SEED BORNE MYCOFLORA OF DIFFERENT VARIETIES OF COTTON (*GOSSYPIUM HIRSUTUM* L.) AND THEIR MANAGEMENT

A THESIS
SUBMITTED IN PARTIAL FULFILLMENT FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY IN BOTANY
UNIVERSITY OF DHAKA

SUBMITTED BY

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DHAKA-1000

June 2022

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CERTIFICATE

*This is to certify that the research work embodying the results reported here in this thesis entitled "**Seed borne Mycoflora of Different varieties of Cotton (Gossypium hirsutum L.) and their Management**" by **Amina Khatun** has been carried out in the Laboratory of Mycology and Plant Pathology, Department of Botany, Curzon Hall Campus, University of Dhaka, under our direct supervision and guidance. It is further certified that the work presented here is original and suitable for submission in partial fulfillment of the requirements for the degree of **Doctor of Philosophy in Botany.***

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

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Dedicated To

My Beloved Parents

My Husband

And

My Respected Teachers

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 five papers have been published in scientific journals.

Date: **Amina Khatun**

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ABSTRACT

Cotton “The king of Fibers” is one of the most renowned, reliable fiber yielding cash crops around the world including Bangladesh. It is the most important cash crop next to jute in Bangladesh. The present research work was undertaken to detect, identify the seed borne mycoflora of 14 varieties (CB1-CB14) of upland cotton (*Gossypium hirsutum*) in Bangladesh and to screen out the efficacy of selected antagonistic fungi, plant parts extracts and fungicides against the fungal pathogens associated with cotton seeds. Seed samples were collected from Cotton Research, Training and Seed multiplication Farm, Sreepur under the district of Gazipur. Quality analysis showed that the percentage of pure seeds varied from 97.08-99.92%, the germination percentage of different varieties of cotton seeds were ranged from 80-93%, the mortality percentage were in the range of 16.05-50.30% and the moisture level was varied from 10.0-11.3%.

The fungi associated with the different varieties of cotton seeds were isolated following Tissue planting method, Blotter method and Paper towel method. A total of twenty nine species of fungi namely *Aspergillus aculeatus*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. nidulans*, *A. subramanianii*, *A. tamarii*, *A. toxicarius*, *A. wentii*, *Curvularia lunata*, *Colletotrichum gloeosporioides*, *C. gossypii*, *Chaetomium globosum*, *Fusarium moniliforme*, *F. nivale*, *F. oxysporum*, *F. fujikuroi*, *F. solani*, *Lasiodiplodia theobromae*, *Meyerozyma guilliermondii*, *Mucor* sp., *Penicillium aculeatum*, *P. citrinum*, *Rhizoctonia solani*, *Rhizopus stolonifer*, *R. oryzae*, *Rhizomucor* sp., *Syncephalastrum racemosum* and *Trichoderma viride* were found to be associated with the seeds of cotton. Isolated fungi were characterized and identified following standard literatures. Molecular identification of 19 fungi were conducted by using ITS sequence based analysis. Among the isolated fungi *Aspergillus subramanianii*, *A. toxicarius*, *A. wentii*, *Penicillium aculeatum*, *P. citrinum*, *Rhizomucor* sp. and *Meyerozyma guilliermondii* are the new records for Bangladesh.

In Tissue planting method, the association of fungi varied with duration of storage periods. *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Chaetomium globosum*, *Penicillium citrinum* and *Rhizopus stolonifer* were the most predominant fungi in terms of prevalence and their frequency were also gradually increased with the increase of storage period. *Chaetomium globosum* was the most predominant fungi noticed in Blotter and Paper Towel method. Maximum number of fungi were found to be externally seed borne i.e. isolated from seed coat. Interrelationships among seed germination, purity percentage, seed moisture, fungi

frequency and seedling mortality of cotton seeds were measured through correlation and regression analysis. Five positive correlations and 2 negative correlations were found among these 5 quality factors.

Out of these 29 fungal species, nine were found to be pathogenic to cotton. They were *Aspergillus flavus* Link, *A. niger* Van Tiegh, *A. tamarii* Kita G., *Colletotrichum gloeosporioides* Penz & Sacc., *Curvularia lunata* (Wakker) Boedijn, *Fusarium nivale* (Fr.) Sorauer, *F. moniliforme* J. Shelden, *Mucor* sp. P. Micheli ex L. and *Rhizoctonia solani* Khun. These pathogenic fungi had remarkable effect on seed germination, vigor index, root-shoot length and mortality of cotton seedlings. All the pathogenic fungi showed transmission nature i.e. fungi transferred from seeds to seedlings in both earthen pot and water agar culture. In pot experiment, percentage of seed to seedling transmission of fungi varied from 17.85 (*A. niger*) to 46.56% (*F. moniliforme*) and in water agar test, *Rhizoctonia solani* showed highest percentage of seed to seedling transmission (35.27%).

The antagonistic potentials of four soil fungi against pathogenic fungi were evaluated by "dual culture colony interaction", volatile and non-volatile metabolites. In "dual culture colony interaction", out of four soil fungi, the highest growth inhibition (84.0%) was observed by *A. fumigatus* against *C. gloeosporioides*. Volatile substances from soil fungi inhibited radial growth of the test pathogens varied from 33.3-72.4%. The highest inhibition (72.4%) was found in *A. fumigatus* against *R. solani*. Non-volatile substances showed inhibition of mycelial growth of cotton pathogens range from 47.06 to 81.82% at 20% concentration. The highest inhibition was observed owing to *A. niger* against *F. nivale.*

Ten fungicides with different active ingredients *viz.*, Capvit 50 WP, Contaf 5 EC, Dithane M-45, Knowin 50 WP, Nativo 75 WP, Ridomil MZ Gold, Score 250 EC, Secure, Silica 80 WG and Tilt 250 EC were selected to evaluate their *in vitro* efficacy at 100, 200, 300, 400 and 500 ppm concentrations against the nine pathogenic test fungi. Contaf and Tilt were found most efficient inhibitor which completely inhibited the radial growth of the test pathogens which was followed by Nativo, Knowin, Score, Dithane, Capvit, Secure, Ridomil and Silica. Antifungal properties of ethanol extracts of ten higher plants namely *Adhatoda vasica* L., *Aegle mermelos* L., *Azadirachta indica* A. Juss., *Citrus lemon* L., *Datura metel* L., *Mangifera indica* L., *Moringa oleifera* Lam., *Ocimum sanctum* L., *Psidium guajava* L. and *Tagetes patula* L. at 5, 10, 15 and 20% concentrations were evaluated against the test pathogens. As regards the plant extracts, the *P. guajava* and *A. indica* were found most efficient inhibitor of

the test pathogens followed by *C. lemon, A. vasica*, *D. metel*, *A. mermelos, T. patula*, *M. oleifera, O. sanctum* and *M. indica* at 20% concentration.

In field experiment out of 19 treatments, T1 (Tilt) and T11 (Tilt + *A. indica* + *T. viride*) showed highest seed germination, seedling vigor index against *A. flavus, A. niger, C. lunata, F. nivale, Mucor* sp. and *A. tamarii, C. gloeosporioides, F. moniliforme* and *R. solani,* respectively. On the other hand, T5 (*T. viride*), T10 (Tilt + *P. guajava* + *T. viride*) and T14 (Tilt + *A. indica*) showed promising germination percentage and seedling vigor index against *A. flavus, R. solani* and *A. tamarii, F. nivale* and *A. niger, C. gloeosporioides, C. lunata, F. moniliforme, Mucor* sp., respectively.

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CHAPTER: 1
INTRODUCTION

INTRODUCTION

Clothing is one of the primary requirements of human beings along with food and shelter. Cotton (*Gossypium* spp.) is unique among all agricultural crops which provides us food and fibre. It is called "The King of Fibers" which is one of the most renowned, reliable fiber yielding cash crops around the world including Bangladesh. Millions of people use cotton for their livelihood at farms, ginning factories, textile mills, edible oil, fish and livestock feed and soap industry. Cotton is therefore, rightly called the life blood of economy of many countries in Asia. It is harvested as seed cotton and then ginning is done to separate seed and lint (Tripathi *et al*. 2014).

Cotton is the major textile fiber used by man in the world and playing a key role in economic and social welfare (Munro 1994). It is cultivated in more than seventy countries and grows mostly in tropical and subtropical regions of the world. China, India, USA and Pakistan are the major cotton producing countries in the world with a total share of 70% of the world cotton production and area, respectively.

Cotton belongs to the genus *Gossypium* under the family Malvaceae. It has four species namely *G. hirsutum* L., *G. arboreum* L., *G. herbaceum* L. and *G. barbadense* L. which are domesticated all over the world as the elementary source of textile fiber. Out of these *G. hirsutum* and *G. barbadense* are economically most important (Percival and Kohel 1990). *G. hirsutum*, widely known as upland cotton or sometimes American, Mexican or Acala cotton, accounts for over 90% of the production. *G. barbadense*, which accounts for some 5%, is commonly known as extra-long-staple cotton or Pima or Egyptian cotton.

Cotton is the second important cash crop next to jute in Bangladesh (Hussain 2013). It is the main raw materials of textile industry and in Bangladesh, the annual requirements of raw cotton for this

sector is 4.2 million bales. Around 3% of the national requirement is fulfilled through the local production from 42 thousand hectares of land and the remaining 97% is imported from foreign countries (BBS 2010). The garments industry has been flourishing in Bangladesh. Readymade garments accounts for about 75% of the total export earnings. But cotton production in Bangladesh did not increase as expected due to several constrains.

Now a days, seed has become an international commodity which is used to exchange germplasm around the world. Seed is also an efficient means of introducing plant pathogens into a new area as well as providing a means of their survival from one cropping season to another (Walcott *et al*. 2003). The lack of high quality seeds and the prevalence of seed borne organisms are the main constraints in maintaining the crop production. More than 400 seed borne diseases in 72 crops inflicting an estimated yield loss amounting to around Tk 1000 million i.e. 200 million US dollars annually was estimated by Fakir (1998).

Seed-borne fungi affect the quality of seeds at all stages of production, from the cropping stages until post-harvest, processing, storage and marketing (Machado *et al*. 2002). These are a serious problem worldwide causing diseases and poor quality of many imported crops. Seeds free from fungal infection are very much essential for good plant production. Investigators reported that fungal pathogens are responsible for the re-emergence of diseases of the past as well as the introduction of diseases into new areas (Gitaitis and Walcott 2007). Seed borne pathogens are a serious threat to seedling establishment (Walcott 2003). The quality of planted seeds has a critical influence on the ability of crops to become established and to realize their full potential of yield and value (McGee 1995).

Seed-borne fungi are responsible for both pre and post-emergence death of grains, affect seedling vigor, reduction in germination and also variation in plant morphology (Van Du *et al*. 2001, Rajput

et al. 2005, Niaz and Dawar 2009). The seed borne pathogens may results loss in germination, discoloration and shriveling of seeds, development of plant diseases, distribution of pathogen to new areas, introduction of new strains of the pathogen along with new germplasm from other countries and toxin production in infected seed (Agarwal and Gaur 2015). Fungal pathogens cause a very serious economic impact on agricultural production due to their ability to induce diseases of cultivated crops that result in important yield losses (Paplomatas 2006)

Most cotton diseases are transmitted through seeds and in most cases they affect the quality of the fibre and seed. Seed diseases cause seed rot and damping-off of the seedlings. Many seed borne fungal pathogens have been reported by different workers that reduce germination percentage and seedling vigour of cotton seeds (Jeyalakshmi *et al*. 1999, Eisa *et al*. 2007, Tomar *et al*. 2012).

Each year, cotton production is being reduced due to the presence of some injurious pathogens and most of the seed-transmitted pathogens are fungi. A number of seed borne pathogenic fungi such as species of *Alternaria, Colletotrichum, Fusarium, Macrophomina, Rhizopus, Rhizoctonia, Fusarium, Helminthosporium, Curvularia, Mucor, Penicillium, Aspergillus, Sclerotium, Cephalosporium, Myrithecium, Trichoderma* and *Xanthomonas* are the most frequently identified from cotton seeds and seedlings (Khan and Kausar 1967, Roy and Bourland 1982, Minton and Garber 1983, Seneewong *et al.* 1991, Asran-Amal *et al*. 2005, Colyer and Vernon 2005, Mikhail *et al.* 2009, Fard and Mojeni 2011).

Alternaria alternata, Aspergillus niger, Fusarium oxysporum, F. moniliforme, F. semitectum, F. acuminatum, F. solani, Pythium ultimum, Rhizopus arrhizus, Rhizoctonia solani and several other fungi were also isolated from cotton seeds and most of these fungi were pathogenic to cotton (Fulton and Bollenbacher 1959, Mansoori and Hamdolahzadeh 1995). Wang *et al*. (1992) recorded high frequency of *Fusarium moniliforme* and *F. semitectum* from cotton seedlings and bolls.

Till now 20 types of symptoms and 52 species of fungi have been reported on cotton plants from various cotton growing areas of the world (Wikipedia 2022). In Bangladesh, a total of 14 diseases of cotton were recorded from which 12 diseases are caused by fungal pathogens (BARI 1990).

Fungi involved in causing leaf diseases namely, anthracnose, leaf spot, mildew and leaf blight are caused by *Colletotrichum gossypii, Cercospora gossypii, Alternaria macrospora, Rhizoctonia solani, Ramularia areola* and *Sphaeropsis*. Seven different fungi *viz*., *Alternaria macrospora, Aspergillus niger, Chaetarium globosus*, *Colletotrichum gossypii*, *Curvularia lunata, Furarium* spp. and *Macrophomina phaseolina* were found to be associated with the seeds of cotton (BARI 1990).

The control of seed borne pathogens is the major step in any agricultural crop production and protection program. Proper seed treatment measures can substantially improve the quality of seeds and significantly increase the yield. Fungicides are known to be the supreme defensive component to control cotton disease and they have broad spectrum activities with protectant and systemic capabilities against most fungal pathogens.

Protecting fungicides are used in a limited amount to control seed-borne fungi (Thomas and Sweetingham 2003). Increasing knowledge and concerns about the environmental hazard due to intensive and repeated applications fungicide have prompted the research scientists to hunt for antifungal substances that are non-toxic, cost-effective, eco-friendly and which are highly performant in eliminating or reducing the incidence of pathogens and improve seed germination and seedling vigor. Biological control of plant diseases using antagonistic microorganisms has been suggested as an alternative to the hazardous and expensive chemical pesticides (Emmert and Handelsman 1999).

Among these substances, plant extracts have proven to be a potential source for the natural pesticide development for the control of seed-borne fungi (Tripathi and Shukla 2010, Al-Reza *et al*. 2010, Veloz-Garcia *et al*. 2010, Kuri *et al*. 2011, Malkhan *et al*. 2012). Plant extracts have been reported to reduce the appearance of fungi transmitted by seeds and to increase the percentage of germination and seedling emergence (Hasan *et al*. 2005). The effectiveness of plant extracts against seed infection have reported by many researchers from various countries in order to increase cotton seedlings performance (Gustavo and Mariana 2010, Tomar *et al*. 2012, Rathinavel 2013).

The study of seed borne pathogens is most essential to determine seed health and to improve the germination potential of seeds which finally leads to increase the crop production. Seed health testing to detect seed-borne pathogens is an important step in the management of crop diseases (Hajihasani *et al*. 2012). Seed health is a well-recognized factor in the modern agricultural science for desired plant population and good harvest (Rahman *et al*. 2008).

Good seed is a fundamental input in agricultural production. Successful agriculture depends on quality of seeds used for sowing. Majority of diseases in cotton such as *Alternaria* blight, bacterial blight, *Fusarium* wilt, *Myrothecium* blight, *Cercospora* blight, *Exserohilum* blight etc are seed-borne. The review of literature indicates that lot of research has been done in home and abroad on cotton plant diseases and their control but information on seed borne pathogenic mycoflora of upland cotton and their management is inadequate. So considering the importance of this valuable fiber crop, present research work was undertaken to detect and identify the seed borne mycoflora of 14 varieties (CB1-CB14) of upland cotton and to find out the possible control of the seed borne fungal pathogens.

Accordingly, the present investigation is being proposed with the following objectives:

- Determination of seed health and quality status of the seeds of fourteen cotton varieties (CB1-CB14).
- Study of histopathology of seeds for the association of the pathogens.
- Isolation, purification, characterization and preservation of fungi associated with cotton seeds of the selected varieties.
- Morphological and molecular identification of the fungi isolated from cotton seeds.
- Determination of pathogenic potentiality of the isolated fungi.
- Evaluation of seed to seedling transmission of the isolated pathogenic fungi of cotton seeds.
- Study of colony interaction between the test pathogens and some selected soil antagonists.
- Study of the effects of volatile and non-volatile culture filtrates of the selected antagonists on the growth of test pathogens.
- *In vitro* evaluation of fungitoxicity of some fungicides against the test pathogens.
- Screening of locally available some plant parts extracts of some selected higher plants against the selected pathogens.
- Integrated approach to control the selected test pathogens of cotton seeds.

CHAPTER: 2
REVIEW of LITERATURE

REVIEW OF LITERATURE

2.1. Cotton diseases and fungal association with cotton seeds

The cotton plant is a shrub and native to tropical and subtropical regions throughout the universe. The crop is attacked by an oversized number of diseases. The diseases can attack cotton at any stage of plant growth and from the seed through the seedling stage (Terrence and Rockroth 2001).

Dikson (1956) stated that the foremost common fungal diseases of cotton are threat to cotton cultivation. Cotton seedling disease complex caused by generally *Colletotrichum gossypii*, *Fusarium* spp., *Pythium* spp., *R. solani, M. phaseolina* and *Thielaviopsis basicola* are main cause of low plant stand (Hillocks 1992, Terrence and Rockroth 2001, Anon. 2010a, b). Diseases cause significant losses in cotton production throughout the cotton growing countries of the universe (Anon 2010c, d).

Diseases are major constraints for cotton cultivation. One of the most common diseases of cotton is boll rot caused by different fungi like *A. niger*, *F. oxysporum, Rhizopus oryzae*. In Bangladesh *Fusarium* wilt, angular leaf spot, damping off, leaf spots and anthracnose have been identified and due to death of cotton plants yield is severely reduced.

Among the cotton diseases, seedling diseases caused severe losses in plant stand and significant losses in cotton production throughout the cotton growing countries of the world (Michael *et al*. 2007, Koenning 2008, Anon. 2010d). Atkinson (1892) first reported the issues of seedlings diseases of cotton from the USA.

In California, an estimated loss of 58491 bales of cotton were lost yearly for boll rot diseases in 1991 to 1993 (Blasingame 1994). The diseases reduced cotton production in Missouri an estimated 8.7, 19.6 and 39.7 million pounds in 2001, 2002 and 2003 respectively (Anon. 2010c). Damping-off diseases of cotton is a disease complex and in SW Spain, post-emergence damping-off seems to be mainly associated with *R. solani* and *Thielaviopsis basicola* (Arndt 1943, Huisman 1988, Delgado *et al.* 2006, Rothrock *et al*. 2007).

Cotton is a cash crop of all over the world. Cotton is attacked by bacteria, fungi, parasitic nematodes, virus, Phytoplasma and spiroplasma. List of diseases of cotton are given below:

Bacterial diseases:

Name of diseases	**Pathogens**
Bacterial blight of cotton	*Xanthomonas citri* sub sp. *malvacearum*
Crown gall	*Agrobacterium tumefaciens*
Lint degradation	*Erwinia herbicola = Pantoea agglomerans*

Fungal diseases:

Name of diseases	**Pathogens**
Anthracnose	*Glomerella gossypii* *Colletotrichum gossypii*
Areolate mildew	*Ramularia gossypii = Cercosporella gossypii* *Mycosphaerella areola*
Anita blight Anita stalk borer	*Ascochyta gossypii*
Black root rot	*Thielaviopsis basicola* *Chalara elegans*

Boll rot	*Ascochyta gossypii* *Colletotrichum gossypii* *Glomerella gossypii Fusarium* spp. *Lasiodiplodia theobromae* *= Diplodia gossypina* *Botryosphaeria rhodina = Physalospora rhodina* *Phytophthora* spp. *Rhizoctonia solani*
Charcoal rot	*Macrophomina phaseolina*
Escobilla	*Colletotrichum gossypii* *Glomerella gossypii*
Fusarium wilt	*Fusarium oxysporum* f. sp. *vasinfectum*
Leaf spot	*Alternaria macrospora* *Alternaria alternata* *Cercospora gossypina* *Mycosphaerella gossypina* *Cochliobolus spicifer* *Bipolaris spicifera* *= Curvularia spicifera* *= Cochliobolus spicifer* *Myrothecium roridum* *Rhizoctonia solani* *Stemphylium solani*
Lint contamination	*Aspergillus flavus* *Nematospora* spp. *Nigrospora oryzae*
Phymatotrichum root rot = cotton root rot	*Phymatotrichopsis omnivora* *= Phymatotrichum omnivorum*
Powdery mildew	*Leveillula taurica* *Oidiopsis sicula* *= Oidiopsis gossypii* *Salmonia malachrae*
Stigmatomycosis	*Ashbya gossypii* *Eremothecium coryli* *= Nematospora coryli* *Aureobasidium pullulans*
Cotton rust	*Puccinia schedonnardii*
Southwestern cotton rust	*Puccinia cacabata*
Tropical cotton rust	*Phakopsora gossypii*

Sclerotium stem and root rot = Southern blight	*Sclerotium rolfsii* *Athelia rolfsii*
Seedling disease complex	*Colletotrichum gossypii* *Fusarium* spp. *Pythium* spp. *Rhizoctonia solani* *Thanatephorus cucumeris* *Thielaviopsis basicola* *Chalara elegans*
Stem canker	*Phoma exigua*
Verticillium wilt	*Verticillium dahliae*

Nematodes, parasitic:

Name of diseases	**Pathogens**
Lance, Columbia nematode	*Hoplolaimus columbus*
Reniform nematode	*Rotylenchulus reniformis*
Root-knot nematode	*Meloidogyne incognita*
Sting nematode	*Belonolaimus longicaudatus*

Viral diseases:

Name of diseases	**Pathogens**
Abutilon mosaic = Malvaceous chlorosis virus	suspect Begomo virus (*Bemisia tabaci* transmitted)
Anthocyanosis	suspect virus
Blue disease	suspect virus or phytoplasma
Leaf crumple	genus *Bigemini* virus, Cotton leaf curl virus (CLCuV)
Leaf curl	genus *Bigemini* virus, Cotton leaf curl virus (CLCuV)
Leaf mottle	suspect virus (etiology unknown)
Leaf roll	suspect virus (etiology unknown)

Mosaic	suspect virus (etiology unknown)
Psylosis	suspect virus (etiology unknown)
Terminal stunt	suspect virus (GTP; Cicadellidae associated)

Phytoplasmal and spiroplasmal diseases:

Name of diseases	**Pathogens**
Phyllody	suspect phytoplasma
small leaf	suspect phytoplasma

Source: Wikipedia (2022) the free encyclopedia 25 April, 2022.

2.2. Seed mycoflora on cotton

Seeds are the efficient medium for survival, dissemination of plant pathogens, large scale and long distance spread of pathogens. Infected or contaminated seeds works as major source of inoculum for great number of plant pathogens which may infect the seeds and survive as spore or resting structures on or within the seeds (Neergaard 1977).

