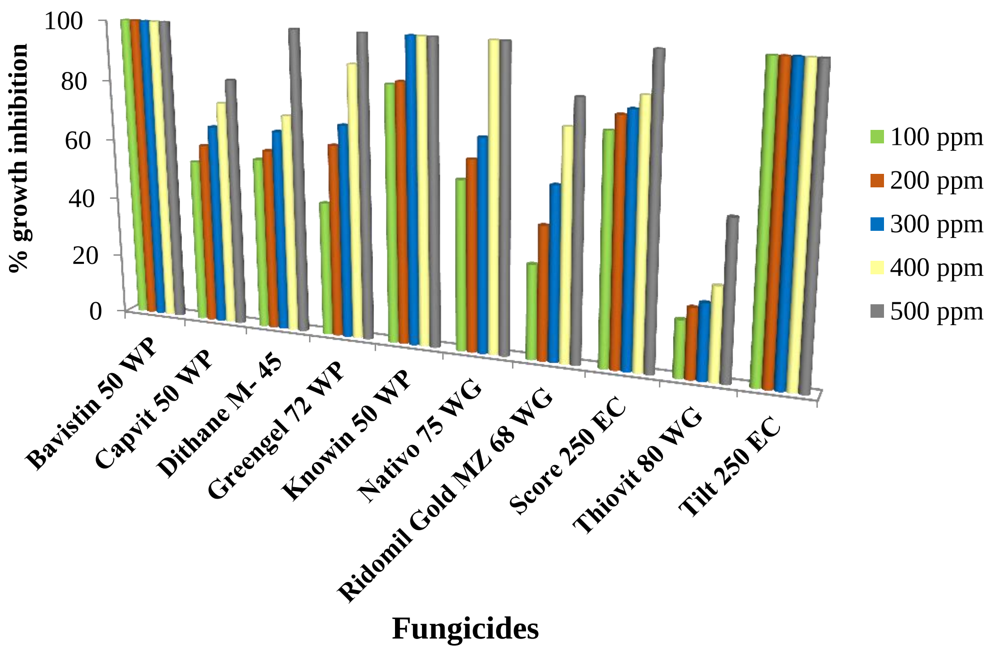


Fig. 18. Per cent growth inhibition of *Bipolaris oryzae* at different concentrations of fungicides.

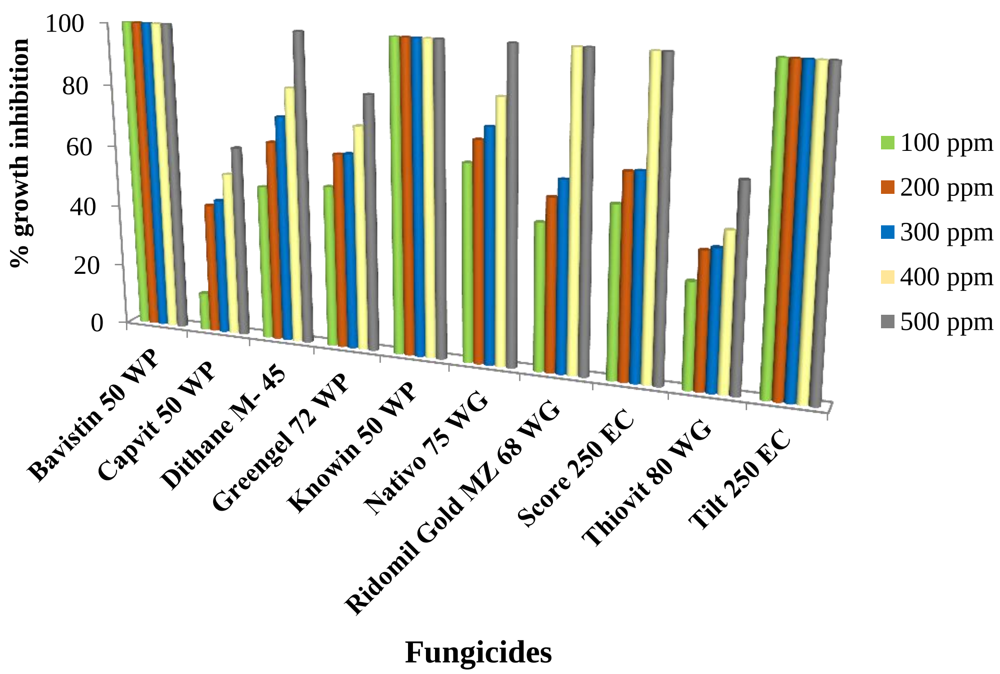


Fig. 19. Per cent growth inhibition of *Curvularia lunata* at different concentrations of fungicides.

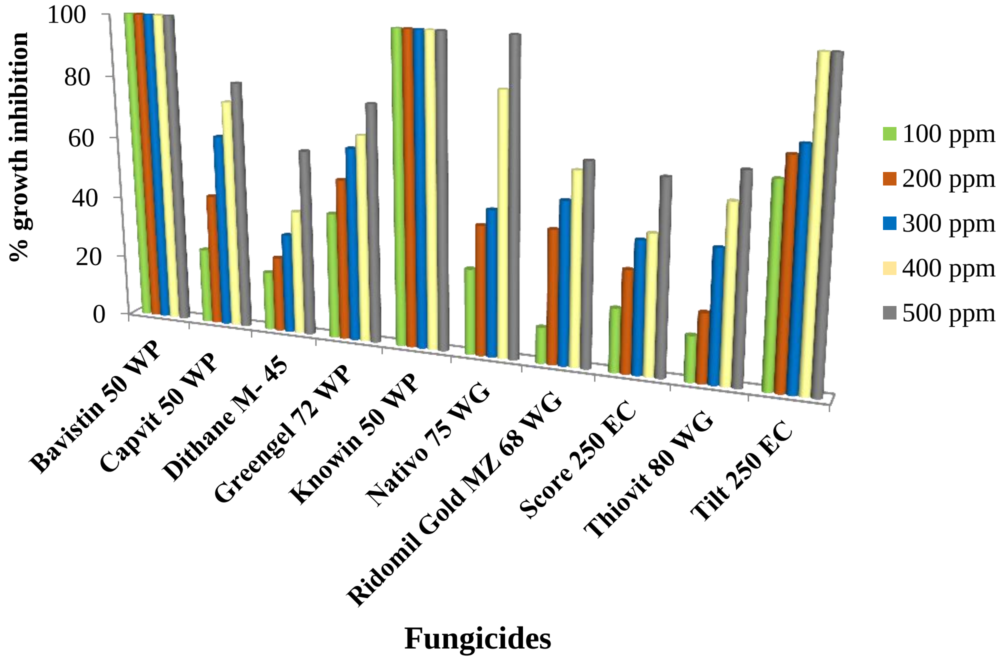


Fig. 20. Per cent growth inhibition of *Fusarium equiseti* at different concentrations of fungicides.

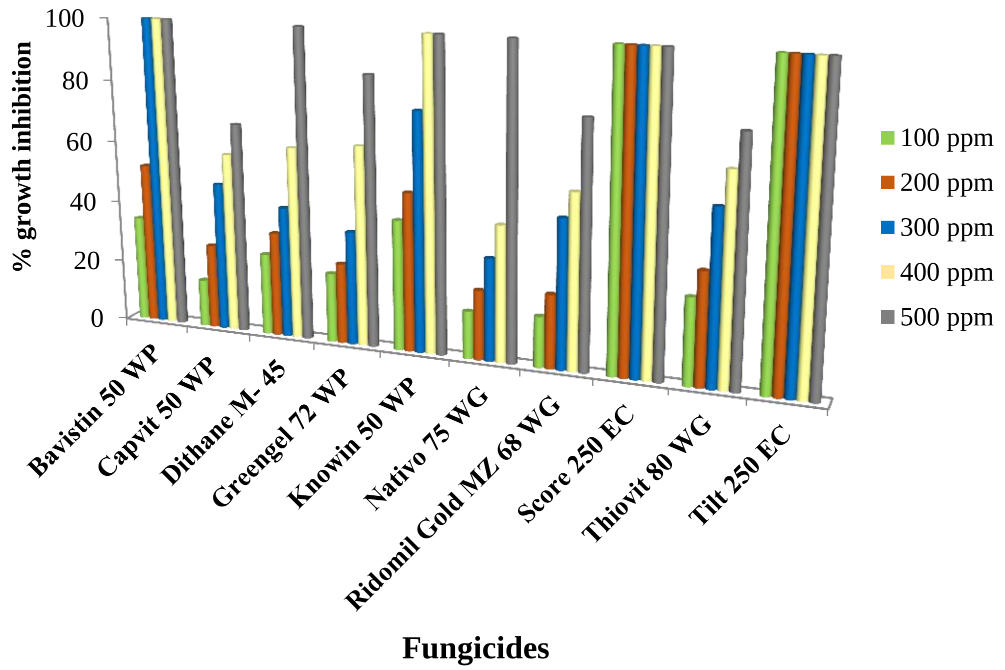


Fig. 21. Per cent growth inhibition of *Fusarium fujikuroi* at different concentrations of fungicides.

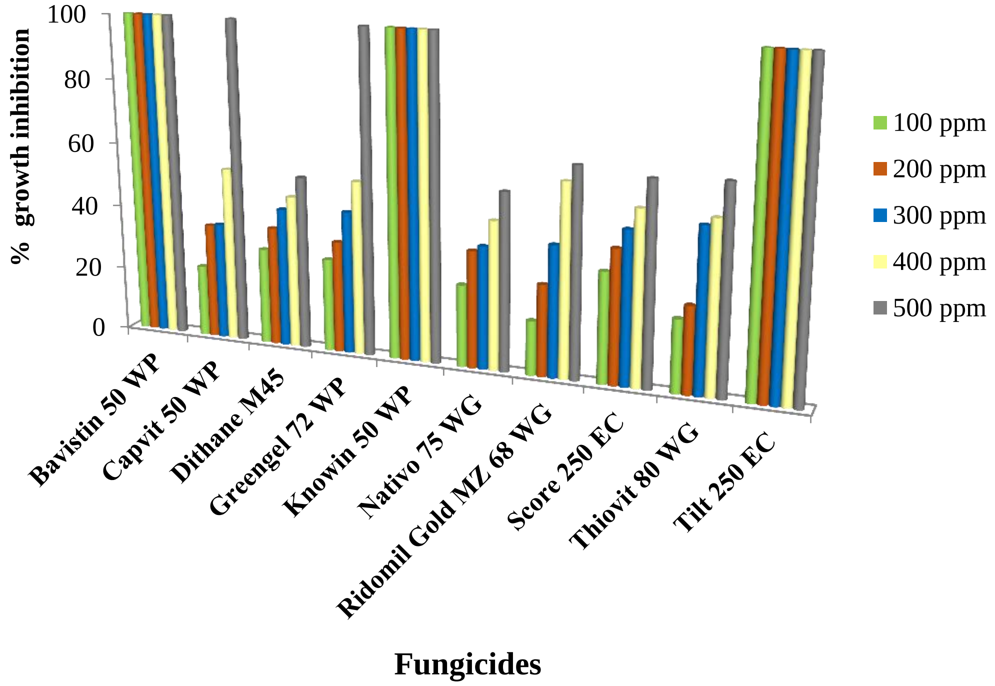


Fig. 22. Per cent growth inhibition of *Microdochium fisheri* at different concentrations of fungicides.

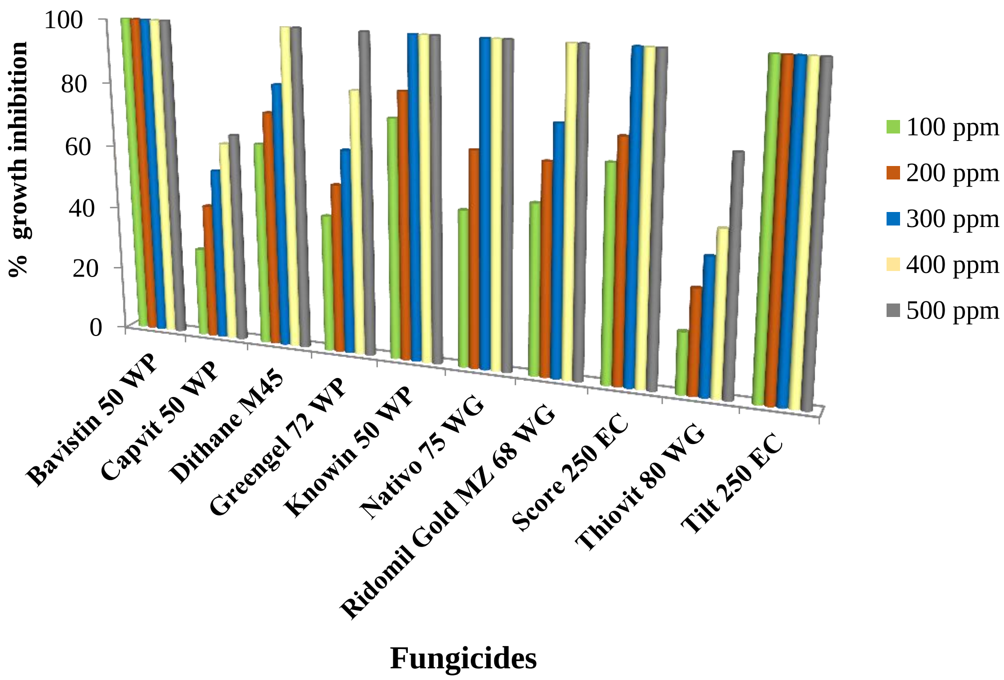


Fig. 23. Per cent growth inhibition of *Nigrospora oryzae* at different concentrations of fungicides.

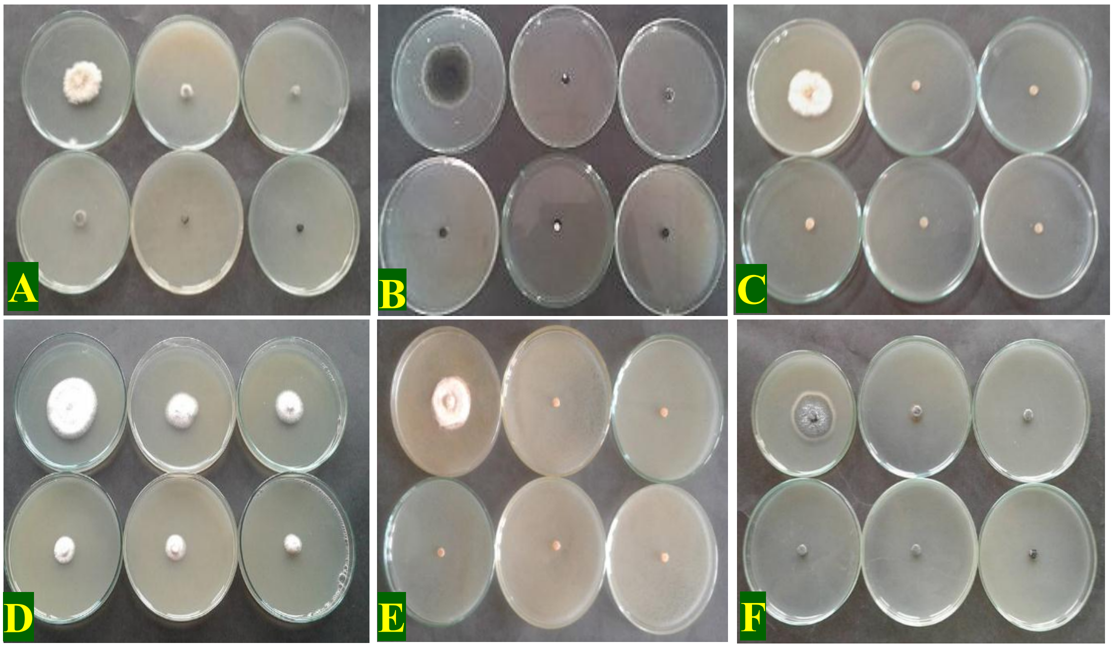


Plate 7. Per cent inhibition of radial growth of **A.** *Bipolaris oryzae*, **B**. *Curvularia lunata*, **C**. *Fusarium equiseti*, **D.** *Fusarium fujikuroi*, **E.** *Microdochium fisheri* and **F.** *Nigrospora oryzae* at 100, 200, 300, 400 and 500 ppm concentrations of Bavistin 50WP.

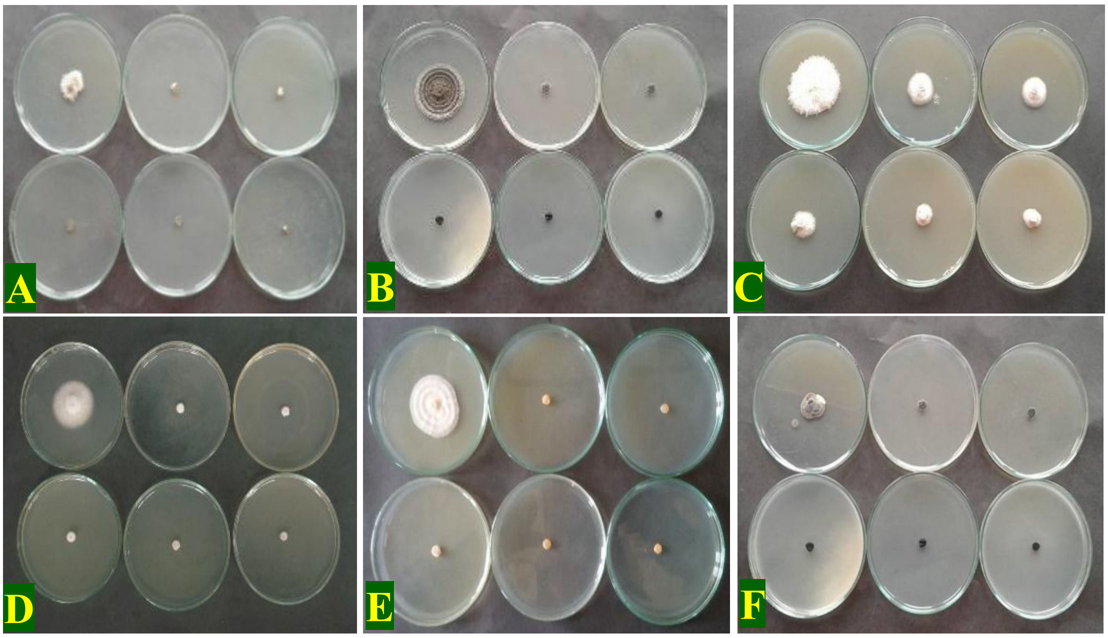


Plate 8. Per cent inhibition of radial growth of **A.** *Bipolaris oryzae*, **B.** *Curvularia lunata*, **C.** *Fusarium equiseti*, **D.** *Fusarium fujikuroi*, **E**. *Microdochium fisheri* and **F.** *Nigrospora oryzae* at 100, 200, 300, 400 and 500 ppm concentrations of Tilt 250 EC.

4.10. Effect of leaf extract of different plants on the radial growth of the test pathogens

A total of ten different plant extracts had been used in the present experiment. The plants were *Adhatoda vasica, Azadirachta indica, Cassia alata, Citrus limon, Datura metel, Heliotropium indicum, Mangifera indica, Moringa oleifera, Psidium guajava* and *Vitex negundo.* Results of plant extracts on the radial growth of *Bipolaris oryzae, Curvularia lunata, Fusarium equiseti, F. fujikuroi, Microdochium fisheri* and *Nigrospora oryzae* are presented in Tables 22-27, Figs 24-29 and Plates 9-10. All the plant extracts showed varied degree of growth inhibition of the test pathogens at 5, 10, 15 and 20% concentrations.

4.10.1. Effect of leaf extracts of ten plants against *Bipolaris oryzae*

Out of the ten plant extracts, *Citrus lemon* showed complete growth inhibition of *Bipolaris oryzae* at 5, 10, 15 and 20% concentrations whereas *Moringa oleifera* showed 100% radial growth inhibition at 10, 15 and 20% concentrations. *Psidium guajava* exhibited 100% radial growth inhibition which was followed by *Datura metel* (84.42%), *Azadirachta indica* (84.02%), *Vitex negundo* (65.20%), *Mangifera indica* (64.51%), *Heliotropium indicum* (61.25%), *Adhatoda vasica* (47.59%) and *Cassia alata* (32.53%) at 20% concentration (Table 22). The inhibition of the pathogen increased with the increase of the concentration of the plant extracts in culture medium.

The order of effectiveness of leaf extracts of ten plants against *Bipolaris oryzae* at 20% concentration was *C. lemon* = *M. oleifera* > *P. guajava* > *D. metel* > *A. indica* > *V. negundo* > *M. indica* > *H. indicum* > *A. vasica* > *C. alata* (Table 22, Fig. 24 and Plates 9-10).

Chowdhury *et al.* (2015) reported that ethanol extract of *D. metel*, *M. indica*, *S. alata* and *A. indica* showed complete radial growth inhibition of *Drechslera oryzae* at 10 and 20% concentrations.

Table 22. Per cent inhibition of radial growth of *Bipolaris oryzae* at different concentrations of plant extracts.

Plants	% inhibition of radial growth at different concentrations			
	5	**10**	**15**	**20**
Adhatoda vasica	26.62[a]	31.87[b]	37.12[b]	47.59[b]
Azadirachta indica	62.63[c]	72.42[b]	76.40[b]	84.02[b]
Cassia alata	12.87[h]	18.27[f]	27.92[e]	32.53[d]
Citrus lemon	100[a]	100[a]	100[a]	100[a]
Datura metel	52.68[d]	62.46[c]	71.42[b]	84.42[b]
Heliotropium indicum	19.72[g]	35.03[e]	42.83[d]	61.25[c]
Mangifera indica	27.07[f]	37.17[e]	45.90[cd]	64.51[c]
Moringa oleifera	82.55[b]	100[a]	100[a]	100[a]
Psidium guajava	47.07[a]	54.39[a]	62.23[a]	100[a]
Vitex nigundo	34.98[e]	42.84[d]	50.10[c]	65.20[c]
CV%	3.27	2.75	2.94	2.25

Means followed by the same letter within a column did not differ significantly at 5% level by DMRT

Remarks of efficiency gradient of *Bipolaris oryzae*: *Citrus lemon = Moringa oleifera > Psidium guajava > Datura metel > Azadirachta indica > Vitex negundo > Mangifera indica > Heliotropium indicum > Adhatoda vasica > Cassia alata.*

Miah *et al* (2017) reported that BARI Gom-26 variety showed lowest fungal infection (6%) owing to *A. indica* and *Thuja occidentalis* plant extract followed by *Citrus limon* (8%), *Allium sativum* (10%) and *Datura metel* (10%). Jadon and Shah (2012) found *A. indica* as the best mycelial growth inhibitor among the perennials against the *Drechslera bicolor*.

Farid *et al.* (2002) evaluated four plant extracts *viz.*, Biskatali, Onion, Garlic and Neem against *Bipolaris oryzae* and found neem and garlic as the most effective plant extracts against *B. oryzae* at 1:1 dilution. Miah *et al.* (1990) reported that *Drechslera oryzae* was best controlled by *Allium sativum* as well as *Azadirachta indica* also inhibited this pathogen significantly over control.

Manimegalai and Ambikapathy (2012) tried to control brown spot disease of rice pathogen by biological active compounds of various plant species such as *Adhatoda vasica*, *Azadirachta indica*, *Datura metal*, *Ocimum sanctum* and *Vitex negundo*. The inhibitory effect of *A. vasica*, *A. indica* and *V. negundo* was more efficient at 20% concentration against *Bipolaris oryzae.* The best inhibitory effect of neem extract against *Bipolaris oryzae* was also observed by Bisht and Khulbe (1995). Similarly, Ganguly (1994) obtained good inhibitory effect of *Azadirachta indica* against *Helmithosporium oryzae.* In the present investigation most of the plant extracts were found active against *Bipolaris oryzae* which is in agreement of the findings of the above mentioned workers.

4.10.2. Effect of leaf extracts of ten plants against *Curvularia lunata*

Among the ten plant extracts, *Azadirachta indica* showed 100% radial growth inhibition of *Curvularia lunata* at all the tested concentrations. At 20% concentrations *Datura metel* showed (95.73%) inhibition of *C. lunata* which was followed by *Citrus lemon* (90%), *Adhatoda vasica* (88.66%), *Cassia alata* (86.40%), *Moringa oleifera* (85.46%), *Psidium guajava* (82.13%), *Mangifera indica* (65.44%), *Heliotropium indicum* (63.10%) and *Vitex negundo* (62.52%) (Table 23). The inhibition of the test pathogen increased with the increase of the concentration of the plant extracts in the culture medium.

The order of effectiveness of leaf extracts of ten plants against *Curvularia lunata* at 20% concentration was *A. indica* > *D. metel* > *C. lemon* > *A. vasica* > *C. alata,* > *M. oleifera* > *P. guajava* > *M. indica* > *H. indicum* > *V. negundo* (Table 23, Fig. 25 and Plates 9-10). Tamuli *et al.* (2014) reported the antifungal activity of ethanolic leaf extract of *V. negundo* against *C. lunata* and showed that the antifungal activity increased with the increase in concentration of the extract which is in agreement with the present findings.

Khatun and Shamsi (2016) found complete inhibition of radial growth of *Curvularia lunata* with plant extract of *A. indica* and *D. metel* at 20% concentration. Similar result was also found in the present investigation.

Table 23. Per cent inhibition of radial growth of *Curvularia lunata* at different concentrations of plant extracts.

Plants	% inhibition of radial growth at different concentrations (%)			
	5	10	15	20
Adhatoda vasica	52.55^{c}	66.60^{d}	84.18^{b}	88.66^{a}
Azadirachta indica	100^{a}	100^{a}	100^{a}	100^{a}
Cassia alata	66.33^{b}	77.66^{c}	82.33^{bc}	86.40^{a}
Citrus lemon	38.25^{d}	52.70^{e}	75.67^{d}	90.00^{a}
Datura metel	55.67^{b}	78.63^{a}	82.06^{a}	95.73^{a}
Heliotropium indicum	20.56^{e}	33.24^{f}	42.52^{f}	63.10^{c}
Mangifera indica	23.00^{e}	32.88^{f}	44.09^{ef}	65.44^{c}
Moringa oleifera	73.67^{b}	78.74^{b}	80.00^{a}	85.46^{a}
Psidium guajava	45.87^{c}	72.27^{cd}	76.92^{cd}	82.13^{b}
Vitex nigundo	22.62^{e}	32.77^{f}	48.40^{e}	62.52^{c}
CV%	4.81	3.89	2.68	1.45

Means followed by the same letter within a column did not differ significantly at 5% level by DMRT

Remarks of efficiency gradient of *Curvularia lunata*: *Azadirachta indica* > *Datura metel* > *Citrus lemon* > *Adhatoda vasica* > *Cassia alata* > *Moringa oleifera* > *Psidium guajava* > *Mangifera indica* > *Heliotropium indicum* > *Vitex negundo.*

Chowdhury *et al.* (2015) reported that 10% ethanol extract of *Mangifera indica* were responsible for complete inhibition of growth of *C. lunata*. Ten per cent ethanol extract of *Datura metel* showed 74% inhibition of radial growth of *C. lunata*. *Datura metel* and *M. indica* showed 52 and 33.33% inhibition of radial growth of *C. lunata* at 5% concentration, respectively. These findings are in agreement with the present investigation.

Extract of neem was reported to be effective in inhibiting mycelial growth of *C. lunata* (Khan and Kumar 1992, Howlader 2003, Mondall *et al.* 2009 which is in agreement with the findings of the present research work.

4.10.3. Effect of leaf extracts of ten plants against *Fusarium equiseti*

The 100% inhibition of mycelial growth of *Fusarium equiseti* was observed with *Azadirachta indica* and *Cassia alata* at 20% concentration which was followed by *Vitex negundo* (81%), *Adhatoda vasica* (80.48%), *Datura metel* (80%), *Heliotropium indicum* (74.29%), *Moringa oleifera* (60.97%), *Psidium guajava* (56.64%), *Mangifera indica* (54.16%) and *Citrus limon* (46.98%) (Table 24). The inhibition of the test pathogen increased with the increase of the concentration of the plant extracts in the culture medium.

The order of effectiveness of leaf extracts of ten plants against *Fusarium equiseti* at 20% concentration was *A. indica* > *C. alata* > *V. negundo* > *A. vasica* > *D.* metel > *H. indicum* > *M. oleifera* > *P. guajava* > *M. indica* > *C. lemon* (Table 24, Fig. 26 and Plate 9-10).

Table 24. Per cent inhibition of radial growth of *Fusarium equiseti* at different concentrations of plant extracts.

Plants	% inhibition of radial growth at different concentrations			
	5	10	15	20
Adhatoda vasica	55.55^{b}	66.66^{b}	75.60^{a}	80.48^{a}
Azadirachta indica	58.53^{a}	65.66^{a}	100^{a}	100^{a}
Cassia alata	53.33^{b}	65.44^{a}	86.66^{a}	100^{a}
Citrus lemon	25.50^{f}	32.52^{f}	40.37^{d}	46.98^{e}
Datura metel	25.83^{f}	38.46^{ef}	44.74^{cd}	80.00^{b}
Heliotropium indicum	41.48^{d}	52.17^{c}	65.54^{b}	74.29^{c}
Mangifera indica	22.51^{f}	44.17^{de}	48.18^{c}	54.16^{d}
Moringa oleifera	36.58^{b}	43.90^{b}	51.20^{b}	60.97^{a}
Psidium guajava	31.65^{e}	41.77^{e}	44.51^{cd}	56.64^{d}
Vitex negundo	42.90^{d}	50.39^{cd}	65.16^{b}	81.00^{b}
CV%	2.68	3.45	2.94	2.82

Means followed by the same letter within a column did not differ significantly at 5% level by DMRT

Remarks of efficiency gradient of *Fusarium equiseti*: *Azadirachta indica* > *Cassia alata* > *Vitex negundo* > *Adhatoda vasica* > Datura metel > *Heliotropium indicum* >*Moringa oleifera* > *Psidium guajava* > *Mangifera indica* > *Citrus lemon*.

Chowdhury *et al*. (2016) reported that 10 and 20% ethanol extracts of ten plants completely inhibited the radial growth of *F. moniliforme*. *Azadirachta indica* and *Citrus medica* showed complete inhibition at 5% concentration against the same pathogen. Madhanraj *et al.* (2010) studied the antifungal ability of some plant extracts against *Fusarium solani* causing wilt disease of banana. The leaves of medicinal plant extract such as *Adhathoda vasica, Azadirachta indica* and *Vitex negundo* was more effective at 20% concentration against the pathogen which is in agreement with the present findings.

4.10.4. Effect of leaf extracts of ten plants against *Fusarium fujikuroi*

The highest growth inhibition of *Fusarium fujikuroi* was observed with *Cassia alata* (82.51%) at 20% concentration which was followed by *Citrus lemon* (75%), *Adhatoda vasica* (74.65%), *Vitex negundo* (73.67%), *Psidium guajava* (66.96%), *Heliotropium indicum* (65.69%), *Moringa oleifera* (64.95%), *Mangifera indica* (56.0%), *Azadirachta indica* (44.89%) and *Datura metel* (44.29%) (Table 25). The inhibition of the pathogen increased with the increase of concentration of the plant extracts in culture medium.

The order of effectiveness of leaf extracts of ten plants against *Fusarium fujikuroi* at 20% concentration was *C. alata* > *C. lemon* > *A. vasica* > *V. negundo* > *P. guajava* > *H. indicum* > *M. oleifera* > *M. indica* > *A. indica* > *D. metel* (Table 25, Fig. 27 and Plates 9-10).

Mamun *et al.* (2016) and Hossain *et al.* (2013) reported the antifungal activity of *Azadirachta indica* and *Datura metel* against *Fusarium* spp. which is in agreement with the findings of the present investigation.

Table 25. Per cent inhibition of radial growth of *Fusarium fujikuroi* at different concentrations of plant extracts.

Plants	% inhibition of radial growth at different concentrations			
	5	**10**	**15**	**20**
Adhatoda vasica	42.33[d]	55.11[c]	62.74[c]	74.65[c]
Azadirachta indica	31.45[a]	36.02[bc]	39.52[b]	44.89[b]
Cassia alata	62.00[b]	66.43[b]	69.88[b]	82.51[b]
Citrus lemon	49.61[a]	57.42[a]	64.84[a]	75.00[a]
Datura metel	15.00[f]	32.50[e]	35.62[f]	44.29[g]
Heliotropium indicum	22.50[e]	42.56[d]	46.64[e]	65.69[e]
Mangifera indica	26.33[e]	33.55[e]	40.00[f]	56.00[f]
Moringa oleifera	41.24[b]	49.48[b]	54.64[b]	64.95[b]
Psidium guajava	22.33[e]	44.98[d]	53.63[d]	66.96[de]
Vitex negundo	40.64[c]	52.00[c]	63.62[c]	73.67[cd]
CV%	3.21	3.52	3.36	3.14

Means followed by the same letter within a column did not differ significantly at 5% level by DMRT.

Remarks of efficiency gradient of *Fusarium fujikuroi*: *Cassia alata* = *Citrus lemon* > *Adhatoda vasica* > *Vitex negundo* > *Psidium guajava* > *Heliotropium indicum* > *Moringa oleifera* > *Mangifera indica* > *Azadirachta indica* > *Datura metel*.

Waris *et al.* (2018) reported the inhibition of radial growth of *F. fujikuroi* with plant extract of *Datura* and Neem which is in agreement with the results of the present investigation.

4.10.5. Effect of leaf extracts of ten plants against *Microdochium fisheri*

The complete growth inhibition of *Microdochium fisheri* was observed with *Citrus lemon* and *Psidium guajava* at all the tested concentrations. *Adhatoda vasica, Cassia alata, Datura metel* and *Vitex negundo* were also responsible for complete inhibition of radial growth at 20% concentration which was followed by *Moringa oleifera* (92.33%), *Heliotropium indicum* (82.42%), *Mangifera indica* (74.50%) and *Azadirachta indica* (74.10%). The inhibition of the pathogen increased with the increase of the concentration of plant extracts in culture medium (Table 26).

The order of effectiveness of leaf extracts of ten plants against *Microdochium fisheri* at 20% concentration was *C. lemon* = *P. guajava* > *A. vasica* > *C. alata* > *V. negundo* > *D. metel* > *M. oleifera* > *H. indicum* > *M. indica* > *A. indica* (Table 26, Fig. 28 and Plates 9-10).

Table 26. Per cent inhibition of radial growth of *Microdochium fisheri* at different concentrations of plant extracts.

Plants	% inhibition of radial growth at different concentrations (%)			
	5	**10**	**15**	**20**
Adhatoda vasica	50.33^{d}	62.63^{d}	75.21^{d}	100^{a}
Azadirachta indica	14.39^{f}	23.05^{f}	45.47^{f}	74.10^{d}
Cassia alata	72.00^{b}	82.67^{b}	92.33^{b}	100^{a}
Citrus lemon	100^{a}	100^{a}	100^{a}	100^{a}
Datura metel	26.67^{e}	51.98^{e}	72.48^{d}	100^{a}
Heliotropium indicum	52.63^{d}	60.72^{d}	74.23^{d}	82.42^{c}
Mangifera indica	52.52^{d}	64.69^{d}	66.29^{e}	74.50^{d}
Moringa oleifera	54.09^{d}	72.71^{c}	82.90^{c}	92.33^{b}
Psidium guajava	100^{a}	100^{a}	100^{a}	100^{a}
Vitex negundo	66.44^{c}	75.00^{c}	92.00^{b}	100^{a}
CV%	2.67	3.09	2.47	1.38

Means followed by the same letter within a column did not differ significantly at 5% level by DMRT.

Remarks of efficiency gradient of *Microdochium fisheri*: *Citrus lemon* = *Psidium guajava* > *Adhatoda vasica* > *Cassia alata* > Datura metel > *Vitex negundo* > *Moringa oleifera* > *Heliotropium indicum* > *Mangifera indica* > *Azadirachta indica.*

Extracts of *C. limon* and *P. guajava* were moderately effective while extracts of *A. indica* and *M. indica* were not so effective in controlling the test pathogen (Fig. 28). Rahman *et al.* (1999) also found moderate effect of neem extract against fungi associated with wheat seeds.

4.10.6. Effect of leaf extracts of ten plants against *Nigrospora oryzae*

The highest inhibition of radial growth of *Nigrospora oryzae* was observed with *Moringa oleifera* (100%) at 20% concentration which was followed by *Vitex negundo* (72.59%), *Azadirachta indica* (65.90%), *Cassia alata* (64.35%), *Heliotropium indicum* (64.00%), *Datura metel* (60.83%), *Psidium guajava* (50.00%), *Mangifera indica* (48.00%), *Adhatoda vasica* (47.15%) and *Citrus lemon* (44.02%) (Table 27). The inhibition of the test pathogen increased with the increase of concentration of plant extracts in the culture medium.

The order of effectiveness of leaf extracts of ten plants against *Nigrospora oryzae* at 20% concentration was *M. oleifera* > *V. negundo* > *A. indica* > *C. alata* > *H. indicum* > *D. metel* > *P. guajava* > *M. indica* > *A. vasica* > *C. lemon* (Table 27, Fig. 29 and Plate 9-10).

Table 27. Per cent inhibition of radial growth of *Nigrospora oryzae* at different concentrations of plant extracts.

Plants	% inhibition of radial growth at different concentrations			
	5	10	15	20
Adhatoda vasica	25.15^{c}	30.07^{cd}	35.44^{c}	47.15^{c}
Azadirachta indica	36.16^{b}	40.29^{bc}	49.85^{c}	65.90^{b}
Cassia alata	26.67^{d}	40.36^{de}	53.50^{c}	64.35^{c}
Citrus lemon	21.94^{c}	26.34^{d}	30.34^{d}	44.02^{c}
Datura metel	32.28^{b}	38.16^{b}	42.50^{bc}	60.83^{b}
Heliotropium indicum	27.83^{cd}	36.70^{e}	50.21^{c}	64.00^{c}
Mangifera indica	20.83^{c}	20.30^{d}	30.45^{d}	48.00^{c}
Moringa oleifera	47.36^{a}	55.45^{a}	76.90^{a}	100^{a}
Psidium guajava	26.52^{d}	40.75^{de}	45.50^{d}	50.00^{d}
Vitex negundo	33.00^{c}	42.94^{d}	51.33^{c}	72.59^{b}
CV%	3.58	2.70	2.63	1.31

Means followed by the same letter within a column did not differ significantly at 5% level by DMRT.

Remarks of efficiency gradient of *Nigrospora oryzae*: *Moringa oleifera* > *Vitex negundo* > *Azadirachta indica* > *Cassia alata* > *Heliotropium indicum* > *Datura metel* > *Psidium guajava* > *Mangifera indica* > *Adhatoda vasica* > *Citrus lemon.*

In contrast to the present study, Bashar and Chakma (2014) reported that the plant extract of *Cassia alata*, *Azadirachta indica* showed 74.78 and 62.03% growth inhibition of *Fusarium oxysporum* at 20% concentration, respectively. The same plant extract also showed different effects on different pathogens in the present investigation. This variation might be due to selection of different test pathogens.

Chakraborty et al. (2009) reported the efficacy of various cell free extracts of the plants against the growth inhibition of the pathogen. The effectiveness of extracts varied significantly with dosage, where 100% inhibition of the pathogen was achieved with neem extracts. Several researches on the fungitoxicity of extracts of various higher plants have indicated the possibility of their exploitation as natural toxicants of fungi for controlling plant diseases (Bashar and Rai 1991, Hossain 1993, Anwar *et al.* 1994, Salma 1995).

Tamuli *et al.* (2014) tested the antifungal activity of ethanolic leaf extract of *V. negundo* against *C. lunata.* The results showed that the antifungal activity increased with the increase in concentration of the extract which is in agreement with the present findings.

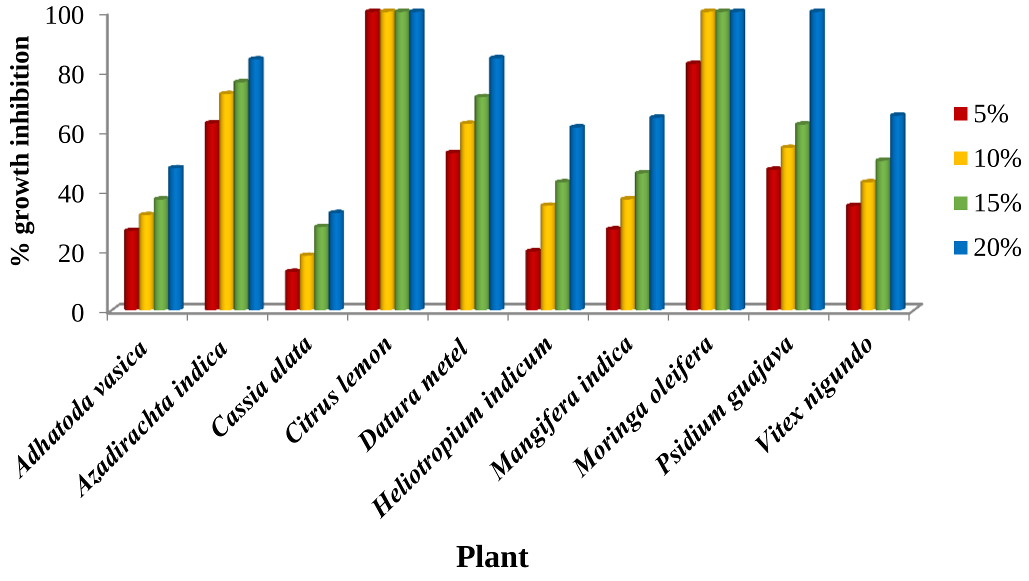


Fig. 24. Effects of plant extracts on the radial growth of *Bipolaris oryzae* at different concentrations.

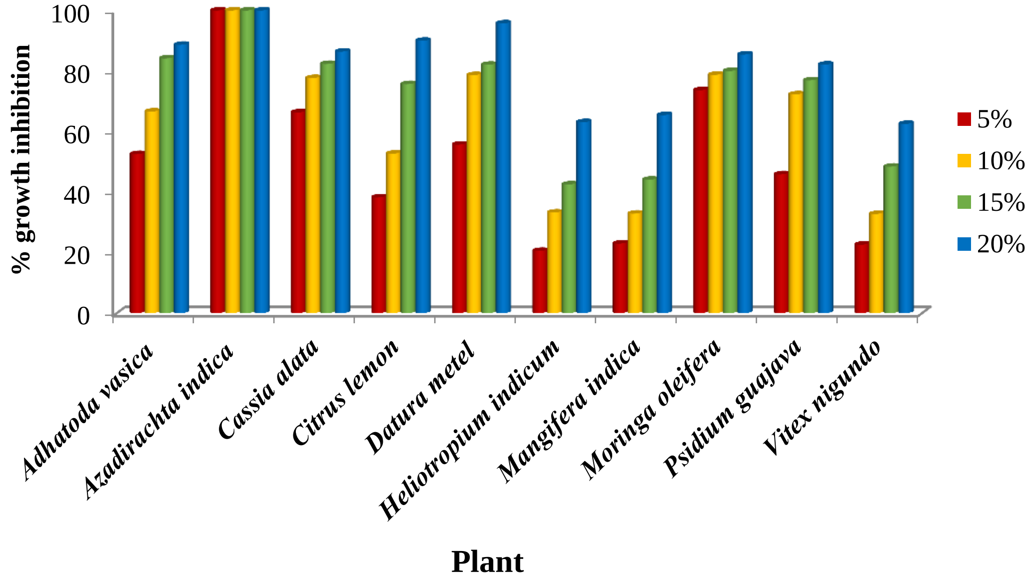


Fig. 25. Effects of plant extracts on the radial growth of *Curvularia lunata* at different concentrations.

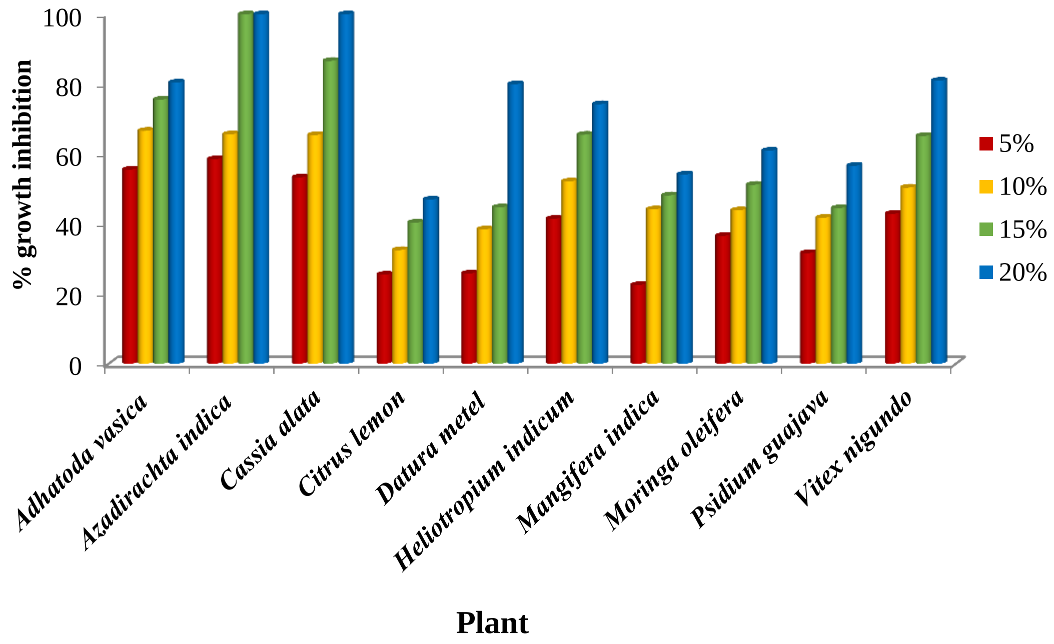


Fig. 26. Effect of plant extracts on the radial growth of *Fusarium equiseti* at different concentrations.

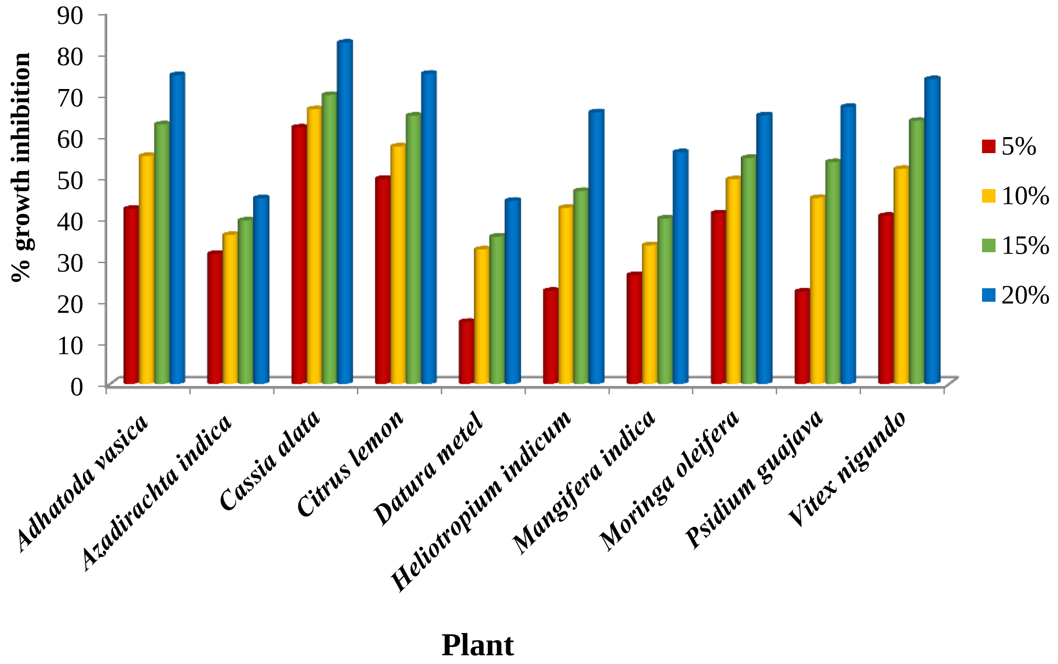


Fig. 27. Effects of plant extracts on the radial growth of *Fusarium fujikuroi* at different concentrations.

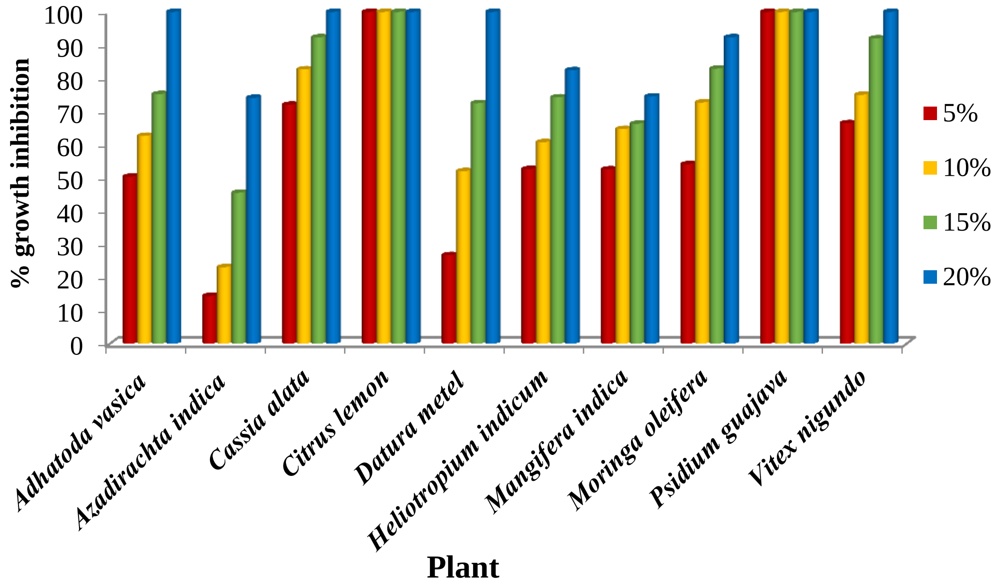


Fig. 28. Effect of plant extracts on the radial growth of *Microdochium fisheri* at different concentrations.

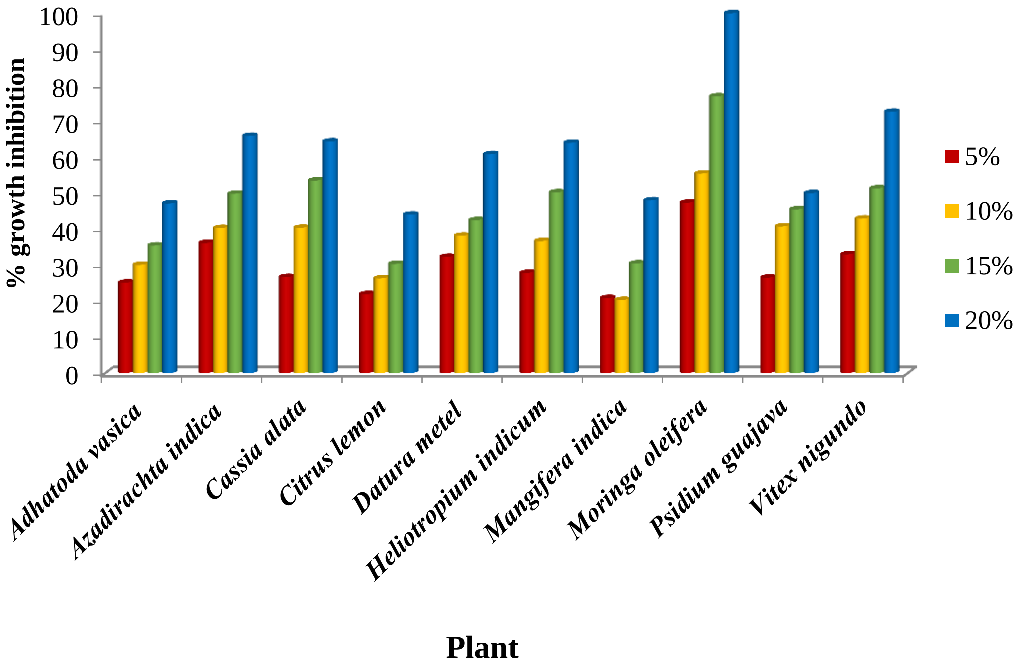


Fig. 29. Effects of plant extracts on the radial growth of *Nigrospora oryzae* at different concentrations of plant extracts.