Fungi are the largest group of the seed-borne pathogens investigated by seed pathologists due to its capacity of multiplication and survival in nature (Neergaard 1977, Richardson 1990, Machado *et al*. 2002). Seed borne fungi may effect on uniform emergence, vigorous and uniform stand of healthy seedlings. If the fungi become virulant in seedling, germination may be delayed or may not occur (Arndt 1953, Lima *et al*. 1988, Roncadori *et al*. 1971, Smith 1950). The fungi involved contaminate the seed before harvest (Hillocks 1992).

A number of seed borne fungi can infect cotton seedling individually or association as disease complex (Hillocks and Waller 1997). A good range of fungi may participate within the process of seed deterioration but some of those fungi causes pre and post emergence damping off in seedlings (Kirkpatrik and Rotrock 2001).

Cotton is usually propagated by seeds and these seeds are potential harbor of various micro-fungi which impair seed germination resulting in the production of abnormal seedlings (Bateman and Kwasna 1999, Khanzada *et al*. 2002). Mazen *et al.* (1990) isolated 39 species and 16 fungal genera from Egyptian cotton seeds. The most common species were *A. niger, A. flavus, A. fumigatus, A. terreus* and *Rhizopus stolonifer. Fusarium semitectum* was found to be the major seed colonizing fungus in the commercial acid delinted cotton (*Gossypium hirsutum*) according to Maria *et al.* (2005).

Templetion *et al*. (1967) reported *Alternaria alternata* from seed coat of cotton. *Rhizoctonia solani, Pythium ultimum, F. oxysporum, F. moniliforme, F. semitectum* and several other fungi were isolated from cotton seeds and seedlings by Fulton and Bollenbacher (1959) and they found that most of the isolated fungi were pathogenic to cotton seedlings. Also, Alfred (1963) reported that fungi belonging to *Alternaria, Aspergillus, Diploidia, Fusarium* and *Rhizoctonia* were associated with the seeds of cotton during boll development. In southern USA, Kuch (1986) isolated *Fusarium equiseti* and *F. semitectum* from cotton seeds.

Wang *et al*. (1992) recorded that *F. moniliforme* and *F. semitectum* from cotton seedlings and bolls were high frequent while, *F. oxysporum, F. solani, F. equiseti* and *F. compactum* were less frequent. Mansoori and Hamdolahzadeh (1995) isolated *Alternaria alternata, Aspergillus niger, F. acuminatum, F. solani, Pythium ultimum, Rhizopus arrhizus* and *R. solani* from cotton seeds. Padaganur (1979) found *Alternaria macrospora* on cotton seeds. Gawade *et al*. (2006) isolated *A. macrospora* from cotton seeds.

In Iran the surveys from 1995 to 2011 for recognize and distribution of the causal agent of diseases on cotton were done, showed that species of fungi, includes *Alternaria alternata, Aspergillus niger, Fusarium accuminatum, F. solani, Pythium ultimum, Rhizopus arrhizus, Aspergillus* spp.,

Rhizoctonia solani, *Penicillium* spp. are the causal agent of seed decay and pre emergence damping off and *F. solani, F. buharicum, F. equiseti, F. proliferatum, A. alternata, Sclerotium rolfsii, Pythium ultimum, Rhizoctonia solani* are the causal agent of post emergence damping off.

Lutfunnessa and Shamsi (2011) reported that in Bangladesh, the most common diseases of cotton are anthracnose, *Alternnaria* leaf spot, *Cercospora* leaf spot, Rust, *Sclerotium* rot, Wilt, and Boll rot. Fungi those are mostly associated with cotton diseases in the field are the species of *Fusarium, Colletotrichum, Rhizopus* and *Pythium* (Roy and Bourland 1982). Seven different fungi *viz*., *Alternaria macrospora, Aspergillus niger, Chaetarium globosum, Colletotrichum gossypii*, *Curvularia lunata, Furarium* spp. and *Macrophomina phaseolina* were found to be associated with the seeds of cotton. Among these fungi *A. macrospora* and *C. gossypii* are causing leaf spots and *M. phaseolina* is associated with seedling blight (BARI 1990).

According to Nahar *et al*. (2019), twelve species of fungi were isolated from the seeds of three varieties of Hill cotton (*Gossypium arboreum* L.) in Bangladesh. Among these, *Aspergillus niger* (Type-I), *A. niger* (Type-II) and *Rhizoctonia solani* were predominant fungi. Many seed borne fungal pathogens have been reported to reduce germination percentage and seedling vigour in cotton seeds by Jeyalakshmi *et al*. (1999), Eisa *et al*. (2007) and Tomar *et al*. **(**2012). The fungi involved contaminate the seed prior to harvest (Hillocks 1992).

A number of seed borne fungi can infect cotton seedling individually or association as disease complex (Hillocks and Waller 1997). Tomar *et al.* (2012) reported eleven fungal flora *viz*., *Aspergillus niger, A. flavus, Penicillium* sp., *Alternaria alternata, Chaetomium* spp, *Rhizopus niger, Fusarium solani, Macrophomina phaseolina, Myrothecium roridum, Trichothecium roseum* and *Curvularia lunata* from JK 4 cotton cultivar growing in the locations of Madhya Pradesh by Blotter method.

In cotton, most of the dangerous diseases are transmitted through seeds and 9 fungal pathogens were isolated from cotton seed and also observed that these seed borne pathogens reduced the germination ability of seed and vigor of the seedlings (Jeyalakshmi *et al.* 1999). Devay *et al*. (1997) studied the development of *Fusarium* wilt in upland cotton (*Gossypium hirsutum*). Bennett *et al.* (2008) reported that *Fusarium oxysporum* f. sp. *vasinfectum* (Atk.) race 4, a biotype highly virulent on certain Pima cotton (*Gossypium barbadens* L.) cultivars which was detected in California in 2001.

2. 3. Cotton seed quality

According to Seshu and Dadlani (1988), there are three major aspects of seed quality. They are genetic and physical purity, germination percentage and vigor and free from seed-borne diseases and insects. Seed health is a well-recognized factor in the modern agricultural science for desired plant population and good harvest (Rahman *et al*. 2008). Seed borne pathogens present a serious threat to seedling establishment (Walcott 2003).

Bhutta (1986) found less number of pathogenic fungi in agar plate method as compared to blotter method using some cotton varieties. Bhutta and Ahmad (1990) found seventeen species of fungi *viz. F. equiseti, F. solani, F. oxysporum, F.semitectum, Macrophomina phaseolina, Myrothesium roridum, M. verrucaria, P. malvacearum, Phoma* spp., *Verticillium* sp. by Blotter method. Mukewar and Mayee (2001) reported the presence of *Macrophomina phaseolina* in seed health testing of cotton at CICR, Nagpur. Kiran *et al.* (2005) reported that the standard blotter method was best method in terms of number of fungal species isolated from chickpea seed followed by agar plate method.

Vinaya Hemmanavar *et al.* (2009) reported that among the different seed health testing methods tested, Standard Blotter Method as the most efficient method for the quick and accurate diagnosis

of *Colletotrichum capsici* in chilli seeds. Dry inspection indicated that the percentage of pure seeds ranged from 90 - 100 in 20 rice varieties by Sultana *et al.* (2018). The maximum percentage of pure seed was observed in BRRI dhan 66 and BRRI dhan 70 (100) and minimum was observed in BRRI dhan 68 (90).

2.4. Molecular identification of seed borne fungi of cotton

Isolation of total genomic DNA from fungi suitable for PCR (polymerase chain reaction) amplification and other molecular application was stated by Amer *et al*. (2011). The main advantages of this method are given below:

- No liquid nitrogen is required for preparation of fungal DNA.
- The mycelium can be directly recovered from petri dish cultures.
- The quality and quantity of DNA obtained from this method are suitable for molecular assays.
- This method is rapid and relatively easy to perform.
- It can be applied to filamentous fungi from soil and from other environmental sources and
- This technique does not require the use of expensive and specialized equipment or hazardous reagents.

An experiment was conducted to analyze the fungal occurrence with contaminated rice grains in local market by Sohaib *et al*. (2015). Eight strains were isolated by them on the basis of phenotypic characters and further subjected to molecular analysis. ITS1 and ITS4 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* and other four strains were identified as *A. flavus*.

EL- shafey *et al*. (2018) identification *Cochliobolus carbonum* based on morpho-pathological characteristics and Internal Transcribed Spacer (ITS) region sequencing analysis. The molecular variation using ITS markers reflected a high level of genetic variation between the isolates. The data suggested that ITS region analysis was a potential instrument for phylogenetic reconstruction of the new isolates and as was DNA barcode for identification of the fungal species.

Molecular identification of fungal isolates by PCR utilizing ITS region universal primers was approved by Mohamed and Gomaa (2019). For confirmation of the species identification, ITS region was amplified. The phylogenetic tree exposed different levels of molecular variation among the fungal species compared to the international isolates deposited in the GenBank.

Shamsi *et al*. (2019) isolated ten pathogenic fungi of deuteromycetes from seven angiospermic plants such as tomato, rice, pointed gourd, wheat, maize, chickpea and jute. Morphological identification and molecular analysis were performed for accurate identification of the isolated pathogenic fungi. ITS1 and ITS4 primers were used to get the sequence results and compared with NCBI Gene Bank and BOLD database using BLAST analysis.

According to Sultana (2021), twenty five fungal species were isolated from twenty rice varieties and among these fungi, 13 were confirmed up to species level through ITS sequence based molecular analysis.

2.5. Seed borne pathogenic fungi of cotton and seed to seedling transmission.

A number of pathogenic fungi such as species of *Alternaria*, *Fusarium*, *Rhizopus* and *Aspergillus* are the most frequently identified seed borne pathogens in cotton (Minton and Garber 1983). Seed borne pathogenic fungi are a major problem which is responsible for the re-emergence of diseases of the past as well as the introduction of diseases into new areas (Gitaitis and Walcott 2007).

Fungi attack plants and cause a very serious economic impact on agricultural production owing to their capacity to induce diseases of cultivated crops that result in important yield losses (Paplomatas 2006).

Aspergillus flavus, A. niger (Type-I), *Curvularia lunata*, *Fusarium moniliforme* var. *subglutinans*, *F. sporotrichioides* and *Rhizoctonia solani* were found to be pathogenic for 3 hill cotton (*Gossypium arboreum*) seeds in Bangladesh (Naznin and Shamsi 2018). Palmateer *et al.* (2004) observed that *Fusarium moniliforme, F. semitectum* and *F. solani* were the most pathogenic fungi responsible for mortality of cotton plants. They also found that In Alabama, *Fusarium oxysporum*, *F. solani* and *F. equiseti* were the most common fungi at the seedling stage of the upland cotton.

According to Asran-Amal *et al*. (2005), *Rhizoctonia solani* is the most important pathogen involved in cotton seedling disease in Egypt. Pre or post emergence cotton seedling damping off, caused by *R. solani*, can be quite serious in the United States and often results in a substantial stand loss (Brown and Carter 1976).

Fusarium moniliforme, F. semitectum, F. oxysporum, F. solani, F. equiseti and *F. compactum* were isolated from cotton seedlings and bolls by Wang *et al*. (1992) and they found that *Fusarium moniliforme* was the predominant pathogen causing seedling and boll red rot of cotton. Bashan (1984) studied about the transmission of *Alternaria macrospora* in cotton seeds.

Devay *et al*. (1997) studied the development of *Fusarium* wilt in upland cotton. Sousa and Viana (2006) evaluated the effectiveness of inoculation procedures and periods of exposition of cotton seeds to *Fusarium oxysporum* f. sp. *vasinfectum*, through water restriction technique and to establish a reliable and a simple methodology of detection of *Fusarium oxysporum* f. sp. *vasinfectum* in cotton seeds for routine seed health analysis.

Grewal *et al*. (1974) first time reported *Fusarium solani* as one of the pathogenic fungi of Gram. Westerlund *et al*. (1973) reported 37 isolates of *Fusarium solani* and the five isolates of *F. oxysporum* were found to be pathogenic to chickpea. Hasanzade *et al*. (2008) carried out the *in vitro* pathogenicity test to prove the pathogenicity of *Fusarium solani* f. sp. *pisi* on susceptible chickpea cultivar Jam under artificial inoculation.

Nahar and Mushtaq (2007) studied the pathogenic effect of six *Fusarium* spp. *viz*., *F. equiseti, F. longipes, F. scirpi, F. oxysporum, F. pallidoroseum* and *F. solani* in sunflower plant by artificial inoculation. Wilting and seedling rot were found to be the most prominent symptom produced by all *Fusarium* spp. Basak and Lee (2002) observed that six pathogenic fungi *viz., Alternaria alternate, Aspergillus niger, Fusarium moniliforme, Fusarium* sp., *Penicillium* sp. and *Ustilago zeae* were associated with maize seeds. Prevalence of seed borne fungi were also varied and all the pathogens transferred from seed to seedlings detected by test tube seedling symptom test.

According to Chowdhury *et al.* (2021), *Alternaria alternata, Aspergillus flavus, Curvularia lunata, Drechslera oryzae, Fusarium moniliforme, F. solani, Microdochium oryzae, Pestalotiopsis guepinii* and *Sarocladium oryzae* were found to be pathogenic to rice seeds and all of them showed seed transmission nature i. e. transferred from seed to seedlings. Sultana (2021) found that, six pathogenic fungi *viz. Bipolris oryzae, Curvularia lunata, Fusarium equiseti, F. fujikuroi, Microdochium fisheri* and *Nigrospora oryzae* were associated with rice seeds and all the fungi transferred from seed to seedlings.

2.6. Management of seed-borne diseases of cotton through novel seed dressing fungicides, bioagents and botanicals.

2.6.1 Evaluation of fungicides

Tomar and Shastry (2006) found that seed treatment with Carbendazim (0.1%) was the most effective fungicide in controlling seed borne *Myrothecium roridum* and increasing the seedling vigour in cotton. Rajput *et al.* (2006) found that Carbendazim significantly reduced the colonization of *Fusarium oxysporum* f. sp. *vasinfectum* on root pieces of Cris-9 variety. Shakoor *et al.* (2011) studied the seed borne fungi associated with bitter gourd seeds under *in vitro* condition and they found that among Ridomil Gold MZ, Bavistin and Score; Ridomil gold MZ gave best results at all concentrations tested against all the isolated fungi.

Ibaiam *et al.* (2008) used seed-dressing fungicides (Bavistin, Benlate, Fernasan-D, Apron plus 50 DS and Dithane–M 45) for the control of seed-borne fungi of rice variety Faro 29 *in vitro*. The results obtained showed that all the fungicides significantly inhibited the seed-borne fungi associated with the seeds of the variety at concentrations of 40 g/ml, and 50mg/ml ($P<0.05$). Tomar *et al.* (2012) reported maximum per cent association of *Aspergillus niger*, *Curvularia lunata* and *Myrothecium roridum* with the cotton seed (JK4).

Verma (1975) treated the chickpea seeds with Bavistin, Vitavax @ 0.25 per cent and reported better protection. Copper fungicide was very handy to control *Aspergillus* spp in *in vitro* condition (Belly *et al.* 2006). Shah *et al.* (2010) reported that Mancozeb was found most effective against *Fusarium* spp. growth.

Paksha *et al.* (2003) used different fungicides to control *Sclerotium rolsfii* in *in vitro* experiment named Carbendazim, Tridemormg, Propiconazol, Captan, Thirum, Copper oxychloride and

Mancozeb and reported that Mancozeb @ 0.4% showed promising efficacy against growth of *Sclerotium* spp. in leaves of cotton.

Syed *et al.* (2001) reported that seed treatment with Mancozeb and Cupravit along with foliar spray with these two chemicals reduced the incidence of seed borne fungi of cotton. Therefore Mancozeb + Cupravit (0.4%) could be used as seed treating agent as well as foliar spray to control boll rot disease of cotton effectively.

Chowdhury *et al.* (2015) observed that the presence of 5 pathogenic fungi *viz*., *Alternaria alternata, Curvularia lunata, Drechslera oryzae, Fusarium moniliforme* and *Pestalotiopsis guepinii* associated with rice grains were completely controlled *in vitro* at different concentrations of Tall 25 EC or Tilt 250 EC. Nahar and Shamsi (2020) reported that Nativo 75 WP and Autostin 50 WDG showed best results to control the pathogenic fungi of cotton (*Gossypium arboreum* L.).

Tilt 250 EC was responsible for complete inhibition of the radial growth of *B. oryzae, C. lunata, F. fujikuroi, M. fisheri* and *N. oryzae* whereas Bavistin showed complete inhibition of the radial growth of *B. oryzae, C. lunata, F. equiseti, M. fisheri* and *N. oryzae* at all the tested concentrations according to Sultana (2021). According to Hosen and Shamsi (2017), Bavistin DF and Tilt 250 EC found to be the best inhibiting fungicides against *A. niger* and *F. merismoides*.

2.6. 2. Evaluation of botanicals

Plant extracts reduce the occurrence of fungi transmitted by seeds and to increase the percentage of germination and seedling emergence reported by Hasan *et al*. (2005). The effectiveness of plant extracts against seed infection in order to increase cotton seedlings performance have been reported by many researchers from various countries (Gustavo and Mariana 2010, Tomar *et al*. 2012, Rathinavel 2013).

Shekhawat and Prasad (1971) reported that, *Allium cepa*, *Allium sativum*, *Mentha piperita*, *Ocimum sanctum* and *Beta vulgaris* showed strong inhibitory effect against *Alternaria tenuis* from bean. Senthilnathan and Narasimhan (1994) found that the leaf extracts of *Aegle marmelos (*10%) was effective in inhibiting the spore germination and mycelial growth of *Alternaria tenuissima* the causal agent of leaf blight of onion.

Amaresh (2000) reported that, leaf extract of neem (5%), *Ocimum canum* (5%) and *Bougainvillea* sp. were observed to be most effective in controlling both *Alternaria* blight and rust. Alpa Chavi *et al.* (2010) revealed that neem extract showed 93.7% inhibition of the seed mycoflora. Pawar (2011) tested the efficacy of stem extracts of nine plants were screened against five seed borne pathogenic fungi *viz. A. alternata*, *A. niger*, *C. lunata*, *F. moniliforme* and *T. viride*. Out of them, six showed antifungal activity.

The extract of *A. indica* showed maximum activity; while minimum was observed with *Callistemon rigidus*. Gupta *et al.* (1981) reported that, conidial germination of *Colletotrichum capsici* was inhibited by phytonoids of *Allium cepa* L., *Allium sativum* L., *Azadirachta indica* juss, *Ocimum basilicum* L. and *Leucas* spp.

According to Nahar and Shamsi (2020), *Azadirachta indica* was most active to inhibit the growth of *Aspergillus niger* (Type-I) and *Fusarium moniliforme* var. *subglutinans, Psidium guajava* was most active against *Aspergillus flavus* and *Curvularia lunata* and *Datura metel* was most active against *Fusarium sporotrichioides* and *Rhizoctonia solani* in *Gossypium arboreum* L. in Bangladesh.

The ethanol extracts of *Tagetes erecta, Datura metel, Senna alata, Azadirachta indica, Citrus medica, Mangifera indica, Artocarpus heterophyllus, Asparagus racemosus, Nerium indicum* and *Allium sativum* completely inhibited the radial growth of the 5 pathogenic fungi *viz.*,

Alternaria alternata, Curvularia lunata, Drechslera oryzae, Fusarium moniliforme and *Pestalotiopsis guepinii* associated with rice grains at 20% concentration according to Chowdhury *et al*. (2015).

Sultana (2021) reported that, *Citrus limon* completely inhibited the radial growth of *B. oryzae* and *M. fisheri* at all the concentrations. *Azadirachta indica* and *Psidium guajava* showed the complete growth inhibition of *Curvularia lunata* and *M. fisheri*. On the other hand, *Azadirachta indica, cassia alata* and *Moringa oleifera* showed highest radial growth inhibition of *F. equiseti, F. fujikuroi* and *N. oryzae* at 20% concentration.

Ethanol extracts of *Allium sativum* showed complete growth inhibition against *A. niger* and *F. merismoides* according to Hosen and Shamsi (2017). *A. sativum, A. indica, C. limon, G. procumbens* and *T. occidentalis* plants extracts were reported as best botanicals for increasing seed germination and inhibiting fungal infection of wheat varieties according to Miah *et al*. (2017).

2.6. 3. Evaluation of bioagents

Charati *et al.* (1998) studied the effect of seed treatment with *Trichoderma* spp. on the incidence of *Fusarium* wilt of cotton. The seed was treated with talc-formulated *T. viride* and *T. harzianum* at 2, 4, and 6 g/kg seed and all the treatments showed significant results.

Farzana *et al.* (1991) observed that infection of 30 and 60 days old soybean plants by root infecting fungi (*R. solani, S. rolfsii* and *Fusarium* spp.) was significantly reduced following seed treatment with *T. harzianum*. Harman *et al.* (1989) reported increased plant stand in soils infested with *F. graminarum* and *Pythium ultimum* by priming of wheat seeds with *Trichoderma harzianum*. *Trichoderma* spp. a biocontrol agent of *Fusarium* spp. and *Sclerotium rolfsii* by seed treatment was reported by Monaco *et al.* (1991).

Liu (1992) controlled cowpea diseases due to *R. solani* and *F. oxysporum* with the help of *Trichoderma*. Elias *et al*. (1993) observed the control of mycelial growth of *F. oxysporum* f. sp. *cucumerinum* and *R. solani* using antagonistic *T. harzianum* species. Ercole and Gennari (1993) used *T. harzianum* for the biological control of *F. oxysporum* f. sp. *melonis* on melon.

Sunder *et al.* (1995) found that *T. viride* was most effective among the tested five *Trichoderma* spp. against *Macrophomina phaseolina* and *F. oxysporum* of castor in dual culture. Katragadda and Murugesan (1996) found that *T. harzianum* potentially reduced the radial growth *F. oxysporum* f. sp. *vasinfectum.* According to Ellil *et al.* (1998) *T. harzianum* reduced root rot infection by 6.7-45.0% in bean.

Bernal *et al*. (2001) observed that 70% inhibition of radial growth of *F. oxysporum* was observed due to *Trichoderma* spp. Goswami and Islam (2002) reported that antagonists such as *Trichoderma* spp. showed greater inhibition of tomato wilt pathogen *F. oxysporum* f. sp. *lycopersici*. Jeyalakshmi *et al*. (2009) investigated the induction of plant defense response against *Fusarium oxysporum* f. sp. *ciceri* by inoculating the roots of chickpea cv. JG 62 with the bio-control agent *Trichoderma harzianum*.

Sunil *et al*. (2007) evaluated 10 isolates belonging to three species of *Trichoderma* (*T. viride, T. harzianum* and *T. virens*) against four different races of *Fusarium oxysporum* f. sp. *ciceri* commonly prevalent in India Dharwad. Sunita and Kurundkar (2007) evaluated the efficacy of *Trichoderma* isolates against *Fusarium oxysporum* f. sp. *ciceri* under laboratory condition by employing dual culture technique on PDA and found that, in general *Trichoderma* isolate inhibited growth of the pathogen.

Zote *et al*. (2007) reported that soil / seed application of *T. viride* was found to be most effective recording lowest wilt incidence (19.04 to 33.33%) with highest wilt reduction (66.67 to 80.86%)

and maximum seed germination (86.73 to 90.00%) over untreated control. Singh *et al.* (2012) reported that seed cotton yield was significantly higher (2290kg/ha) in seed treatment with *Pseudomonas fluorescens* than untreated control (1890 kg/ha).

2.7. Integrated management of seed borne cotton pathogens

Integration of biological and chemical control seems to be a promising way of controlling many pathogens with minimum interference in the biological equilibrium in soil (Papavizas 1985). Tewari and Mukhopadhyay (2003) reported application of Carboxymethyl cellulose (CMC) with *Gliocladium virens* (Gv) in combination with Vitavax provided maximum protection (81.9%) to the crop against chickpea root rot and collar rot pathogens in glass house. Chickpea seed treated with Gv powder + CMC + Vitavax significantly increased seedling emergence, final plant stand and grain yield.

Kapoor *et al*. (2006) reported soil amendment with *Lantana camera* (10 t/ha) + bioagent Tricoguard @ 2.5 kg/62 kg FYM /ha + spray with Carbendazim at pre flowering stage was most effective in managing the root rot-wilt complex disease in pea. Gade *et al.* (2007) observed that soil solarization alone and in combination of seed treatment with Thiram + Benomyl 1:1 @ 3g / kg of seed reduced wilting to the extent of 22.8 and 22.6 % during first year and 16.3 and 15.7% during second year, respectively in case of pigeon pea.

Nikam *et al*. (2007) reported chemical seed treatment with Thiram (0.15%) + Carbendazim (0.1%) is proved to be the most effective fungicide combination against *Fusarium oxysporum* f. sp. *ciceri.* Masum *et al*. (2009) reported that, five seed treatment practices *viz*., hot water treatment, garlic tablet, neem leaf extract, BAU- Biofungicide and vitavax-200 significantly reduced the total seed-borne fungal infections as well as the population of individual six target pathogenic fungi *Agrostis tenuis, Bipolaris sorghicola, Botrytis cinerea, Crinum graminicola, Curvularia lunata* and

Fusarium moniliforme. They observed that combination of Mancozeb and Cupravit 50 WP both @ 0.4% significantly reduced the mycelial growth of *Fusarium* spp, *Alternaria* spp, *Sclerotium* spp, *Aspergillus flavus* and *A. niger*. This result is in accordance with a vast amount of the research findings of Muthomi *et al*. (2007), Hussain *et al*. (2001), Nisa *et al*. (2011) and Shah *et al*. (2010).

Phillip *et al.* (2003) reported that combination of Mancozeb and Fludioxonil up to ten days prior to planting can control *Fusarium* decay of seeds. Fravel *et al.* (2005) conducted an experiment to find out the efficacy of Mancozeb and Cupravit against the radial growth of *Fusarium oxysporum* and found that Mancozeb and Cupravit both reduced the growth of *Fusarium* spp. This finding was supported by Minamor (2013), Belly *et al.* (2006) and Wani and Nisa (2011). Jeyalakshmi *et al*. (1999) reported that the application of bioagents and Bavistin increased the seed germination percentage. Seed treatment with bioagents and fungicides reduced root rot incidence (Patil *et al.* 2003).

Sultana (2021) observed that, in field experiment out of twelve treatments T10 (Tilt + *Azadirachta indica* + *Trichoderma viride*), T3 (Bavistin + Tilt) and T7 (*Trichoderma viride*) showed highest germination percentage and highest seedling vigor index against *Bipolaris oryzae, Curvularia lunata* and *Fusarium fujikuroi*. On the other hand, T3 (Bavistin + Tilt), T7 (*Trichoderma viride*) and T1 (Bavistin) showed promising germination percentage and seedling vigor index.

According to Chowdhury *et al.* (2022), amongst the 13 treatments only T6 (Bavistin + *Azadirachta indica* + *Trichoderma harzianum*) showed highest percentage of seed germination and seedling vigor index of seeds. T10 (Bavistin + Tall + *A. indica* + *Citrus medica*) showed promising result against *Drechslera oryzae, Fusarium moniliforme, Microdochium oryzae* and *Sarocladium oryzae.* T3 treatment (*A. indica*) showed highest percentage of seed germination and seedling vigor index

in case of *Fusarium solani* inoculated seeds. Among the treatments, integrated use of Bavistin, *A. indica* and *T. harzianum* showed the best results for growth reduction of test pathogens and increased germination percentage of seeds.

CHAPTER: 3
MATERIALS AND METHODS

MATERIALS AND METHODS

3.1. Collection of Cotton Seed Samples

Gossypium hirsutum seeds of fourteen varieties namely CB1-CB14 were collected from Cotton Research, Training and Seed multiplication Farm, Sreepur, Gazipur. Samples were collected during the tenure of July 2017 to June 2018.

3.2. Preservation of Seed Samples

The collected seed samples were kept in clean glass jars, labeled properly and preserved at room temperature (25 ± 2°C) in the Mycology and Plant Pathology Laboratory, Department of Botany, University of Dhaka for subsequent uses.

3.3. Seed Quality Analysis

3.3.1. Dry Seed Inspection

Seed quality analysis observation is very important for seed. The seeds were subjected to visual observation and microscopic examination under stereoscopic microscope. Seed quality analysis was performed using 100g seeds for each sample. The ratio of pure seeds, abnormal seeds, inert matter and weed seeds of collected samples were determined. Seeds with distinct symptoms and abnormalities were selected and categorized into different groups. Seed contaminants and abnormal seeds were separated and recorded from each sample as follows:

- Inert matter (Stones, soil and sand particles, etc.)
- Varietal mixture (Plant debris, sunken seed, sclerotium, etc.)
- Other crop seeds

Abnormal seeds:

- Discolored seeds
- Wrinkled seeds
- Spotted seeds
- Undersized seeds

Determination of purity percentage of seeds was calculated with the following formula:

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

3.3.2. Determination of seed germination

For determination of germination, 300 surface sterilized seeds of each sample were taken and 10 seeds were placed in each petri plate according to the rules of ISTA (1996). Filter papers were soaked in distilled water and placed in 9 cm diameter petri plate. Then 10 seeds were placed in each petri plates on the top of filter paper. The lid of the petri plates were tightly fitted to remove the evaporation of water. The petri plates were then incubated at room temperature (25 ± 2°C) for 7 days. Seeds producing both plumule and radical were deliberated as germinated seeds. Germination was recorded after 7 days and expressed as percentage according to Shamsi and Khatun (2016).

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

3.3.3. Determination of seedling mortality

Seedling mortality were determined after ten days of incubation according to Shamsi and Khatun (2016) with the formula given below:

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

3.3.4. Determination of seedling height

Seedling height (root and shoot length) of different varieties of cotton seeds were determined according to the method described by Shamsi and Khatun (2016).

3.3.5. Determination of seedling vigor index

The seedling vigor index of different cotton varieties were recorded with the formula of Lee *et al*. (2008) which is given below:

Vigor index (VI) = (Mean of root length + Mean of shoot length) × Percent of seed germination

3.3.6. Per cent determination of seed moisture content (%)

Moisture content of the seeds of different varieties of cotton was measured by using moisture meter (Model: GMK - 303RS, Korea) and calculated according to Christensen and Lopez (1965).

3.4. Isolation, purification and identification of fungi associated with the seeds of *Gossypium hirsutum* L.

3.4. 1. Isolation of fungi

Fungi associated with the seeds of *Gossypium hirsutum* L. were isolated separately following methods:

(a) Tissue planting method (CAB 1968)

(b) Blotter method (ISTA 1996) and

(c) Paper towel method (ISTA 1996).

For these methods of isolation, approximately 300 seeds with three replications were used for 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 again washed with sterile water for three times. Finally, the seeds were placed on the sterilized filter paper to remove the excess surface water and kept in room condition. The surface sterilized seeds used as inocula for isolation purpose.

(a). Tissue planting method

In tissue planting method surface sterilized seeds were placed on sterilized potato dextrose agar (PDA) medium (Appendix 1) in Petri plate. For this method, 300 seeds were washed in sterile water and then surface sterilized by 10% Chlorox solution. Each Petri plate contained 15 ml of PDA medium with 1 drop of lactic acid (0.03 ml) and 10 cotton seeds. PDA was used as growth medium and lactic acid to prevent the bacterial growth. Inoculated Petri plates were incubated for 10 days at 25 ± 2ºC. After incubation, the fungi associated with the inoculum were recorded. Per cent frequency of occurrence of the fungi was calculated by adopting the formula of Spurr and Wetly (1972) as given below:

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

(b) Blotter method

Moist chamber was made by placing 2 layers of filter paper at the bottom of 9 cm diameter Petri plate, sufficient water was added to soak the blotting papers and then covered with the upper part of the Petri plate. The Petri plates were sterilized within an autoclave at 121°C for 20 minutes. Surface sterilized seeds were inoculated in Petri plates and each Petri plate contained 10 seeds.

The inoculated Petri plates were incubated in an incubator (25±2°C) for 10 days. After incubation, the fungi associated with the seeds were recorded carefully. Per cent frequency of occurrence of the fungi was calculated by following the formula of Spurr and Wetly (1972).

(c) Paper Towel method

For this method, firstly, moist paper towel chambers were prepared by using 3 layers of moist newsprint papers. The papers were moistened by sterile or distilled water. The paper towel was then rolled and covered by foil paper and autoclaved at 15 lbs pressure and 121°C temperature for 20 minutes. The surface sterilized seed inocula were placed in moist paper towel chamber. 10 seeds were placed in each chamber. Then they were incubated at 25 ± 2 °C for 15 days. After 7, 10 and 15 days the fungi were observed and recorded.

3.4.2. Isolation of fungi from different parts of cotton seeds

The location of fungi in cotton seeds was studied by employing "Component plating technique" described by Shamsi *et al*. (1995). Seed coat, embryo and endosperm are the three parts of Cotton seeds. The seed samples with highest (CB 10), lowest (CB 8), moderate fungal frequency (CB 3) (shown in Table 10) and seeds selected randomly from all varieties (CB1-14) were used for the study. Three hundred seeds of each samples were taken for this experiment. The seed parts were separated and then surface sterilized with 10% Chlorox solution for five minutes. These seed parts were again washed with distilled water for three times and soaked with sterilized filter paper. The separated seed parts were then placed in Petri plates containing sterilized PDA medium. Each Petri plate contained 15 ml of PDA medium with an addition of one drop of lactic acid which was used to check the bacterial growth. Then the inoculated plates were incubated at room temperature (25

± 2 °C) for 5-7 days. The fungi isolated from different seed parts were examined under electron microscope (Appendix III).

Fig. 1. Different parts of cotton seeds: A. Germinating seeds, **B.** Embryo with endosperm **C.** Embryo, **D.** Endosperm and **E.** Seed coat.

3.4.3. Identification of fungi associated with the seeds of *Gossypium hirsutum* L.

(a) Morphological identification

Morphological studies of the isolated fungi were made to determine their identity. Fungal structures 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 (Appendix I) over a clean slide for microscopic observation. A little amount of Cotton Blue (Appendix I) was added to the mounted fluid in case of hyaline structures. 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 high resolution digital camera. Conidia and conidiophores of the important fungi were drawn with the aid of a Camera Lucida for the identification of that fungus. The entire specimen, included in the present study was preserved in the Mycology and Plant Pathology Laboratory, Department of Botany, University of Dhaka, Bangladesh.

Morphological Identification of the isolates was determined following standard literatures (Barnett and Hunter 1972, Benoit and Mathur 1970, Booth 1971, Ellis 1971, 1976, Rapper and Thom 1949, Subramanian 1971, Sutton 1980, Thom and Rapper 1945).

(b) Molecular identification

Molecular identification of the isolates were done according to Amer *et al.* (2011) with some modification.

DNA extraction

For DNA extraction, the fungi were grown on PDA medium at 25± 2ºC for 15 days. With a sterile spatula one gm fungal mycelia were taken in 1.5 ml Eppendorf tubes from the petri plates. The

mycelia were immediately grinded with a homogenizer machine in each Eppendorf with 400 µl sterile extraction buffers (200mM Tris- HCL, 250mM NaCl, 25mM EDTA, 0.5% SDS). Then 6 µl of 20 mg/ml RNase was added in each Eppendorf. The tubes were placed in 65ºC preheated water bath for 10 minutes. The samples were taken from the water bath and cooled down to room temperature. In each sample, 130 µl of 3M sodium acetate, pH 5.2 was added. Samples were vortexed for 30s at maximum speed to mix well and incubated at -20º C for 10 minutes. The samples were centrifuged at 13,000 rpm, 4º c for 15 minutes. The supernatants were transferred to fresh tubes and an equal volume of isopropanol was added to each sample, mixed well and were incubated at 4°C for one night. Samples were then centrifuged at 6000 rpm, 4ºc for 20 minutes. White coloured pellet was formed. The supernatant was discarded and the pellet was washed with 700 µl of 70% ethanol in two times. The DNA pellets were then 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 dissolved overnight at 4 ºC in the refrigerator.

PCR amplification

Molecular identification of the isolates was completed 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 performed 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 micro centrifuge. The PCR was introduced 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. PCR amplified products were stored in – 20º C freezer for analysis by resolving in 1% agarose gel. The gel was prepared using

1.0g agarose powder containing 8µl ethidium bromide. Agarose gel electrophoresis was conducted in 1× TAE buffer at 90 Volts and 300 mA for 60 minutes. Alongside the ITS reactions, one molecular weight marker 1kb DNA ladder was electrophoresed. DNA bands were then photographed by a Gel Documentation system (model: DI-HD, UK).

Sequencing Analysis

The PCR amplified products were purified by alcohol precipitation and sequenced through automated sequencer in Centre for Advanced Research in Sciences (CARS), University of Dhaka. The obtained sequences were compared with already available sequences in the National Center for Biotechnology Information (NCBI, Bethesda, MD, USA) using BLAST program (http://blast.ncbi.nlm.nih.gov) to identify the genus and species of the isolates.

3.5. Pathogenicity test of the isolated fungi

Pathogenicity test of isolated fungi were done following seed inoculation technique described by Chowdhury *et al*. (2015). Three hundred seeds of each variety were taken and separately soaked in distilled water in the beaker for 30 minutes and then surface sterilized with 10% Chlorox solution for 5 minutes. Spore suspension of the test fungi (10^4 /ml conc.) was prepared in a sterilized beaker. Seeds were placed in beakers with spore suspension and then left undisturbed condition for 2 hours. One hundred of each healthy, spotted and inoculated seeds of each cotton varieties were then selected and single seed was placed in sterilized 8 inch cotton plugged test tubes which contains 10 ml 2% water agar medium. Healthy seeds served as control. Observation was made for 3 weeks at 3 days intervals. Germination percentage of seeds, development of disease symptoms, seedling mortality and shoot, root length of seedling were recorded on healthy and inoculated seeds of each cotton varieties (Appendix III). Pathogenic fungi were re-isolated from

diseased and inoculated cotton seeds after 15 days of inoculation and confirmed their identity following Koch's postulates where healthy seeds and seedlings remained fresh.

3.6. Transmission of pathogenic fungi from seed to seedling

3.6.1. Seedling symptoms test of fungi in test tubes

The seedling symptom test in test tube was used for this study which was developed by Khare *et al*. (1977). Nine pathogenic seed borne fungi of cotton *viz., Aspergillus flavus, A. niger, A. tamarii, Colletotrichum gloeosporioides, Curvularia lunata, Fusarium nivale, F. moniliforme, Mucor* sp. and *Rhizoctonia solani* were selected for seedling symptoms test. Three hundred seeds of each variety were selected and soaked in distilled water in the beaker for 30 minutes separately and then surface sterilized with 10% Chlorox for 5 minutes. Spore suspension of the test fungi at 10^4 /ml concentration was prepared in a sterilized beaker. Seeds from each variety were placed in beakers with spore suspension and then left undisturbed condition for 2 hours.

Seeds were placed in sterilized 8 inch cotton plugged test tubes containing 10 ml 2% water agar medium. Healthy seeds served as control. The test tube with seeds were then incubated in the laboratory at room temperature (25 ± 2 °C). Observation was made for 3 weeks at 3 days intervals. For the presence of visible symptoms (seed rot, germination failure and infection or death of emerged seedlings) developed by the pathogens present in the seeds, the germinating seeds and seedlings were examined (Appendix III). Germination percentage of seeds, development of disease symptoms, seedling mortality and shoot root length of seedlings were recorded from healthy and inoculated seeds of each cotton varieties. The symptoms produced on the germinating seeds and seedlings by the associated pathogens were confirmed by examining the seeds under stereo-binocular microscope. Seedling symptoms of pathogenic fungi were recorded according to Hansraj *et al*. (2017).

3.6.2. Seedling symptoms test of fungi in pot culture

Seeds were selected randomly from all varieties and were grown in pots filled with sterilized soil (Hansraj *et al*. 2017). Nine pathogenic seed borne fungi of cotton were also selected for seedling symptoms test. Two hundred seeds of each variety were selected and soaked in distilled water for 30 minutes and then surface sterilized with 10% Chlorox solution for 5 minutes. Spore suspension of the test fungi was prepared in a sterilized beaker. Seeds were placed in beakers with spore suspension and then left undisturbed for 2 hours. One hundred of each healthy, spotted and inoculated seeds of each cotton varieties were selected and fifty seeds were sown per pot. In case of control, surface sterilized healthy seeds were inoculated in the pot. The pots were kept in pot house and watered regularly. Observation was made for 3 weeks at 7 days intervals and symptoms were recorded after 21 days of germination. For confirmation the organisms were examined under electron microscope.

3.7. Evaluation of antagonistic potentials of some soil fungi against the test pathogens.

Some soil fungi were isolated from the cotton field soil following serial dilution method described by Krieg 1981. Initially, one gm of soil was added with 99 ml of distilled water in a conical flask and mixed it very well with a glass rod and marked as mother suspension. Then five test tubes containing 9 ml of sterilized distilled water in each were taken. One ml of mother suspension was added into the 1^{st} test tube and made it 10 ml. So, into the first test tube the mother suspension was diluted 10 times. It was mixed well and then one ml of the suspension from the 1^{st} test tube was added into the 2^{nd} test tube and made it 10 ml. So, into the 2^{nd} test tube the mother suspension was diluted 100 times. This process was performed for rest of the test tubes and the mother suspension was diluted into 10, 100, 1000, 10000 and 100000 times.

One ml of suspension was poured into a sterilized Petri plate for each dilution, and then about 15 ml of sterilized melted PDA medium was added. The Petri plate was then moved gently on the laminar air flow table to get a homogenous distribution of the suspension. For each dilution, five replications were maintained. All the Petri plates were incubated into 25±2˚C temperature. After 3 days of incubation, the *Aspergillus* and *Trichoderma* genera were sub-cultured on PDA slants randomly, from the culture plates and stored at 4^{o} C in an incubator for future studies. Identification of the soil fungi were determined following the standard literature (Barnett and Hunter 1972, Benoit and Mathur 1970, Booth 1971, Ellis 1971, 1976, Thom and Raper 1945, Raper and Thom 1949, Subramanian 1971, Sutton 1980, Gilman 1967). Cultures were maintained by sub-culturing them after four weeks intervals. From the isolated soil fungi, *Aspergillus flavus* Link, *A. fumigatus* Fresenius, *A. niger* Tiegh and *Trichoderma viride* Pers were selected randomly to study colony interactions against the test pathogens.

3.7.1. Colony interactions

Colony interactions between the selected soil fungi and the test pathogens were studied in dual culture techniques on PDA medium. A Petri plate with 15 ml solidified PDA medium was inoculated with 5 mm mycelial agar disc of a test pathogen and a soil fungus, 3 cm apart from each other. In each case, three replications were maintained. The inoculated plates were incubated at 25±2˚C temperature for 5 days. The colony growth of the pathogen was measured at both sides, i.e. towards and opposing each other from their central loci. The radial growth of the fungi, intermingled and inhibition zone was measured after 5 days of incubation. Measurements of colony interactions between the soil fungi and test pathogens were also done in terms of grades which were discerned by Skidmore and Dickinson (1976) (Appendix II). The grades and types are as follows:

Grade 1 (Type A): Mutually intermingling growth where both the fungi grew into one another without showing any 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 fungus and test pathogen frightened each other until almost in contact with a narrow demarcation line (1-2mm).

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

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

$$\% \text{ growth inhibition} = \frac{r_1 - r_2}{r_1} \times 100$$

Where,

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

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

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

3.7.2. 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 3.7.1. For this study, the method described by Dennis and Webster (1971 b) was followed. The soil fungi were grown in 9 cm Petri plates on PDA medium for 5 days. The lid of each Petri plate was replaced by the same sizes bottom plate after the inoculation at 25±2˚C, containing 15 ml PDA medium with 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 III). 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 fungi were measured in all the sets and the per cent inhibition or stimulation in the colony diameter of the test fungi was also calculated after 7th day of incubation. The formula of per cent growth inhibition as follows:

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

Where, I= Per cent growth inhibition

C= Growth in control

T= Growth in treatment

3.7.3. 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 3.7.2. Three equal size blocks of each 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. The culture of a soil fungus was filtered first through a Whatman filter paper and then centrifuged at 3000 rpm for 20 minutes after 10 days of incubation at 25±2˚C. Then 5, 10, 15 and 20 ml metabolites of each fungus were added in 95, 90, 85 and 80 ml sterilized PDA medium separately. To get the homogenous distribution of the supplemented medium, the conical flask containing the PDA medium and culture filtrates was moved in different directions gently on the laminar air flow table. This concentration was found to be most suitable for such studies by Singh and Webstar (1978). Each Petri plate contained 15 ml of PDA medium and metabolites with an addition of 1 drop (ca 0.03) of lactic acid. 5, 10, 15 and 20 ml of supplemented medium was poured in a sterilized Petri plate 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 a test pathogen. In the control, Petri plate containing PDA medium without culture filtrates were inoculated with a test pathogen as described above. Equal amount of sterilized water was added with the PDA medium instead of culture filtrate in the control set. Three replications were maintained for each treatment. All the Petri plates were incubated at 25±2˚C. After 5 days of incubation, the radial growth of the colonies was measured (Appendix III).

The percent inhibition of each test pathogen was calculated with the following formula:

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

Where,

I= Per cent growth inhibition

C= Growth in control and

T= Growth in treatment

3.8. Fungitoxicity assessment of fungicides against the test pathogens.

Ten fungicides with different active ingradients, *viz*. Capvit 50 WP, Contaf 5 EC, Dithane M-45, Knowin 50 WP, Nativo 75 WP, Ridomil MZ Gold, Score 250 EC, Secure, Silica 80 WG and Tilt 250 EC were selected for evaluating their effect on the radial growth of *Aspergillus flavus*, *A. niger*, *A. tamarii*, *Colletotrichum gloeosporioides*, *Curvularia lunata*, *Fusarium nivale*, *F. moniliforme*, *Mucor* sp. and *Rhizoctonia solani* (Table 1). These fungicides were collected from the Krishi Upokoron Biponi Kendro, Khamarbari, Farmgate, Dhaka. A stock solution having the concentration of 10,000 ppm was prepared for each fungicide. The calculated amount of the stock solution of a fungicide was supplemented with sterilized PDA medium to get the final concentrations of 100, 200, 300, 400 and 500 ppm (Appendix III). Twenty ml of the medium of a particular concentration was poured in sterilized Petri plates and allowed to solidify. The concentrations of fungicides were expressed in terms of its active ingredients. In the control set, required amount of sterile water was added to the PDA medium instead of adding fungicide. Then it was inoculated in the centre of the 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 plates were incubated at 25±2°C in an incubator. The radial growth of control and treatment plates were measured at 5 days of incubation.

The growth inhibition of each test pathogen 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

Table 1. Particulars of fungicides used in the study.