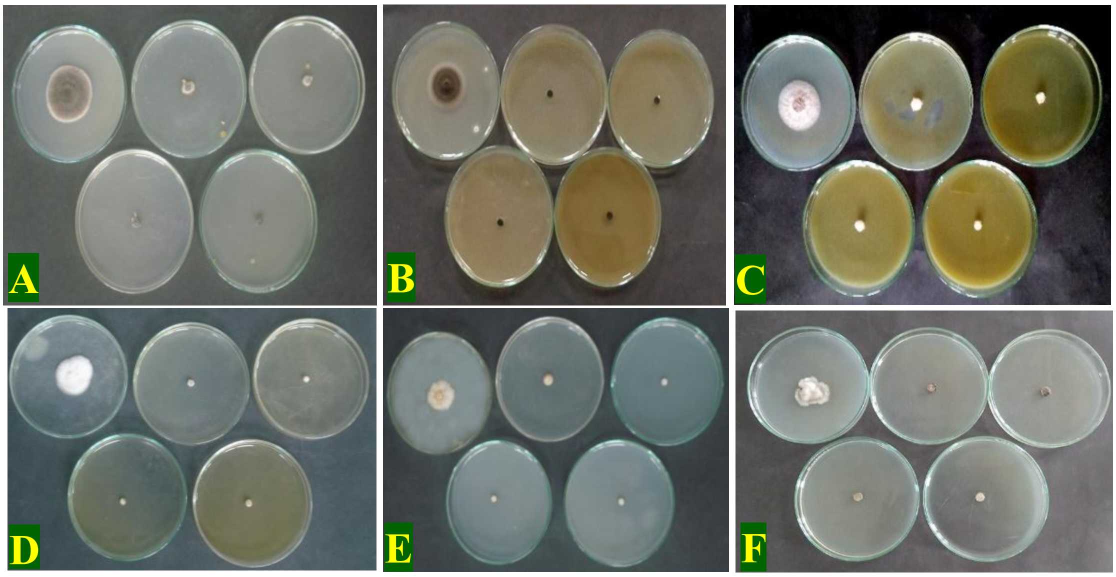


Plate 9. Fungitoxicity of leaf extracts of **A.** *Bipolaris oryzae,* **B.** *Curvularia lunata,* **C.** *Fusarium equiseti*, **D.** *Fusarium fujikuroi*, **E.** *Microdochium fisheri* and **F.** *Nigrospora oryzae* at 5, 10, 15 and 20% concentrations of *Azadirachta indica.*

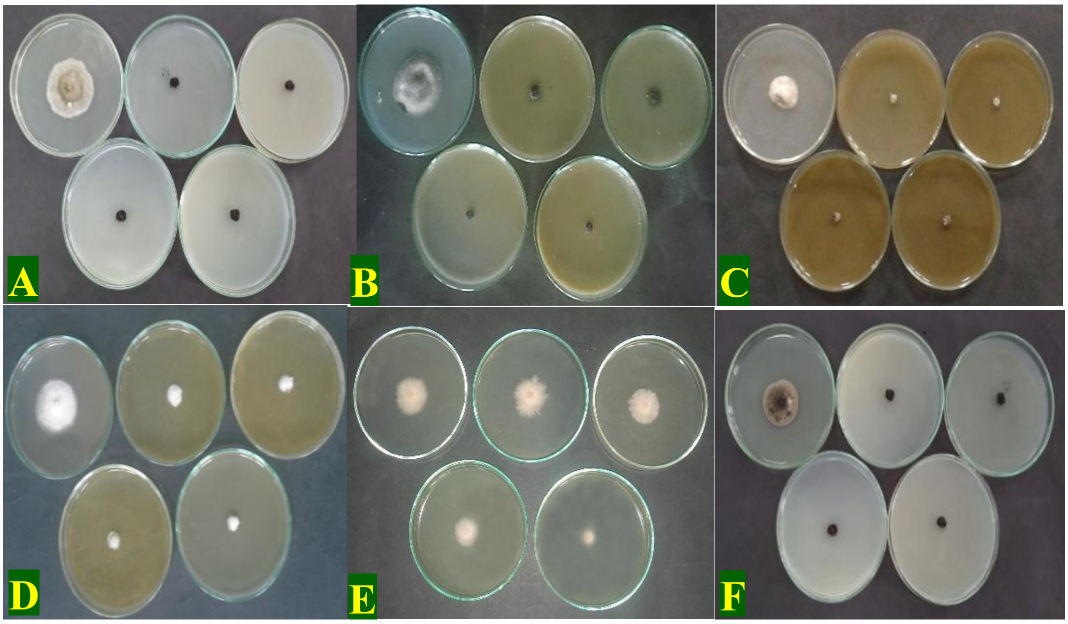


Plate 10. Fungitoxicity of leaf extracts of **A**. *Bipolaris oryzae,* **B**. *Curvularia lunata,* **C.** *Fusarium equiseti*, **D.** *Fusarium fujikuroi*, **E.** *Microdochium fisheri* and **F.** *Nigrospora oryzae* at 5, 10, 15 and 20% concentrations of *Citrus lemon.*

4.11. Colony interactions between the test pathogens and antagonistic fungi.

The colony interaction includes the grading, per cent inhibition of growth of the test pathogens due to the presence of antagonists, intermingling zone and zone of inhibition (Skidmore and Dickinson 1976). The interactions were recorded after 5 days of inoculation. Assessment of colony interaction between the fungi was done in terms of 'grades' with the help of colony interaction model of Skidmore and Dickinson (1976) presented in the appendix IV which is primarily based on the observation of Porter (1924), and Dickinson and Boardman (1970).

The results of colony interactions have been summarized in Tables 28-33, Figs 30 and Plates 11-16. Different antagonistic effects of the antagonistic fungi were noted against the test pathogens. Grade Bi type was found to be the most commonly encountered type of colony interaction and grade C type was found in rare case. From the results it is evident that *Aspergillus flavus, A. fumigatus, A. niger* and *T. viride* exhibited strong antagonistic effect against the test pathogens.

The results of colony interactions between *Bipolaris oryzae* and antagonistic fungi are presented in Table 28, Figs 30 and Plate 11. It is evident that grade Bi was very common. *Aspergillus fumigatus* showed C type of interaction. The maximum inhibition of radial growth of *B. oryzae* was observed due to *Trichoderma viride* (61.67%) followed by *Aspergillus flavus* (60%), *A. fumigatus* (44.44%) and *A. niger* (40%). Lowest intermingled zone was found in *A. flavus* and *A. niger* (0.2cm).

The results of colony interactions between *Curvularia lunata* and antagonistic fungi are presented in Table 29, Figs 30 and Plate 12. Grade Bi type of interactions was common in all the cases. The highest inhibition of radial growth of *C. lunata* was observed with *Trichoderma viride* (63.64%) which was followed by *Aspergillus fumigatus* (45.46%), *A.*

flavus (42.86%) and *A. niger* (37.50%). Intermingled zone was common in all the cases and lowest was noticed in *A. niger* (0.15 cm).

The data obtained due to colony interactions between *Fusarium equiseti* and antagonistic fungi are shown in Table 30, Figs 30 and Plate 13. Most of the antagonists exhibited grade Bi type of colony interaction (Plate 13). *Aspergillus fumigatus* showed C type of interaction. The maximum inhibition of radial growth of *F. equiseti* was recorded with *Trichoderma viride* (70.58%) followed by *Aspergillus flavus* (36.37%), *A. niger* (30.76%) and *A. fumigatus* (30%). Inhibition zone was notice in case of *A. niger*.

The results of colony interactions between *Fusarium fujikuroi* and antagonistic fungi are presented in Table 31, Figs 30 and Plate 14. *Aspergillus niger* showed C type of interaction. The maximum inhibition of radial growth of the pathogen was exhibited by *Trichoderma viride* (87.15%) which was followed by *Aspergillus flavus* (46.67%), *A. fumigatus* (30.76%) and *A. niger* (13.33%).

The results of colony interactions between *Microdochium fisheri* and antagonistic fungi are presented in Table 32, Figs 30 and Plate 15. *Aspergillus flavus* and *A. niger* showed grade C type of interaction. The highest inhibition of radial growth of *M. fisheri* was observed with *Trichoderma viride* (65.35%) followed by *Aspergillus fumigatus* (52%), *A. flavus* (50%) and *A. niger* (48.25%).

The data obtained due to colony interactions between *Nigrospora oryzae* and antagonistic fungi are shown in Table 33, Figs 30 and Plate 16. Grade Bi type of interactions was common in all the cases. The maximum inhibition of radial growth of *N. oryzae* was recorded with *Trichoderma viride* (60.64%) followed by *Aspergillus fumigatus* (45.00%), *A. flavus* (40%) and *A. niger* (35.50%).

The intermingled zone between the antagonistic fungi and test pathogens *viz*., *Bipolaris oryzae, Curvularia lunata, Fusarium equiseti, F. fujikuroi, Microdochium fisheri* and

Nigrospora oryzae were very common (Tables 28-33). The maximum intermingled zone was observed in *Trichoderma viride* (Tables 28-33, Plates 11-16). *Trichoderma viride* grew over the colony of the *B. oryzae, C. lunata, F. equiseti, F. fujikuroi, M. fisheri* and *N. oryzae*. The inhibition zone between the antagonistic fungi and test pathogens *viz., F. fujikuroi, M. fisheri, B. oryzae* and *F. equiseti* were found in rare cases. The maximum inhibition zone was observed in case of *Aspergillus flavus* (Tables 28-33 and Plates 11-16). It is evident from the results that *Trichoderma viride* was found to be the most antagonistic fungi against the test pathogens.

Table 28. Colony interactions between *Bipolaris oryzae* and antagonistic fungi.

Antagonists	Grade*	% inhibition of colony of the pathogen	Intermingled zone (cm)	Inhibition zone (mm)
Aspergillus flavus	Bi	60.00	0.2	-
A. fumigatus	C	44.44	-	0.2
A. niger	Bi	40.00	0.2	-
Trichoderma viride	Bi	61.67	0.3	-

'–' = absent

Grade A: Mutually intermingling growth were both fungi grew into one another without any showing sign of interaction.

Grade Bi: Intermingling growth where the test fungus grew over the test pathogen either above or below or both resulting in suppression of growth of the test pathogen.

Grade Bii: Intermingled growth where the test pathogen grew over the test fungus resulting in reduction of growth of the test fungus.

Grade C: Slight inhibition where both the test pathogen and test fungus approached each other until almost in contact, leaving a narrow demarcation line (1-2) mm.

Table 29. Colony interactions between *Curvularia lunata* and antagonistic fungi.

Antagonists	Grade*	% inhibition of colony of the pathogen	Intermingled zone (cm)	Inhibition zone (mm)
Aspergillus flavus	Bi	42.86	0.2	-
A. fumigatus	Bi	45.46	0.2	-
A. niger	Bi	37.50	0.15	-
Trichoderma viride	Bi	63.63	0.3	-

'–' = absent, Abbreviations are similar as in Table 28.

Table 30. Colony interactions between *Fusarium equiseti* and antagonistic fungi.

Antagonists	Grade*	% inhibition of colony of the pathogen	Intermingled zone (cm)	Inhibition zone (mm)
Aspergillus flavus	Bi	36.37	0.1	-
A. fumigatus	C	30.00	0.3	-
A. niger	Bi	30.76	-	0.1
Trichoderma viride	Bi	70.58	0.25	-

'–' = absent, Abbreviations are similar as in Table 28.

Table 31. Colony interactions between *Fusarium fujikuroi* and antagonistic fungi.

Antagonists	Grade*	% inhibition of colony of the pathogen	Intermingled zone (cm)	Inhibition zone (mm)
Aspergillus flavus	Bi	46.67	-	0.2
A. fumigatus	Bi	30.76	0.3	-
A. niger	C	13.33	-	0.1
Trichoderma viride	Bi	87.15	0.25	-

'–' = absent, Abbreviations are similar as in Table 28.

Table 32. Colony interactions between *Microdochium fisheri* and antagonistic fungi.

Antagonists	Grade*	% inhibition of colony of the pathogen	Intermingled zone (cm)	Inhibition zone (mm)
Aspergillus flavus	C	50.00	-	0.2
A. fumigatus	Bi	52.00	0.2	-
A. niger	C	48.25	-	0.1
Trichoderma viride	Bi	65.35	0.6	-

'–' = absent, Abbreviations are similar as in Table 28.

Table 33. Colony interactions between *Nigrospora oryzae* and antagonistic fungi.

Antagonists	Grade*	% inhibition of colony of the pathogen	Intermingled zone (cm)	Inhibition zone (mm)
Aspergillus flavus	Bi	40.00	0.2	-
A. fumigatus	Bi	45.00	0.1	-
A. niger	C	35.50	-	-
Trichoderma viride	Bi	60.64	0.2	-

'–' = absent, Abbreviations are similar as in Table 28.

In contrast to the present study, Akter *et al.* (2014) reported that in dual culture colony interaction *Aspergillus niger, Trichoderma viride, A. flavus* and *A. fumigatus* showed 68.66, 57.24, 54.19 and 50.25% growth inhibition of *Colletotrichum* sp., respectively. Again *Aspergillus niger, Trichoderma viride, A. flavus* and *A. fumigatus* showed 75.87, 75.5, 51.78 and 45.52 % growth inhibition of *Curvularia lunata*, respectively. Further, *T. viride, A. niger, A. flavus* and *A. fumigatus* showed 56.52, 50.70, 47.36 and 46.15% growth inhibition of *Fusarium semitectum*, respectively.

Bashar and Chakma (2014) reported that in dual culture colony interaction *A. niger, T. viride, A. flavus* and *A. fumigatus* showed 65.21, 64.24, 57.14 and 34.78% growth inhibition of *F. oxysporum*, respectively. Tapwal *et al*. (2015) reported that in dual culture colony interaction *T. viride* showed 12.50% growth inhibition of *C. gloeosporioides.*

Bhale *et al*. (2013) reported that in dual culture colony interaction *T. viride* showed 74.40% growth inhibition of *Geotrichum candidum* causing fruit rot diseases on Sapodilla (*Manilkara zapota* L.). The same antagonists also showed different effects on different fungi in the present investigation. This variation might be due to selection of different test pathogens.

Khair and Subash (2018) reported that seed-borne fungi cause enormous losses in rice production. Six antagonistic fungi comprising three species of *Trichoderma viz*. *T. viride, T. harzianum* and *T. hamatum* and three species of *Aspergillus viz*., *A. flavus*, *A. niger* and *A. terreus*, were used against five important rice pathogenic fungi i.e., *Bipolaris oryzae, Curvularia lunata, Fusarium moniliformae, Sarocladium oryzae* and *Trichocoins padwickii* for the purpose of biological control. The highest radial growth inhibition exhibited by *A. niger* and *T. harzianum* against *Sarocladium oryzae* (48.07%) and *Trichocoins padwickii* (65.40%). *Aspergillus terreus* produced distinct inhibition zone against all the five rice pathogens in dual culture.

Four antagonistic fungi inhibited the growth of all the test pathogens in varied degrees in dual cultures experiments on agar plates (Tables 28-33). Papavizas (1985) has reviewed the biology of *Trichoderma*, which is a fast growing and antagonistic fungus to many pathogenic and non-pathogenic fungi. Due to fast growing nature, rapid sporulation and toxic metabolite producing capacity, the antagonistic activity of *Trichoderma* sp. is potential (Garrett 1981). Hence, high antagonistic activity of the *Trichoderma viride* observed against the test pathogens might be due to the above reasons.

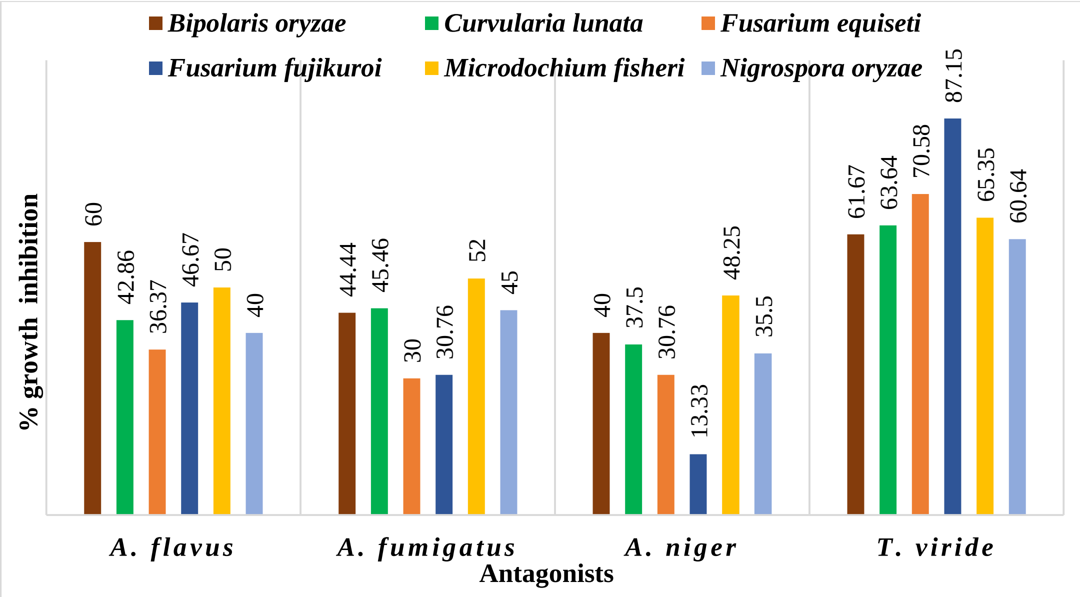


Fig. 30. Colony interactions between the test pathogens and antagonistic fungi.

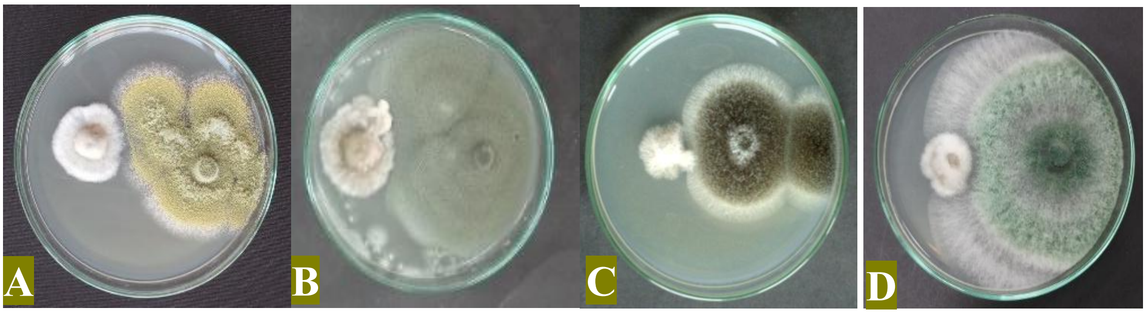


Plate 11. Colony interactions between *Bipolaris oryzae* and antagonists.

A. *Bipolaris oryzae* and *Aspergillus flavus*
B. *B. oryzae* and *A. fumigatus*
C. *B. oryzae* and *A. niger*
D. *B. oryzae* and *Trichoderma viride*.

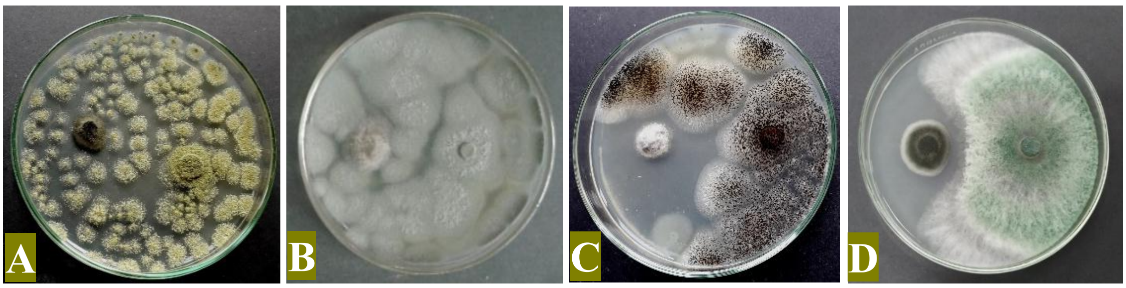


Plate 12. Colony interactions between *Curvularia lunata* and antagonists.

A. *Curvularia lunata* and *Aspergillus flavus*
B. *C. lunata* and *A. fumigatus*
C. *C. lunata* and *A. niger*
D. *C. lunata* and *Trichoderma viride*.

Plate 13. Colony interactions between *Fusarium equiseti* and antagonists.

A. *Fusarium equiseti* and *Aspergillus flavus*
B. *F. equiseti* and *A. fumigatus*
C. *F. equiseti* and *A. niger*
D. *F. equiseti* and *Trichoderma viride*.

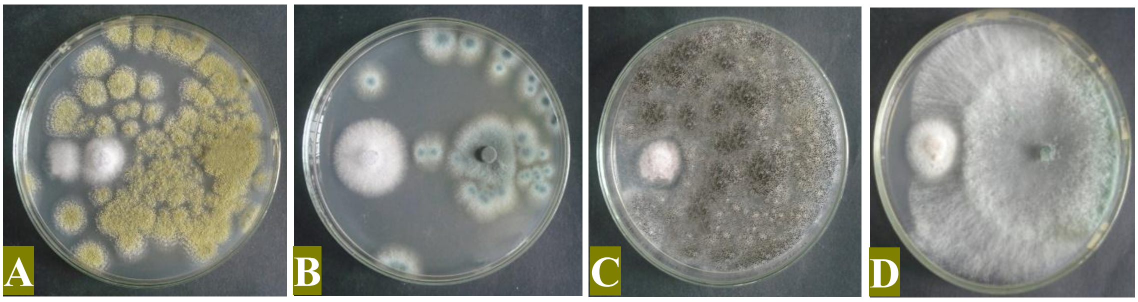


Plate 14. Colony interactions between *Fusarium fujikuroi* and antagonists.

A. *Fusarium fujikuroi* and *Aspergillus flavus*
B. *F. fujikuroi* and *A. fumigatus*
C. *F. fujikuroi* and *A. niger*
D. *F. fujikuroi* and *Trichoderma viride*.

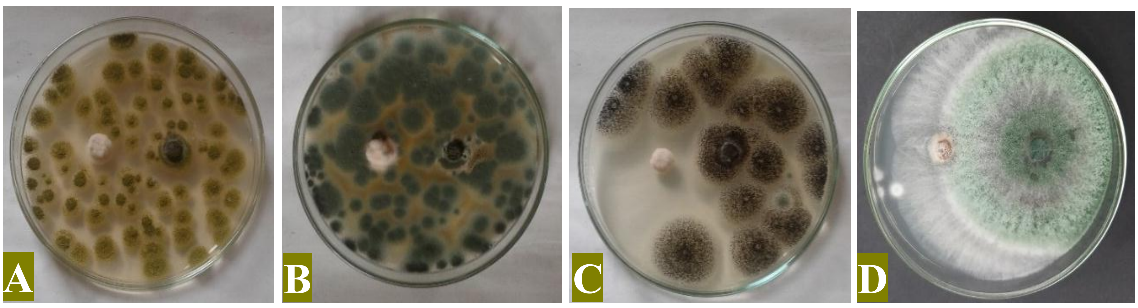


Plate 15. Colony interactions between *Microdochium fisheri* and antagonists.

A. *Microdochium fisheri* and *Aspergillus flavus*
B. *M. fisheri* and *A. fumigatus*
C. *M. fisheri* and *A. niger*
D. *M. fisheri* and *Trichoderma viride*.

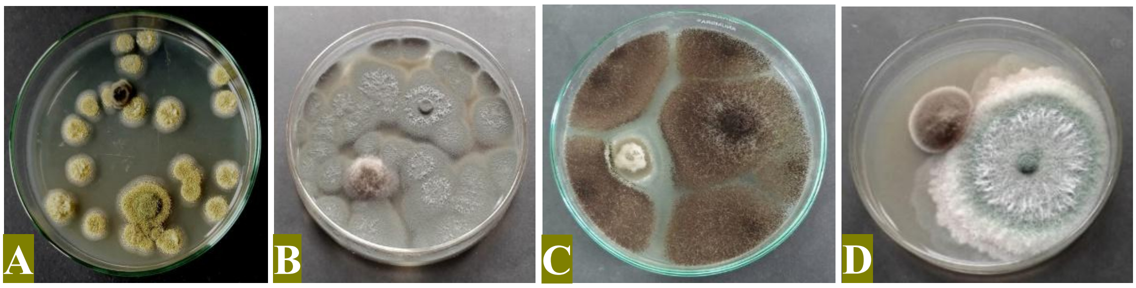


Plate 16. Colony interactions between *Nigrospora oryzae* and antagonists.

A. *Nigrospora oryzae* and *Aspergillus flavus* C. *N. oryzae* and *A. niger*
B. *N. oryzae* and *A. fumigatus* D. *N. oryzae* and *Trichoderma viride*.

4.12. Effect of volatile substances emanating from the cultures of the antagonistic fungi on the growth of the test pathogens

Effect of volatile metabolites of antagonistic fungi on rice pathogens are presented in Table 34, Figs 31 and Plates 17-22. It is clear from the result that the volatile substances emanating from the cultures of *Aspergillus flavus, A. fumigatus, A. niger* and *Trichoderma viride* inhibited the radial growth of the test pathogens i.e., *Bipolaris oryzae, Curvularia lunata, Fusarium equiseti, Fusarium fujikuroi, Microdochium fisheri* and *Nigrospora oryzae* to varied degrees. Any stimulation in the growth due to volatiles was not observed during this study.

The maximum inhibition of radial growth of *Bipolaris oryzae* was observed in case of *Trichoderma viride* (65.35%) which was followed by *Aspergillus flavus* (23.81%), *A. niger* (23.72%) and *A. fumigatus* (20.18%) owing to the volatile metabolites after 4 days of incubation at 25±2° C (Table 34, Figs 31 and Plate 17).

The highest inhibition of radial growth of *Curvularia lunata* was observed in case of *Trichoderma viride* (54.37%) followed by *A. niger* (44.08%), *A. flavus* (33.87%) and *A. fumigatus* (13.88%) owing to the volatile metabolites after 4 days of incubation at 25±2° C (Table 34, Figs 31 and Plate 18).

The maximum inhibition of radial growth of *Fusarium equiseti* was also observed in case of *Trichoderma viride* (63.90%) which was followed by *A. niger* (62.64%), *A. fumigatus* (58.17%) and *A. flavus* (52.32%) due to their volatile metabolites after 4 days of incubation at 25±2° C (Table 34, Figs 31 and Plate 19).