Sl. No.	Fungicides	Active ingredient (s)	Manufacturer
1.	Capvit 50 WP	Copper oxychloride	Crop Touch Pesticide Ltd, India
2.	Contaf 5 EC	Hexaconajol	Rallis India Ltd
3.	Dithane M-45	80% Mancozeb	Dow Agro Science, India
4.	Knowin 50 WP	50% Carbendazim	Mc Donald Bangladesh (Pvt) Limited
5.	Nativo 75 WG	50% Tebuconajol + 25% trifloxistrobin	Bayer crop Science, UK
6.	Ridomil MZ Gold	64% Mancozeb + 4% Metalexil	Synzenta (BD) Ltd
7.	Score 250 EC	Diphenoconajole	Synzenta (BD) Ltd
8.	Secure	64% Mancozeb + 8% Fenamidan	Synzenta (BD) Ltd
9.	Silica 80 WG	Sulphur	Synzenta (BD) Ltd
10.	Tilt 250 EC	Propiconazole	Synzenta (BD) Ltd

3.9. Effect of plant extracts on the radial growth of the test pathogens.

A total of ten plants *Viz.*, *Adhatoda vasica* L., *Aegle mermelos* L., *Azadirachta indica* A. Juss., *Citrus lemon* L., *Datura metel* L., *Mangifera indica* L., *Moringa oleifera* Lam., *Ocimum sanctum* L., *Psidium guajava* L. and *Tagetes patula* L. were selected to evaluate their effect on the radial growth of *Aspergillus flavus*, *A. niger*, *A. tamarii*, *Colletotrichum gloeosporioides*, *Curvularia lunata*, *Fusarium nivale*, *F. moniliforme*, *Mucor* sp. and *Rhizoctonia solani* (Table 2). The plant parts were collected from the Botanical Garden, Curzon Hall Campus, Dhaka University.

(a) Preparation of aqueous plant extracts

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

II. Leaf extract was prepared by crushing known weight of fresh leaves with distilled water in the 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 the particulate matter.

IV. The supernatants were filtered through Whatman filter paper and the filtrate was collected in 250 ml conical flasks.

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

(b) Inoculation of the test pathogens

I. The medium thus prepared was poured into sterilized Petri plates and allowed to concentrate. 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 the control set, a Petri plate containing PDA medium with the required amount of distilled water instead of plant extract was also inoculated with agar disc of the test pathogen in the same procedure as mentioned above.

III. For both the experimental and control sets, three replications were maintained. The inoculated Petri plates were incubated at 25±2˚ C. After 5 days of incubation, the radial growth of the colonies of the test pathogen was measured.

Table 2. Particulars of angiospermic plant species used in the present study.

Sl. No.	Plant species	Native name	Family	Plant parts used
1.	*Adhatoda vasica* L.	Basak	Acanthaceae	Leaf
2.	*Aegle mermelos* L.	Bel	Rutaceae	Leaf
3.	*Azadirachta indica* A. Juss.	Neem	Meliaceae	Leaf
4.	*Citrus lemon* L.	Lebu	Rutaceae	Leaf
5.	*Datura metel* L.	Datura	Solanaceae	Leaf
6.	*Mangifera indica* L.	Aam	Anacardiaceae	Leaf
7.	*Moringa oleifera* Lam.	Sojne	Moringaceae	Leaf
8.	*Ocimum sanctum* L.	Tulshi	Lamiaceae	Leaf
9.	*Psidium guajava* L.	Peyera	Myrtaceae	Leaf
10.	*Tagetes patula* L.	Gada	Asteraceae	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 and

T = Growth in treatment

3.10. Integrated approach to control the test pathogens

Integrated approach was done following Waris *et al*. (2018) with some modification. The experiment was carried out in the earthen pot in Botanical garden, Department of Botany, University of Dhaka. Best performing two fungicides, leaf extracts of two plants and one antagonistic fungus were tested against the test pathogens in the pot. The seeds were surface sterilized with 10% chlorox for 5 minutes. The seeds were then washed with sterile distilled water for 4-5 times. Spore suspensions were prepared from 10 days old culture using sterile distilled water. 10^7- 10^8 cfu / ml spores was contained by each spore suspension.

Then the seeds were inoculated with equal volume of spore suspension of each test fungus separately and left for two hours in sterilized petri plates. Then the inoculated seeds were treated with the various combination of fungicides, plant extracts and biocontrol agents enlisted in Table 3. The fungicides were mixed with appropriate amount of water for their respective dose. Spore suspension of antagonistic fungus was made with sterile distilled water. Plant extracts were also prepared as described above and treated to the pre-inoculated seeds. The seeds were then sown in 12″ X 8″ size pots containing sterile soil. Experimental design was CRD and RBD having 3 replications. Observations and data were recorded after 7, 14 and 21 days of seed germination. Final data were recorded after 21 days of germination. The results of seed germination percentage, seedling mortality, shoot length, root length and seedling vigor index were recorded carefully (Appendix III).

Table 3. Components of different treatments with their doses.

Treatments	Component of treatments	Dose
T1	Tilt	100 ppm conc.
T2	Contaf	100 ppm conc.
T3	*Psidium guajava*	100 ppm conc.
T4	*Azadirachta indica*	10% conc.
T5	*Trichoderma viride*	10% conc.
T6	Contaf + Tilt	10% conc.
T7	*Psidium guajava* + *Azadirachta indica*	10% conc.
T8	Contaf + *P. guajava* + *T. viride*	10% conc.
T9	Contaf + *A. indica* + *T. viride*	10% conc.
T10	Tilt + *P. guajava* + *T. viride*	10% conc.
T11	Tilt + *A. indica* + *T. viride*	10% conc.
T12	Contaf + *A. indica*	10% conc.
T13	Contaf + *P. guajava*	10% conc.
T14	Tilt + *A. indica*	10% conc.
T15	Tilt + *P. guajava*	10% conc.
T16	Contaf + Tilt + *P. guajava*+ *A. indica*	10% conc.
T17	Contaf + Tilt + *P. guajava*+ *A. indica*+ *T. viride*	10% conc.
T18	Inoculated without treatment	
Control	Uninoculated healthy seeds	

3.11. Analysis of data

Data on different parameters were analyzed following computer package MSTAT-C and means were compared using Duncan's Multiple Range Test (DMRT). The data were collected as inhibition percentage of the radial growth of the test pathogen in mm in each replication and then evaluated by analysis of variance (ANOVA) by using STAR statistical program.

CHAPTER: 4
RESULTS AND DISCUSSION

Results and Discussion

4.1. Seed quality analysis

For seed quality analysis the percentage of pure seeds, other seeds and abio or non-seeds are presented in Table 4. Seed quality analysis revealed that the maximum purity percentage was 99.92 found in CB13 and minimum was 97.08 found in CB12. The highest per cent of the other seed was recorded in CB12 (0.91%) and lowest (0.0%) in 9 other cotton varieties. The non or abio seed percentage was maximum (1.0) in CB5 and minimum (0.10) in CB13 (Table 4).

Quality of good cotton seeds are as follows:

- Purity: Minimum 98%
- Abio or non seeds: Maximum 1%
- Mixture of other seeds: Maximum 1%
- Amount of moisture: Maximum 8-12% and
- Rate of germination: Minimum 80%

Source: The Annual Report and Work plan 2016-2017 (CDB) (Anon. 2017).

Table 4 expressed that, in case of purity, all the cotton varieties individually did not follow the standard of CDB rules (Anon. 2017), but the mean value have followed the standard value. In respect of other seeds and non-seeds, they have followed the standard. Maximum moisture percentage (11.3) was recorded in CB12 and minimum (10) in CB5. All these factors followed the standard of CDB rules (Anon. 2017).

Table 4. Percentage of pure seeds, other seeds, abio seeds, moisture and germination of different varieties of cotton seeds.

Cotton varieties	Pure seeds (%)	Other seeds (%)	Abio or non seeds (%)	Moisture (%)	Germination (%)
CB1	99.55^{abc}	0.0^{c}	0.79^{d}	10.9^{b}	82^{efg}
CB2	98.78^{cde}	0.03^{c}	0.61^{e}	10.6^{cd}	83de
CB3	99.0^{cde}	0.0^{c}	0.54^{ef}	11.2^{a}	85^{cd}
CB4	98.45^{abcd}	0.0^{c}	0.95^{b}	10.5^{d}	80^{g}
CB5	99.91^{ab}	0.0^{c}	1.0^{a}	10.0^{e}	81^{fg}
CB6	97.47^{de}	0.0^{c}	0.56^{ef}	10.7^{bc}	87^{c}
CB7	98.77^{abcd}	0.0^{c}	0.40^{g}	10.7^{bc}	81^{fg}
CB8	98.41^{abcde}	0.10^{bc}	0.82^{cd}	10.4^{d}	92^{ab}
CB9	98.39^{abcde}	0.0^{c}	0.89^{bc}	10.8^{bc}	81^{fg}
CB10	99.80^{abc}	0.0^{c}	0.90^{bc}	10.6^{bcd}	93^{a}
CB11	97.91^{cde}	0.0^{c}	0.49^{f}	10.7^{b}	92^{ab}
CB12	97.08^{e}	0.91^{a}	0.91^{b}	11.3^{a}	84^{ef}
CB13	99.92^{a}	0.10^{c}	0.10^{h}	11.2^{a}	83^{ef}
CB14	98.19^{bcde}	0.49^{b}	0.49^{f}	11.2^{a}	91^{b}
Mean	98.69	0.12	0.67	10.78	85.36
CV (%)	0.5320	49.37	4.16	0.9297	1.17

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

4.2. Effects of seed borne fungi on germination, seedling mortality, seedling height and vigor index of cotton seeds.

The per cent germination and seedling mortality, seedling height and vigor index of 14 varieties of cotton seeds is presented in Table 5. The germination percentage of seeds was maximum in CB 10 (93) and minimum in CB4 (80). The percentage of seedling mortality was highest in CB11 (50.30) and lowest in CB7 (16.05). The highest shoot length was observed in CB 2 (19.8 mm) and

the lowest in CB 4 (9.0 mm) whereas root length was highest in CB 5 (4.6 mm) and lowest in CB 4 (2.9 mm). The maximum vigor index was observed in CB10 (2064.6) and the minimum in CB4 (952.0).

Table 5. Effects of seed borne fungi on germination, seedling mortality, seedling height and vigor index of cotton seeds.

Cotton varieties	% Germination (5^{th} day)	% Mortality (7^{th} day)	Seedling height (mm) (5^{th} day)		Vigor index
			Root	Shoot	
CB 1	82	25.39	4.4	15.0	1590.8
CB 2	83	27.71	3.1	19.8	1892.4
CB 3	85	35.29	3.2	11.3	1232.5
CB 4	80	31.25	2.9	9.0	952.0
CB 5	81	33.33	4.6	16.6	17717.2
CB 6	87	43.93	2.3	10.3	1096.2
CB 7	81	16.05	3.8	16.3	1628.1
CB 8	92	23.91	3.9	13.2	1573.2
CB 9	81	45.89	3.0	12.9	1287.9
CB10	93	26.88	3.7	18.5	2064.6
CB11	92	50.30	3.8	17.4	1950.4
CB12	845	21.43	3.5	14.9	1545.6
CB13	83	22.98	3.7	15.0	1552.1
CB14	91	47.88	4.5	17.8	2029.3

The prevalence of seed-borne infection is responsible for low rate of gerrmination (Fakir 1998, Islam *et al.* 2003). This is similar with the findings of present results. Most of the pathogens are transmitted through seeds in cotton plants, which impair seed germination resulting in the production of abnormal seedlings (Bateman and Kwasna 1999, Khanzada *et al*. 2002). The results of seed germination of the research work also in agreement with the findings of Jeyalakshmi *et al*. 1999, Eisa *et al*. 2007, Tomar *et al*. 2012 who reported that various seed borne fungal pathogens in the world reduce germination percentage and seedling vigor of cotton.

4.3. Detection of seed borne fungi associated with cotton seeds

A total of twenty nine species of fungi belonging to 14 genera were found to be associated with the seeds of 14 varieties (CB 1- CB 14) of cotton seeds (Figs. 2-4, Table 6). The name of species of fungi are as follows:

1. *Aspergillus aculeatus* Lizuka,
2. *A. flavus* Link,
3. *A. fumigatus* Fresenius,
4. *A. niger* Van Tiegh,
5. *A. nidulans* Eidam,
6. *A. subramanianii* Visagie, Frisvad & Samson,
7. *A. tamarii* Kita G.,
8. *A. toxicarius* Murak,
9. *A. wentii* Wehmer,
10. *Curvularia lunata* (Wakker) Boedijn,
11. *Colletotrichum gloeosporioides* Penz & Sacc,
12. *C. gossypii* Southw.,
13. *Chaetomium globosum* Kunze.,
14. *Fusarium moniliforme* J. Shelden,
15. *F. nivale* (Fr.) Sorauer,
16. *F. oxysporum* Schlechtendal,
17. *F. fujikuroi* Nirenberg,
18. *F. solani* (Mart.) Sacc.,
19. *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl.
20. *Meyerozyma guilliermondii* (Wick.) Kurtzman & M. Suzuki,
21. *Mucor* sp. P. Micheli ex L.,
22. *Penicillium aculeatum* Raper & Fennell,
23. *P. citrinum* Thom,
24. *Rhizoctonia solani* Khun.,
25. *Rhizopus stolonifer* (Ehrenb.) Vuill.,
26. *R. oryzae* Went & Prins. Geerl.,
27. *Rhizomucor* sp. Lucet & Costantin,
28. *Syncephalastrum racemosum* Cohn and
29. *Trichoderma viride* Pers.

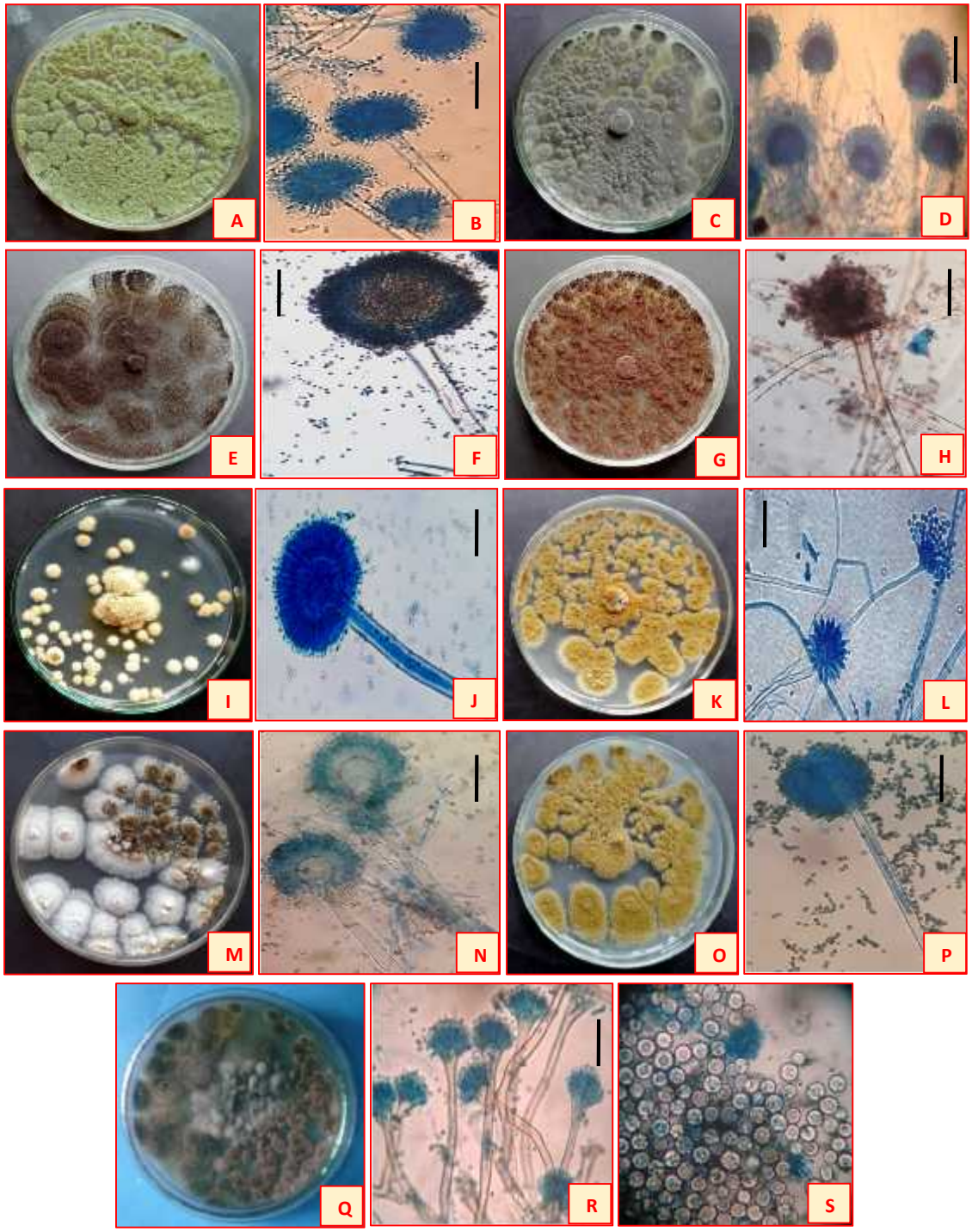

Fig. 2. Colony, conidiophore and conidia of *Aspergillus* spp associated with cotton seeds.

A-B: *Aspergillus flavus*, **C-D:** *A. fumigatus*, **E-F:** *A. niger*, **G-H:** *A. aculeatus*, **I-J:** *A. subramanianii*, **K-L:** *A. tamarii*, **M-N:** *A. wentii*, **O-P:** *A. toxicarius*, **Q-R:** *A. nidulans* and **S:** Hulle cell of *A. nidulans* (Bar = 50 µm).

Fig. 3. Colony, conidiophores and conidia of different fungi associated with cotton seeds.

A-B: *Colletotrichum gloeosporioides*, **C-D:** *C. gossypii*, **E-F:** *Curvularia lunata*, **G-H:** *Chaetomium globosum*, **I-J:** *Fusarium oxysporum*, **K-L:** *F. nivale*, **M-N:** *F. moniliforme*, **O-P:** *F. fujikuroi*, **Q-R:** *F. solani* and **S-T:** *Lasiodiplodia theobromae* (Bar = 50 µm).

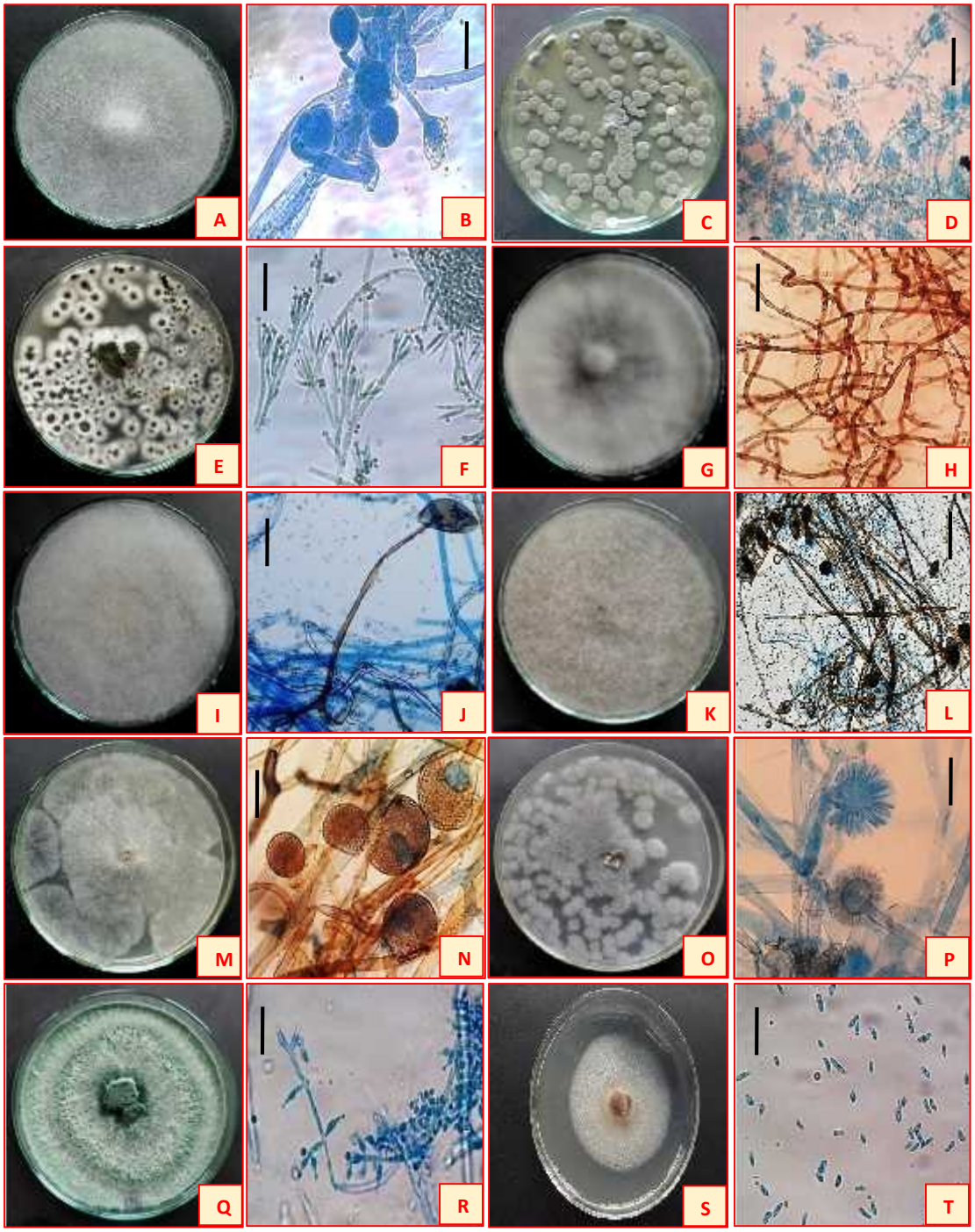

Fig. 4. Colony, conidiophores and conidia of different fungi associated with cotton seeds.
A-B: *Mucor* sp., **C-D:** *Penicillium citrinum*, **E-F:** *P. aculeatum*, **G-H:** *Rhizoctonia solani*, **I-J:** *Rhizopus oryzae*, **K-L:** *R. stolonifer*, **M-N:** *Rhizomucor* sp., **O-P:** *Syncephalastrum racemosum*, **Q-R:** *Trichoderma viride* and **S-T:** *Meyerozyma guilliermondii* (Bar = 50 µm).

Table 6. Fungi associated with different varieties of cotton seeds.

Sl No	Fungal species	CB 1	CB 2	CB 3	CB 4	CB 5	CB 6	CB 7	CB 8	CB 9	CB 10	CB 11	CB 12	CB 13	CB 14
1.	*Aspergillus aculeatus*	-	-	-	-	+	-	-	+	-	-	+	+	+	+
2.	*A. flavus*	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3.	*A. fumigatus*	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4.	*A. nidulans*	+	-	-	+	-	-	-	-	-	-	-	-	-	+
5.	*A. niger*	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6.	*A. subramanianii*	+	-	-	+	+	-	+	+	+	-	-	+	-	+
7.	*A. tamarii*	+	+	+	-	+	-	-	-	-	+	-	+	-	+
8.	*A. toxicarius*	+	-	+	+	-	+	+	+	+	+	-	-	-	-
9.	*A. wentii*	+	-	+	+	+	+	+	+	+	+	-	+	+	+
10.	*Colletotrichum gossypii*	-	-	-	+	-	-	-	-	-	-	-	-	-	+
11.	*C. gloeosporioides*	-	+	+	-	-	-	+	+	+	-	-	-	-	+
12.	*Chaetomium globosum*	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13.	*Curvularia lunata*	-	-	-	+	+	+	-	+	-	-	+	+	-	+
14.	*Fusarium moniliforme*	+	-	-	-	+	-	-	-	-	-	-	+	-	-
15.	*F. nivale*	+	+	+	+	+	+	-	+	+	+	+	-	+	+
16.	*F. oxysporum*	-	-	-	-	-	-	-	-	-	-	-	+	-	-
17.	*F. solani*	-	-	-	+	-	-	-	+	-	-	-	-	-	-
18.	*F. fujikuroi*	+	-	-	-	-	-	-	+	-	-	-	-	+	-
19.	*Lasiodiplodia theobromae*	-	-	-	-	-	-	-	+	-	-	-	+	-	+
20.	*Meyerozyma guilliermondii*	-	-	-	-	-	-	+	+	-	-	-	-	-	-
21.	*Mucor* sp.	-	-	+	-	-	-	-	+	+	+	-	+	+	+
22.	*Penicillium aculeatum*	-	+	-	-	-	-	-	+	-	-	-	-	+	+
23.	*P. citrinum*	+	+	+	+	+	+	+	+	+	+	+	+	+	+
24.	*Rhizoctonia solani*	+	+	-	-	+	-	+	-	-	-	+	+	+	+
25.	*Rhizomucor* sp.	-	-	-	-	-	-	-	-	+	-	-	-	-	-
26.	*Rhizopus stolonifer*	+	+	+	+	+	+	+	+	+	+	+	+	+	+
27.	*R. oryzae*	-	-	-	+	-	-	+	-	-	-	-	+	-	-
28.	*Syncephalastrum racemosum*	+	-	-	-	-	-	-	-	-	-	-	-	-	-
29.	*Trichoderma viride*	-	+	+	+	+	+	+	+	+	-	-	-	+	+

'+' and '–' represent the presence and absence of fungi, respectively.

4.4. Frequency percentage of association of fungi with different varieties of cotton seeds in Tissue planting method of different intervals.

4.4.1. Frequency percentage of fungi within two months of harvest.

The fungi associated with cotton seeds within two months of harvest is presented in Table 7, Fig. 5 and Plate 1. Twenty three species of fungi belonging to 11 genera were found to be attached with 14 varieties of cotton seeds. The isolated fungi were *Aspergillus flavus, A. fumigatus, A. niger, A. aculeatus, A. tamarii, A. wentii, A. toxicarius, Curvularia lunata, Colletotrichum gossypii, C. gloeosporioides, Chaetomium globosum, Fusarium oxysporum, F. nivale, F. fujikuroi, F. solani, Lasiodiplodia theobromae, Mucor* sp., *Penicillium citrinum, P. aculeatum, Rhizopus stolonifer, R. oryzae, Rhizoctonia solani* and *Trichoderma viride.* Prevalence of *A. niger* and *A. toxicarius* was highest (100%) found in CB12 and CB9, respectively, whereas appearence of *C. lunata, Mucor* sp. and *P. aculeatum* were lowest (2%). The total association was highest in CB12 (277) and lowest in CB8 (68). *Aspergillus flavus, A. niger* and *Rhizopus stolonifer* were common in most of the varieties whereas, *Chaetomium globosum, Colletotrichum gossypi* and *Fusarium oxysporum* were found only in one variety.

4.4.2. Frequency percentage of fungi after six months of storage.

Table 8, Fig. 6 and Plate 2 represent the association of fungi with cotton seeds after six months of storage. The isolated fungi were *Aspergillus flavus, A. fumigatus, A. niger, A. aculeatus, A. subramanianii, A. tamarii, A. wentii, A. toxicarius, Chaetomium globosum, Fusarium nivale, F. moniliforme, Lasiodiplodia theobromae, Penicillium citrinum, Mucor* sp., *Meyerozyma guilliermondii, Rhizopus stolonifer, Rhizoctonia solani, R. oryzae* and *Syncephalastrum racemosum.* The frequency percentage of *R. stolonifer* was highest (100%) recorded in CB5 and CB10. On the other hand, lowest (2%) was recorded in *A. tamarii, A. subramanianii, P. citrinum,*

C. globosum and *S. racemosum*. The whole association of fungi was maximum in CB10 (208) and minimum in CB8 (60). *Aspergillus flavus, A. niger, Penicillium citrinum* and *Rhizopus stolonifer* were common in most of the varieties whereas, *Lasiodiplodia theobromae, Rhizoctonia solani, R. oryzae* and *Syncephalastrum racemosum* were exclusively found in only one variety.

4.4.3. Frequency percentage of fungi after ten months of storage.

Appearance of fungi with the cotton seeds after 10 months is presented in Table 9, Fig. 7 and Plate 3. Sixteen types of fungi were isolated from 14 types of cotton seeds. The isolated fungi were *Aspergillus flavus, A. fumigatus, A. niger, A. nidulans, A. subramanianii, A. wentii, A. toxicarius, Curvularia lunata, Chaetomium globosum, Fusarium nivale, Mucor* sp., *Penicillium citrinum, Rhizopus stolonifer, Rhizoctonia solani, Rhizomucor* sp. and *Trichoderma viride.* The most predominant fungi were *A. flavus, A. fumigatus, A. niger, C. globosum, P. citrinum* and *R. stolonifer* in terms of prevalence and their frequency were also gradually increased with the increase of storage period. The prevalence of *A. toxicarius* and *R. stolonifer* was highest (100%) recorded in CB10 and CB11 respectively. *Aspergillus subramanianii, A. wentii, C. lunata* and *T. viride* was observed the lowest (2%) in CB12, CB1, CB6 and CB14, respectively. The total fungal association was maximum in CB11 (185) and minimum in CB2 (53). The most frequent fungi in this category were *Aspergillus flavus, A. fumigatus, A. niger, A. wentii* and *Penicillium citrinum*. On the other hand, *Mucor* sp., *Rhizoctonia solani* and *Rhizomucor* sp. were found in rare case.

Table 7. Frequency percentage of association of fungi with different varieties of cotton seeds within two months of harvest.

Fungal species	Frequency percentage of fungi with different varieties of cotton seeds													
	CB 1	**CB 2**	**CB 3**	**CB 4**	**CB 5**	**CB 6**	**CB 7**	**CB 8**	**CB 9**	**CB 10**	**CB 11**	**CB 12**	**CB 13**	**CB 14**
Aspergillus flavus	4	-	12	16	-	36	8	3	-	12	4	23	3	3
A. fumigatus	12	26	6	10	18	-	-	18	-	16	3	10	-	-
A. niger	28	10	16	8	16	4	34	4	6	8	34	100	52	96
A. aculeatus	-	-	-	-	10	-	-	4	-	-	20	48	50	24
A. tamari	8	-	4	-	6	-	-	-	-	-	-	-	-	-
A. wentii	-	-	-	-	-	-	86	-	-	4	-	-	-	-
A. toxicarius	-	-	-	34	-	-	44	-	100	86	-	-	-	-
Chaetomium globosum	-	-	-	-	-	-	-	-	-	20	-	-	-	-
Colletotrichum gloeosporioides	-	7	9	-	-	-	7	3	5	-	-	-	-	3
C. gossypii	-	-	-	4	-	-	-	-	-	-	-	-	-	-
Curvularia lunata	-	-	-	2	3	-	-	-	-	-	2	2	-	6
Fusarium fujikuroi	8	-	-	-	-	-	-	6	-	-	-	-	4	-
F. oxysporum	-	-	-	-	-	-	-	-	-	-	-	6	-	-
F. nivale	-	14	4	5	-	7	-	8	-	6	3	-	3	3
F. solani	-	-	-	3	-	-	-	7	-	-	-	-	-	-
Lasiodiplodia theobromae	-	-	-	-	-	-	-	-	-	-	-	4	-	5
Mucor sp.	-	-	-	-	-	-	-	-	-	2	-	8	4	8
Penicillium citrinum	8	-	4	-	10	-	-	-	-	14	3	-	-	-
P. aculeatum	-	4	-	-	-	-	-	4	-	-	-	-	2	8
Rhizoctonia solani	8	6	-	-	5	-	-	-	-	-	-	52	10	8
Rhizopus stolonifer	30	30	50	-	8	10	22	8	20	12	35	20	-	4
R. oryzae	-	-	-	3	-	-	10	-	-	-	-	4	-	-
Trichoderma viride	-	4	4	24	12	12	10	4	6	-	-	-	3	-
Total fungi	106	101	109	101	88	69	221	68	137	180	102	277	131	167

'-' = No fungal growth

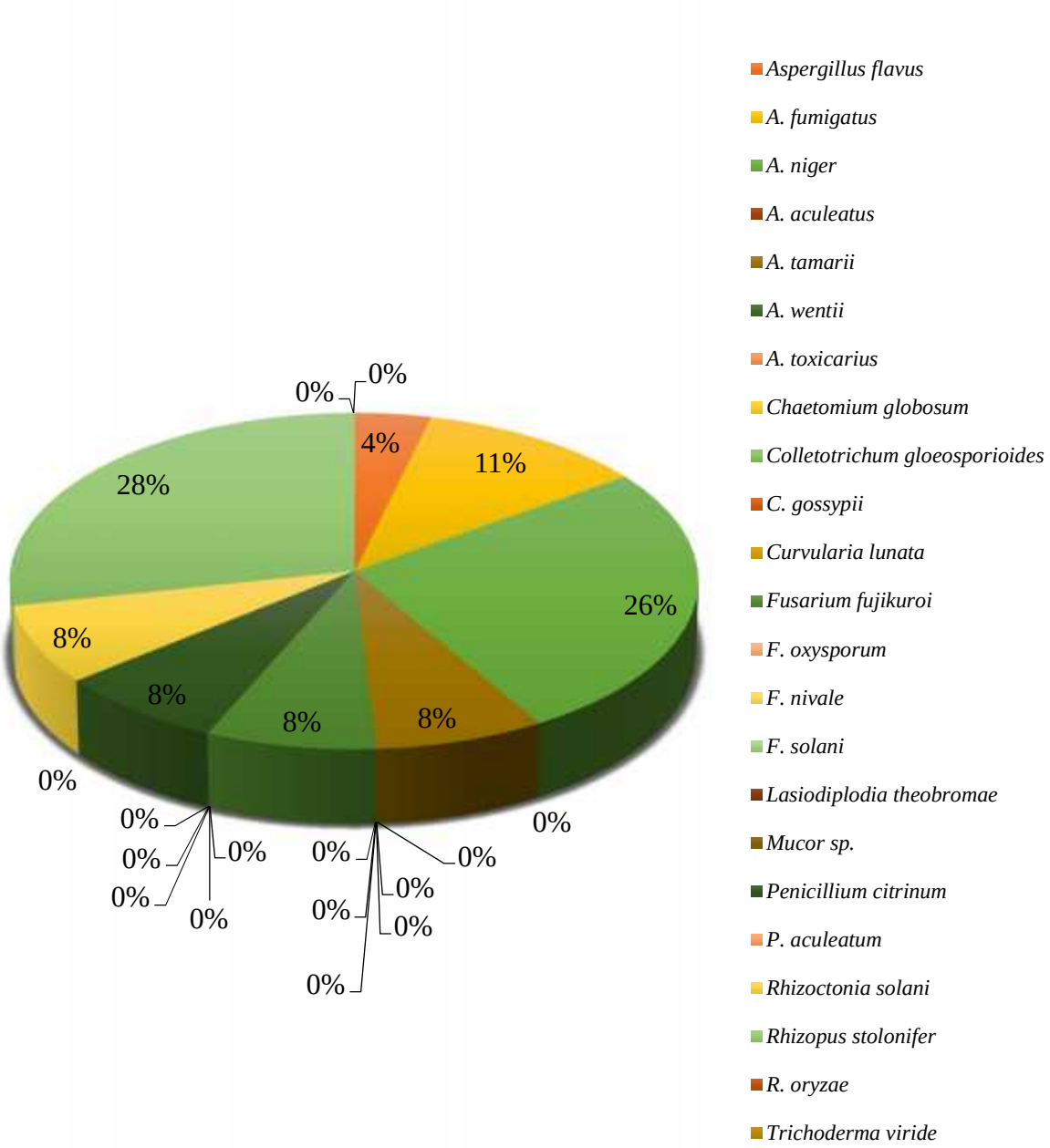

Fig. 5. Mean per cent frequency of fungi associated with different varieties of cotton seeds within two months of harvest.

Plate 1. Fungi associated with germinating seeds of different varieties of cotton within two months of harvest.

Table 8. Frequency percentage of association of fungi with different varieties of cotton seeds after six months of storage.

Fungal species	Frequency percentage of fungi with different varieties of cotton seeds													
	CB 1	**CB 2**	**CB 3**	**CB 4**	**CB 5**	**CB 6**	**CB7**	**CB 8**	**CB 9**	**CB 10**	**CB 11**	**CB 12**	**CB 13**	**CB 14**
Aspergillus flavus	6	12	6	16	20	10	20	6	12	24	12	12	24	48
A. fumigatus	3	10	-	-	14	8	-	8	4	6	3	22	10	28
A. niger	4	22	16	10	30	6	14	12	4	36	60	20	84	10
A. aculeatus	-	-	-	-	-	-	-	-	-	-	33	14	16	6
A. subramanianii	12	-	-	2	-	-	-	4	-	-	-	-	-	-
A. tamari	-	4	-	-	2	-	-	-	-	28	-	4	-	3
A. wentii	14	-	-	-	-	-	-	-	-	14	-	-	10	4
A. toxicarius	60	-	46	34	-	56	34	6	36	-	-	-	-	-
Chaetomium globosum	-	2	-	-	-	-	-	-	-	-	3	12	-	-
Fusarium moniliforme	4	-	-	-	3	-	-	-	-	-	5	-	-	-
F. nivale	-	4	-	5	-	-	-	-	-	-	-	-	-	6
Lasiodiplodia theobromae	-	-	-	-	-	-	-	10	-	-	-	-	-	-
Mucor sp.	-	-	3	-	-	-	3	4	-	-	-	4	-	-
Meyerozyma guilliermondii	-	-	-	-	-	-	7	2	-	-	-	-	-	-
Penicillium citrinum	10	8	-	6	4	6	-	10	-	-	2	2	-	3
Rhizoctonia solani	-	-	-	-	-	-	-	-	-	-	10	-	-	-
Rhizopus stolonifer	10	70	62	52	100	40	4	-	52	100	20	12	10	10
R. oryzae	-	-	-	5	-	-	-	-	-	-	-	-	-	-
Syncephalastrum racemosum	2	-	-	-	-	-	-	-	-	-	-	-	-	-
Total fungi	124	132	133	130	173	126	75	60	108	208	148	102	154	117

'-' = No fungal growth

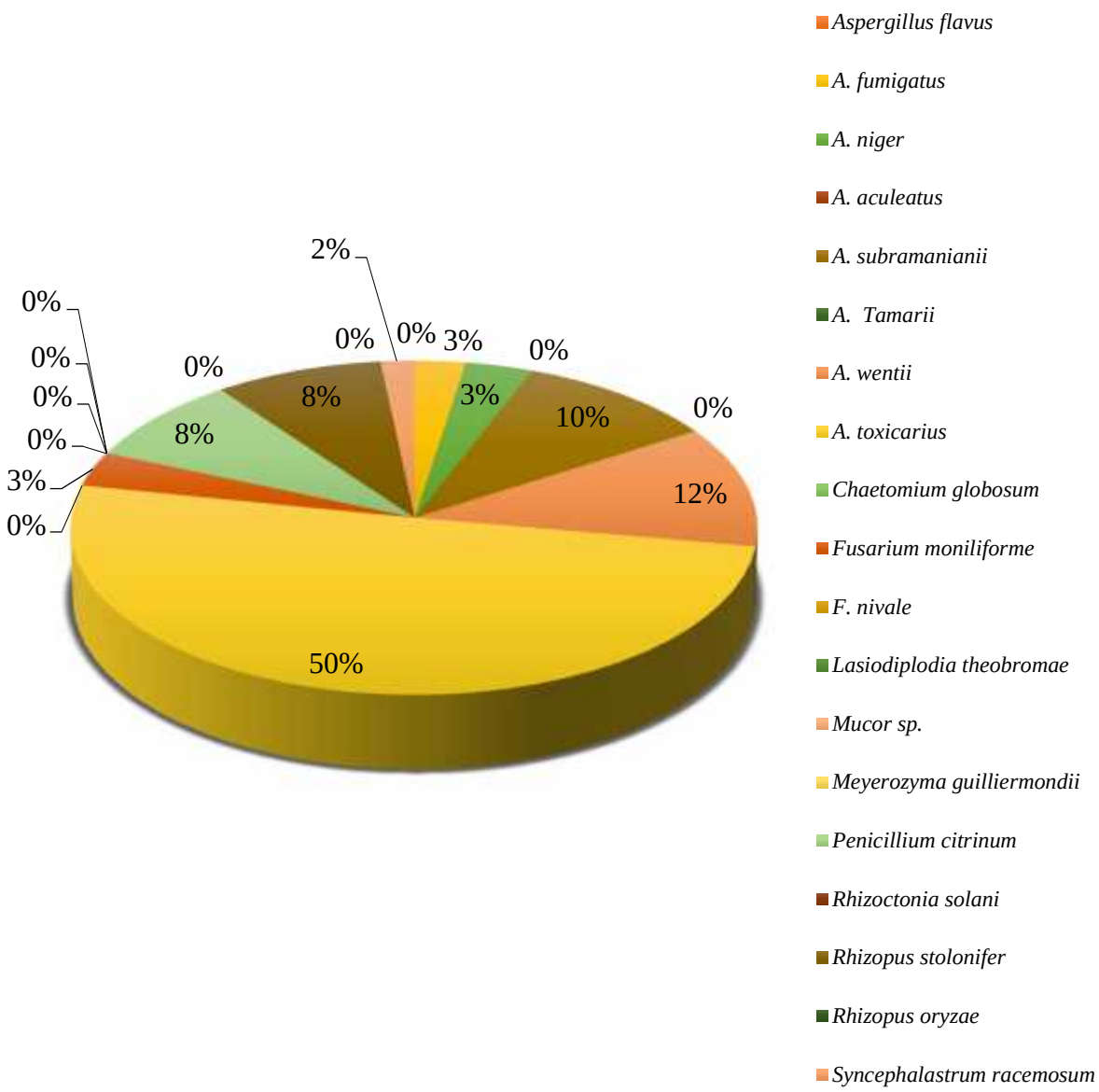

Fig. 6. Mean per cent frequency of fungi associated with different varieties of cotton seeds after six months of storage.

Plate 2. Fungi associated with germinating seeds of different varieties of cotton after six months of storage.

Table 9. Frequency percentage of association of fungi with different varieties of cotton seeds after ten months of storage.

Fungal species	Frequency percentage of fungi with different varieties of cotton seeds													
	CB 1	**CB 2**	**CB 3**	**CB 4**	**CB 5**	**CB 6**	**CB 7**	**CB 8**	**CB 9**	**CB 10**	**CB 11**	**CB 12**	**CB 13**	**CB 14**
Aspergillus flavus	2	7	24	9	12	-	4	25	10	3	8	12	17	24
A. fumigatus	6	4	8	8	4	10	12	9	8	12	4	4	7	6
A. nidulans	4	-	-	6	-	-	-	-	-	-	-	-	-	-
A. niger	-	7	16	-	30	24	9	6	10	16	70	24	78	34
A. subramanianii	-	-	-	-	3	-	4	-	19	-	-	2	-	4
A. wentii	2	-	14	10	10	22	17	4	18	4	-	5	3	4
A. toxicarius	6	-	38	46	-	-	96	-	-	100	-	-	-	-
Chaetomium globosum	18	25	16	22	5	28	7	4	20	-	-	13	-	52
Curvularia lunata	-	-	-	-	-	2	-	3	-	-	-	-	-	-
Fusarium nivale	10	-	-	-	5	-	-	6	-	-	3	-	3	-
Mucor sp.	-	-	-	-	-	-	-	-	3	-	-	-	-	-
Penicillium citrinum	30	-	4	14	10	10	14	25	4	32	-	36	15	38
Rhizoctonia solani	-	-	-	-	-	-	10	-	-	-	-	-	-	-
Rhizomucor sp.	-	-	-	-	-	-	-	-	3	-	-	-	-	-
Rhizopus stolonifer	-	10	-	11	12	-	-	-	45	10	100	21	20	10
Trichoderma viride	-	-	-	-	-	-	-	4		-	-	-	-	2
Total fungi	83	53	120	126	117	116	173	96	139	177	185	119	148	174

'-' = No fungal growth

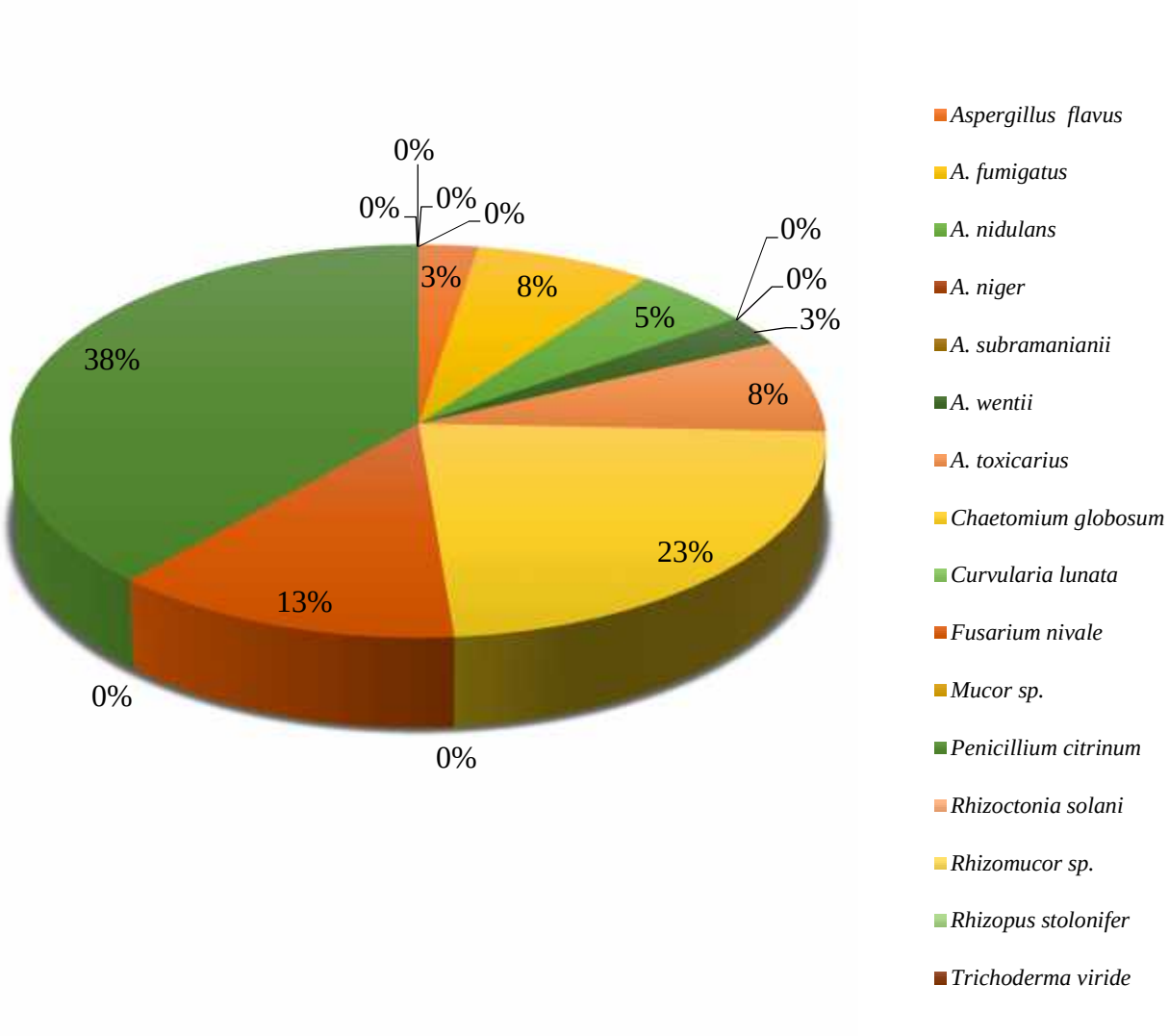

Fig. 7. Mean per cent frequency of fungi associated with different varieties of cotton seeds after ten months of storage.