The maximum inhibition of radial growth of *Fusarium fujikuroi* was also observed in case of *Trichoderma viride* (57.12%) which was followed by *Aspergillus fumigatus* (47.63%), *A. niger* (40.75%) and *A. flavus* (32.96%) due to the volatile metabolites after 4 days of incubation at 25±2° C (Table 34, Figs 31 and Plate 20).

The maximum inhibition of radial growth of *Microdochium fisheri* was also observed in case of *Trichoderma viride* (62.40%) which was followed by *Aspergillus fumigatus* (46.65%), *A. niger* (36.29%) and *A. flavus* (30.42%) owing to their volatile metabolites after 4 days of incubation at 25±2° C (Table 34, Figs 31 and Plate 21).

The maximum inhibition of radial growth of *Nigrospora oryzae* was also observed in case of *Trichoderma viride* (82.63%) followed by *A. niger* (75%), *A. flavus* (42.87%) and *A. fumigatus* (35.60%) owing to their volatile metabolites after 4 days of incubation at 25±2° C (Table 34, Figs 31 and Plate 22).

In contrast to the present study, Aktar *et al*. (2014) reported that volatile metabolites produced by *Aspergillus niger*, *A. flavus*, *A. fumigatus* and *Trichoderma viride* inhibited the mycelial growth of *Colletotrichum* sp. by 14.68, 11.78, 11 and 11%, respectively. Again the volatile metabolites produced by an isolate of *T. viride, A. niger*, *A. flavus* and *A. fumigatus* inhibited the mycelial growth of *Curvularia lunata* by 20.86, 14.85, 10.5 and 14.85%, respectively. Further, the volatile metabolites produced by an isolate of *T. viride*, *A. niger*, *A. flavus* and *A. fumigatus* inhibited the mycelial growth of *Fusarium* *semitectum* by 13.5, 9.5, 8 and 7.75%, respectively. Differences in per cent inhibition with the present study might be due to the different isolates involved in the interaction.

Bashar and Chakma (2014) reported that volatile substances produced by *T. viride, A. niger, A. flavus* and *A. fumigatus* showed 29.75, 20.15, 15.78 and 12.25% growth inhibition of *Fusarium oxysporum*. Thakur and Harsh (2014) reported that volatile metabolites produced from the culture of *Aspergillus niger* showed 42.43% inhibition of mycelial growth of *Colletotrichum gloeosporioides.*

Bashar and Al-Ameen (2017) reported that the maximum inhibition of radial growth of *Curvularia brachyspora* was observed in *T. viride* (54.87%) followed by *A. niger* (40%), *A. flavus* (36%) and *A. fumigatus* (10%) owing to volatile metabolites, respectively.

Hosen and Shamsi (2019) reported that volatile metabolites produced by an isolate of *T. viride, A. niger, A. flavus* and *A. fumigatus* inhibited mycelial growth of *F. merismoides* by 67.69, 64.62, 61.54 and 56.57%, respectively. Similar observation was also noticed in the present investigation but the inhibition data varied due to differences in isolates involved in the interaction.

The growth inhibition of the test pathogens may be attributed owing to the presence of growth inhibitory substances in the metabolites (Bilai 1966, Dick and Hutchinson 1966, Marshall and Hutchinson 1970). The gross effect may also depend on the interaction between the volatile factors of two fungi as some sort of chemical reaction may occur there, which may include the nullification of the metabolites by each other.

Table 34. Per cent inhibition of radial growth of test pathogens by volatile metabolites of antagonistic fungi.

Antagonists	% inhibition of radial growth of test pathogens					
	Bipolaris oryzae	*Curvularia lunata*	*Fusarium equiseti*	*Fusarium fujikuroi*	*Microdochium fisheri*	*Nigrospora oryzae*
Aspergillus flavus	23.81[d]	33.87[c]	52.32[a]	32.96[c]	30.42[cd]	42.87[b]
A. fumigatus	20.18[d]	13.88[d]	58.17[a]	47.63[b]	46.65[b]	35.60[c]
A. niger	23.72[e]	44.08[c]	62.64[b]	40.75[cd]	36.29[d]	75.00[a]
Trichoderma viride	65.35[b]	54.37[d]	63.90[b]	57. 12[cd]	62.40[bc]	82.63[a]

Mean followed by the same letter (s) within a column did not differ significantly and dissimilar letter (s) within a column differ significantly at 5% level by DMRT.

Remarks of efficiency of antagonists: *T. viride > A. niger > A. flavus > A. fumigatus.*

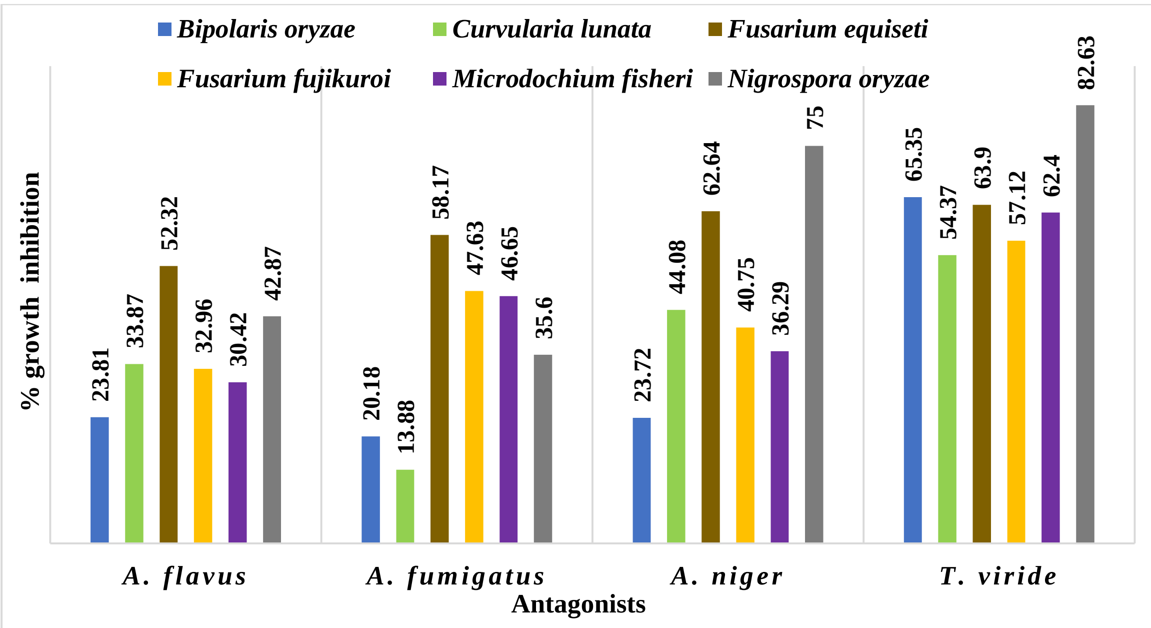


Fig. 31. Per cent inhibition of radial growth of test pathogens owing to volatile metabolites of antagonistic fungi.

The present investigation suggests that there were qualitative and quantitative differences in the volatile substances produced by the different antagonistic fungi. So, they exhibited different degrees of growth inhibition of the test pathogens. Dennis and Webster (1971) noted that certain *Trichoderma* spp. produced volatile antibiotics. These compounds inhibited the growth of *Rhizoctonia solani, Pythium ultimum* and *Fusarium oxysporum*. No lethality to any of the test fungi was reported by these authors and comprehensive chemical analysis of the volatile components of fungal cultures were not performed, although acetaldehyde was suggested as one of the volatiles. Some protective compounds isolated from endophytes are taxol, oocydin A, cryptocin, ambuic acid and jesteron (Stirele *et al*. 1993, Strobel *et al*. 2001, Li *et al*. 2000, 2001, Li and Strobel 2001). However, Hutchinson (1771, 1973) gave direct evidence by quantitative analysis of these volatiles. Fries (1973) has described the modes of action of volatile compounds by (a) the activation of enzymes, (b) removal or neutralization of the inhibitors, (c) influence of nutrient uptake from the medium and (d) stimulation of a limiting factor in intermediary metabolites.

Dennis and Webster (1971) detected the several substances in the volatile fraction of culture filtrates of fungi *viz*., acetaldehyde, n-propanol, propionaldehyde, isobutanol, n-butyraldehyde, ethyl acetate, isobutyl acetate and acetone. In the volatile fraction of culture Alcohols, esters, ketones of which 1-butanol, 3-methyl acetate, styrene, methyl isobutyl ketone, naphthalene, butylatedhydroxytoluene, 1-butanol, 3-methyl- followed by 1-butanol, 3- methyl-acetate were detected from *Muscodor albus*, a novel endophytic fungus (Gray *et al*. 2001).

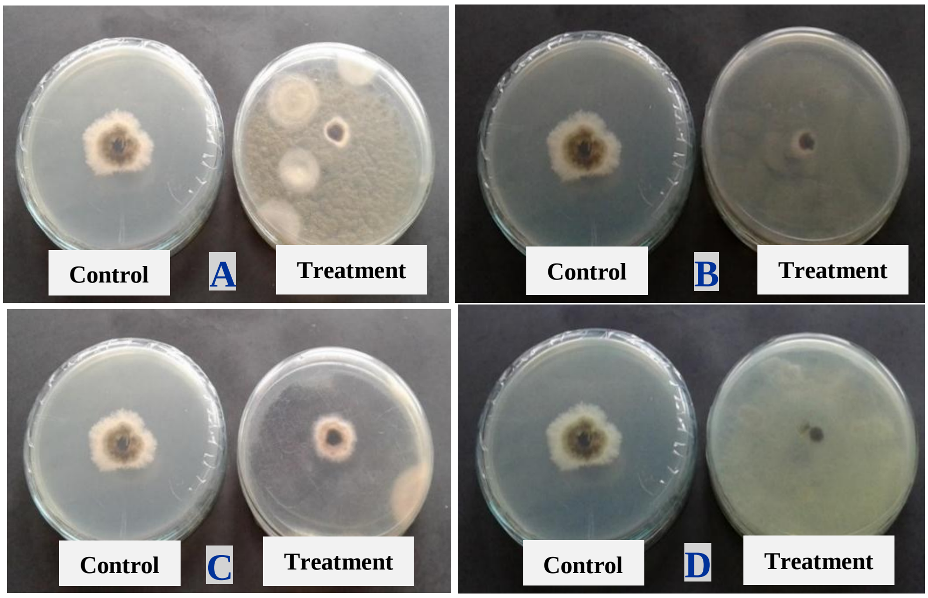


Plate 17. Growth inhibition of *Bipolaris oryzae* owing to volatile metabolites of antagonists.

A. *Bipolaris oryzae*: *Aspergillus flavus*

B. *B. oryzae*: *A. fumigatus*

C. *B. oryzae*: *A. niger*

D. *B. oryzae*: *Trichoderma viride*

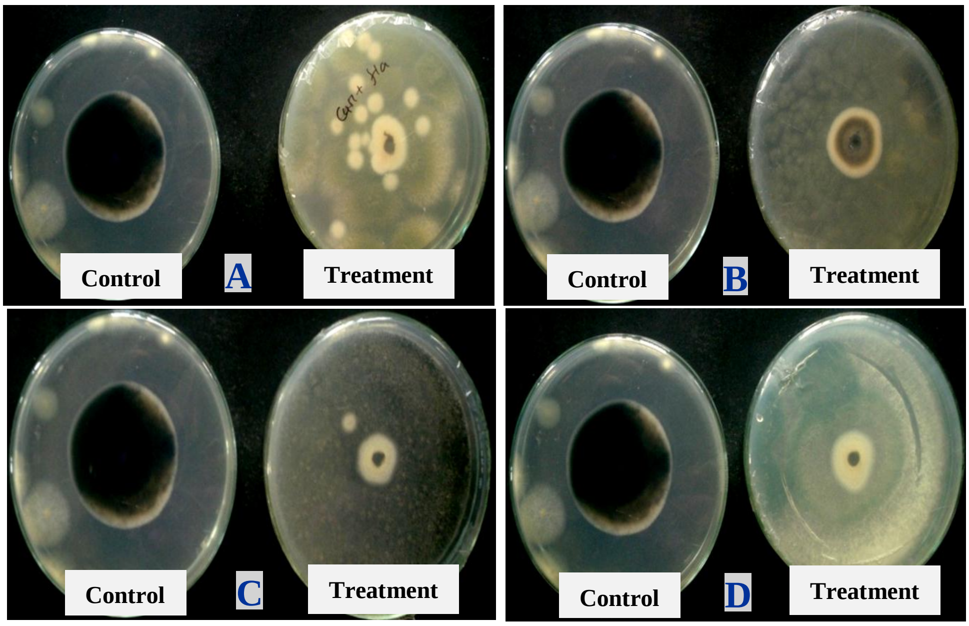


Plate. 18. Growth inhibition of *Curvularia lunata* owing to volatile metabolites of antagonists.

A. *Curvularia lunata*: *Aspergillus flavus*

B. *C. lunata*: *A. fumigatus*

C. *C. lunata*: *A. niger*

D. *C. lunata*: *Trichoderma viride*

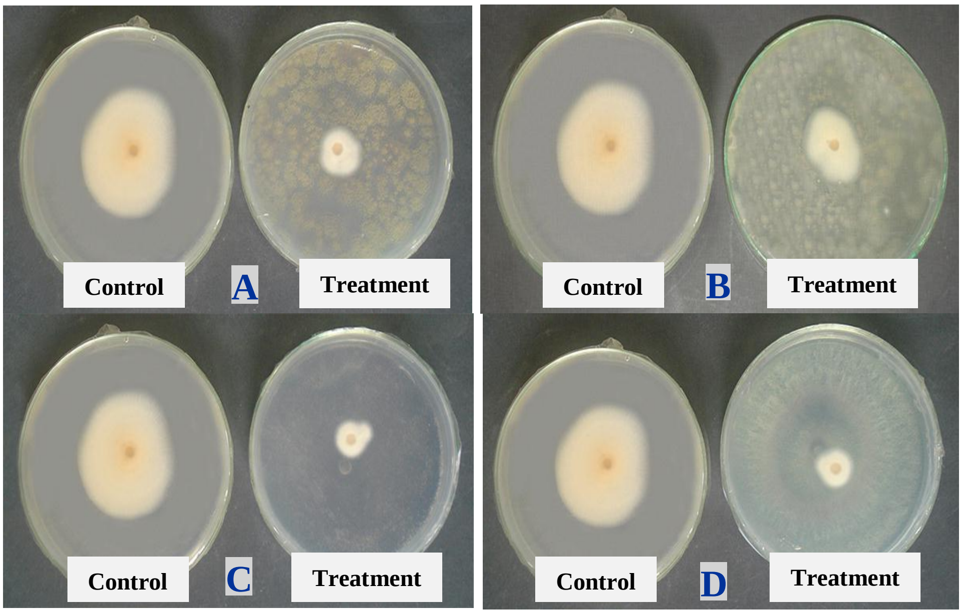


Plate. 19. Growth inhibition of *Fusarium equiseti* owing to volatile metabolites of antagonists.

A. *Fusarium equiseti*: *Aspergillus flavus* **C.** *F. equiseti*: *A. niger*

B. *F. equiseti*: *A. fumigatus* **D.** *F. equiseti*: *Trichoderma viride*

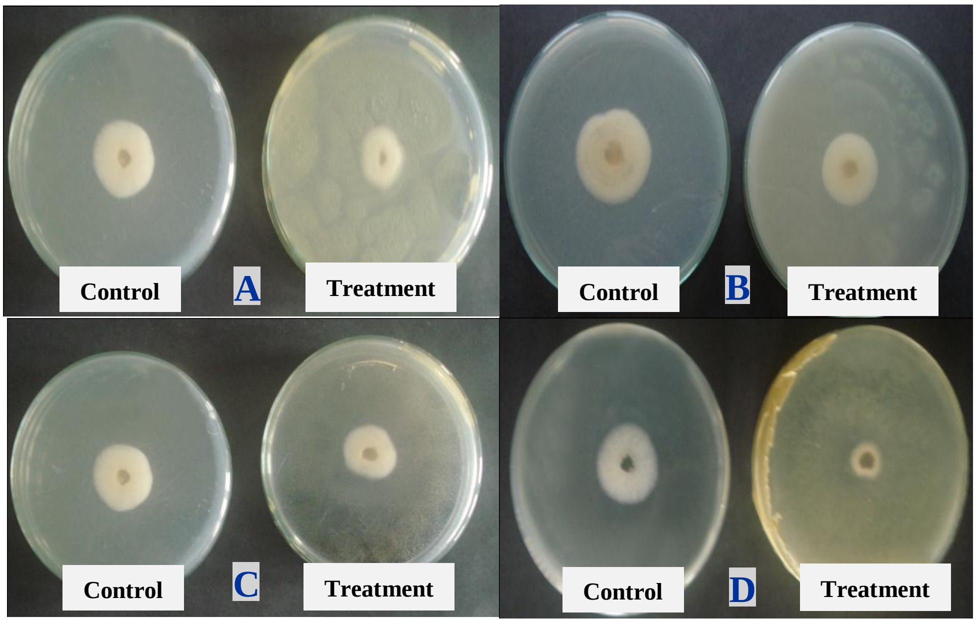


Plate. 20. Growth inhibition of *Fusarium fujikuroi* owing to volatile metabolites of antagonists.

A. *Fusarium fujikuroi*: *Aspergillus flavus* **C.** *F. fujikuroi*: *A. niger*

B. *F. fujikuroi*: *A. fumigatus* **D.** *F. fujikuroi*: *Trichoderma viride*

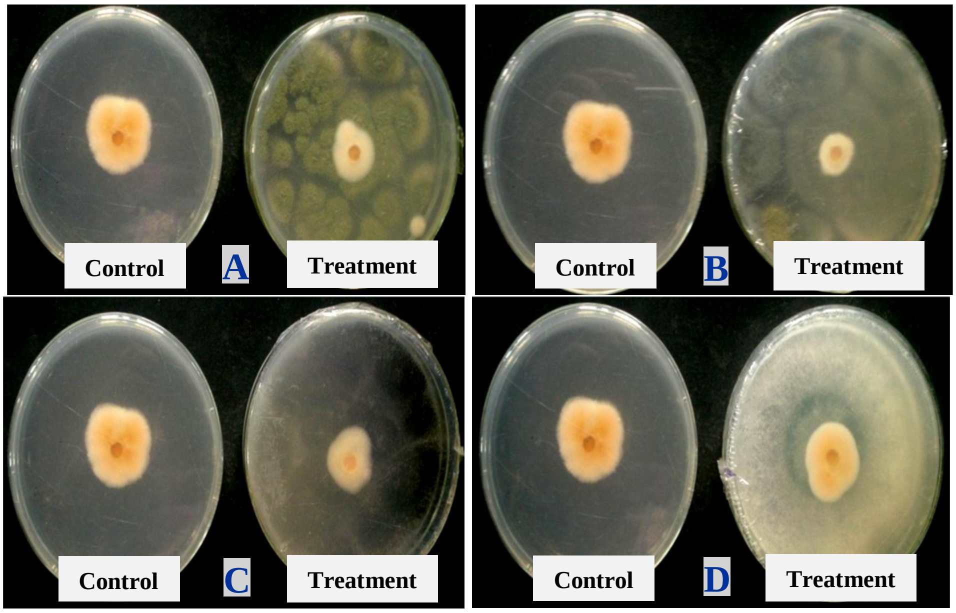


Plate. 21. Growth inhibition of *Microdochium fisheri* owing to volatile metabolites of antagonists.

A. *Microdochium fisheri*: *Aspergillus flavus* **C.** *M. fisheri*: *A. niger*

B. *M. fisheri*: *A. fumigatus* **D.** *M. fisheri*: *Trichoderma viride*

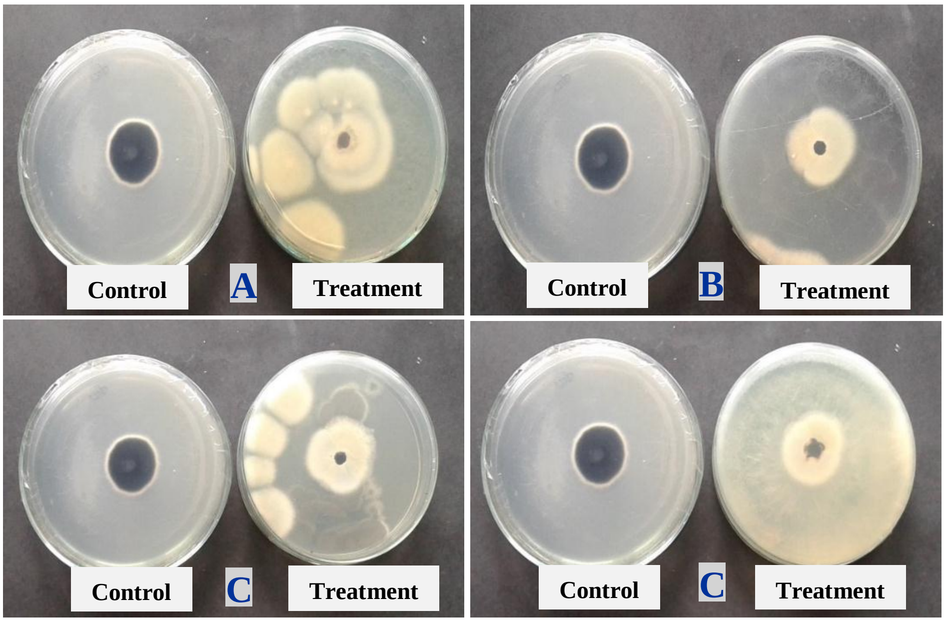


Plate. 22. Growth inhibition of *Nigrospora oryzae* owing to volatile metabolites of antagonists.

A. *Nigrospora oryzae*: *Aspergillus flavus* **C.** *N. oryzae*: *A. niger*

B. *N. oryzae*: *A. fumigatus* **D.** *N. oryzae*: *Trichoderma viride*

4.13. Effects of culture filtrates (Non-volatile metabolites) of antagonistic fungi on the growth of the test pathogens.

The Table 35, Figs 32-37 and Plates 23-25 showed the effects of non-volatile metabolites on the mycelial growth of the test pathogens *viz*., *Bipolaris oryzae, Curvularia lunata, Fusarium equiseti*, *F. fujikuroi*, *Microdochium fisheri* and *Nigrospora oryzae*. All the selected antagonists showed varied degree of growth inhibition of the test pathogens at different concentrations. None of the test antagonistic fungi could check the growth of the test pathogens completely even at 20% concentration.

The maximum inhibition of radial growth of *Bipolaris oryzae* was observed with the culture filtrates of *Aspergillus niger* (86.55%) which was followed by *A. fumigatus* (77.78%), *Trichoderma viride* (65.11%) and *A. flavus* (55.35%) at 20% concentration (Table 35, Fig.32 and Plate 23) after 4 days of incubation at 25±2°C. The inhibition of the pathogen increased with the increase of the concentration of the culture filtrates in culture medium. The order of effectiveness against *Bipolaris oryzae* at 20% concentration was *Aspergillus niger* > *A. fumigatus* > *Trichoderma viride* > *A. flavus.*

The highest inhibition of radial growth of *Curvularia lunata* was observed with the culture filtrates of *Trichoderma viride* (88.36%) followed by *A. flavus* (78.30%), *A. fumigatus* (72.32%) and *A. niger* (58.02%) at 20% concentration (Table 35, Fig. 33 and Plate 24) after 4 days of incubation at 25±2˚C. The inhibition of the pathogen increased with the increase of the concentration of the culture filtrates in culture medium. The order of effectiveness against *Curvularia lunata* at 20% concentration was *Trichoderma viride* > *Aspergillus flavus* > *A. fumigatus* > *A. niger*.

The maximum inhibition of radial growth of *Fusarium equiseti* was observed with the culture filtrates of *Aspergillus niger* (86.80%) which was followed by *Trichoderma viride*

(83.54%), *A. fumigatus* (71.14%) and *A. flavus* (42.75%) at 20% concentration (Table 35, Fig. 34 and Plate 23) after 4 days of incubation at 25±2° C. The inhibition of the pathogen increased with the increase of concentration of the culture filtrates in culture medium. The order of effectiveness against the pathogen at 20% concentration was *Aspergillus niger* > *Trichoderma viride* > *A. fumigatus* > *A. flavus.*

The highest inhibition of radial growth of *Fusarium fujikuroi* was observed with the culture filtrates of *Trichoderma viride* (90.81%) which was followed by *Aspergillus flavus* (73.87%), *A. niger* (72.93%) and *A. fumigatus* (64.83%) at 20% concentration (Table 35, Fig. 35 and Plate 24) after 4 days of incubation at 25±2° C. The inhibition of the pathogen increased with the increase of concentration of the culture filtrates in culture medium. The order of effectiveness against *Fusarium fujikuroi* at 20% concentration was *Trichoderma viride* > *Aspergillus flavus* > *A. niger* > *A. fumigatus.*

The maximum inhibition of radial growth of *Microdochium fisheri* was observed with the culture filtrates of *Trichoderma viride* (68.40%) which was followed by *Aspergillus niger* (62.27%), *A. fumigatus* (55.33%) and A. *flavus* (44.59%) at 20% concentration (Table 35, Fig. 36 and Plate 24) after 4 days of incubation at 25±2°C. The inhibition of the pathogen increased with the increase of concentration of the culture filtrates in culture medium. The order of effectiveness against *Microdochium fisheri* at 20% concentration was *Trichoderma viride* > *Aspergillus niger* > *A. fumigatus* > *A. flavus.*

The maximum inhibition of radial growth of *Nigrospora oryzae* was observed with the culture filtrates of *Aspergillu*s *flavus* (94.10%) which was followed by *Trichoderma viride* (93.24%), *A. niger* (82.81%) and *A. fumigatus* (80.49%) at 20% concentration (Table 35, Fig. 37 and Plate 25) after 4 days of incubation at 25±2° C. The inhibition of the pathogen increased with the increase of concentration of the culture filtrates in culture medium. The order of effectiveness against *Nigrospora oryzae* at 20% concentration was *Aspergillus flavus* > *Trichoderma viride* > *A. niger* > *A. fumigatus.*

In contrast to the present study, Aktar *et al.* (2014) reported that non-volatile metabolites produced by *Aspergillus niger, Trichoderma viride, A. flavus* and *A. fumigatus* inhibited mycelial growth of *Colletotrichum* sp.. Again the non-volatile metabolites produced by *Trichoderma viride, A. niger, A. flavus* and *A. fumigatus* inhibited mycelial growth of *Curvularia lunata* by 60.07, 52.5, 40.32 and 28.5%, respectively. Further the non-volatile metabolites produced by *Trichoderma viride, A. niger, A. flavus* and *A. fumigatus* inhibited mycelial growth of *Fusarium semitectum* by 50, 45, 8 and 7.75%, respectively. Differences in per cent inhibition with the present study might be due to the difference in fungal isolates involved in the interaction.

Hosen and Shamsi (2019) reported that culture filtrates of *T. viride, A. fumigatus, A. flavus* and *A. niger* showed 75.00, 60.61, 56.82 and 54.55% growth inhibition of *F. merismoides* at 20% concentration owing to non-volatile metabolites. Hosen *et al*. (2016) also reported that non-volatile metabolites produced by *T. viride* and *A. niger* inhibited mycelial growth of *Colletotrichum gloeosporioides* also.

Bashar *et al.* (2017) reported that the maximum inhibition of radial growth of *Curvularia brachyspora* owing to non-volatile metabolites was produced by *A. flavus* and *A. niger* (73.33%) which was followed by *T. viride* (57.65%) and *A. fumigatus* (54.55%) at 20% concentration. Madhanraj *et al*. (2010) reported that culture filtrates of *T. viride* and *A. niger* inhibited the mycelial growth of *F. solani* by 85 and 70%, respectively at 20 per cent concentration.

In contrast to the present study, Bashar and Chakma (2014) reported that culture filtrates of *T. viride, A. fumigatus, A. niger* and *A. flavus* showed 82.05, 80.56, 72.22 and 66.66% growth inhibition of *F. oxysporum* at 20% concentration owing to non-volatile metabolites. Similarly Dikshit *et al.* (2011) reported that cell free culture filtrate of *T. viride* inhibited 100% radial growth of *Sclerotium rolfsii* at 20% concentration, whereas 81.25, 58.32 and 31.45% inhibition of radial growth was noticed at 15, 10 and 5% concentrations. Tapwal *et al.* (2015) reported that culture filtrates of *T. viride* showed 13.33% growth inhibition of *Colletotrichum gloeosporioides*.

The inhibition of radial growth of the test pathogens owing to non-volatile metabolites have been attributed to the production of toxic substances in the culture filtrates (Brian 1957, 1960, Gottlieb 1957, Gottlieb and Shaw 1970, Dennis and Webster 1971a, Singh and Webster 1973, Skidmore and Dickinson 1976, Kexiang *et al.* 2002, Krupke *et al.* 2003), nutrient impoverishment (Diem 1969, Fokkema 1976, Skidmore 1976, Howell *et al.* 2003, Wool and Larito 2007) and alteration of pH of the culture medium resulting from staling growth products (Newhook 1951, 1957, Bhatt and Vaughan 1962, Bier 1966).

The results also show that the test pathogens have the ability to tolerate the effect of culture filtrates of antagonistic fungi to some extent. Growth of a fungus in the culture filtrates depends directly on its ability to tolerate the toxicity of fungal metabolites (Park 1963).

Table 35. Per cent inhibition of radial growth of test pathogens owing to non-volatile metabolites of antagonistic fungi.

Test pathogen	Concentation (%)	% inhibition of radial growth of test pathogens			
		A. flavus	***A. fumigatus***	***A. niger***	***T. viride***
Bipolaris oryzae	5	24.35^{c}	50.64^{b}	54.43^{a}	17.47^{d}
	10	32.30^{d}	54.32^{b}	60.22^{a}	36.02^{c}
	15	42.48^{d}	72.30^{b}	78.34^{a}	60.22^{c}
	20	55.35^{d}	77.78^{b}	86.55^{a}	65.11^{c}
Curvularia lunata	5	54.05^{c}	42.24^{b}	24.42^{c}	22.50^{c}
	10	62.16^{a}	55.47^{b}	32.26^{d}	42.61^{c}
	15	64.01^{b}	62.86^{b}	47.10^{c}	78.38^{a}
	20	78.30^{b}	72.32^{c}	58.02^{d}	88.36^{a}
Fusarium equiseti	5	15.27^{c}	21.31^{b}	50.05^{a}	22.83^{b}
	10	31.78^{c}	32.50^{c}	64.37^{a}	42.07^{b}
	15	36.65^{c}	36.24^{c}	72.09^{a}	54.55^{b}
	20	42.75^{b}	71.14^{a}	86.80^{a}	83.54^{a}
Fusarium fujikouri	5	54.50^{b}	30.82^{c}	31.72^{c}	62.97^{a}
	10	62.11^{ab}	57.05^{b}	31.88^{c}	75.03^{a}
	15	64.04^{b}	63.05^{b}	64.35^{b}	82.73^{a}
	20	73.87^{b}	64.83^{c}	72.93^{b}	90.81^{a}
Microdochium fisher	5	23.95^{b}	14.62^{c}	23.54^{b}	44.57^{a}
	10	32.50^{b}	4.10^{c}	36.09^{b}	55.98^{a}
	15	36.94^{d}	42.70^{c}	55.26^{b}	65.43^{a}
	20	44.59^{d}	55.33^{c}	62.27^{b}	68.40^{a}
Nigrospora oryzae	5	63.93^{ab}	48.04^{c}	60.36^{b}	66.64^{a}
	10	73.12^{a}	58.81^{b}	70.75^{a}	75.13^{a}
	15	82.82^{a}	66.96^{b}	72.15^{b}	84.62^{a}
	20	94.10^{a}	80.49^{b}	82.81^{b}	93.24^{a}

Mean followed by the same letter (s) within a column did not differ significantly and dissimilar letter (s) within a column differ significantly at 5% level by DMRT.

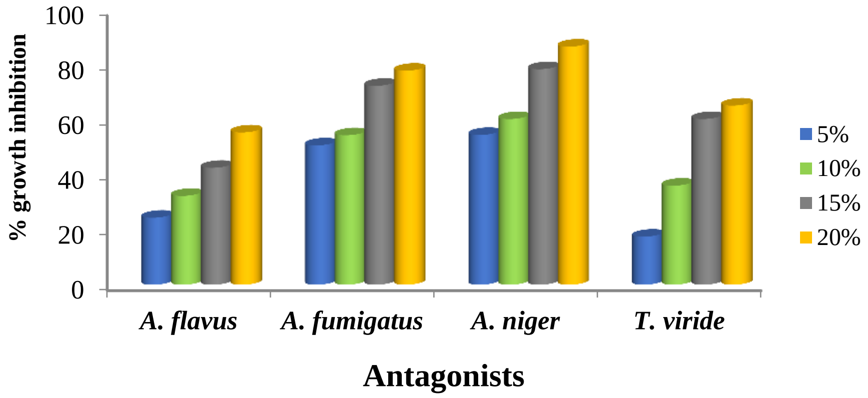


Fig. 32. Per cent inhibition of radial growth of *Bipolaris oryzae* owing to non-volatile metabolites of antagonistic fungi.

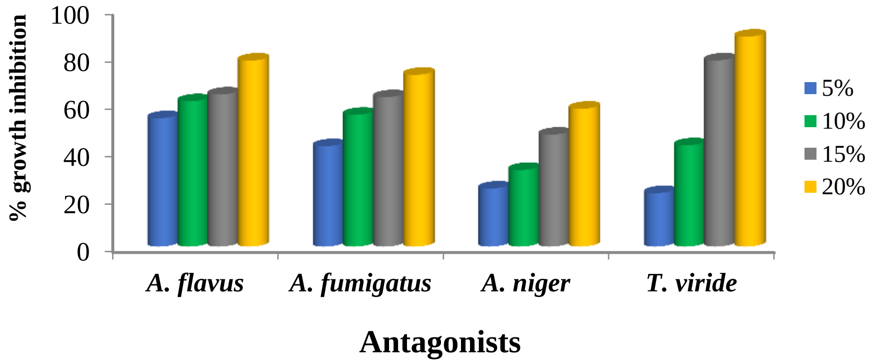


Fig. 33. Per cent inhibition of radial growth of *Curvularia lunata* owing to non-volatile metabolites of antagonistic fungi.

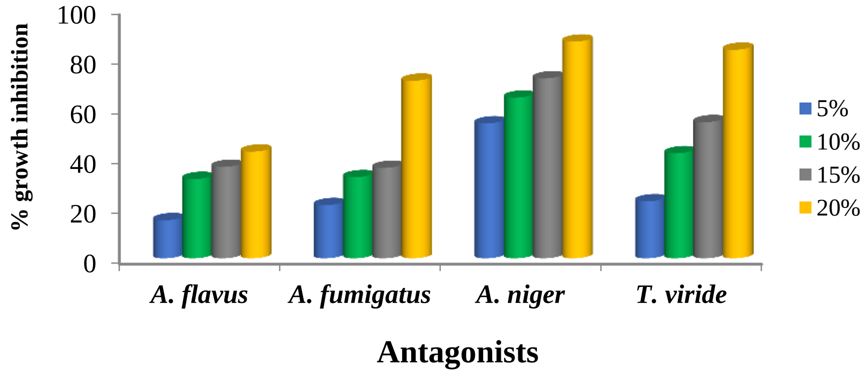


Fig. 34. Per cent inhibition of radial growth of *Fusarium equiseti* owing to non-volatile metabolites of antagonistic fungi.

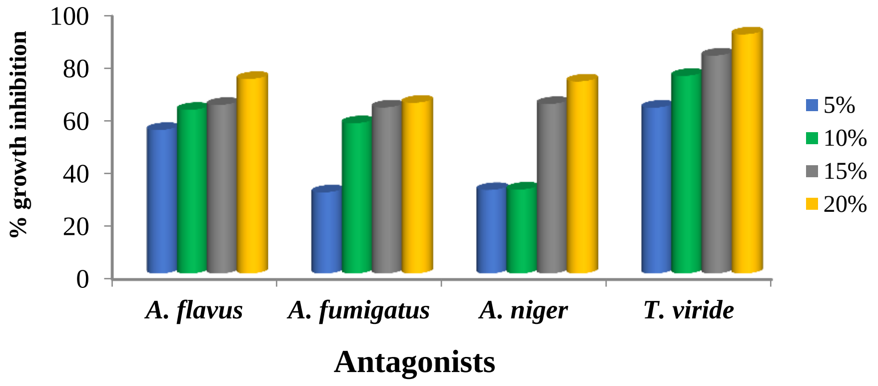


Fig. 35. Per cent inhibition of radial growth of *Fusarium fujikuroi* owing to non-volatile metabolites of antagonistic fungi.

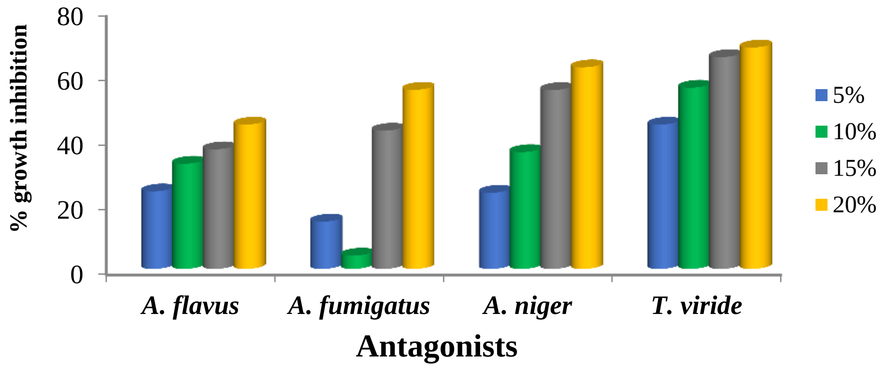


Fig. 36. Per cent inhibition of radial growth of *Microdochium fisheri* owing to non-volatile metabolites of antagonistic fungi.

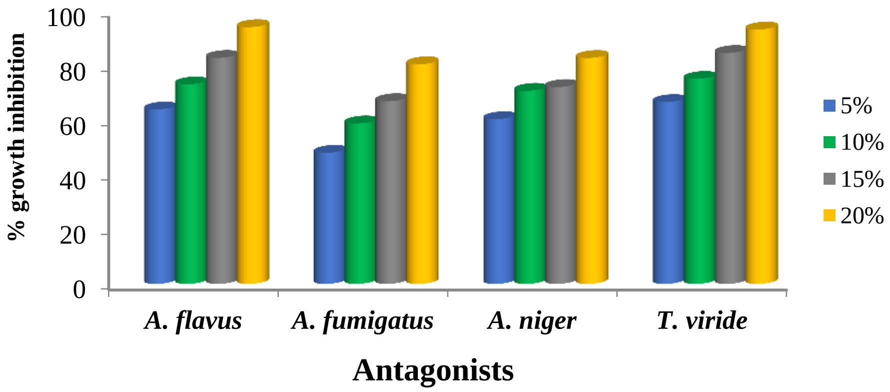


Fig. 37. Per cent inhibition of radial growth of *Nigrospora oryzae* owing to non-volatile metabolites of antagonistic fungi.

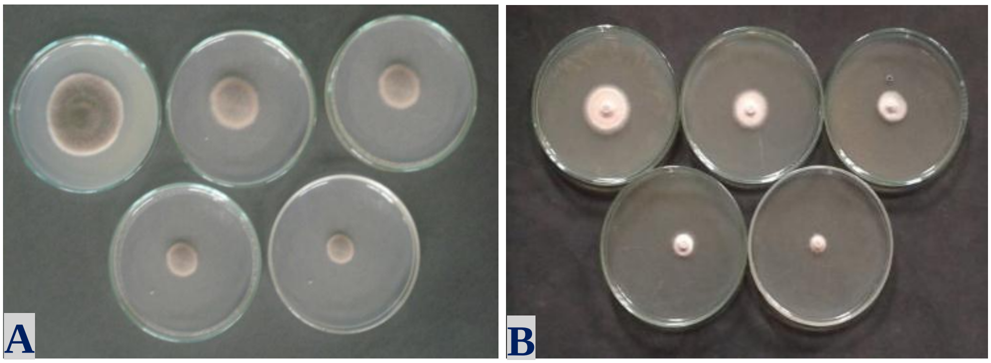


Plate 23. Growth inhibition of *Bipolaris oryzae* (A) and *Fusarium equiseti* (B) owing to non-volatile metabolites of *Aspergillus niger* at 5, 10, 15 and 20% concentrations.

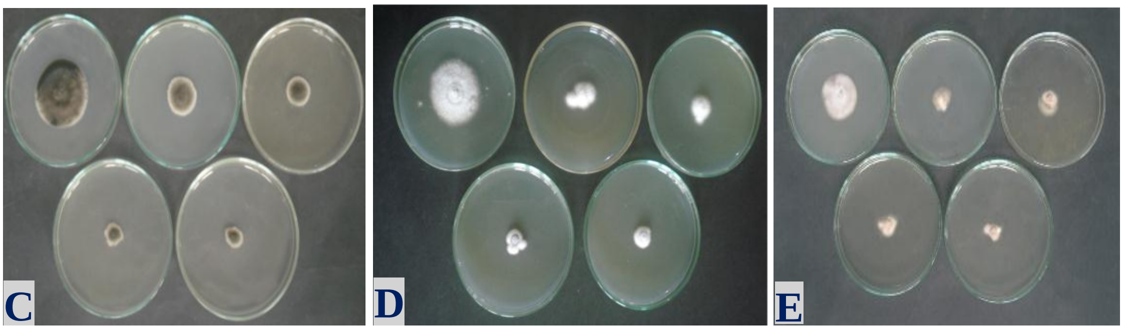


Plate 24. Growth inhibition of *Curvularia lunata* (C), *Fusarium fujikuroi* (D) and *Microdochium fisheri* (E) owing to non-volatile metabolites of *Trichoderma viride* at 5, 10, 15 and 20% concentrations.

Plate 25. Growth inhibition of *Nigrospora oryzae* (F) owing to non-volatile metabolites of *Aspergillus flavus* at 5, 10, 15 and 20% concentrations.

4.14. Evaluation of combined effects of fungicides, plant extracts and bioagents against the rice pathogens

An investigation was undertaken to screen out the most feasible seed treatment method with fungicides, plant extracts and biocontrol agents against *B. oryzae, C. lunata, F. equiseti*, *F. fujikuroi*, *M. fisheri* and *N. oryzae*. In the experiment it was found that all the treatments could significantly reduce the seed borne diseases and improve the quality status of the artificially inoculated seeds. Different seed treatments were compared with control set on the basis of seed germination, seedling mortality, root length, shoot length and vigor index (Table 36).

4.14.1. Effects of different treatments on *Bipolaris oryzae*

Seeds inoculated with *Bipolaris oryzae* showed 41.67-82% germination. Table 36 showed that out of 11 treatments, 7 treatments *viz.,* T3, T6, T7, T8, T9, T10 and T11 exhibited promising results compared to control. Among 11 treatments, T10 showed the highest germination percentage (82) whereas the lowest germination (41.67%) was recorded in T4 (Table 36, Fig. 38). Highest seedling vigor index (1771.08) was observed in T10 followed by T8 (1562.03) and lowest vigor index was found in T5 (574.20). Seedling mortality was also counted after 21 days of germination. The maximum seedling mortality (16.17%) was found in T5 and minimum (3.93%) in T10 treatments. In the present investigation maximum shoot length (16.50 cm) was recorded in T10 and minimum (8.42 cm) in T6 whereas highest root length (5.27 cm) was noticed in T10 and lowest (2.89 cm) in T2.

4.14.2. Effects of different treatments on *Curvularia lunata*

Out of 11 treatments, 8 treatments *viz.,* T1, T2, T3, T4, T6, T8, T9 and T11 exhibited best results compared to control. Among 11 treatments, T3 showed highest germination percentage (75.67) whereas lowest germination (49%) was recorded in T10 (Table 36, Fig

38). Maximum seedling vigor index (1694.80) was observed in T3 and minimum vigor index was found in T5 (575.87). The maximum seedling mortality (14.33%) was found in T5 and minimum (4.27%) in T3 treatments after 21 days of germination. In the present investigation maximum shoot length (17 cm) was recorded in T3 and minimum (9 cm) in T5 whereas highest root length (5.38 cm) was noticed in T3 and lowest (2.39 cm) in T7.

4.14.3. Effects of different treatments on *Fusarium fujikuroi*

Out of 11 treatments, 8 treatments *viz.*, T1, T3, T4, T5, T6, T7, T10 and T11 exhibited promising results compared to control. Among 11 treatments, T7 showed highest germination (80%) whereas lowest (42.67%) was recorded in T9 (Table 36, Fig 38). Maximum seedling vigor index (1773.83) was observed in T7 and minimum was found in T9 (438.80). Seedling mortality was also counted after 21 days of germination where maximum seedling mortality (21.67%) was recorded in T9 and minimum (7.17%) in T7 treatments. In the present investigation maximum shoot length (15.42 cm) was recorded in T7 and minimum (7.17 cm) in T9 whereas highest root length (6.77 cm) was noticed in T7 and lowest (2.65 cm) in T1.

4.14.4. Effects of different treatments on *Fusarium equiseti*

All the treatments exhibited good results compared to control. Among the 11 treatments, T3 showed highest germination percentage (81.33) and lowest (60) in T10. The maximum seedling mortality (13.88 %) was found in T10 and minimum (4.50%) in T3 treatments after 21 days of germination. Maximum seedling vigor index (2382.60) was observed in T3 and minimum in T10 (718.70) (Table 36, Fig 38). The maximum shoot length (23.67cm) was recorded in T3 and minimum (8.75 cm) in T10. The maximum root length (5.63cm) was recorded in T3 followed by T2 (5.37 cm), T8 (5.12 cm) and T4 (4.79 cm) and minimum (2.90 cm) was found in T9.

Table 36. Combined effects of seed treatment with fungicides, leaf extracts and biocontrol agents on seed quality parameters of BRRI rice varieties.

Treatments	Seed quality parameters on different pathogens														
	Bipolaris oryzae					*Curvularia lunata*					*Fusarium fujikuroi*				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
T1	43.67 fg	10.17bc	3.91abcd	9.77ef	596.32ef	64.33bcd	6.08cd	4.04bcd	12.83cde	1086.55cd	57.67de	18.33abc	2.65c	9.00cde	669.37fgh
T2	50.00 f	7.30 cde	2.89d	10.92de	690.81def	67.67abc	6.15cd	4.56abc	14.64 bc	1299.23bc	47.67fg	18.00bcd	4.08bc	10.18bcde	680.12fg
T3	63.67de	6.50def	3.35cd	13.67bc	1084.90bc	75.67a	4.27d	5.38a	17.00a	1694.80a	66.00bcd	15.83cdef	3.95bc	10.93abcde	976.07de
T4	41.67g	12.50b	3.42bcd	10.85def	593.65ef	57.67defg	6.33cd	2.98efg	11.66def	843.98ef	62.33cd	16.50cde	3.95bc	9.83bcde	856.27ef
T5	46.00fg	16.17a	3.63abcd	8.94ef	574.20f	50.00g	14.33a	2.54fg	9.00g	575.87g	66.33bcd	12.25fg	6.59a	14.58ab	1404.32bc
T6	62.67e	8.13cd	4.33abcd	8.42f	802.33de	64.00bcd	6.04cd	4.60abc	13.10bcd	1135.40cd	72.33ab	9.17gh	6.44a	14.41ab	1506.81b
T7	62.00e	8.17cd	3.27cd	11.01de	884.95cd	52.00fg	12.00ab	2.39fg	10.04fg	647.04fg	80.00a	7.17h	6.77a	15.42a	1773.83a
T8	76.33ab	5.00ef	5.02abc	15.13ab	1562.03a	72.00 ab	4.81d	4.83ab	15.17ab	1438.67b	44.67fg	18.00bcd	2.80c	8.83de	516.60gh
T9	70.67bcd	5.47def	4.41abcd	14.00bc	1301.13b	64.67bcd	6.77cd	3.90cde	12.97cd	1075.21d	42.67g	21.67a	3.14c	7.17e	438.80h
T10	82.00a	3.93f	5.27a	16.50a	1771.08a	49.00g	13.83a	3.02efg	9.33g	604.82 g	60.67cde	13.83ef	6.17a	13.77abc	1209.67cd
T11	71.33bc	5.53def	4.79abc	12.55cd	1236.35b	61.00cde	7.27cd	3.30def	11.93def	930.08de	58.00de	14.43def	5.98a	12.82abcd	1091.03de
T12	61.67e	6.43def	5.17ab	13.31bcd	1138.85b	53.33efg	11.80ab	2.35g	10.27fg	673.07fg	52.0ef	21.00ab	4.00bc	6.67e	551.67gh
Control	68.67cde	6.00def	4.09abcd	12.43cd	1135.02b	60.00cdef	9.47bc	2.95fg	10.82efg	826.23ef	68.00bc	13.50ef	5.6ab	7.33e	869.03ef
CV%	4.05	13.13	14.42	6.91	7.43	4.82	17.40	8.69	5.75	7.36	4.98	7.94	13.17	15.31	8.39

Table 36. contd.

Treatments	Seed quality parameters on different pathogens														
	Fusarium equiseti					***Microdochium fisheri***					***Nigrospora oryzae***				
	A	**B**	**C**	**D**	**E**	**A**	**B**	**C**	**D**	**E**	**A**	**B**	**C**	**D**	**E**
T1	74.33abcd	7.50bcd	3.97cd	15.60de	1453.09cd	59.33ef	12.33a	4.28cd	20.93a	1498.13fg	80.00a	3.83g	7.18a	25.35a	2601.97a
T2	80.00ab	6.17de	5.37ab	22.33ab	2213.81a	66.33cde	5.14def	5.47a	23.73a	1937.63cde	67.67ab	6.64efg	5.15b	23.40ab	1932.20bc
T3	81.33a	4.50e	5.63a	2367a	2382.60a	60.67ef	4.67def	5.43ab	21.82a	1652.37efg	67.67ab	8.17defg	4.17c	23.50ab	1873.18bcd
T4	74.33abcd	6.04de	4.79abc	20.07bc	1848.71b	71.67bcd	4.67def	5.42ab	23.37a	2062.95bcd	71.00ab	4.83fg	6.47a	24.63a	2214.10ab
T5	65.00efg	8.42bcd	3.03def	13.50ef	1076.37ef	78.00ab	3.50ef	5.73a	25.39a	2427.97ab	51.33cd	13.67abc	3.11d	15.65e	962.52fg
T6	71.67bcde	6.37cde	4.43bc	17.50cd	1571.53c	74.33bc	4.33ef	5.65a	23.73a	2176.45abc	62.67bc	10.17bcde	3.33d	18.98d	1396.17ef
T7	63.67efgh	9.17bc	2.95ef	11.15fgh	897.95fg	83.67a	2.47f	5.52a	24.95a	2548.82a	60.00bc	7.29defg	4.08c	21.67bc	1550.53cde
T8	78.00abc	5.54de	5.12ab	23.00ab	2192.99a	57.67fg	6.83cde	4.14cd	21.55a	1477.89fg	68.00ab	7.30defg	5.32b	24.08a	1999.40bc
T9	62.33fgh	10.3b	2.90ef	9.67ghi	782.75gh	63.67def	6.30def	5.38ab	23.50a	1837.40cdef	59.33bc	8.9cdef	3.52cd	20.72cd	1440.48de
T10	60.3gh	13.883a	3.15def	8.75hi	718.70gh	55.67fg	8.33bcd	4.15cd	20.25a	1358.63g	60.33bc	11.83abcd	3.15d	16.01e	1156.52efg
T11	67.67defg	7.97bcd	3.233def	12.77eg	1082.37ef	61.00ef	11.50ab	4.40c	23.50a	1358.63g	51.67cd	14.00ab	2.80de	15.16e	928.40fg
T12	55.67h	13.67a	2.50f	7.50i	556.17h	50.33g	13.67a	4.50bc	13.95b	1713.53defg	42.33d	15.00a	2.11e	14.33e	696.10g
Control	70.67cdef	7.05cde	3.47de	14.57def	1275.07de	59.33ef	10.33abc	3.40d	21.20a	934.30h	72.33ab	6.00efg	5.32b	24.43a	2151.23ab
CV%	4.30	12.31	8.33	7.70	6.45	4.51	18.58	6.58	8.11	7.28	7.38	17.76	5.89	3.80	9.89

Mean followed by the same letter (s) within a column did not differ significantly and dissimilar letter (s) within a column differ significantly at 5% level by DMRT.

T1: Bavistin, **T2:** Tilt, **T3:** Bavistin+Tilt, **T4:** *Azadirachta indica*, **T5:** *Citrus lemon*, **T6:** *A. indica+C. lemon*, **T7:** *Trichoderma viride*, **T8:** Bavistin+ *A. indica+ T. viride*, **T9:** Bavistin+ *C. lemon* + *T. viride*, **T10:** Tilt + *A. indica+ T. viride*, **T11:** Tilt + *C. lemon* + *T. viride*, **T12: Control. A**= % Germination, **B**= % Mortality after 21 days of germination, **C**= Root length (cm), **D**= Shoot length (cm) and **E**= Seedling vigor index.