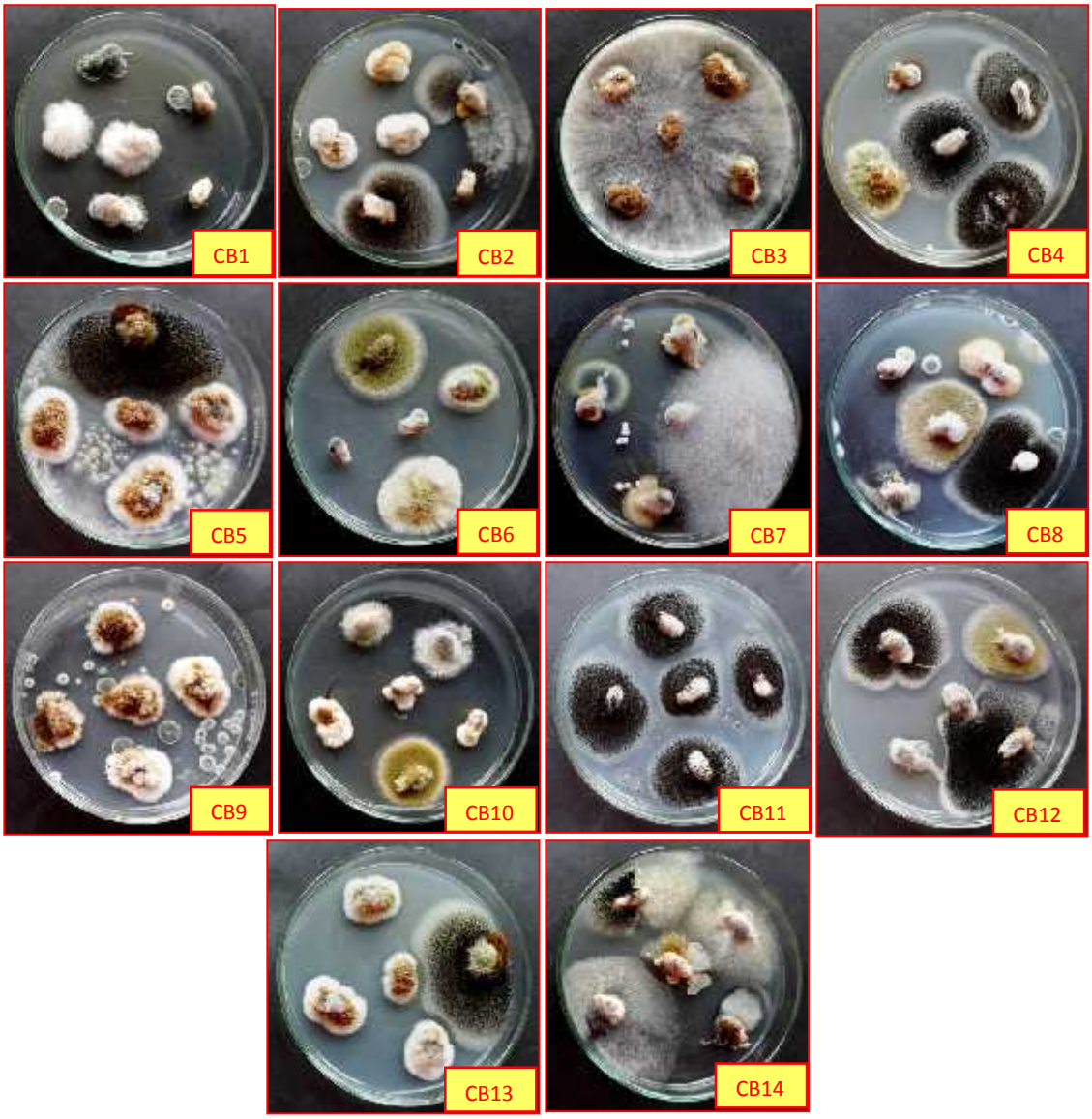

Plate 3. Fungi associated with germinating seeds of different varieties of cotton after ten months of storage.

The results of Table 9 are similar with the findings of Mansoori and Hamdolahzadeh (1995), who isolated *Alternaria alternata*, *Aspergillus niger*, *Fusarium acuminatum*, *F. solani*, *Pythium ultimum*, *Rhizopus arrhizus* and *Rhizoctonia solani* from cotton seeds. They found that *Fusarium oxysporum* and *F. solani* were the predominant species of fungi which gradually decreased with the increase of storage fungi like the species of *Aspergillus*, *Penicillium* and *Rhizopus*.

4.4. 4. Average frequency of fungi after ten months of storage.

Table 10 shows that twenty nine species of fungi were found to be associated with fourteen varieties of cotton seeds. *Aspergillus flavus, A. fumigatus, A. niger, Chaetomium globosum, Penicillium citrinum* and *Rhizopus stolonifer* were found in all varieties examined and frequency percentage of these fungi were also higher. In terms of prevalence these were the most predominant fungi. These six predominant fungi varied in prevalence with respect to variety and time duration whereas, *Colletotrichum gossypii, Fusarium oxysporum, Syncephalastrum racemosum* and *Rhizomucor* sp. were exclusively isolated from only one variety. The frequency percentages of these fungi were very low. The total association of fungi in cotton seeds was highest in CB10 (204) and lowest in CB8 (92) (Fig. 8). On the other hand, the total association of *Aspergillus niger* in 14 varieties was the highest (359) and *Colletotrichum gossypii* the lowest (1) was in (Fig. 9).

The present findings are in accordance with the results of Lutfunnessa and Shamsi (2011) who reported *Alternaria tenuis, Aspergillus flavus, A. niger, A. fumigatus, Fusarium moniliforme* and *Rhizopus nigricans* with cotton seeds. Similarly, according to Nahar *et al.* (2019), twelve species of fungi were isolated from the seeds of three varieties of Hill cotton (*Gossypium arboreum* L.) in Bangladesh. Among these, *Aspergillus niger* (Type-I), *A. niger* (Type-II) and *Rhizoctonia solani* were predominant fungi and the fungal associations were varied with duration of storage periods.

Many seed borne fungal pathogens have been reported to reduce germination percentage and seedling vigour in cotton seeds by Jeyalakshmi *et al*. (1999), Eisa *et al*. (2007) and Tomar *et al*. **(**2012). Similarly, Arabsalmani (2015) isolated *Rhizoctonia solani, Aspergillus flavus, Alternaria macrospora, A. alternata* and *Fusarium* spp from the seed of cotton in Tissue planting method.

Table 10. Average frequency percentage of the association of fungi with 14 varieties of cotton seeds after ten months of storage.

Fungal species	Frequency percentage of fungi with different varieties of cotton seeds														Total fungi
	CB 1	CB 2	CB 3	CB 4	CB 5	CB 6	CB 7	CB 8	CB 9	CB 10	CB 11	CB 12	CB 13	CB 14	
Aspergillus aculeatus	-	-	-	-	4	-	-	2	-	-	18	21	22	10	77
A. flavus	4	6	14	14	11	16	20	12	7	13	8	16	15	25	181
A. fumigatus	7	14	5	6	12	6	4	12	6	11	6	12	6	12	119
A. nidulans	2	-	-	3	-	-	-	-	-	-	-	-	-	-	5
A. niger	11	13	16	6	25	12	19	8	7	20	55	48	72	47	359
A. subramanianii	4	-	-	2	1	-	2	2	6	-	-	2	-	2	21
A. tamarii	3	2	2	-	3	-	-	-	-	10	-	2	-	1	23
A. toxicarius	22	-	28	38	-	19	58	2	45	74	-	-	-	-	286
A. wentii	5	-	5	3	3	7	6	2	6	9	-	5	5	3	53
Chaetomium globosum	6	9	6	8	2	9	3	2	7	7	2	8	2	18	89
Colletotrichum gloeosporioides	-	3	3	-	-	-	2	1	2	-	-	-	-	1	12
C. gossypii	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1
Curvularia lunata	-	-	-	1	1	1	-	1	-	-	1	2	-	2	9
Fusarium moniliforme	2	-	-	-	3	-	-	-	-	-	3	-	-	-	8
F. nivale	3	6	2	3	2	3	-	5	2	2	3	-	2	3	36
F. oxysporum	-	-	-	-	-	-	-	-	-	-	-	4	-	-	4
F. solani	-	-	-	3	-	-	-	7	-	-	-	-	-	-	10
F. fujikuroi	3	-	-	-	-	-	-	2	-	-	-	-	2	-	7
Lasiodiplodia theobromae	-	-	-	-	-	-	-	10	-	-	-	4	-	5	19
Meyerozyma guilliermondii	-	-	-	-	-	-	7	2	-	-	-	-	-	-	9
Mucor sp.	-	-	1	-	-	-	-	2	2	2	-	4	2	3	16
Penicillium aculeatum	-	2	-	-	-	-	-	2	-	-	-	-	2	3	9
P. citrinum	18	3	2	7	5	6	5	12	2	16	2	13	5	14	110
R. oryzae	-	-	-	5	-	-	10	-	-	-	-	4	-	-	19
Rhizoctonia solani	3	2	-	-	2	-	3	-	-	-	5	17	4	3	39
Rhizomucor sp.	-	-	-	-	-	-	-	-	2	-	-	-	-	-	2

Rhizopus stolonifer	14	37	38	21	40	17	9	3	39	41	52	18	3	5	337
Syncephalastrum racemosum	2	-	-	-	-	-	-	-	-	-	-	-	-	-	2
Trichoderma viride	-	2	2	8	4	4	3	3	2	-	-	-	1	1	31
Total fungi	109	99	124	129	118	101	151	92	133	204	155	180	143	158	

'-' = No fungal growth

Bhat *et al.* (1999) reported the presence of *Aspergillus niger*, *A. flavus* and *Penicillium* sp. with the spoilage of beni seed. They are either surface contaminants or seed borne which could cause biodeterioration and reduce the vigour. Nasira *et al*. (2004) reported some of the seed borne fungi of beni seed as *Penicillium*, *Alternaria, Fusarium* and *Curvularia*. These results are also resembling with the results of present investigation.

Storage fungi deteriorate the quality and quantity of seeds in storage condition. In this experiment, fungal population of 14 varieties of cotton seeds have been extensively studied up to 10 months of storage. The occurrence and abundance of fungi were different with duration of storage period. The association of *Aspergillus subramanianii, A. toxicarius, A. wentii, Penicillium aculeatum, P. citrinum, Rhizomucor* sp. and *Meyerozyma guilliermondii* with cotton seeds are new records for Bangladesh (Siddiqui *et al*. 2007, Shamsi 2017 and Nahar *et al.* 2019). These findings clearly exhibited the diversity of the fungi on different varieties of cotton seeds.

In the present investigation many saprophytic fungi were also found in all the samples. Saprophytic fungi include *Aspergillus* spp., *Penicillium* sp. and *Rhizopus stolonifer* (Tables 7-10). The saprophytic fungi have the ability to secrete toxic metabolites which damages the quality of seed in storage. This is in agreement with the findings of Christensen (1972).

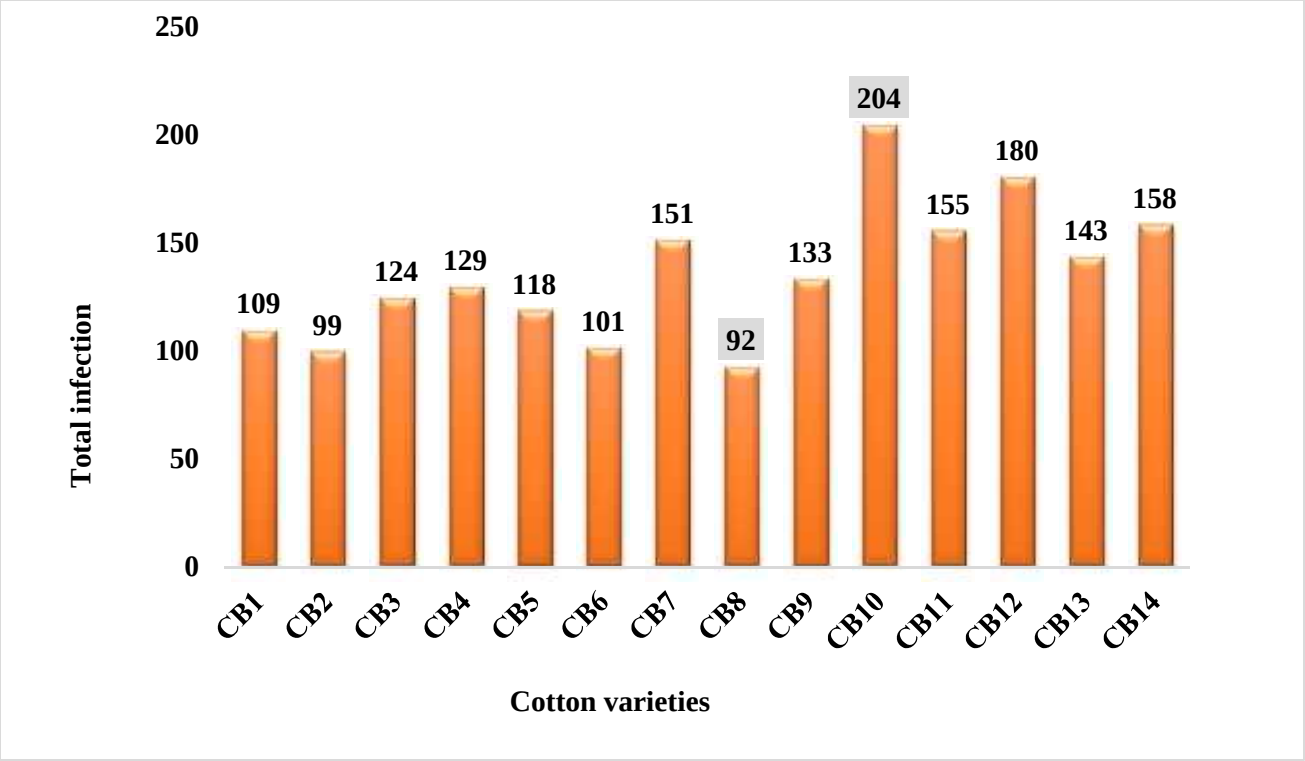

Fig. 8. Average total associations of fungi with different varieties of cotton varieties (CB1-CB14) in Tissue planting method.

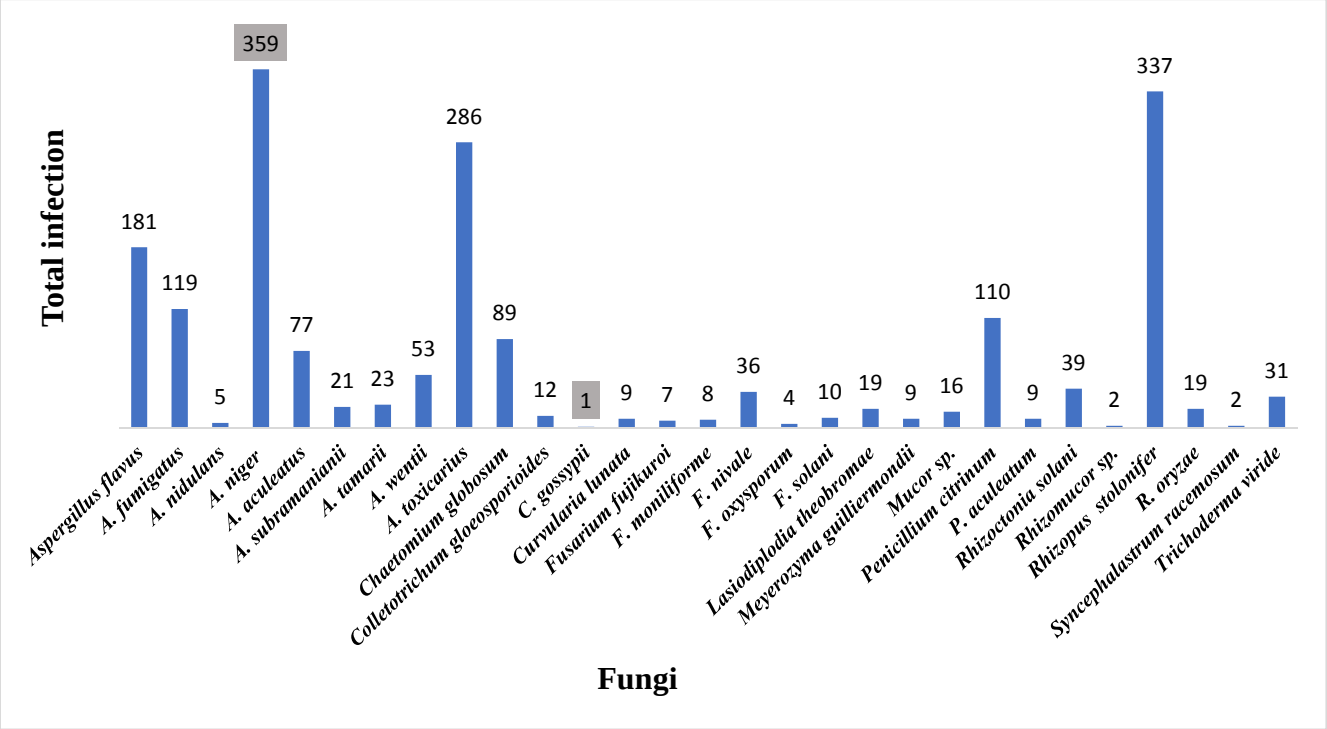

Fig. 9. Average per cent incidence of fungi of three isolations in Tissue planting method.

4.5. Blotter method

In blotter method a total of eight species of fungi and sterile mycelia were isolated (Table 11 and Plate 4). The isolated fungi were *Aspergillus flavus, A. fumigatus, A. niger, A. tamarii, Chaetomium globosum, Rhizopus stolonifer, Mucor* sp. and *Trichoderma viride*. *Chaetomium globosum* was the most predominant fungi which was found in all the cotton varieties examined. The highest frequency of this fungi (94%) was recorded in CB12 and lowest (4%) was recorded in CB5. Here, the frequency percentage of *Chaetomium globosum* was maximum (94%) and *A. fumigatus* was minimum (2%) (Table 11).

This results are in agreement with the results of Nahar *et al.* (2019) who reported that, in Blotter method, a total of 5 species of fungi were isolated from 3 varieties of Hill cotton (*Gossypium arboreum* L.) in Bangladesh. The highest association percentage was recorded for *Chaetomium globosum* in all the varieties of cotton seeds. Similarly, Tomar *et al*. (2012) reported eleven fungal flora *viz*., *Aspergillus niger, A. flavus, Penicillium* sp., *Alteraria alternata, Chaetomium* spp, *Rhizopus niger, Fusarium solani, Macrophomina phaseolina, Myrothecium roridum, Trichothecium roseum* and *Curvularia lunata* from JK 4 cotton cultivar growing in the locations of Madhya Pradesh by Blotter method.

4.6. Paper Towel method

Table 12 and Plate 5 showed the association of fungi with cotton seeds isolated from Paper Towel method. The associated fungi were *Aspergillus flavus, A. fumigatus, A. niger, A. tamarii, Chaetomium globosum* and sterile mycelia. After 7 days, *Chaetomium globosum, A. tamarii* and sterile mycelia were found. *C. globosum* was observed in all the varieties, *A. tamarii* was observed in only 2 varieties and sterile mycelia was found in 4 cotton varieties. After 10 days of incubation, there were found *A. niger*, *A. fumigatus*, *A. tamarii* and *Chaetomium globosum*. Here also *C.*

globosum was observed in all the varieties tested, *A. tamarii* was observed in only 2 varieties, *A. fumgatus* was observed in 2 varieties and *A. niger* was found in only CB14 (20%). After 15 days of incubation, there were found *A. flavus*, *A. niger*, *Chetomium globosum* and *A. tamarii*. *A. flavus* was recorded in only CB4 (10%). The most predominant fungi of this method was *Chaetomium globosum* which was found in all the cotton varieties examined and the highest frequency of this fungus was 100%. The percentage of *Chaetomium globosum* was varied from 20-100% after 7 days of isolation but after 15 days of inoculation, it was turned into 100% in all the varieties.

Table 11. Frequency percentage of fungi associated with different varieties of cotton seeds in blotter method.

Cotton varieties	Percentage of fungi								
	Chaetomium globosum	***Trichoderma viride***	***Aspergillus niger***	***A. fumigatus***	***A. flavus***	***A. tamarii***	***Rhizopus stolonifer***	***Mucor* sp.**	**Sterile mycelia**
CB1	52	-	-	-	-		-	-	-
CB2	30	-	-	-	-	-	-	-	-
CB3	74	-	-	-	-	-	12	-	5
CB4	32	-	-	-	-	-	-	-	-
CB5	4	4	-	12	-	18	-	-	-
CB6	22	10	5	30	-	36	-	-	-
CB7	36	-	10	-	14	8	-	-	14
CB8	14	-	-	-	6	-	-	-	-
CB9	36	-	-	-	-	-	-	-	24
CB10	30	-	-	-	-	-	-	-	20
CB11	91	-	3	2	8	4	-	-	8
CB12	94	-	-	-	-	-	-	-	3
CB13	50	-	10	-	6	-	40	-	-
CB14	84	-	6	-	-	-	-	4	-
Mean	46.36	1.0	2.43	3.15	2.43	4.72	3.71	0.29	5.29

Plate 4. Fungi associated with cotton seeds (CB1-CB14) in Blotter method.

Table 12. Frequency percentage of association of fungi with different varieties of cotton seeds in Paper Towel method.