4.14.5. Effects of different treatments on *Microdochium fisheri*

Different trends were observed in treated seeds inoculated with *Microdochium fisheri*. All the treatments exhibited promising results compared to control. Among them, T7 treatment exhibited significantly highest (83.67%) germination and lowest (55.67%) in T10 compared to other treatments. Seedling vigor index was highest (2548.82) in T7 and lowest (1358.63) in T10 (Table 36). In the present investigation maximum shoot length (25.39 cm) was recorded in T5 and minimum (20.25cm) in T10 whereas highest root length (5.73 cm) was noticed in T5 and lowest (4.14 cm) in T8. Seedling mortality was also counted after 21 days of germination where maximum seedling mortality (12.33%) was recorded in T1 and minimum (2.47%) in T7 treatments. All the chemicals, plant extracts and biocontrol agents significantly reduced the mortality of seeds inoculated with *Microdochium fisheri* in a similar pattern (Table 36, Fig 38).

4.14.6. Effects of different treatments on *Nigrospora oryzae*

Different trends were noticed in treated seeds inoculated with *Nigrospora oryzae* for the efficacy of different treatments. All the treatments exhibited good results compared to control. Among 11 treatments, T1 showed highest germination percentage (80) whereas lowest germination (51.33) was recorded in T5 (Table 36, Fig 38). Maximum seedling vigor index (2601.97) was observed in T1 and minimum was found in T11 (928.40). Seedling mortality was also counted after 21 days of germination where maximum seedling mortality (14%) was recorded in T11 and minimum (3.83%) in T1 treatment. In the present investigation maximum shoot length (15.16 cm) was recorded in T11 and minimum (25.35 cm) in T1 whereas highest root length (7.18 cm) was noticed in T1 and lowest (2.80 cm) in T11.

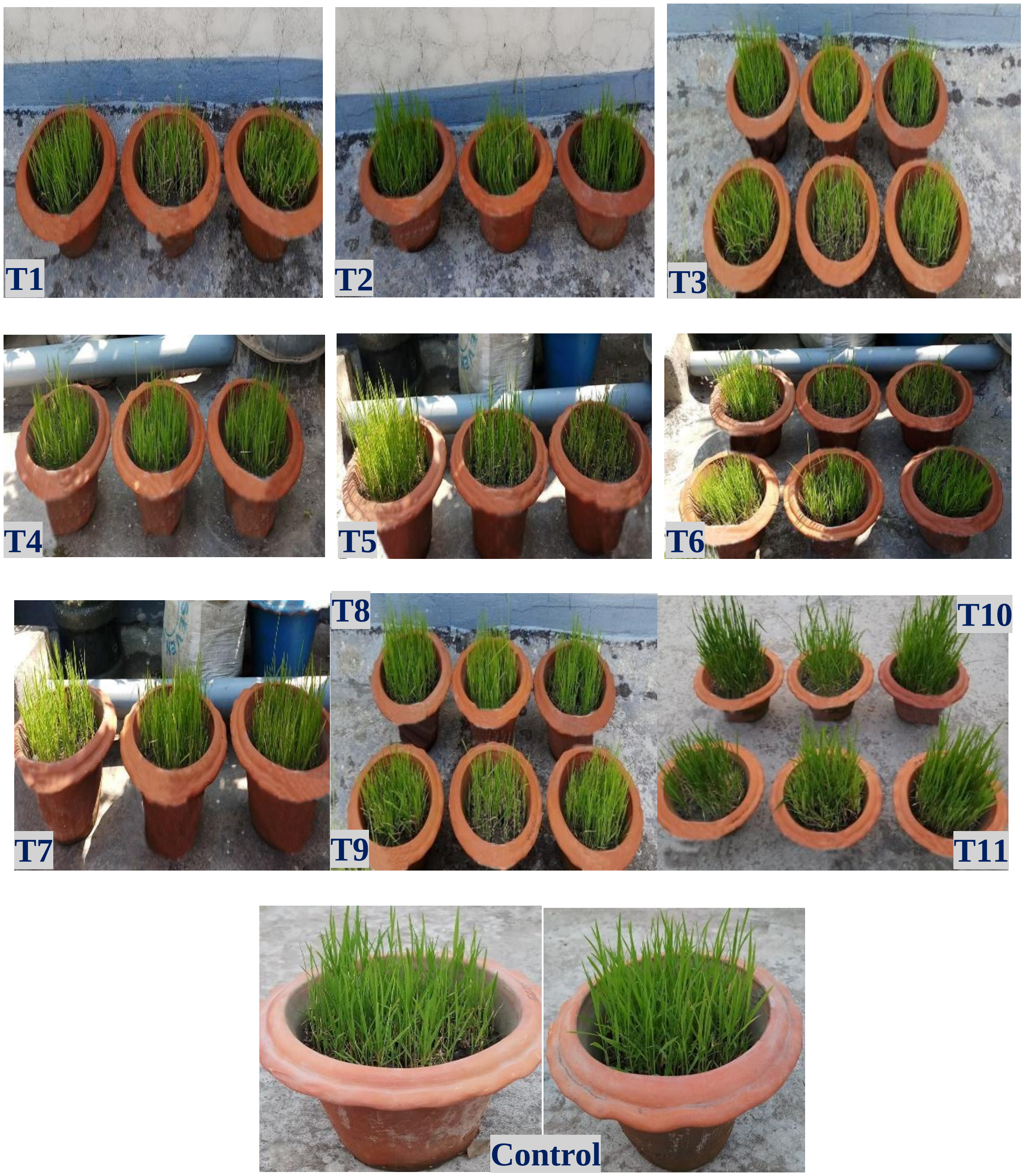


Fig. 38. Combined effects of seed treatment with fungicides, leaf extracts and biocontrol agent on seed quality parameters of BRRI rice varieties (BRRI dhan 63, 65, 70, and 74).

CONCLUSION

Based on the findings of the present study, following conclusions were done-

- The present investigation suggested that out of 20 BRRI rice varieties BRRI dhan 66, BRRI dhan 68 and BRRI dhan 74 showed better performances on the basis of percentage of pure seed, fungal association, vigor index value, germination percentage, root and shoot length and minimum seedling mortality.
- Association of 25 species of fungi with 20 BRRI rice varieties was observed during 2016 to 2018. Of which 13 fungal isolates were identified by sequence analysis of the ITS region.
- Among these 25 fungi, *Bipolaris multiformis, Microdochium fisheri* and *Pestalotiopsis oxyanthi* are new record for Bangladesh.
- Ten species of fungi were isolated from empty glume, flowering glume, embryo and endosperm with different parts of seeds of selected BRRI rice varieties.
- *Bipolaris oryzae, Aspergillus flavus* and *Microdochium fisheri* were predominant in most of the rice varieties whereas *Bipolaris multiformis*, *Phanerochaete chrysosporium* and *Alternaria tenuissima* were recorded only with a few varieties of rice seeds.
- The highest fungal infection was observed in BRRI dhan 65 followed by BRRI dhan 63, BRRI dhan 57 and the lowest was in BRRI dhan 73.
- Among the isolated fungi, six were found to be pathogenic to BRRI rice seeds. They were *Bipolaris oryzae, Curvularia lunata, Fusarium equiseti, Fusarium fujikuroi, Microdochium fisheri* and *Nigrospora oryzae.* These six fungi showed seed to seedling transmission nature in water agar test tube and earthen pot experiment.

- Bavistin 50 WP and Tilt 250 EC identified as the best inhibiting fungicides against the tested pathogenic fungi of rice.
- *Azadirachta indica* and *Citrus limon* showed complete growth inhibition of the test pathogens.
- *Aspergillus niger* and *Trichoderma viride* showed promising inhibitory effect on the growth of the test pathogens.
- *In vivo* experiment, out of twelve treatments, T10 (Tilt + *Azadirachta indica* + *Trichoderma viride*), T3 (Bavistin+Tilt) and T7 (*Trichoderma viride*) showed highest seed germination, seedling vigor index against *Bipolaris oryzae, Curvularia lunata* and *Fusarium fujikuroi*.
- On the other hand, out of 12 treatments T3 (Bavistin+Tilt), T7 (*Trichoderma viride*) and T1 (Bavistin) showed promising seed germination and seedling vigor index against *Fusarium equiseti*, *Microdochium fisheri* and *Nigrospora oryzae.* These three test pathogens were completely controlled by the treatments used in the experiment.

RECOMMENDATIONS

Since rice a staple food, better seed health management is a prerequisite for successful rice production because pathogenic fungi are known to cause huge economic losses by reducing rice yield. In connection to the main findings from this study the following are recommended:

- ✓ This work should be repeated by using a sensitive method like agar and blotter methods to authenticate the results of the present work.
- ✓ Application of Bavistin 50 WP and Tilt 250 EC at 100, 200, 300, 400 and 500 ppm concentrations may be commercially used for managing pathogens of rice seeds. For more confirmation the above mentioned fungicides also need 2-3 years trial in nursery bed and in field condition.
- ✓ In small scale, *Azadirachta indica* and *Citrus limon* at 10% concentration can be used for controlling diseases and production of healthy seeds.
- ✓ *Trichoderma viride* may be exploited commercially as a bio-control agent against pathogens of rice.
- ✓ Molecular identification of the fungal species using ITS sequence analysis may be the best complement to conventional detection method.
- ✓ Combined application of Bavistin, Tilt, *Azadirachta indica*, *Citrus limon* and *Trichoderma viride* in seed treatments may be used commercially to control rice pathogens. For more confirmation the above mentioned treatments also need to test 2-3 years in field condition.
- ✓ Findings of this research work will be helpful for designing a proper management of pathogenic fungi of rice.

REFERENCES

Ahmed M, Hossain M, Hassan K and Dash CK 2013. Efficacy of different plant extract on reducing seed borne infection and increasing germination of collected rice seed sample. Universal Journal of Plant Science **1**(3): 66-73.

Ahmed MF, Khalequzzaman KM, Islam MN, Anam MK and Islam MT 2002. Effect of plant extracts against *Bipolaris oryzae* of rice under *in vitro* conditions. Pakistan Journal of Biological Sciences **5**(6): 442-445.

Ahmed SN, Siddique NV and Khan MQ 1989. Seed borne fungi associated with seed lots of different paddy cultivars in Pakistan. Pakistan Journal of Botany **21**(2):309.

Aktar MT, Hossain KS and Bashar MA 2014. Antagonistic potential of rhizosphere fungi against leaf spot and fruit rot pathogens of brinjal. Bangladesh J. Bot. **43**(2): 213-217.

AL-Ameen MD, Hosen S, Shamsi S and Bashar MA 2017. Antagonistic potential of soil fungi against post-harvest pathogenic fungi of *Musa sapientum* L. Bangladesh J. Bot. **46**(2): 733-738.

Alemu K, Ayalew A and Woldetsadic K 2014. Effect of aqueous extracts of some medicinal plants in controlling anthracnose disease and improving postharvest quality of mango fruit. Persian Gulf Crop Protection **3**(3): 84-92.

Ali MS and Deka B 1996. Role of fungi causing grain discoloration of rice and management under storage. Indian J. Plant Pathol. **26**(1): 79-85.

Amer OE, Mahmoud MA, El-Samawaty AMA and Sayed SRM 2011. Non liquid nitrogen-based-method for isolation of DNA from filamentous fungi. Afr. J. Biotechnol. **10**(65): 14337-14341.

Anonymous 1990. Annual report 1980-81. Plant Pathology Division, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur. pp. 81-84 and 89-90.

Anwar MN, Singh P, Begum J and Chowdhury JU 1994. Antifungal activity of some selected plants on phytopathogenic fungi. Bangladesh J. Life Science **6:** 23-26.

Archana B and Prakash HS 2013. Survey of seed-borne fungi associated with rice seeds in India. International Journal of Research in Pure and Applied Microbiology **3**(1): 25-29.

Ashrafuzzaman MH and Hossain I 1992. Antifungal activity of crude extracts of plants against *Rhizoctonia solani* and *Bipolaris sorokiniana*. Proc. BAU Res. Prog. **6:** 188-192.

Ashrafuzzaman MH and Khan AR 1992. Antifungal activity *in vitro* of some plant extract on *Rhizoctonia solani*. Bangladesh Journal of Science and Research **10** (2): 243-244.

Azher M, Yasin SI, Mahmood S, Hannan A and Akhtar M 2013. Field evaluation of new fungicides against rice (*Oryza sativa*) diseases. Pak. J. Phytopathol. **25**(2):141-145.

Baki AA and Anderson JD 1972. Physiological and biological deterioration of seeds. *In*. Seed Biology II, Academic Press, New York, USA. pp. 283-315.

Barnett HL and Hunter BB 1972. Illustrated Genera of Imperfect Fungi. Burgess Pub. Co. U.S.A. pp. 241.

Basak AB and Lee MW 2002. Prevalence and transmission of seed-borne fungi of maize grown in a farm of Korea. The Korean Society of Mycobiology **30**(1):47-50.

Bashar MA 1992. Laboratory evaluation of some pesticides on *Fusarium oxysporum* f. sp. *ciceri* causing wilt of chickpea. Bangladesh J. Bot. **21**(1): 157-159.

Bashar MA and Chakma M 2014. *In vitro* control of *Fusarium solani* and *F. oxysporum,* the causative agent of brinjal wilt. Dhaka Univ. J. Biol. Sci. **23**(1): 53-60.

Bashar MA and Rai B 1991. Antifungal activity of extracts of some plant parts against *Fusarium oxysporum* f. sp. *ciceri*. Bangladesh J. Bot. **20**: 219-222.

BBS 2012. Statistical Year Book of Bangladesh. Bangladesh Bureau of Statistics. Ministry of Planning, Government of the People's Republic of Bangladesh. Pp. 33-36.

BBS 2016. Statistical Pocket Book of Bangladesh. Bangladesh Bureau of Statistics, Statistics Division, Ministry of Planning, Government of People's Republic of Bangladesh. Pp. 207.

Bhale UN, Wagh PM and Rajkonda JN 2013. Antagonistic confrontations of *Trichoderma* spp. against fruit rot pathogens on Sapodilla (*Manilkara zapota* L.). Journal of Yeast and Fungal Research **4**(1): 5-11.

Bhandari H, Mohanty S and Hossain M 2011. Hybrid rice in Bangladesh: Current status and future project. *In* Proceedings of the 7th ASAE Conference 2011, Hanoi, Vietnam. pp. 13-15.

Bhatt DD and Vaughan EK 1962. Preliminary investigations on biological control of gray mold (*Botryotis cinerea*) of strawberries. Pl. Dis. Reptr. **46**: 342-345.

Bhuiyan MR, Rashid MM, Khan MAI, Hoque M, Nessa B, Rafii MY and Latif MA 2013. Eco-friendly management of seed borne fungi for sustainable crop production. Life Science Journal **10**(4): 1640-1650.

Bhuiyan NI, Paul DNR and Jabber MA 2002. Feeding the extra millions by 2025- challenges for rice research and extension in Bangladesh. A key note paper presented in the National Workshop on Rice Research and Extension-2002 held at BRRI, Gazipur, Bangladesh.

Bhutta AR and Hussain SA 1998. Seed borne fungi associated with rice seed lots in Pakistan. Int. Rice Res. Notes **23**(3): 26-27.

Bicca FM, Bandet L and Zimmer GJ 1998. Separation of discolored seed from rice seed lots using the gravity table and influence on seed health. Revista, Brasileria de Sementes **20**(1):106-111.

Bier JE 1966. *In*: Breeding Pest Resistant Trees. Henry D. Gerhold, Pergamon Press, London.

Bilai VI 1966.Volatile antibiotics in fungi of the genus *Trichoderma*. Microbiologia **25**: 458-465.

Bisht GS and Khulbe RD 1995. *Indian phytopathol*., **4**:480-482.

Bodalka C and Awadhiya GK 2009. Assessment of percent grain discoloration in important rice varieties. International Journal of Current Research in Biosciences and Plant Biology. **1**(4): 61-64.

Bokhary HA 1991. Seed borne fungi of rice from Saudi Arabia. Zeitschrift fiir Ptlanzenkran Kheiten and Pflanzenschutz **98**(3): 287-292.

Booth C 1971. The Genus *Fusarium.* The Commonwealth Mycological Institute, England. pp. 267.

Brian PW 1957. The ecological significance of antibiotic production. *In*: Microbial Ecology. 7th Symposium. Soc. Gen. Microbial. Cambridge Univ. Press. pp. 168-188.

Brian PW 1960. Griseofulvin. Trans. Br. Mycol. Soc. **43:** 1-13.

Butt AR, Yaseen SI and Javaid A 2011. Seed borne mycoflora of stored rice grains and its chemical control. The Journal of Animal and Plant Sci. **21**(2): 193-196.

CAB (Commonwealth Agricultural Bureau) 1968. Plant Pathologist’s Pocket Book. 1st edn. The Commonwealth Mycological Institute, England. pp. 267.

Caratelli A and Saponaro A 1983. Mycoflora of rice seeds from Maranhao state, Brazil. Rice Abs. **8**(1):112-114.

Chai RY, Jin MZ and Zhang QS 1991. The inhabiting fungi of discolored paddy rice grains and their pathogenicity. Rev. Plant Path. **72** (2): 964.

Chakraborty MR, Chatterjee NC and Quimio TH 2009. Integrated management of *Fusarium* wilt of eggplant (*Solanum melongena*) with soil solarization. Micologia Aplicada International **21**(1):25-36.

Chalupová J, Raus M, Sedlářová M, Šebela M. 2014. Identification of fungal microorganisms by MALDI-TOF mass spectrometry. Biotechnol Adv. **32**(1):230–241.

Chowdhury P, Bashar MA and Shamsi S 2015. *In vitro* evaluation of fungicides and plant extracts against pathogenic fungi of two rice varieties in Bangladesh. Bangladesh J. Bot. **44**(2): 251-259.

Chowdhury P and Shamsi S 2016. *In vitro* evaluation of fungicides and some plant extracts against rice sheath rot pathogen *Sarocladium oryzae.* Bangladesh J. Sci. Res. **29**(1): 47-54.

Christensen CM and Lopez LC 1965. Relation of moisture content and length of storage to changes in the microflora and germination percentage of rough rice. Phytopathology **55**: 953-956.

Dennis C and Webster J 1971a. Antagonistic properties of species groups of *Trichoderma.* I. Production of non-volatile antibiotics. Trans. Brit. Mycol. Soc. **57:** 25-29.

Dennis C and Webster J 1971b. Antagonistic properties of species groups of *Trichoderma.* II. Production of volatile antibiotics. Trans. Brit. Mycol. Soc. **57:** 41-48.

Dick CM and Hutchinson SA 1966. Biological activity of volatile fungal metabolites. Nature **211**: 868.

Dickinson CH and Boardman F 1970. Physiological studies of some fungi isolated from peat. Trans. Brit. Mycol. Soc. **55**: 293-305.

Diem HG 1969. Microorganisms de la surface des feuilies II. Interactions entre quelqueschampignous parasites at divers' saprophytes filamenteux de la phyllosphere de l′ org. III. Effect de la competition nutritive sur la germination des spores d′ unesouched′ *Helminthosporium sativum.* Bull. Ecol. Nat. Sup. Agron. Nanacy **11**:12-25.

Dikshit A, Mishra BK, Mishra RK, Mishra RC, Tiwari AK and Yadav RS 2011. Biocontrol efficacy of *Trichoderma viride* isolates against fungal plant pathogens causing disease in *Vigna radiata* L. Archives of Appl. Sci. Res. **3(**2): 361-369.

Ellis MB 1971. Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, England. pp. 608.

Ellis MB 1976. More Dematiaceous Hyphomycetes. The Commonwealth Mycological Institute, England. pp. 507.

Ellis MB and Ellis JP 1997. Micro Fungi on Land Plants. An identification Handbook. The Commonwealth Mycological Institute, England. pp. 868.

EL-Shafey RAS, Attia K, Elamawi M and Mostafa FA 2018. Incidence and molecular identification of *Cochliobolus carbonum* a causal organism of rice seedling blight. Beni-Suef University

Esuruoso OF and Joaqui MA 1975. Control of some important seed-borne fungi of rice (*Oryzae sativa* L.) in Nigeria. African J. Plant Protection **2**(1): 29-42.

Fakir GA 1998. First National Workshop on Seed Pathology. Seed Pathology Laboratory, Dept. of Plant Pathology, BAU, Mymensingh.

Fakir GA 2000. An annotated list of seed-borne disease in Bangladesh. Seed Pathology Laboratory. Dept. of Plant Pathology, BAU, Mymensingh. pp. 41.

Fakir GA, Hossain I, Ahmed MU, Anam MK, Alam MN and Rahtnan M 2003: Effect of ash, chalk powder and neem leaf on the quality of Boro rice seed stored in gunny bag, motka, plastic drum and tin. Proceeding of review and planning meeting of the rice seed health improvement sub-project held at BRRI, Gazipur, Bangladesh. 21-22 April, 2003. pp. 1-37.

Fakir GA, Hossain I, Ahmed MU, Asad-ud doula M and Alam MN 2002. Quality of farmer's Boro and T. aman rice seeds collected from Bogra, Rajshahi and Rangpur district of Bangladesh. Proceeding: A report for presentation in the review and planning meeting of the rice seed health improvement sub-project held at BRRI Gazipur, Bangladesh. pp.1-16.

Fakir GA, Islam MR and Islam MF 1990. Survey on the health status of jute and rice seeds of farmers of Sadar Thana, Mymensingh. BAU Research Programme **4:** 42-47.

FAO 2014. Food and Agriculture Organization of the United Nation, XVII (3).

FAO 2016. Food and Agriculture Organization of the United Nations. www. fao.org/ world food situation/csdb/en.

Farid AKM, Khalequzzaman, Islam N, Anam MK and Islam MT 2002. Effect of fungicides against *Bipolaris oryzae* of rice under *in vitro* condition. Pak. J. Plant Pathol. **1**(1): 4 -7.

Faruq AN, Rahman MA, Aminuzzman FM, Mamun-ur-Rashid MD and Hoque S 2014. *Pyricularia oryzae* causal agent of rice blast disease. American Journal of Plant Sciences. **6**: 602-611.

Fokkema NJ 1976. Antagonism between fungal saprophytes and pathogens on aerial plant surfaces. *In*: Microbiology of Aerial Plant Surfaces, Dickinson CH and PreeceTF(eds.) Academic Press, London, pp. 486-506.

Fravel DR 2005. Commercialization and implementation of biocontrol 1. Ann. Rev. Phytopathol. **43**: 337-359.

Fries N 1973. Effect of volatile organic compounds on the growth and development of fungi. Trans. Brit. Mycol. Soc. **60**: 1-21.

Ganguly LK 1994. Fungitoxic effect of certain plant extracts against rice blast and brown spot pathogen. Environ. Ecol. **12:** 731-733.

Garrett RN 1981. A preliminary report of studies on great barrier reef *Octocorallia. In:* Crown-of-Thorns Starfish Seminar Proceedings Canberra. Australian Government Publishing Service. Pp. 135-147.

Geetha D and Sivaprakasam K 1993. Treating rice seeds with fungicides and antagonist to control seed-borne diseases. Rice Res. Notes. **18**: 30-31.

Gilman JC 1967. A Manual of Soil Fungi. Oxford and IBH Publishing Co., New Delhi, 2nd edition. pp. x + 450.

Gill, MA, Wahid A, Javed MS and Khan TZ 1999. Major diseases of rice crop in the Punjab and their management strategies. *In*: Proc. 2nd Nat. Conf. Plant Pathol., Sept. 27-29, 1999, Univ. Agri. Faisalabad.

Gopalakrishnan C, Kamalakannan A and Valluvaparidasan V 2010. Effect of seed-borne *Sarocladium oryzae*, the incitant of rice sheath rot on rice seed quality. J. Plant Prot. Res. **50**(1):98-110.

Gottlieb D 1957. The effect of metabolites on antimicrobial agent. Phytopathol. **47:** 59-67.

Gottlieb D and Shaw PD 1970. Mechanism of action of antifungal antibiotics. Ann. Rev. Phytopathol. **8:** 371-402.

Gray A, Strobel, Dirkse E, Sears J and Markworth C 2001.Volatile antimicrobials from *Muscodor albus*, a novel endophytic fungus. J. Microbiol. **147**: 2943-2950.

Guerrero FC, Mathur SB and Neergaard P 1972. Seed health testing of rice. Seed borne fungi associated with abnormal seedlings of rice. Proc. Int. Seed Test. Assoc. **37**: 985-997.

Gupta V, Shamas N, Razdan VK, Sharma BC, Sharma R, Kaur K, Singh I, John D and Kumar A 2013. Foliar application of fungicides for the management of brown spot disease in rice (*Oryza sativa* L) caused by *Bipolaris oryzae*. African Journal of Agricultural Research **8**(25): 3303-3309.

Habib A, Javed N, Sahi ST and Waheed M 2012. Detection of seed-borne mycoflora of different coarse and fine rice varieties and their management through seed treatment. Pakistan Journal of Phytopathology **24**(2):133 -136.

Hajano JU, Pathan MA, Rajput QA and Lodhi MA 2011. Rice blast-mycoflora, symptomatology and pathogenicity. International Journal of Agriculture & Biology **5**(1): 53-63.

Halgekar NY, Giri GK and Ashwini C 2014. Efficacy of bio-agents against seed borne fungi of rice. International Journal of Applied Biology and Pharmaceutical Technology **5**: 157-158.

Hamamura H, Kawahara M and Shimoda S 1989. Some characteristics of *Gibberella fujikuroi* (*Fusarium moniliforme*) isolates less sensitive to triflumizole. Annals Phytopathol. Soc. Japan **55**: 275-80.

Hansraj D, Ratnoo RS and Jat A 2018. Detection and identification of seed borne mycoflora of wheat (*Triticum aestivum* L.) seed samples. Journal of Pharmacognosy and Phytochemistry **7**(3): 3164-3170.

Haque AHMM, Akon H, Islam MA, Khalequzzaman KM and Ali MA 2007: Study of seed health, germination and seedling vigor of farmers produced rice seeds. International Journal of Sustainable Crop Production **2**(5): 34-39.

Hasan MM, Hossain I and Fakir GA 2001. Effect of seed cleaning and washing on germination and seedling disease of rice BR 11 (Mukta). Bangladesh J. Seed Sci. and Tech. **5**(1&2):1-6.

Hasan NA, Rafii MY, Rahim HA, Ali NS, Mazlan N and Abdullah S 2016. Morphological and molecular characterization of fungal pathogen, *Magnaphorthe oryzae*. Paper presented at the AIP Conference Proceedings.

Helal BR, Hosen S and Shamsi S 2018. Mycoflora associated with post-harvest disease of papaya (*Carica papaya* L.) and their pathogenic potentiality. Bangladesh J. Bot.47(3): 389-395.

Hosen D and S Shamsi 2019. *In vitro* antagonism of *Trichoderma viride* and *Aspergillus* spp. against a pathogenic seed borne fungus of sesame. J. Bangladesh Acad. Sci. **43** (1): 17-23.

Hossain I and Dey P 2011. Annual Report. Seed Pathology Centre, BAU, Mymensingh, Bangladesh. pp. 5-6.

Hossain KS 1993. A study on the fungi associated with damping-off infected seedling of cabbage, cauliflower and kohlrabi of nursery beds of the Dhaka city and the effect of some fungicides, antibiotics and plant extracts on the growth of three isolates. M.Sc. Thesis, Dept. of Botany, Dhaka University, Dhaka. pp. 58.

Hossain KS and Taher Mia MA 2015. Management of bakanae disease of rice; Bangladesh Journal of Botany **44**(2): 277-283.

Hossain MM, Hossain N, Sultana F, Islam SMN, Islam MS and Bhuiyan MKA 2013. Integrated management of Fusarium wilt of chickpea (*Cicer arietinum* L.) caused by *Fusarium oxysporum* f. sp. *ciceri* with microbial antagonist, botanical extract and fungicide Afr. J. Biotechnol. **12**: 4699-4706.

Hossain MS, Ayub MA, Mollah MIU, Khan MAI and Sajjadul AKM 2015. Evaluation of fungicides for the control of bakanae disease of rice caused by *Fusarium moniliforme*. Bangladesh Rice J. **19**(1):49 -55.

Howell CR 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: The history and evolution of current concepts. Plant Dis. **87**: 4-10.

Howlader AN 2003. Effect of seed selection and seed treatment on the development of *Phomopsis* blight and fruit rot of eggplant. M.S. Thesis. Dept. of Plant Pathology, BAU, Mymensingh, Bangladesh.

Hutchinson SA 1971. Biological activity of volatile fungal metabolites. Trans. Brit. Mycol. Soc. **57**: 185-200.

Hutchinson SA 1973. Biological activity of volatile fungal metabolites. Ann. Rev. Phytopathol. **11**: 223-246.

Huynh Van N and Ashok G 2005. Efficacy of seed treatment in improving seed quality in rice (*Oryza sativa* L.) **13**: 42-51.

Iqbal Z, Pervez MA, Ahmad S, Iftikhar Y, Yasin M, Nawaz A, Ghazanfar MU, Dasti AA and Saleem A 2010. Determination of minimum inhibitory concentrations of fungicides against fungus *Fusarium mangiferae*. Pak. J. Bot. **42**(5): 3525-3532.

Ishii H and Takeda H 1989. Differential binding of a N-phenyl from amidoxime compound in cell free extract of benzimidazole resistant and sensitive isolates of *Venturia nashicola, Botrytis cinerea* and *Gibberella fujikuroi*. Netherland J. Plant Pathol. **95** (1): 99-108.

Islam MK, Rahman AJMM and Mia MAT 2000. Significance of seed-borne fungal pathogens of rice with emphasis on *Bipolaris oryzae*. Bangladesh Journal of Plant Pathology **16**(1-2): 27-30.

Islam MK, Rahman AJMM and Mia MAT 2007. Study of seed health, germination and seedling vigor of farmers produced rice seeds. International Journal of Sustainable Crop Production **2**(5): 34-39.

Islam MM, Sultana K, Mostafa MG, Begum HA, Rahman MM and Nabi MN 2003. Effect of different level of seed-borne infections on fibre yield contributing characters of jute (*Corchorus capsularis* L.). Pakistan J. Plant Pathol. **2**(3): 129-135.

Islam MN and Mukherjee SK 2011. Construction of MYMIV based gene silencing vector and its use. ISBN: 978-3-8443-8820-6. LAP-LAMBERT Academic Publishing GmbH & Co KG. Dudweiler Landstr. 99, 66123 Saarbrücken, Germany.

Islam MR, Ali A and Mia MAR 1994. Survey on seed-borne pathogens of rice in fifteen districts of Bangladesh. Bangladesh Journal of Plant Pathology **10** (1&2): 23-26.

Islam MS, Jahan QSA, Bunnarith K, Viangkum S and Merca SD 2000. Evaluation of seed health of some rice varieties under different conditions. Bot. Bull. Acad. Sin. **41**:293-297.

ISTA 1993. International rules for seed testing. Seed Science and Technology **21**: 141-146.

ISTA 1996. International rules for seed testing rules. Seed Science and Technology **4**(1): 3-177.

ISTA 2001. International Rules for Seed Testing Association 31: 107-115.

Jadon KS and Shah R 2012. Antifungal activity of different plant extracts against *Drechslera bicolor* causing leaf blight of bell pepper. Archives of Phytopathology and Plant Protection **45**: 1417-1428.

Jha MM, Kumar S and Hasan S 2004. Effect of botanicals on Maydis leaf blight of maize *in vitro.* Ann. Biol. **20**:173-176.

Kexiang G, Xiaogaung L, Youghong L, Tianbo Z and Shuliang W 2002. Potential of *Trichoderma harzianum* and *T. atroviride* to control *Botryosphaeria berengeriana* f. sp. *piricola*, the causal organism of apple ring rot. Phytopathol. **150**: 271-276.

Khair A and Subash CD 2018. Control of seed borne fungi by *Aspergillus* and *Trichoderma*. SSRG International Journal of Agriculture & Environment Science (SSRG-IJAES) **5**(I): 34-39.

Khalili E, Sadravi M, Naeimi S and Khosravi V 2012. Biological control of rice brown spot with native isolates of three *Trichoderma* species. Brazilian Journal of Microbiology **43**(1): 297-305.

Khan MI and Kumar R 1992. Antifungal activity of leaf extracts neem on seed mycoflora of wheat. Indian J. Seed Abs. **15**(7): 299.

Khan SA, Anwar SA and Bhutta AB 1990. Studies on seed borne fungi, bacteria and nematodes of rice in the Punjab. Pak. J. Sci. Ind. Res. **33**(11): 489-492.

Khan TZ, Gill MA, Nasir MA and Bokhari SAA 1999. Fungi associated with the seeds of different rice varieties/lines. Pak. J. Phytopathol. **1**(11): 22-24.

Khare MN 1999: Seed health care in seed quality control. Indian Journal of Phytopathology **52**(3):305.

Khare MN, Mathur SB and Neergaard P 1977. A seedling symptom test for the detection of Septoria nodorum in wheat seeds. Seed Sci. Technol. **5**: 613-617.

Khatun A and Shamsi S 2016. *In vitro* evaluation of fungicides and plant extracts against the fungi associated with seeds of nine chickpea varieties. Dhaka University Journal of Biological Sciences **25**(1): 83-90.

Khatun A and Shamsi S 2016. Estimation of interrelationship among seed germination, purity, seedling mortality and association of fungi with seeds of chickpea. Bangladesh J. Bot. **45**(3): 693-698.

Khush GS and Brar DS 2002: The application of biotechnology to rice. *In*: Ives C. and Bedford B. (ed). Agricultural Biochemistry in International Development Wallingford (UK), CAB International pp. 83-108.

Krieg NR 1981. Enrichment and isolation. *In*: Manual of Methods for General Bacteriology, Gerhardt P, Murray RGE, Costilow RN, Nester EW, Wood WA, Krieg NR, Phillips GB (eds) Amer. Soc. Microbiol., Washington D.C. pp. 112-142.

Krupke AO, Castle AJ and Rinker DL 2003. The North American mushroom competitor, *Trichoderma aggressivum* f. *aggressivum*, produces antifungal compounds in mushroom compost that inhibit mycelia growth of the commercial mushroom *Agaricus bisporus*. Mycol. Res. **107**: 1467-1475.

Kuepper G 2003. Down mildew control in cucurbits ATTRA (National Sustainable Agriculture).

Kumar V, Jariwala S and Rai B 1997. Effect of physico-chemical factors on growth of *Drechslera oryzae.* J. Mycopathol. Res. **35**:131-136.

Li JY, Harper J, Grant DM, Tombe BO, Bashyal B, Hess WM and Strobel GA 2001. Ambuic acid, a highly functionalized cyclohexenone with antifungal activity from *Pestalotiopsis* spp. and *Monochaetia* sp. Phytochemistry **56**: 463-468.

Li JY and Strobel GA 2001. Jesterone and hydroxyl-jesterone antioomycete cyclohexenone epoxides from the endophytic fungus *Pestalotiopsis jester.* Phytochemistry **57**: 261-265.

Li JY, Strobel G, Harper J, Lobkovsky E and Clardy J 2000. Cryptocin, a potent tetramic acid antimycotic from the endophytic fungus *Cryptosporiopsis* cf. *quercina.* Org. Lett. **2**(6): 767-770.

Madbouly AK, Ibrahim MIM and Abdel-Wahhab MA 2014. Efficacy of corn and rice seed-borne mycoflora in controlling aflatoxigenic *Aspergillus flavus*. Comunicata Scientiae **5**(2):118-130.

Madhanraj P, Ambikapathy V and Panneerselvam A 2010. Biological control of banana wilt caused by *Fusarium solani* (Mart.) Sacc. **I** (3):1032-1039.

Mamun MA, Shamsi S and Bashar MA 2016. *In vitro* evaluation of fungicides and plant extracts against pathogenic fungi of jute seeds. Bioresearch Communications **2**(1):189-192.

Mamun MA, Shamsi S and Bashar MA 2016. Estimation of interrelationship among some quality factors of jute seeds. Dhaka Univ. J. Biol. Sci. **25**(1): 9- 17.

Manandhar HK, Jorgensen HJL, Smedegaard-Petersen V and Mathur SB 1998. Seed borne infection of rice by *Pyricularia oryzae* and its transmission to seedlings. The American Phytopathological Society **82**(10): 1093-1099.

Manimegalai V and Ambikapathy V 2012. Evaluation of inhibitory effects of medicinal plants extract against *Bipolaris oryzae* of rice. Der Pharmacia Sinica **3**(4):507-510.

Mansur A, Hossain M, Hassan K and Dash CK 2013. Seed health and quality test of three rice varieties for the detection of fungi associated with seed sample. Universal J. Plant Sci. **1**(2): 37-42.

Mansur A, Hossain M, Hassan K and Dash CK 2013. Efficacy of different plant extract on reducing seed borne infection and increasing germination of collected rice seed sample. Universal J. Plant Sci. **1**(3): 66 -73.

Marshal AM and Hutchinson SA 1970. Biological activity of volatile metabolites from the culture of *Fomes scutellatus.*Trans. Br. Mycol. Soc. **55**: 239-251.

Mendoza AM and Molina RP 1980. A study on seed-borne fungi associated with rice seed and their effects on rice seedlings. Journal of Araneta Research **27** (14): 50-69.

Mew TW and Gonzales P 2002. A Hand Book of Rice Seed-borne Fungi. IRRI Science Publishers. **83**: 187-191.

Mia MAT, Ali A, Nahar N and Shajahan AKM 1994. Incidence of grain spot disease of rice in Bangladesh. Bangladesh Journal of Plant Pathology **10** (1&2):27-30.

Mia MAT and Mathur SB 1983. Study on seed mycoflora of rice in Bangladesh. Seed Research **11**(2): 254-257.

Miah A, Shamsi S, Hosen S and Morshed MS 2017. *In vitro* efficacy of plant extracts on seed germination and fungal infection of six varieties of wheat (*Triticum aestivum* L.). Bioresearch Communications **3**(2): 415-421.

Miah AT, Ahmed MU, Sharma NR, Ali A and Miah SA 1990. Antifungal activity of some plant extracts. Bangladesh J Bot. **19**(1): 5-20.

Mian IH and Fakir GA 1989. Fungi, moisture content and germinability of rough rice grains during storage. Seed Res. **17**: 169-173.

Mishra AK and Dharam V 1991. Wild rice and its crosses, the alternative hosts of paddy grain discoloring fungi. Int. J. Trop. Plant. Dis. **9**(1): 123-125.

Misra AP and Singh TB 1972. Effect of some copper and organic fungicides on the germination of seeds and the growth of paddy seedling. Indian Phytopathol. **25**: 297-300.

Misra JK, Gergon E and Mew TW 1994. Occurrence, distribution and phenology of seed borne fungi of rice in certain Provinces of Philippine. Plant Pathol. Bull. **3** (4): 229-239.

Mitchell J. I. and Zuccaro A. 2006, "Sequences, the environment and fungi," *Mycologist*, vol. 20, no. 2, pp. 62–74.

Mohamed AA and Gomaa FH 2019. Molecular characterization and biological control of some rice seed-borne fungal pathogens. J. Phytopathol. Pest Management **6**(1):40-53.

Mondall NK, Mojumdar ASK, Chatterje A, Banerjee JK and Gupta S 2009. Antifungal activities and chemical characterization of neem leaf extracts on the growth of some selected fungal species *in vitro* culture medium. Journal of Applied Sciences and Environmental Management **13**(1): 49-53.

Muthomi JW, Riungu GM and Narla RD 2007. Effect of antagonistic microorganisms on severity of *Fusarium* head blight of wheat and grain yield. African Crop Science Conference Proceedings **8**: 827-832.

Naher L, Ali MA and Sheheli S 2016. Effect of seed treatment on seed borne fungi of rice. Department of Agricultural Extension Education, BAU, Mymensingh. **27**: 48-56.

Nahar MN and Shamsi S 2020. *In vitro* screening of fungicide and plant extracts against six pathogenic fungi isolated from cotton (*Gossypium arboreum* L.) seed. Bangladesh J. Bot. **49**(2): 197-204.

Nazrul ASM and Fakhrul ISM 2010. Factor demand in the healthy rice seed use in Boro and T. Aman: A case study of Bangladesh. Bangladesh Journal of Agricultural Research. **35**(2):297-312.

Neergaard P 1977. Seed Pathology. The MacMillan Press Ltd. London, Vol. 1-2, pp. 1191.

Newhook FJ 1951. Microbiological control of *Botrytis cinerea* Pers. I. The role of pH changes and bacterial antagonism. Ann. Appl. Biol. **39**: 168-184.

Newhook FJ 1957. The relationship of saprophytic antagonism to control *Botryotis cinerea* Pers. on tomato. New Zealand J. Sci. Technol. Sec. A. **38**: 473-481.

Nguefack, Nguikwie J, Fotio SK, Dongmo D, Leth B, Nkengfack AE and Amvam ZPH 2007. Fungicidal potential of essential oils and fractions from *Cymbopogon citratus*, *Ocimum gratissimum* and *Thymus vulgaris* to control *Alternaria padwickii* and *Bipolaris oryzae*, two seed-borne fungi of rice (*Oryza sativa* L.). J. Essential Oil Restitution **19**: 581-587.

Niaz I, Sitara U and Qadri S 2008. Effect of different seed oils and benlate fungicide on *in vitro* growth of four *Drechslera* species. Pak. J. Bot. **40**(1): 397- 401.

Nilsson RH, Abarenkov K, Larsson K-H, Kõljalg U. 2011. Molecular identification of fungi: rationale, philosophical concerns, and the UNITE database. Open Appl Inf J. **5**:81–86.

Nurulhidayah MM and Kalaivani N 2015. Morphological and molecular characterization of *Magnaporthe oryzae* (fungus) from infected rice leaf samples. AIP Conference Proceeding, 1614, pp. 756-760.

Ogawa K 1988. Damage by bakanae disease and its chemical control. Japan Pesticide Information **52**: 13-15.

Ogawa K and Takeda S 1990. Population of benomyl resistant rice "bakanae" fungus in paddy fields. Annals Phytopathol. Soc. Japan **56**: 247- 49.

Omatsu N, Izumi S and Shinyashiki I 1990. Occurrence of benomyl resistant strains of *Gibberella fujikuroi* in Kagoshima prefecture and control of bakanae disease of rice seed by disinfection. Proc. Assoc. Pl. Prot. Kyushu **36:** 5-7.

Ora N, Faruq AN, Islam MT, Akhtar N and Rahman MM 2011. Detection and identification of seed borne pathogens from some cultivated hybrid rice varieties in Bangladesh. Middle-East Journal of Scientific Research **10** (4): 482-488.

Papavizas GC 1985. *Trichoderma* and *Gliocladium* biology, ecology and the potential for biocontrol. Ann. Rev. Phytopathol. **23**: 23- 77.

Park D 1963. The ecology of soil-borne fungal disease. Ann. Rev. Phytopathol. Z. **106**: 226-232.

Patel R and Solanki VA 2017. Seed borne mycoflora associated with rice seeds in south Gujarat. International Journal of Plant Protection **10**: 311-319.

Porter CL 1924. Concerning the characters of certain fungi as exhibited by their growth in presence of other fungi. Am. J. Bot. **11**: 168-188.

Poudel NS, Prakash B and Acharya M 2019. Influence of different chemical fungicides against rice brown leaf spot disease caused by *Bipolaris oryzae*. Int. J. Curr. Microbiol. Appl. Sci. **8**(1): 441-446.

Rahman GMM, Islam MR and Wadud MA 1999. Seed treatment with plant extracts and hot water: a potential biophysical method of controlling seed-borne infection of wheat. Bangladesh Journal of Training and Development **12**(1-2): 185-190.

Rana S, Baghela A and Singh SK 2017. Morphology and phylogeny of *Microdochium fisheri*, a new record from India. Plant Pathology and Quarantine **7**(2): 191-200.

Rao KV, Singh SP, Surekha K and Muthuraman P 2010. Site specific integrated nutrient management in rice and rice based cropping systems. Indian Agril. Res., Directorate Rice Res. pp. 1-2.

Rao SS and Ranganathaiah KG 1988. Control of seed-borne infection of *Drechslera oryzae* in paddy by seed treatment. Seed Res. **16**: 193-199.

Rao SS, Reddi KM, Madhusudhan P and Ravindra RB 2018. Evaluation of bio-efficiency of rice based fungicides against rice discoloration causing pathogen *Curvularia lunata* (Wakker) Boedijn. Int. J. Curr. Microbiol. App. Sci **7**(7): 1373-1379.

Raper KB and Thom C 1949. Manual of the Penicillia, Williams and Wilkins, Baltimore, MD. USA. pp. 875.

Rashid AQMB and Fakir GA 2000: Impact of Seed Health on Sustainable Crop Production in Bangladesh. Co-operation, Yearly J. Published by Cooperative Dept. Samabaya Sadan, 9/D, Motijheel Commercial Area, Dhaka-1000, Bangladesh.pp. 24-36.

Rashid MH, Hague SMA, Mosaddeque HQM, Ali MS and Polan MS 2007. Association of seed-borne fungi with T. aman seed in relation to variety and farmers' seed processing activities. International Journal of Sustainable Agricultural Technology **3**(2):7-10.

Rathod LR and Pawar NB 2013. *In-vitro* seed treatment of fungicides for the control of seed borne fungi of soya bean variety Durga. Global Research Analysis **2**(10):15-16.

Reddy AB and Khare MN 1978. Seed-borne fungi of rice in Maddha Pradesh and their significance. Indian Phytopathology **31:** 300-303.

Rossman AY and Palm-Hernandez ME 2008. Systematics of plant pathogenic fungi: Why it matters. Plant Disease **92**: 1376-1386.

Sagar SD and Hegde YR 2006. Management of seed mycoflora of rice by different seed dressing fungicides. International Journal of Plant Sciences **1**(2): 197-199.

Salim M, Akram M, Akhtar MH and Ashraf M 2003. Rice Production Handbook. Pak. Agric. Res. Council, Islamabad.pp.70.

Salma U 1995. Fungitoxicity of extracts of twenty higher plants and four fungicides on three fungal plant pathogens. M.Sc. Thesis, Dept. of Botany, Dhaka University, Dhaka. pp. v+42.

Santos GR, Castro N, Ignacio M, Furtado GQ, Rancel PHN, Silva LM and Riveiro FF 2009. Resistance of upland rice genotypes to rice disease at the south of Tocantins State. Bio. Sci. J. **25**(6): 96 -105.

Saranraj P, Sivasakthivelan P and Sivasakthi S 2013. Prevalence and production of plant growth promoting substance by *Pseudomonas fluorescens* isolated from paddy rhizosphere soil of Cudd lore district, Tamil Nadu, India. African Basic and Applied Sci. **5**: 95-101.

Selvaraj K and Annamalai P 2015. *In vitro* evaluation of fungicides and two species of *Trichoderma* against *Sarocladium oryzae* causing sheath rot of paddy (*Oryza sativa* l.). World Journal of Pharmaceutical Research **4**: 1200-1206.

Seshu DV and Dadlani M 1988. Role of woman in seed management with special reference to rice. IRTP Technical Bulletin 5: 24

Shahjahan AKM, Mia MAT and Miah SA 1988. Rice grain spotting and associated organisms. Bangladesh J. Plant Pathol. **4**(1&2): **1-7.**

Shamima A, Latif MA, Taher MA, Ansari TH, Islam T and Rafii MY 2013. Efficacy of fungicides against grain spot disease in rice (*Oryza sativa*). Life Science Journal **10** (4): 3005-3008.

Shamsi S 1999. Investigations into the sheath rot disease of rice (*Oryza sativa* L.) in Bangladesh. Ph.D. Thesis. Department of Botany, University of Dhaka. pp. i-xii + 1-127.

Shamsi S, Hosen S and Ahmed AS 2018. Fungi associated with leaves of *Sonneratia apetala* Buch. Ham and *Sonneratia caseolaris* (L.) Engler from Rangabali coastal zone of Bangladesh. Dhaka Univ. J. Biol. Sci. **27**(2):155-162.

Shamsi S, Islam NM, Hosen S, Mamun MD, Chowdhury P, Momtaz S, Naher N, Yeasmin Z, Sultana S, Khatun A, Islam AA and Bashar MA 2019. Morphological and molecular identification of ten plant pathogenic fungi. Bangladesh J. Plant Taxon. **26**(2): 169-177.

Shamsi S, Naher N, Chowdhury P and Momtaz MST 2010. Fungal diseases of three aromatic rice (*Oryza sativa* l.). Journal of Bangladesh Academy of Sciences **34** (2): 163-170.

Shamsi S, Nowsher AK AZM, Shajahan AKM and Miah SA 1995. Prevalence of *Sarocladium oryzae* (Sawada) W. Gams and D. Hawksw. in different parts of rice grains. Bangladesh J Bot. **24** (2): 217-219.

Sharma A and Kapoor AS 2016. Detection of seed borne mycoflora associated with some rice varieties grown in Himachal Pradesh. International J Life Sci. **11**(4): 733-739.

Shrestha SK, Mathur SB and Neergard P 1977. Seed borne organisms in some crops in Nepal. Seed Science and Technology **5** (1):111-121.

Siddiquie KU, Islam MA, Ahmed ZU, Begum ZNT, Hasan MA, Khondker M, Rahman MM Kabir SMH, Ahmad M, Ahmed ATA, Rahman AKA and Haque EU (Eds) 2007. Encyclopedia of Flora and Fauna of Bangladesh. Vol. **2**. Cyanobacteria, Bacteria and Fungi. Asiatic Society of Bangladesh, Dhaka. pp. 415.

Singh M, Devi PHS, Singh MSS and Singh MT 2004. Effect of plant extracts on seed mycoflora of rice during storage. Indian Phtopathology Jouranl **57** (2): 205-207.

Singh N and Singh RS 1970. Development of wilt causing species of Fusarium in fungicide treated soils. Indian Phytopath. **23**: 545-552.

Singh N and Webster J 1973. Antagonism between *Stilbella erythrocephala* and other coprophilous fungi. Trans. Brit. Mycol. Soc. **61:** 487- 495.

Sisterna M and Ronco L 1994. Efficacy of three fungicides for controlling growth of five seed-borne fungi associated with rice grain spotting. Int. Rice Res. Notes **19**: 25-26.

Sisterna MN, Lori GA and Marassi JJ 1994. Symptomatology and fungi associated with rice grain spotting in cultivar Igra 409. Revista de la Facultad de Agronomic **70:**13-21.

Sivalingam PN, Vishwakarma SN and Singh US 2006. Role of seed-borne inoculum of *Rhizoctonia solani* in sheath blight of rice. Indian Phytopathology **59**: 445-452.

Skidmore AM 1976. Interaction in relation to biological control of plant pathogens. *In*: Microbiology of Aerial Plant Surfaces (eds. Dickinson CH and Preece TF), Academic Press, London. pp. 507-528.

Skidmore AM and Dickinson CH 1976. Colony interaction and hyphal interference between *Septoria nodorum* and phylloplane fungi. Trans. Brit. Mycol. Soc. **66**: 57-64.

Sohaib A, Shinawar WA, Ahmed A and Rashid M 2015. Molecular characterization of fungal species isolated from rice grains. International conference on chemical, agricultural and biological sciences. Sept. 4-5, Istanbul (Turkey).

Spurr HWJ and Wetly RE 1972. Incidence of tobacco leaf microflora in relation to brown spot disease and fungicidal treatment. Phytopathol. **62**: 916-920.

Srinivas P and Ramakrishnan G 2005. Management of rice seed borne pathogens by nativeative bioagents. Ann. Plant Protection Science **13** (2): 422-426.

Stierle A, Strobel G and Stierle D 1993. Taxol and taxane production by *Taxomyces andreanae,* an endophytic fungus of Pacific yew. Science **260**: 214-216.

Strobel GA, Dirkse E, Sears J and Markworth C 2001. Volatile anti-microbials from *Muscodor albus,* a novel endophytic fungus. Microbiology **147**: 2943-2950.

Suleiman MN and Omafe OM 2013. Activity of three medicinal plants on fungi isolated from stored maize seeds (*Zea mays* L.). Global J. Med. Plant Res. **1**(1): 77-81.

Sutton BC 1980. The Coelomycetes. Fungi Imperfecti with Pycnidia, Acervuli and Stroma. The Commonwealth Mycological Institute, England. pp. 696.

Tamuli P, Das J and Boruah P 2014. Antifungal activity of *Vitex negundo* Linn. against some phytopathogenic fungi. Plant Archives **14**(2): 981-982.