Cotton varieties	% frequency of fungi at different intervals										
	7th day			10th day				15th day			
	C. globosum	*A. tamarii*	Sterile mycelia	*C. globosum*	*A. tamarii*	*A. fumigatus*	*A. niger*	*C. globosum*	*A. tamarii*	*A. flavus*	*A. niger*
CB1	90	-	-	100	-	-	-	100	10	-	-
CB2	60	-	-	70	-	-	-	100	-	-	-
CB3	100	-	-	100	-	20	-	100	-	-	10
CB4	80	-	-	100	-	-	-	100	-	10	10
CB5	100	-	20	100	-	-	-	100	-	-	10
CB6	100	10	-	100	20	-	-	100	20	-	-
CB7	100	10	40	100	10	-	-	100	10	-	20
CB8	80	-	-	100	-	-	-	100	-	-	-
CB9	100	-	-	100	-	-	-	100	-	-	10
CB10	50	-	20	80	-	-	-	100	20	-	-
CB11	20	-	10	30	-	10	-	100	10	-	-
CB12	80	-	-	100	-	-	-	100	-	-	20
CB13	20	-	-	90	-	-	-	100	-	-	-
CB14	70	-	-	90	-	-	20	100	10	-	-

'-' = No fungal growth

Fig. 10. Fungi associated with cotton seeds (CB1-14) in Paper Towel method.

4.7. Fungi associated with different parts of cotton seeds

For histopathological study, associates seed borne fungi were isolated from three parts of cotton seeds *viz*. seed coat, embryo and endosperm. A total of 14 species of fungi were found to be associated with different parts of cotton seeds (Plate 5 and Table 14). Maximum number of fungi were associated with seed coat in all varieties of cotton. Per cent frequency of fungi associated with different parts of cotton seeds (randomly selected seeds of 14 varieties) are shown in Table 13. Ten species of fungi were isolated from these categories, out of which 9 species were found from seed coat. The total frequency of *Aspegillus niger* was maximum (246) and *Penicillium citrinum* was minimum (4). But frequency of total fungi was higher (218) in endosperm and in embryo it was lower (146). *Aspergillus flavus, A. niger* and *R. stolonifer* were found in all the parts of cotton seeds whereas *C. gloeosporioides, C. lunata, F. oxysporum* and *F. fujikuroi* were found exclusively in seed coat and *Penicillium citrinum* was found only in embryo (Table 13).

Cotton variety (CB10) showed maximum outbreak of fungal frequency. Nine species of fungi were isolated from the aforecited cotton varieties, of which 8 were found in seed coat. The total frequency of *R. stolonifer* was highest (94) and *Pencillium citrinum* was lowest (4). On the other hand, total infection was maximum (119) in seed coat and it was minimum (34) in embryo. In this case, *R. stolonifer* was found in all parts of cotton seeds and rests were found in only seed coat except *F. nivale* and *A. flavus* (Table 14).

Plate 5. Fungi associated with different parts *viz*. seed coat, embryo and endosperm of cotton seeds.
A-C. Seeds randomly selected from 14 cotton varieties, **D-F.** CB10 with highest fungal frequency, **G-I.** CB8 with lowest fungal frequency and **J-L**. CB3 with moderate fungal frequency.

Table 13. Frequency percentage of fungi associated with different parts of cotton seeds (randomly selected seeds of 14 varieties).

Fungi	% frequency of fungi in different parts of cotton seeds				
	Seed coat	Embryo	Endosperm	Mean	Total
Aspergillus flavus	17[d]	26[c]	80[b]	41.0	123
A. fumigatus	4[g]	4[d]	0[e]	2.67	8
A. niger	74[a]	78[a]	94[a]	82.0	246
Colletotrichum gloeosporioides	7[f]	0[e]	0[e]	2.33	7
Curvularia lunata	10[e]	0[e]	0[e]	3.33	10
Fusarium fujikuroi	30[c]	0[e]	0[e]	10.0	30
F. nivale	10[e]	0[e]	10[d]	6.67	20
F. oxysporum	7[f]	0[e]	0[e]	2.33	7
Penicillium citrinum	0[h]	4[d]	0[e]	1.33	4
Rhizopus stolonifer	50[b]	34[b]	34[c]	39.34	118
Mean	20.9	14.6	21.8		
Total	209	146	218		
CV (%)	4.56	4.84	2.85		

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

Cotton variety (CB8) showed minimum prevalence of fungal frequency. Eleven fungal species were associated with this cotton variety of which 10 were isolated from seed coat. The total frequency of *Fusarium nivale* was highest (130) and *Penicillium aculeatum* was lowest (3). In seed coat, the total fungal infection was highest (160) and lowest in embryo (81). Here, *R. stolonifer* was observed in all the parts of cotton seeds whereas, *F. fujikuroi*, *P. aculeatum*, *S. racemosum* and *T. viride* were observed only in seed coat and *P. citrinum* only in embryo (Table 14).

Cotton variety (CB3) showed average fungal infection. Seven species of fungi were isolated from this variety and out of which six were isolated from seed coat. The total frequency of *R. stolonifer* was highest (167) and *F. fujikuroi* was lowest (7). Total fungal infection was maximum in seed

coat (99) and minimum in embryo (104). *Aspergillus flavus* and *R. stolonifer* were observed in all the parts of selected cotton seeds whereas, *A. fumigatus, F. fujikuroi* and *R. solani* were noticed only in seed coat (Table 14).

Table 14. Per cent frequency of fungi associated with different parts of cotton seeds.

Fungi	Per cent frequency of fungi in different parts of cotton seeds											
	CB1-14			CB10			CB8			CB3		
	SC	EM	EN	SC	EM	EN	SC	EM	EN	SC	EM	EN
Aspergillus flavus	17^{d}	26^{c}	80^{b}	7^{e}	0^{c}	10^{c}	10^{c}	17^{b}	0^{d}	7^{d}	27^{d}	10^{c}
A. fumigatus	4^{g}	4^{d}	0^{e}	10^{d}	0^{c}	0^{d}	3e	3^{d}	0^{d}	10^{c}	0^{b}	0^{e}
A. niger	74^{a}	78^{a}	94^{a}	40^{a}	0^{c}	0^{d}	12^{c}	7^{c}	0^{d}	0^{e}	10^{c}	7^{d}
Colletotrichum gloeosporioides	7^{f}	0^{e}	0^{e}	0^{f}	0^{c}	0^{d}	0^{f}	0^{e}	0^{d}	0^{e}	0^{d}	0^{e}
Curvularia lunata	10^{e}	0^{e}	0^{e}	7^{e}	0^{c}	0^{d}	7^{d}	0^{e}	3^{c}	0^{e}	0^{d}	0^{e}
Fusarium fujikuroi	30^{c}	0^{e}	0^{e}	4^{e}	0^{c}	0^{d}	11^{c}	0^{e}	0^{d}	7^{d}	0^{d}	0^{e}
F. nivale	10^{e}	0^{e}	10^{d}	24^{b}	0^{c}	17^{b}	60^{a}	0^{e}	70^{a}	11^{c}	0^{d}	34^{b}
F. oxysporum	7^{f}	0^{e}	0^{e}	0^{f}	0^{c}	0^{d}	0^{f}	0^{e}	0^{d}	0^{e}	0^{d}	0^{e}
Penicillium aculeatum	0^{h}	0^{e}	0^{e}	0^{f}	0^{c}	0^{d}	3^{e}	0^{e}	0^{d}	0^{e}	0^{d}	0^{e}
P. citrinum	0^{h}	4^{d}	0^{e}	0^{f}	4^{b}	0^{d}	0^{f}	4^{d}	0^{d}	0^{e}	0^{d}	0^{e}
Rhizoctonia solani	0^{h}	0^{e}	0^{e}	7^{e}	0^{c}	0^{d}	0^{f}	0^{e}	0^{d}	14^{b}	0^{d}	0^{e}
Rhizopus stolonifer	50^{b}	34^{b}	34^{c}	20^{c}	30^{a}	44^{a}	37^{b}	54^{a}	20^{b}	50^{a}	67^{a}	50^{a}
Syncephalastrum racemosum	0^{h}	0^{e}	0^{e}	0^{f}	0^{c}	0^{d}	7^{d}	0^{e}	0^{d}	0^{e}	0^{d}	0^{e}
Trichoderma viride	0^{h}	0^{e}	0^{e}	0^{f}	0^{c}	0^{d}	11^{c}	0^{e}	0^{d}	0^{e}	0^{d}	0^{e}
Total fungi	209	146	218	119	34	71	160	81	93	99	104	101
CV (%)	5.40	5.73	3.37	8.82	15.56	9.13	7.35	9.84	6.97	9.26	6.23	7.41

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

SC = Seed coat, EM = Embryo, EN = Endosperm, "0"= No fungal growth.

The most prevalent fungus was *R. stolonifer* which was found in all the parts of all cotton varieties whereas, *C. gloeosporioide, F. oxysporum, Penicillium aculeatum*, *S. racemosum* and *T. viride* were found only in seed coat. The total infection was highest (218) in endosperm of randomly selected seeds of 14 different cotton varieties and lowest (34) in embryo of CB 10. Out of these 14 fungi, *Aspergillus flavus, A. fumigatus*, *A. niger*, *C. lunata*, *C. gloeosporioides, F. nivale, F. oxysporum, F. fujikuroi, Penicillium aculeatum*, *R. solani*, *R. stolonifer*, *S. racemosum* and *T. viride* were found to be external seed borne fungi; *Aspergillus flavus, A. fumigatus*, *A. niger*, *C. lunata*, *F. nivale, Penicillium citrinum* and *R. stolonifer* were found to be internal seed borne fungi. *Aspergillus flavus, A. fumigatus*, *A. niger*, *C. lunata*, *F. nivale* and *R. stolonifer* were associated as both external and internal seed borne fungi.

This result is in conformity with Roy and Bourland (1982) and Seneewong *et al.* (1991) who also found that *A. niger* was responsible for both external and internal infection in cotton seeds. *Fusarium lateritium, F. udum, F. vasintectum*, *F. bulbigenum, F. moniliforme, F. oxysporum, F. roseum, F. solani* and *F. truncatum* were also reported by them as externally as well as internally seed borne fungi.

Eisa *et al*. (2007) reported *Rhizoctonia solani* the highest pathogenic fungus isolated from seed coat of Giza-86, Giza-89, Giza-86 and Giza-89 cotton varieties. On the other hand, *Alternaria alternata, Fusarium solani, F. tricinectum, Penicillium* spp and *A. niger* were also isolated from cotton seed coat in the lowest numbers. *Alternaria alternata, Aspergillus niger, Fusarium acuminatum, Fusarium solani, Pythium ultimum, Rhizopus arrhizus* and *Rhizoctonia solani* were isolated by Mansoori and Hamdolahzadeh (1995) from different parts of cotton seeds. The findings of present study are also in accordance with the findings of above mentioned workers.

4.8. Morphological identification of the fungi isolated from cotton seeds

Aspergillus flavus Link. **(Fig. 11A)**

Colony colour on PDA medium is grayish powdery and fast growing. Conidial heads are yellow to green became brownish in edge. Conidiophores are less than 1.0 mm length and 10- 20 µm diameter, vesicle was glubose to subglubose. Conidia are glubose minutely accumulate and measured 2.5-3.5 µm. Mycelia well developed, septate, hyaline and profusely branched. Conidiophores 300-600 µm long. Cells are multinucleate Vesicles 10 - 35 µm in diameter. Sterigmata 8 - 14 × 3 - 5 µm.

Aspergillus fumigatus Fresenius. Beitragezur Mykologie **3**:81 (1863) **(Fig. 11B)** Colonies flat, olivaceous green, 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. Surface of vesicle are covered by closely packed more or less clavate branches. Conidia catenulate, dry, usually globose, echinulate and smooth. Colonies of the fungus produced thousands of minute pale green conidia 2-3 µm.

Aspergillus niger van Tieghem Ann. Sci. Nat. Bot. Ser. 5, **8**:240 (1867) **(Fig. 11C)** Colonies are effuse, black. Mycelium are 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. Vesicle surface covered by closely packed more or less clavate branches. Conidia catenulate, dry, usually globose, echinulate, dark brown in color.

Aspergillus aculeatus **Lizuka**, Annls sci. nat. (Bot.), Ser. 5, **8**:240 (1867) **(Fig. 11D)**

Aspergillus aculeatus is a ubiquitous species that usually isolated from rotting fruits and soil. Colonies effuse, brownish 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 is covered by closely packed, more or less clavate shaped branches. Conidia catenulate, dry, usually globose, echinulate, dark brown in colour.

Aspergillus subramanianii Visagie, Frisvad & Samson: 66 (1877) **(Fig. 11E)**

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 and 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 roughen. Sclerotia are pink to vinaceous-purple coloured, irregular shaped and up to 1 mm diam. It is a species with rough walled stipes, biseriate conidial heads, yellow to ochre conidia and sclerotia that do not turn black.

Aspergillus tamarii Kita G, in Centralb. F. Bakt., **37**(17/21): 433-452. (1913)

(Fig. 11F)

Aspergillus tamarii belongs in *Aspergillus* Section *Flavi*, and resembles *A. flavus* and *A. parasiticus*, but conidia colour is olive to brown and are larger, with thick, conspicuously roughened walls. Colonies on Czapek's solution agar spreading broadly at room temperature with vegetative hyphae mostly submerged, fruiting areas at first colourless, then passing through orange yellow shades to brown in old colonies. Not showing true green but often presenting green that is transient and limited to areas of young heads; reverse uncoloured or occasionally pinkish. On AFPA, it produces a deep brown reverse coloration, in contrast to the orange yellow of *A. flavus* and *A. parasiticus*. Conidial heads more or less columnar but not completely globose and upto 300µ in diameter, with radiating chains and columns of conidia. Conidiophores arising from

submerged hyphae upto 1or 2mm in length , colourless with walls becoming abruptly thinner at the base of the vesicle. Vesicles globose to subglobose, 25 to 50300µ in diameter. Sterigmata, in large heads. Conidia more or less pyriform, through subglobose to globose, commonly ranging from 5.0-6.5µ in diameter, occasionally upto 8µ.

Aspergillus wentii Wehmer **(Fig. 11G)**

Aspergillus wentii is an asexual, filamentous, endosymbiotic fungus. It produces single-celled, globose, conidia in unbranched. Spores are smooth, colourless, and ellipsoidal, approximately 4.5–5 µm in diameter. Conidia are darker yellow to brown in colour when mature and have a single wall. The conidial head is yellow to darker coffee-coloured and 500–800 µm in diameter. The conidiophore are 3-5 millimeters in length, has a glassyor hyaline appearance. It produces aerial hyphae, white or sometimes yellow in colour that can grow to a few millimeters in length. Foot cells have dense walls and are branched.

Aspergillus toxicarius Murakami (1971) **(Fig. 11H)**

Aspergillus toxicarius belongs in *Aspergillus* Section *Flavi* and resembles *A. flavus*, but conidia of *A. toxicarius* are coloured olive to yellow, and are larger, with thick, conspicuously roughened walls. Conidiophores are less than 1.2 mm length and 10- 20 µm diameter, vesicle was glubose to subglubose. Conidia are glubose minutely accumulate. Colonies effuse yellowish green. Mycelia well developed, septate, hyaline and profusely branched. Conidiophores 400-600 µm long. Cells are multinucleate Vesicles 15 - 30 µm in diameter. Sterigmata 10 - 14 × 4 - 5 µm. Conidia greenish, catenulate, globose or pyriform, smooth, 4 - 5 µm in diameter. Colonies spreading broadly, dark cress green.

Aspergillus nidulans Eidam, (1883) **(Fig. 11I)**

Colonies spreading broadly, dark cress green. Perithecia developing from the center of the colony, reverse of colony in varying shades of purplish red during the growing period, becoming very dark in age. Perithecia developed separately within or upon the conidial layer, globose ranging from 100 to 175µ in diameter with outer layer a yellowish to cinnamon colored envelop of scattered hyphae bearing hulle cells upto 25 µm in diameter. Head short, columnar, ranging from 40 to 80µ by 25 to 40µ; conidiophores commonly sinuous, with smooth walls. Wall composed of one layer of cells, dark reddish purple in color. Ascospores purple red in color, lenticular, smooth walled with two equal crests.

Colletotrichum gloeosporioides (Penz.) Sacc., Fung. Agrum. **2**:6 (1882)

Vermicularia gloeosporioides penz., *Michelia* **2:**450 (1880)

Teleomorohosis: *Glomerella cingulata* (Stonem) Spauld & Schrenk, *Science* Ser. 2, **17**:751 (1903).

(Fig. 11J)

Colony is white but at maturity become grayish with lighter center. Sclerotia present but setae absent. Conidia comperatively large, straight, obtuse at the apex, 14.8-24.4 × 3.6-5.2 µm in size. Appressoria abundant, Pale to medium brown, circular or slightly irregular in size. In all the criteria that have been used for differentiating taxa than in *Colletotrichum*, it is clear that *Colletotrichum gloeosporioides* showed extensively wide variation.

Colletotrichum gossypii Sowthworth **(Fig. 11K)**

Colonies grayish, cottony with pinkish orange acervuli. Hyphae brown, partly superficial, septate. Acervuli are 100 to 153 cm in diameter. Setae olive or dark brown in colour, straight, curved, flexuous, or rarely branched. Spores are occasionally produced upon the ends of the setae.

Conidiophores are pale brown, 12 - 39 × 3.4 - 4.5 cm. Conidia solitary, straight, hyaline, 10.5-16.8 cm × 4.2 - 5.2 cm. The conidia have only one nucleus.

Curvularia lunata (Wakker) Boedijn. [*Cochliobolus linatus* Nelson & Haasis]. Ellis MB, Mycol. Pap. **106**: 2-43, 1966**.** **(Fig. 11L)**

Colonies are effuse, brown, grey or black, hairy, cottony or velvety. Stromata rarely formed in culture, colonies on PDA markedly zonate. Conidiophores are solitary, mostly unbranched, straight or slightly undulating, mostly flexuous geniculate, mid brown, septate up to 250 µm. Conidia are mostly 3-septate, dark brown, mostly curved, third cell from the base is broader and darker than others, broader cells are mid brown and other cells paler, smooth, 22.5 - 31.2 × 9.3 - 12.6 µm.

Chaetomium globosum Kunze ex Fr., Systema Mycologicum **3**:255 (1829) **(Fig. 11M)**

Colonies golden brown, punctiform, greyish, and numerous on substrate. Hyphae are brown septate, profusely branched, mycelium dark. Perithecia dark brown colour with long hairy wavy appendages. Perithecia dark brown to black and clothed, especially in the upper part, by dark brown setae. Their setae may be simple or branched, straight, wavy or spirally coiled, smooth or ornamented in various ways. Asci soon disappearing and the lemon-shaped, brown ascosporous freed from their asci through the ostiole, 5.2-6×2.8-4 µm.

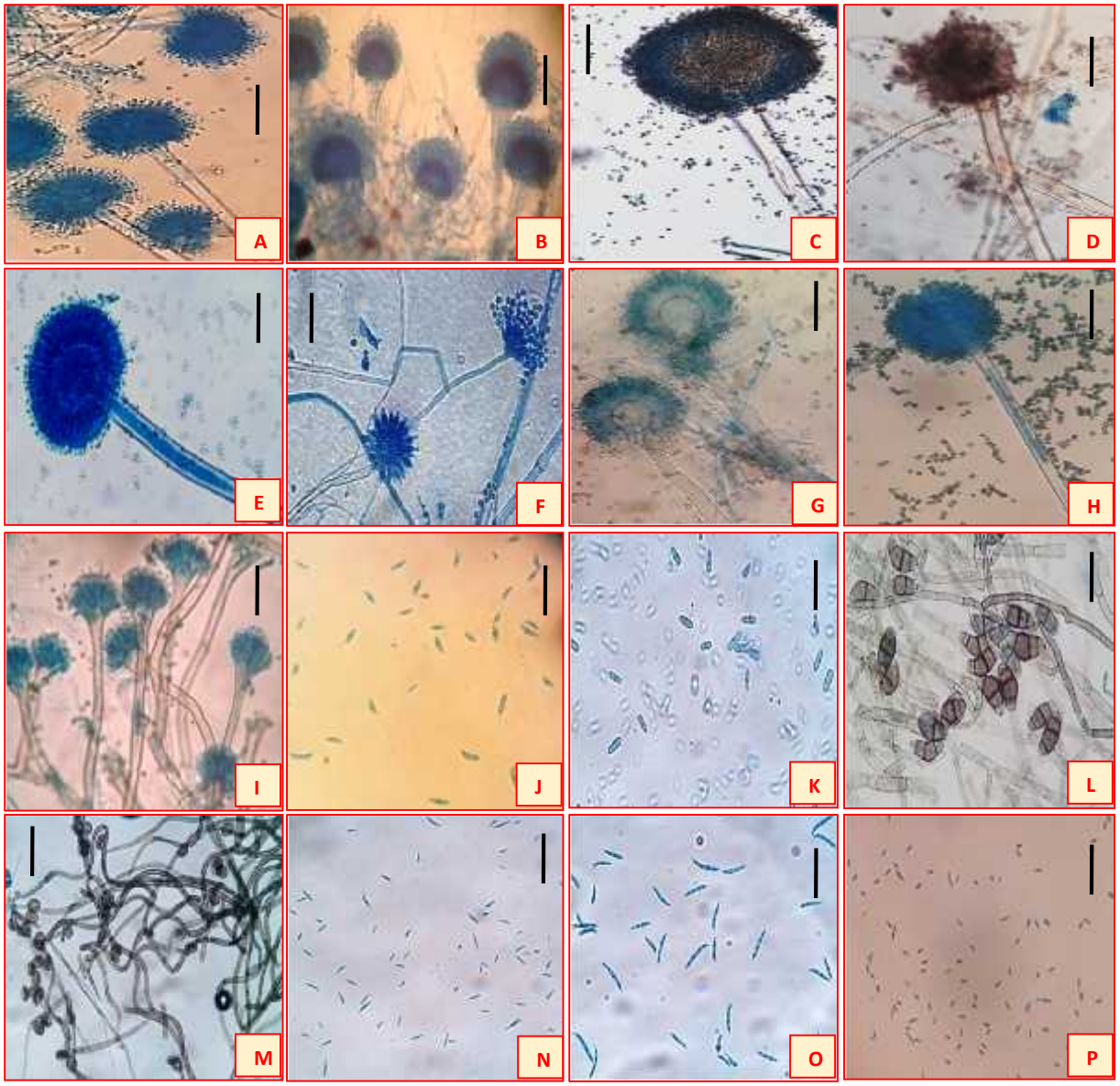

Fig. 11. Conidiophore and conidia of different fungi associated with cotton seeds. A. *Aspergillus flavus*, **B.** *A. fumigatus*, **C.** *A. niger*, **D.** *A. aculeatus*, **E.** *A. subramanianii*, **F.** *A. tamarii*, **G.** *A. wentii*, **H.** *A. toxicarius*, **I.** *A. nidulans* **J.** *Colletotrichum gloeosporioides*, **K.** *C. gossypii*, **L.** *Curvularia lunata*, **M.** *Chaetomium globosum*, **N.** *Fusarium oxysporum*, **O.** *F. nivale* and **P.** *F. moniliforme* (Bar = 50 µm).