Tapwal A, Tyagi A, Thakur G and Chandra S 2015. *In vitro* evaluation of *Trichoderma* species against seed borne pathogens. IJCBS Res. 2349-2724.

Thakur S and NSK Harsh. 2014. *In vitro* potential of volatile metabolites of phylloplane fungi of *Piper longumas* biocontrol agent against plant pathogen. *I.J.S.N.* **5**(1): 33-36.

Thom C and Raper KB 1945. A Manual of the Aspergilli. Williams and Wilkins, Baltimore, M.D. USA. pp. 373.

Tripathi P and Dubey NK 2004. Exploitation of natural products as an alternative strategy to control postharvest fungal rotting of fruit and vegetables. Postharvest Biology and Technology **32**: 235-245.

Tripathi P and Shukla AK 2007. Emerging non-conventional technologies for control of post-harvest diseases of perishables. Fresh Prod. **1**(2): 111-120.

Uddin MJ 2005. The quality of farmers stored rice seed of Begumgonj Upazilla, M. S. Thesis, Department of Plant Pathology. Bangladesh Agricultural University, Mymensingh.

Vachspati P, Agarwal VK and Pandey MP 2000. Location and seed transmission of fungi in discolored seeds of hybrid rice. Indian Phytopathology **53**(1): 45-49.

Waris M, Hemalatha P, Mishra MK and Kumar KA 2018. Management of seed borne pathogens of rice. International Journal of Current Microbiology and Applied Sciences **7**(10): 3638-3648.

Wool SL and Larito M 2007. Exploiting the interactions between fungal antagonists, pathogens and the plant for biocontrol. *In:* Vurro M and Gressel J (Eds.). Novel Biotechnologies for Biocontrol Agent Enhancement and Management. Springer Press, Amsterdam, Netharland. pp.425.

Yasuda Y 1986. Present situation on benzimidazole resistance of *Gibberella fujikuroi* and *Botrytis cinerea* in Japan. ISPP Chemical Control News letter **7**: 8-9.

APPENDICES

Appendix I. Analysis of variance of pathogenic fungi on germination, mortality, root and shoot length

Germination percentage of inoculated seeds

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	6	5390.0000	898.3333	122.50	0.0000
Error	14	102.6667	7.3333		
Total	20	5492.6667			

CV(%)2.99

Mortality percentage of inoculated seeds

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	6	982.9524	163.8254	13.99	0.0000
Error	14	164.0000	11.7143		
Total	20	1146.9524			

CV(%)6.75

Root length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	6	1388.4762	231.4127	31.56	0.0000
Error	14	102.6667	7.3333		
Total	20	1491.1429			

CV(%)4.36

Shoot length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	6	6319.9048	1053.3175	52.79	0.0000
Error	14	279.3333	19.9524		
Total	20	6599.2381			

CV(%)5.49

Appendix II. Analysis of variance of seed to seedling transmission of test pathogens in test tube experiments

Germination percentage of inoculated seeds

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	6	3993.8095	665.6349	55.47	0.0000
Error	14	168.0000	12.0000		
Total	20	4161.8095			

CV(%)4.97

Mortality percentage of inoculated seeds

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	6	784.4762	130.7460	15.60	0.0000
Error	14	117.3333	8.3810		
Total	20	901.8095			

CV(%)10.07

Analysis of variance of seed to seedling transmission of test pathogens in pot experiments

Seed germination of infected seeds (%)

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	6	5860.4762	976.7460	96.75	0.0000
Error	14	141.3333	10.0952		
Total	20	6001.8095			

CV(%)5.91

No. of seedlings exhibiting symptoms

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	6	127.1429	21.1905	9.67	0.0003
Error	14	30.6667	2.1905		
Total	20	157.8095			

CV(%)28.10

Seed to seedling transmission of disease (%)

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	6	603.3333	100.5556	25.44	0.0000
Error	14	55.3333	3.9524		
Total	20	658.6667			

CV(%)13.46

Appendix III. Analysis of variance for fungitoxicity of fungicides against *Bipolaris oryzae*

100 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	21938.0860	2437.5651	2223.87	0.0000
Error	20	21.9218	1.0961		
Total	29	21960.0078			

CV(%)1.70

200 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	18945.3709	4736.3427	1093.14	0.0000
Error	20	43.3277	4.3328		
Total	29	18988.6986			

CV(%)2.33

300 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	15445.7002	1716.1889	667.35	0.0000
Error	20	51.4326	2.5716		
Total	29	15497.1328			

CV(%)2.12

400 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	13324.3007	1480.4779	839.97	0.0000
Error	20	35.2505	1.7625		
Total	29	13359.5512			

CV(%)1.58

500 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	6584.5878	731.6209	572.12	0.0000
Error	20	25.5757	1.2788		
Total	29	6610.1635			

CV(%)1.23

Analysis of variance for fungitoxicity of fungicides against *Curvularia lunata*

100 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	24409.3039	2712.1449	893.11	0.0000
Error	20	60.7350	3.0368		
Total	29	24470.0389			

CV(%)2.84

200 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	13224.3459	1469.3718	334.23	0.0000
Error	20	87.9268	4.3963		
Total	29	13312.2727			

CV(%)2.97

300 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	12805.0706	1422.7856	427.30	0.0000
Error	20	66.5939	3.3297		
Total	29	12871.6645			

CV(%)2.52

400 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	10615.1963	1179.4663	346.90	0.0000
Error	20	68.0000	3.4000		
Total	29	10683.1963			

CV(%)2.19

500 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	6515.5469	723.9497	774.54	0.0000
Error	20	18.6936	0.9347		
Total	29	6534.2405			

CV(%)1.06

Analysis of variance for fungitoxicity of fungicides against *Fusarium equiseti*

100 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	31210.5308	3467.8368	1332.29	0.0000
Error	20	52.0582	2.6029		
Total	29	31262.5890			

CV(%)3.83

200 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	21881.1035	2431.2337	1050.03	0.0000
Error	20	46.3078	2.3154		
Total	29	21927.4113			

CV(%)2.88

300 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	15183.4568	1687.0508	665.31	0.0000
Error	20	50.7151	2.5358		
Total	29	15234.1719			

CV(%)2.60

400 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	13985.5343	1553.9483	847.59	0.0000
Error	20	36.6673	1.8334		
Total	29	14022.2015			

CV(%)1.87

500 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	8377.6167	930.8463	775.71	0.0000
Error	20	24.0000	1.2000		
Total	29	8401.6167			

CV(%)1.35

Analysis of variance for fungitoxicity of fungicides against *Fusarium fujikuroi*

100 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	28994.9339	3221.6593	1330.62	0.0000
Error	20	48.4235	2.4212		
Total	29	29043.3573			

CV(%)3.89

200 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	23806.6801	2645.1867	966.97	0.0000
Error	20	54.7109	2.7355		
Total	29	23861.3910			

CV(%)3.50

300 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	20493.5230	2277.0581	942.23	0.0000
Error	20	48.3331	2.4167		
Total	29	20541.8561			

CV(%)2.43

400 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	13459.9998	1495.5555	332.44	0.0000
Error	20	89.9752	4.4988		
Total	29	13549.9750			

CV(%)2.83

500 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	13459.9998	1495.5555	332.44	0.0000
Error	20	89.9752	4.4988		
Total	29	13549.9750			

CV(%)1.38

Analysis of variance for fungitoxicity of fungicides against *Microdochium fisheri*

100 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	35505.6192	3945.0688	1710.60	0.0000
Error	20	46.1251	2.3063		
Total	29	35551.7443			

CV(%)3.17

200 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	27790.8371	3087.8708	1367.36	0.0000
Error	20	45.1655	2.2583		
Total	29	27836.0026			

CV(%)2.79

300 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	21158.3923	2350.9325	1291.08	0.0000
Error	20	36.4181	1.8209		
Total	29	21194.8103			

CV(%)2.25

400 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	14720.2857	1635.5873	720.03	0.0000
Error	20	45.4308	2.2715		
Total	29	14765.7165			

CV(%)2.26

500 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	12573.7914	1397.0879	334.34	0.0000
Error	20	83.5725	4.1786		
Total	29	12657.3639			

CV(%)2.56

Analysis of variance for fungitoxicity of fungicides against *Nigrospora oryzae*

100 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	19889.4407	2209.9379	663.42	0.0000
Error	20	66.6231	3.3312		
Total	29	19956.0637			

CV(%)3.05

200 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	13447.0051	1494.1117	557.93	0.0000
Error	20	53.5587	2.6779		
Total	29	13500.5638			

CV(%)2.36

300 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	13002.8678	1444.7631	852.25	0.0000
Error	20	33.9046	1.6952		
Total	29	13036.7724			

CV(%)6.04

400 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	9118.3070	1013.1452	667.82	0.0000
Error	20	30.3418	1.5171		
Total	29	9148.6488			

CV(%)1.50

500 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	4633.5441	514.8382	16.01	0.0000
Error	20	643.0303	32.1515		
Total	29	5276.5744			

CV(%)1.37

Analysis of variance for antifungal activity of plant extracts against *Bipolaris oryzae*

5% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	32755.0612	3639.4512	967.56	0.0000
Error	20	75.2294	3.7615		
Total	29	32830.2906			

CV(%)3.27

10% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	27826.1571	3091.7952	1373.33	0.0000
Error	20	45.0263	2.2513		
Total	29	27871.1834			

CV(%)2.25

15% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	21323.9523	2369.3280	615.42	0.0000
Error	20	76.9990	3.8499		
Total	29	21400.9513			

CV(%)2.94

20% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	14080.1019	1564.4558	288.35	0.0000
Error	20	108.5127	5.4256		
Total	29	14188.6147			

CV(%)2.25

Analysis of variance for antifungal activity of plant extracts against *Curvularia lunata*

5 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	24451.5966	2716.8441	398.29	0.0000
Error	20	136.4243	6.8212		
Total	29	24588.0209			

CV(%)4.81

10 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	18941.3076	2104.5897	325.07	0.0000
Error	20	129.4844	6.4742		
Total	29	19070.7920			

CV(%)3.89

15 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	14201.1544	1577.9060	386.63	0.0000
Error	20	81.6225	4.0811		
Total	29	14282.7769			

CV(%)2.68

20 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	8016.4321	890.7147	553.60	0.0000
Error	20	32.1789	1.6089		
Total	29	8048.6111			

CV(%)1.45

Analysis of variance for antifungal activity of plant extracts against *Fusarium equiseti*

5 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	26456.1014	2939.5668	1369.87	0.0000
Error	20	42.9173	2.1459		
Total	29	26499.0187			

CV(%)2.68

10 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	18757.7429	2084.1937	444.62	0.0000
Error	20	93.7525	4.6876		
Total	29	18851.4954			

CV(%)3.45

15 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	18834.3839	2092.7093	482.30	0.0000
Error	20	86.7802	4.3390		
Total	29	18921.1641			

CV(%)2.94

20 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	12961.3076	1440.1453	273.82	0.0000
Error	20	105.1905	5.2595		
Total	29	13066.4989			

CV(%)2.82

Analysis of variance for antifungal activity of plant extracts against *Fusarium fujikuroi*

5 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	33918.3307	3768.7034	1344.09	0.0000
Error	20	56.0781	2.8039		
Total	29	33974.4089			

CV(%)3.21

10 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	20524.2851	2280.4761	466.93	0.0000
Error	20	97.6788	4.8839		
Total	29	20621.9639			

CV(%)3.52

15 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	16833.3784	1870.3754	419.38	0.0000
Error	20	89.1973	4.4599		
Total	29	16922.5757			

CV(%)3.36

20% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	10110.3541	1123.3727	170.73	0.0000
Error	20	131.5989	6.5799		
Total	29	10241.9529			

CV(%)3.14

Analysis of variance for antifungal activity of plant extracts against *Microdochium fisheri*

5 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	20411.0823	2267.8980	918.69	0.0000
Error	20	49.3723	2.4686		
Total	29	20460.4547			

CV(%)2.67

10 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	14057.6389	1561.9599	339.53	0.0000
Error	20	92.0069	4.6003		
Total	29	14149.6457			

CV(%)3.09

15 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	7791.2510	865.6946	220.67	0.0000
Error	20	78.4597	3.9230		
Total	29	7869.7107			

CV(%)2.47

20 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	4	3304.4674	367.1630	225.57	0.0000
Error	20	32.5541	1.6277		
Total	29	3337.0215			

CV(%)1.38

Analysis of variance for antifungal activity of plant extracts against *Nigrospora oryzae*

5 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	29535.4494	3281.7166	909.11	0.0000
Error	20	72.1962	3.6098		
Total	29	29607.6456			

CV(%)3.58

10 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	19619.3870	2179.9319	770.69	0.0000
Error	20	56.5706	2.8285		
Total	29	19675.9576			

CV(%)2.70

15 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	12882.4032	1431.3781	428.12	0.0000
Error	20	66.8682	3.3434		
Total	29	12949.2714			

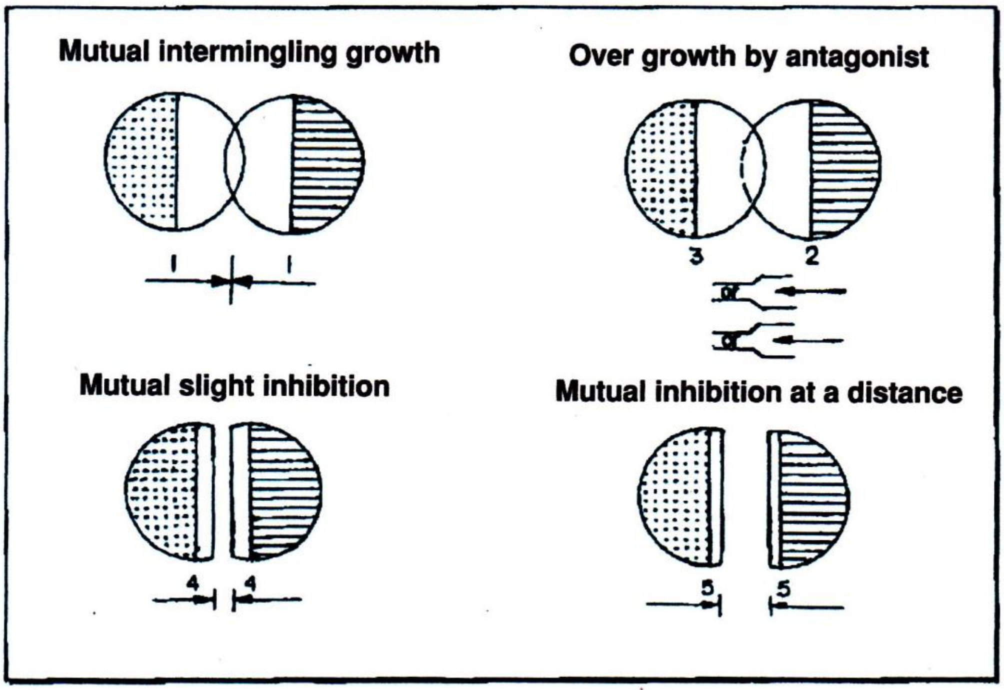
CV(%)2.63

20 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	6235.3382	692.8154	518.78	0.0000
Error	20	26.7092	1.3355		
Total	29	6262.0474			

CV(%)1.31

Appendix IV. The colony interaction model of Skidmore and Dickinson (1976)



Appendix V. Analysis of variance for per cent inhibition of test pathogens owing to volatile metabolites of antagonists

Bipolaris oryzae

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	3829.3415	1276.4472	237.94	0.0000
Error	8	42.9172	5.3646		
Total	11	3872.2587			

CV(%)7.05

Curvularia lunata

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	2832.0217	944.0072	140.92	0.0000
Error	8	53.5900	6.6988		
Total	11	2885.6117			

CV(%)7.02

Fusarium equiseti

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	249.4153	83.1384	19.01	0.0005
Error	8	34.9924	4.3741		
Total	11	284.4077			

CV(%)3.53

Fusarium fujikuroi

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	948.9606	316.3202	55.71	0.0000
Error	8	45.4218	5.6777		
Total	11	994.3824			

CV(%)5.34

Microdochium fisheri

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	1768.7092	589.5697	184.38	0.0000
Error	8	25.5809	3.1976		
Total	11	1794.2902			

CV(%)4.07

Nigrospora oryzae

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	5257.9292	1752.6431	192.03	0.0000
Error	8	73.0150	9.1269		
Total	11	5330.9442			

CV(%)5.18

Analysis of variance for per cent inhibition of *Bipolaris oryzae* owing to non-volatile metabolites of antagonists

5 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	3093.2480	1031.0827	350.83	0.0000
Error	8	23.5120	2.9390		
Total	11	3116.7600			

CV(%)4.67

10 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	1675.0913	558.3638	215.03	0.0000
Error	8	20.7738	2.5967		
Total	11	1695.8651			

CV(%)3.53

15 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	2222.0865	740.6955	194.65	0.0000
Error	8	30.4429	3.8054		
Total	11	2252.5294			

CV(%)3.06

20 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	1701.8004	567.2668	230.27	0.0000
Error	8	19.7076	2.4634		
Total	11	1721.5080			

CV(%)2.20

Analysis of variance for per cent inhibition of *Curvularia lunata* owing to non-volatile metabolites of antagonists

5 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	2042.8819	680.9606	209.16	0.0000
Error	8	26.0460	3.2558		
Total	11	2068.9279			

CV(%)5.04

10 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	1599.4720	533.1573	111.88	0.0000
Error	8	38.1245	4.7656		
Total	11	1637.5965			

CV(%)4.54

15 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	1471.4194	490.4731	156.20	0.0000
Error	8	25.1206	3.1401		
Total	11	1496.5400			

CV(%)2.81

20 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	1448.2604	482.7535	103.71	0.0000
Error	8	37.2379	4.6547		
Total	11	1485.4984			

CV(%)2.91

Analysis of variance for per cent inhibition of *Fusarium equiseti* owing to non-volatile metabolites of antagonists

5 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	2210.0858	736.6953	141.71	0.0000
Error	8	41.5887	5.1986		
Total	11	2251.6745			

CV(%)5.01

10 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	2944.2170	981.4057	10.92	0.0033
Error	8	718.7181	89.8398		
Total	11	3662.9351			

CV(%)16.77

15 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	807.8555	269.2852	89.45	0.0000
Error	8	24.0841	3.0105		
Total	11	831.9397			

CV(%)2.53

20 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	1263.2605	421.0868	89.28	0.0000
Error	8	37.7302	4.7163		
Total	11	1300.9907			

CV(%)2.85

Analysis of variance for per cent inhibition of *Fusarium fujikuroi* owing to non-volatile metabolites of antagonists

5 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	2210.0858	736.6953	141.71	0.0000
Error	8	41.5887	5.1986		
Total	11	2251.6745			

CV(%)5.00

10 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	2944.2170	981.4057	10.92	0.0033
Error	8	718.7181	89.8398		
Total	11	3662.9351			

CV(%)10.70

15 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	807.8555	269.2852	89.45	0.0000
Error	8	24.0841	3.0105		
Total	11	831.9397			

CV(%)2.80

20 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	1263.2605	421.0868	89.28	0.0000
Error	8	37.7302	4.7163		
Total	11	1300.9907			

CV(%)2.55

Analysis of variance for per cent inhibition of *Microdochium fisheri* owing to non-volatile metabolites of antagonists

5 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	1448.7814	482.9271	123.42	0.0000
Error	8	31.3043	3.9130		
Total	11	1480.0857			

CV(%)7.42

10 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	1643.2036	547.7345	134.63	0.0000
Error	8	32.5477	4.0685		
Total	11	1675.7513			

CV(%)5.43

15 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	1468.1228	489.3743	130.45	0.0000
Error	8	30.0117	3.7515		
Total	11	1498.1345			

CV(%)3.87

20 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	938.7277	312.9092	68.15	0.0000
Error	8	36.7338	4.5917		
Total	11	975.4615			

CV(%)3.72

Analysis of variance for per cent inhibition of *Nigrospora oryzae* owing to non-volatile metabolites of antagonists

5 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	606.8782	202.2927	40.52	0.0000
Error	8	39.9369	4.9921		
Total	11	646.8150			

CV(%)3.74

10 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	857.3775	285.7925	31.80	0.0001
Error	8	71.8971	8.9871		
Total	11	929.2746			

CV(%)4.40

15 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	647.2758	215.7586	15.48	0.0011
Error	8	111.5385	13.9423		
Total	11	758.8143			

CV(%)4.87

20 % concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	443.0081	147.6694	25.51	0.0002
Error	8	46.3058	5.7882		
Total	11	489.3139			

CV(%) 2.74

Appendix VI. Analysis of variance for combined effects of seed treatment with fungicides, leaf extracts and biocontrol agents on BRRI rice varieties

Effects of different treatments on *Bipolaris oryzae*

Germination

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	12	5887.5897	490.6325	78.74	0.0000
Error	26	162.0000	6.2308		
Total	38	6049.5897			

CV(%)4.05

Mortality

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	12	415.0744	34.5895	33.05	0.0000
Error	26	27.2133	1.0467		
Total	38	442.2877			

CV(%)13.13

Root length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	12	22.2833	1.8569	5.26	0.0002
Error	26	9.1726	0.3528		
Total	38	31.4559			

CV(%)14.42

Shoot length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	12	208.6844	17.3904	24.80	0.0000
Error	26	18.2349	0.7013		
Total	38	226.9194			

CV(%)6.91

Vigor index

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	12	5245111.2931	437092.6078	74.84	0.0000
Error	26	151847.7545	5840.2982		
Total	38	5396959.0475			

CV(%)7.43

Effects of different treatments on *Curvularia lunata*

Germination

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	12	2492.3590	207.6966	24.11	0.0000
Error	26	224.0000	8.6154		
Total	38	2716.3590			

CV(%)4.82

Mortality

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	12	433.7609	36.1467	16.94	0.0000
Error	26	55.4708	2.1335		
Total	38	489.2317			

CV(%)17.40

Root length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	12	36.8497	3.0708	31.28	0.0000
Error	26	2.5522	0.0982		
Total	38	39.4019			

CV(%)8.69

Shoot length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	12	206.1554	17.1796	34.85	0.0000
Error	26	12.8163	0.4929		
Total	38	218.9718			

CV(%)5.75

Vigor index

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	12	4262850.2889	355237.5241	67.34	0.0000
Error	26	137156.5932	5275.2536		
Total	38	4400006.8821			

CV(%)7.36

Effects of different treatments on *Fusarium equiseti*

Germination

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	12	2278.5641	189.8803	21.22	0.0000
Error	26	232.6667	8.9487		
Total	38	2511.2308			

CV(%)4.30

Mortality

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	12	307.3962	25.6163	25.14	0.0000
Error	26	26.4947	1.0190		
Total	38	333.8909			

CV(%)12.31

Root length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	12	40.6962	3.3913	32.35	0.0000
Error	26	2.7254	0.1048		
Total	38	43.4216			

CV(%)8.33

Shoot length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	12	1107.6009	92.3001	65.66	0.0000
Error	26	36.5500	1.4058		
Total	38	1144.1509			

CV(%)7.70

Vigor index

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	12	13557088.2741	1129757.3562	140.87	0.0000
Error	26	208514.8169	8019.8006		
Total	38	13765603.0910			

CV(%)6.45

Effects of different treatments on *Fusarium fujikuroi*

Germination

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	12	4377.0256	364.7521	41.00	0.0000
Error	26	231.3333	8.8974		
Total	38	4608.3590			

CV(%)4.98

Mortality

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	12	653.1264	54.4272	36.61	0.0000
Error	26	38.6573	1.4868		
Total	38	691.7837			

CV(%)7.94

Root length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	12	83.1623	6.9302	17.48	0.0000
Error	26	10.3097	0.3965		
Total	38	93.4720			

CV(%)13.17

Shoot length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	12	336.6735	28.0561	10.19	0.0000
Error	26	71.5956	2.7537		
Total	38	408.2691			

CV(%)15.31

Vigor index

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	12	6165072.7275	513756.0606	78.38	0.0000
Error	26	170420.4337	6554.6321		
Total	38	6335493.1613			

CV(%)8.39

Effects of different treatments on *Microdochium fisheri*

Germination

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	12	3320.1026	276.6752	32.50	0.0000
Error	26	221.3333	8.5128		
Total	38	3541.4359			

CV(%)4.51

Mortality

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	12	480.2391	40.0199	22.15	0.0000
Error	26	46.9776	1.8068		
Total	38	527.2167			

CV(%)18.58

Root length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	12	20.7620	1.7302	16.76	0.0000
Error	26	2.6844	0.1032		
Total	38	23.4464			

CV(%)6.58

Shoot length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	12	306.6175	25.5515	7.93	0.0000
Error	26	83.7547	3.2213		
Total	38	390.3721			

CV(%)8.11

Vigor index

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	12	7467780.9828	622315.0819	37.40	0.0000
Error	26	432606.6063	16638.7156		
Total	38	7900387.5891			

CV(%)7.28

Effects of different treatments on *Nigrospora oryzae*

Germination

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	12	3684.9744	307.0812	14.36	0.0000
Error	26	556.0000	21.3846		
Total	38	4240.9744			

CV(%)7.38

Mortality

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	12	471.7572	39.3131	15.20	0.0000
Error	26	67.2605	2.5869		
Total	38	539.0177			

CV(%)17.76

Root length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	12	81.5281	6.7940	106.77	0.0000
Error	26	1.6545	0.0636		
Total	38	83.1825			

CV(%)5.89

Shoot length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	12	600.4818	50.0402	81.43	0.0000
Error	26	15.9779	0.6145		
Total	38	616.4598			

CV(%)3.80

Vigor index

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	12	11907537.0426	992294.7536	39.27	0.0000
Error	26	656903.5949	25265.5229		
Total	38	12564440.6375			

CV(%)9.89

Dhaka University Institutional Repository

Published paper from the research work

1. **Sultana T,** S Shamsi and MA Bashar 2018**.** Prevalence of fungi with seeds of twenty BRRI released rice varieties and seed quality analysis. J. Asiatic. Soc. Bangladesh, Sci. **44** (1): 79-89.

2. **Sultana T**, MA Bashar and S Shamsi 2020. Pathogenic potentiality of fungi isolated from seeds of twenty BRRI released rice varieties (*Oryza sativa* L.) ***Biores Comm***. **6** (1): 810-814.

3. **Sultana T**, S Shamsi and MA Bashar 2020. Morphological characterization of seed borne fungi associated with BRRI rice varieties in Bangladesh. The Dhaka University Journal of Biological Science. **29** (1): 75-86.