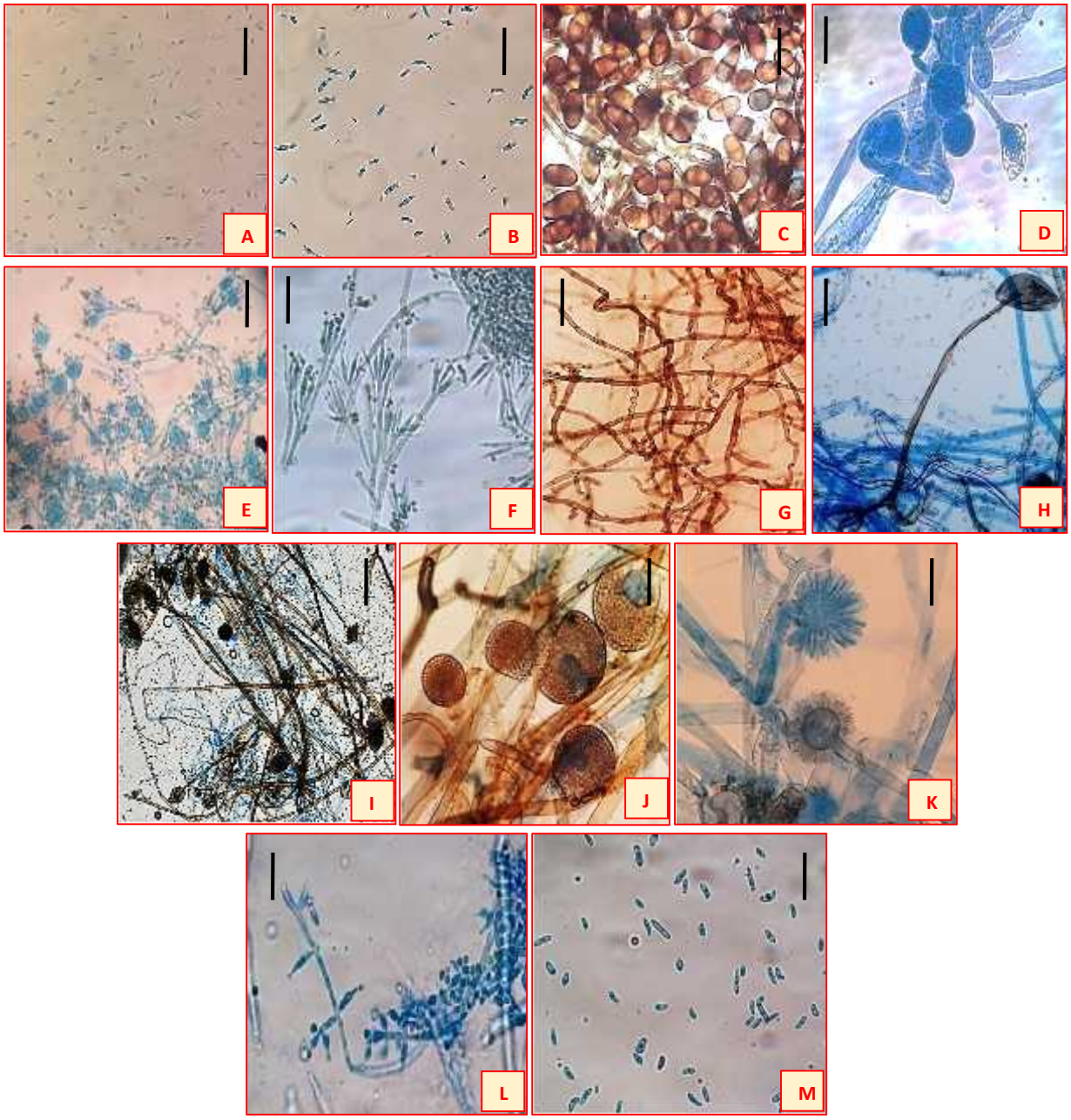

Fig. 12. Conidiophore and conidia of different fungi associated with cotton seeds. A. *Fusarium fujikuroi*, **B.** *F. solani* and **C.** *Lasiodiplodia theobromae*, **D.** *Mucor* sp., **E.** *Penicillium citrinum*, **F.** *P. aculeatum*, **G.** *Rhizoctonia solani*, **H.** *Rhizopus oryzae*, **I.** *R. stolonifer*, **J.** *Rhizomucor* sp., **K.** *Syncephalastrum racemosum*, **L.** *Trichoderma viride* and **M.** *Meyerozyma guilliermondii* (Bar = 50 µm).

Fusarium oxysporum Schlecht, Flora berol. **2**: 139, (1824) **(Fig. 11N)**

Mycelium are delicate, white in color in the 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 in size and macroconidia are 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. The most commonly found spores are 3 septate.

Fusarium nivale (Fr.) Ces., Rabenh. Herb. Myc., Ed. 1, No. 1439.

Lanosa nivalis Fr. pr. p., Summa Veg. Scand.:495, (1849). **(Fig. 11O)**

Colonies are white to pale petch to apricot with little discoloration of agar. Mycelium sparse to densely floccose or felted, individual hyphae irregular, 1.5 µm diam. Conidia are hyaline, variable, principally of two kinds, curved, broadly falcate with a pointed apex and flattened, wedge shape base, 3-septate, 10-30 ×2.5-5 µm. Depending on the position of the widest point of the spore, the shape changes. It may be central, above or below the center. Conidia 1septate, 10-20 × 2.5-5 µm. macroconidia several-celled, slightly curved or bent at the pointed ends, typically canoe-shaped, microconidia 1-celled, ovoid or oblong, borne singly or in chains, some conidia intermediate, 2 or 3 celled, oblong or slightly curved, parasitic or saprophytic. Genarally, chlamydospore are not observed.

Fusarium moniliforme Sheldon 1904. Rep. Neb. Agric. Exp. Stn **17**:23-32 **(Fig. 11P)**

They are extensive and cottony, white, often with some tinge of pink mycelium. Reverse pinkish yellow. Mycelia are hyaline, profusely branched, septate. Conidiophores are hyaline, 0-2 septate. Phialides hyaline, 16 - 20 × 3 - 4 µm in diameter and conidia are hyaline, variable, principally of two kinds. Microconidia and macroconidia. Microconidia hyaline, 1-celled, ovoid or oblong, borne

singly or in chains, 5 - 15 × 2 - 3 µm. Macroconidia hyaline, several-celled, slightly curved or bent at the pointed ends, 3 - 5 septate, conidia 25 - 35 × 3 - 4 µm, 5 - 6 septate conidia 30 - 50 × 3 - 5 µm.

Fusarium fujikuroi *Gibberella fujikuroi* (Sawada) Wollenw. (1931) **(Fig. 12A)**

Colonies are white, floccus to slightly felt. Conidia are hyaline, fusiform, ovate or clavate; one or two celled, measured 26.7-73.6 × 8.1-17.1 µm. Mycelium sparse to densely floccose or felted. Conidiophores hyaline, usually 0-2 septate.

Fusarium solani (Mart.) Sacc., Michelia **2**:296, 1881, emend. Snyder & Hansen pro. parte, Am. J. Bot. **26**:740, 41. **(Fig. 12B)**

Cottony whitish mycelium was found at the coller parts of the plant. Growth rate is 3.2 cm, colony greyish-white and aerial mycelium striate, sparse to dense and floccose. Microconidia develops abundantly in the fresh isolates after 2-3 days. They are also broader and more oval in shape with somewhat thicker walls; they are 8-16×2-4µm. Macroconidia develop after four to seven days from initially simple but later from short multibranched conidiophores which soon merge to form effuse sporodochia. They are inequilaterally fusoid with many of the spores having the widest diameter in the penultimate cell. The apical cell is pointed and some what beaked. They are 35-55×4.5-6 µm and may become 1-2 septate.

Lasiodiplodia theobromae (Pat.) Griff. & Maubl, Bull. Trimest. Soc. Mycol. Fr. **8**:136 (1892) **(Fig. 12C)**

Colonies are greyish brown, cottony, reverse brownish black. Hyphae septate, branched, dark chocolate brown. Pycnidia globose, dark brown, ostiolate. Conidiophore short, hyaline. Conidia dark brown, two-celled, ellipsoidal, 16−22 × 8−12 µm. Pycnidia formed with septate paraphyses

between the conidiogenous cells. The conidia measured 20-21.8 × 9.1-10.9 µm. They are initially hyaline, thin-walled and aseptate, cylindrical to subovoid in shape.

***Mucor* sp.** Fresen **(Fig. 12D)**

Colonies are typically white to beige or grey and are fast-growing. Older colonies become grey to brown in color due to the development of spores. *Mucor* spores or sporangiospores are simple or branched and form apical, globular sporangia that are supported and elevated by a column-shaped columella. *Mucor* can be differentiated from moulds of the genera *Absidia*, *Rhizomucor* and *Rhizopus* by the shape and insertion of the columella and the lack of rhizoids. Some *Mucor* species produce chlamydospores. They produce mold with irregular, non-septate hyphae branching at wide angles. The tip of the sporangiophore swells to form a globose sporangium that contains uninucleate, haploid sporangiospores. An extension of the sporangiophore called the columella. The sporangium walls are easily ruptured to release the spores. They may germinate to form hyphae or a sporangium.

Penicillium citrinum Thom **(Fig. 12E)**

Penicillium citrinum produces septate, hyaline hyphae. Colonies are usually fast growing, in shades of green, sometimes white, mostly consisting of a dense felt of conidiophores. Microscopically, chains of single-celled conidia are produced in basipetal succession from a specialised conidiogenous cell called a phialide.

Penicillium aculeatum Raper & Fennell (1948). **(Fig. 12F)**

It is characterized by very restricted and comparatively deep colonies on Czapek agar, variously buckeled and wrinkled, irregular in outline, medium sporing, often with a limited overgrowth of red- pigmented hyphae, growing margins 2-3 mm wide, white to slightly pink, odor almost lacking, reverse in vinaceous or purplish red in older areas. Conidiophores short, commonly about 50µ,

rarely upto 100µ, with walls appearing somewhat granular. Penicilli are relatively shorter and broader than in the preceding species, usually appearing definitely inflated, sterigmata 7-9µ by 3-3.5µ and conidia are strictly globose to subglobose, 3-3.5µ in diameter.

Rhizoctonia solani J.G. Khun (1858). **(Fig. 12G)**

It is an asexual fruit bodies and spores lacking sclerotia, brown or black in colour, variable in form, frequently small and loosely formed. Formed among and connected by mycelia threads; hyphae of mycelium are brown, with long cells, septa or branch set off from main hyphae; parasitic, chiefly on roots and other underground parts of plants. Its hyphal cells are multinucleated. It produces white to deep brown mycelium when grown on an artificial medium. The hyphae are 4–15 µm wide and tend to branch at right angles. *R. solani* is subdivided into anastomosis groups based on hyphal fusion between compatible strains.

Rhizopus oryzae Went & Prins. Geerl., (1895) **(Fig. 12H)**

Rhizopus oryzae is a filamentous heterothallic microfungus . It differs from *R. oligosporus* and *R. microsporus* by its larger columellae and sporangiospores. It has variable sporangiosphores such as straight or curved, swollen or branched, and the walls can be smooth or slightly rough. sporangiosphores are pale brown to brown in colour. The sporangia are globose or subglobose, wall spinous and black when mature, 60-180 µm in diameter. The columellae are globose, subglobose or oval in shape. The wall is generally smooth and pale brown in colour. It has abundant, root-shaped rhizoids. The stolons are smooth or slightly rough, almost colorless or pale brown, 5-18 µm in diameter. The chlamydospores are abundant, globose ranging from 10-24 µm in diameter, elliptical and cylindrical. Initially colonies are white becoming brownish with age and can grow to about 1 cm thick.

Rhizopus stolonifer (Ehrenb.) Vuill., Revue Mycologique Toulouse **24**:54 (1902) (Ehrenb.:Fr.) Vuillemin. Toney Bot.Clup. **69**:592-616. (1902) **(Fig. 12I)**

Colony on PDA was initially white or grayish, fluffy. Reverse light gray, cottony, mycelium coenocytic, hyaline, aseptate, well developed, branched and fluffy. Rhizoids branched and fluffy, sporangiophores arised in clusters. Mycelium produces many aerial stolons that develop rhizoids at certain points. Mycelium produces long conidiophores, many aerial stolons also develop rhizoids at certain points. Directly above the rhizoids one or more sporangiospores are produced. Sporangium produces non motile, brownish sporangiospores, 4-6 µm in diameter. The central portion of sporangium is called the columella, becomes highly vacuolated and it eventually surrounded by a wall that separates it's from the peripheral zone. Spores are round to ovule, hyaline or grayish brown, one celled smooth, 3.8 to 6.4 µm in diameter.

***Rhizomucor* sp.** Lucet & Costantin, (1900). **(Fig. 12J)**

Colonies of *Rhizomucor* sp. grow very rapidly, fill the Petri dish and mature in 4 days. The texture is typically cotton-candy like. The colony colour is white initially and turns grey to yellowish brown in time from the front but the reverse is white to pale. The microscopic morphology of *Rhizomucor* appears to be intermediate between that of *Rhizopus* and *Mucor*. Nonseptate or sparsely septate broad hyphae, rudimentary rhizoids, sporangiophores, sporangia, and sporangiospores are visualized. Sporangiophores are usually irregularly branched. Sporangia are brown in color, 40-80 µm in diameter and round in shape. Apophysis is absent. Columellae are prominent and spherical to pyriform in shape. Sporangiospores are small, unicellular, 3-4 µm in diameter and round to ellipsoidal in shape.

Syncephalastrum racemosum Cohn ex J. Schrot. Kryptogamen-Flora von Schlesien 3-1 (2): 217 (1886) **(Fig. 12K)**

Colonies transparent, fluffy, grow very rapidly and fill the Petri plate on PDA medium in 48 hours. Mycelium usually grow rapidly, abundantly branched. Sporangiophores are frequently branched and rather short. They end up in a vesicle (85 µm in diameter). Around this vesicle are the merosporangia (4 – 6 × 9-60 µm), which are filled with linear series (chains) of sporangiospores. 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. Reverse is pale or yellowish-brown in colour. Merosporangiospores are one-celled and spherical to cylindrical in shape.

Trichoderma viride Pers. (1794) **(Fig. 12L)**

T. viride is a mold which produces spores asexually, by mitosis. It is the anamorph of *Hypocrea rufa*, its teleomorph, which is the sexual reproductive stage of the fungus and produces a typical fungal fruiting body. Colony effuse, light green in colour. Conidiophores hyaline, much branched that cluster into fascicles, bearing phialides single or in groups. Broad and straight/flexuous branches. They may have conidial pigments that are either white or bright green in colour. Conidia are usually hyaline, powdery mass, 1-celled, ovoid shaped and borne in small terminal clusters.

Meyerozyma guilliermondii (Wick.) Kurtzman & M. Suzuki **(Fig. 12M)**

Meyerozyma guilliermondii (formerly known as *Pichia guilliermondii*) is a species of yeast of the genus *Meyerozyma* whose asexual or anamorphic form is known as *Candida guilliermondii.*

Colonies are flat, moist, smooth and cream to yellow in colour on Sabouraud dextrose agar. It does not grow on the surface when inoculated into Sabouraud broth. Pseudohyphae are short and few in number. Cell reproduces by budding, ellipsoidal, ovoidal and clavate, occur singly and in pairs, or short chains, pseudohyphae is formed. Colony flat, moist, smooth, cream to yellow in colour.

4.9. Molecular Identification of fungi associated with different varieties of cotton seeds

Based on morphological characteristics, 29 fungal isolates were identified provisionally. In the present investigation, some fungal species were unable to identify upto species level based on the morphological features only. Hence, molecular characterization of the fungal species was performed for proper identification using ITS sequence analysis according to Amer *et al.* (2011) with some modifications. For further confirmation of these 29 fungi, ITS sequence based molecular analysis was performed and 19 were confirmed up to species level through ITS sequence based molecular analysis (Fig. 14). All fungal isolates were identified using the sequences obtained through ITS1 and ITS4 primers (Table 15).

Genomic DNA was successfully isolated from the nineteen isolates. PCR was conducted using ITS1 (Forward) and ITS4 (Reverse) primers and ~600 bp DNA band was amplified (Fig 13). Sequence analysis of the amplified DNA through BLAST search in GenBank was conducted and found 85.51-99.81% similarity with partial sequence of 18S ribosomal RNA gene, complete sequence of internal transcribed spacer 1, internal transcribed spacer 2, 5.8S ribosomal RNA gene and partial sequence 28S ribosomal RNA gene of different isolates (Table 15).

Analysis of the nucleotide sequences of the amplified fragments allowed the identification of the isolates at the species level (Table 15, Fig. 13). ITS1 and ITS4 primers depicted isolate species identities more than 90% sequence similarity except the isolate number 7 which showed 85.51%

sequence similarity. All the fungal isolates were identified using the sequences obtained through ITS1 and ITS4 primers. To confirm at the genomic sequence level, PCR amplified bands (600 bp) from ten samples were subjected to automated sequencing followed by BLAST analysis (Fig. 13).

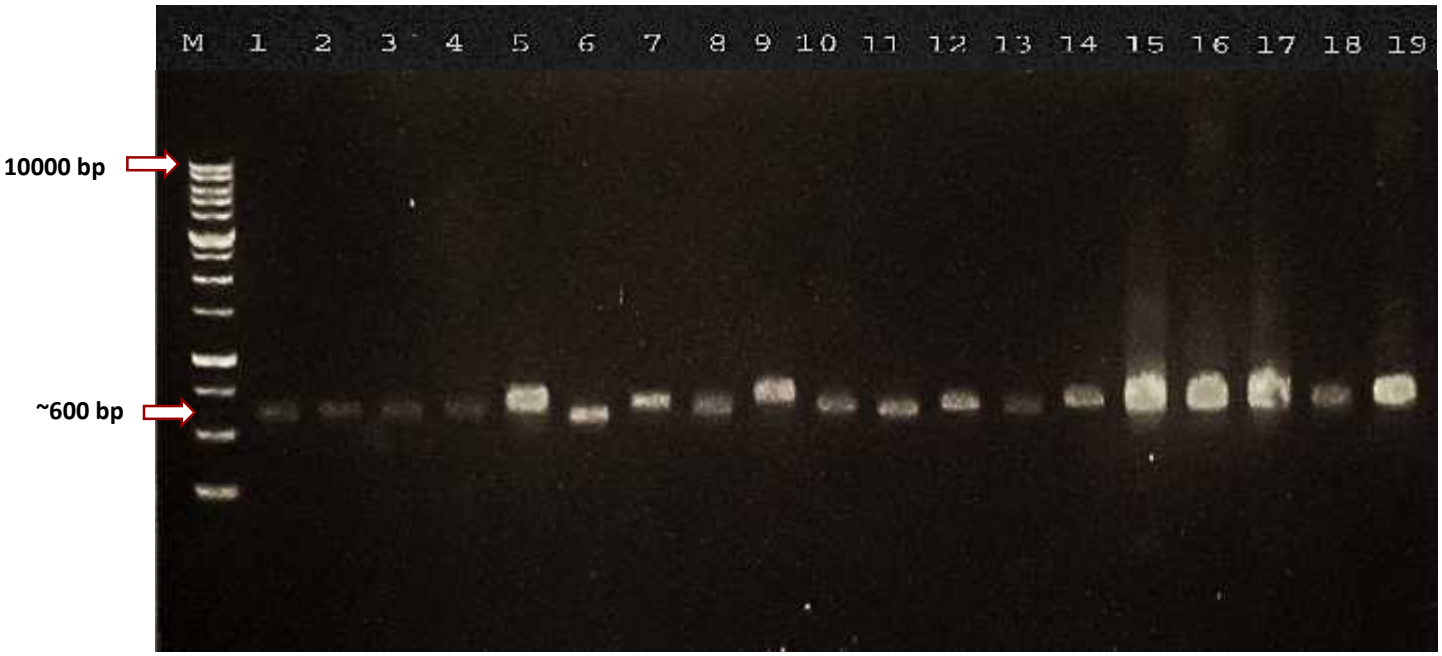

Fig. 13. Gel electrophoresis of the PCR products of 19 fungal isolates performed by ITS1 (F) and ITS4 (R) primers and showing ~600 bp amplification.

ITS sequences of nineteen samples were analyzed through NCBI-BLAST program database search system. Results obtained from the BLAST database showed that 99.81% nucleotide identities with *Trichoderma viride* strain TVJ-S-1; 99.74% nucleotide identities with *Fusarium moniliforme* isolate CJBB12-18; 99.38% nucleotide identities with *Curvularia lunata* strain AME-83; 99.09% nucleotide identities with *Aspergillus aculeatus* isolate KUASN10; 98.93% nucleotide identities with *Aspergillus fumigatus* isolate HF11 and *Aspergillus wentii* strain CBS 131.49; 98.47% nucleotide identities with *Lasiodiplodia theobromae* strain E42F; 98.31% nucleotide identities with *Rhizopus oryzae* isolate EV62; 98.25% nucleotide identities with *Meyerozyma guilliermondii*

strain Q2; 98.15% nucleotide identities with *Fusarium solani* strain GuangX9 and *Fusarium oxysporum* isolate FLS 4; 97.74% nucleotide identities with *Aspergillus flavus* isolate En14; 97.24% nucleotide identities with *Penicillium aculeatum* strain LP65; 96.70% nucleotide identities with *Aspergillus tamarii* isolate MH3; 96.51% nucleotide identities with *Aspergillus toxicarius* strain CBS 129270; 94.97% nucleotide identities with *Penicillium citrinum* isolate 14R-2-F05; 92.60% nucleotide identities with *Fusarium fujikuroi* isolate EFS3; 92.86% nucleotide identities with *Mucor* sp. isolate 580816 and 85.51% nucleotide identities with *Aspergillus subramanianii* strain DTO245E4 (Table 15).

From the comparison between morphological and molecular identification, it was clear that out of 19 fungal isolates morphological identification of one fungal isolate did not match with molecular identification. It was *Aspergillus ochraceous* which was identified as *Aspergillus subramanianii.* by molecular identification (Table 16). Besides, there were four species of *Aspergillus*, two species of *Fusarium* and two species of *Penicillium* which were difficult to identify up to species label by morphological identifications. The species name of these fungi were easily identified by this molecular technique. Furthermore, one unidentified fungi could be detected easily by the analysis of the nucleotide sequences (Table 16).

Among the isolated fungi, *Aspergillus subramanianii, A. toxicarius, A. wentii, Penicillium aculeatum, P. citrinum,* and *Meyerozyma guilliermondii* are the new records for Bangladesh (Siddiqui *et al*. 2007, Shamsi S 2017, Nahar *et al.* 2019). Hence, these fungi are reported here first time from Bangladesh.

The present investigation suggests that molecular technique is more accurate and rapid means of fungal identification. ITS-based molecular identification methods may be an important

complement to conventional mycological detection by culture, which is becoming increasingly essential in mycology as well as plant pathology.

Aspergillus flavus = AACCCAAAACCGAGGGTAGGGGTTCCTAGCGAGCCCAACCTCCCACCAGAAAAAGGCTGGAAGCTT

A. fumigatus = GCTTAAAACCTCTAATTGAATGACCTAGCCGTTTCCAACCTCCCACGGGTGTAAAAAAAAAGGTTGTT

A. subramanianii = GAACATAAATGAAGGAGGGTCCTCGGGGCCCAACCTCCCACCCGAGAAAAGCAGACCTTGTTGCTTC

A. Aculeatus = AGGGGTTGCCCGAAGGGCGTGAGGTCCTTCGTTGCCCAACCTCCCACCCGTGCAAAAAAGAACCCTG

A. tamarii = TACGTTTCATCCTCCGGGTATGGTGCCTCGTTAGCCCAACCTCCCACCAGAAAGAGGGAGGGAACCTT

A. wentii = GATAAAAGGGGGGGGGGCAGTAAGGCGGGAGGACTGTTCCCGCCGAACCTCCACCGGACGAGGCATT

A. toxicarius = GACCACTACGGGTTGGGGCCGGCCGCGCGTGCGCTGCCCGCCGCGAGGTAACTCGAAAGGAGGGGG

Curvularia lunata = GGAAAGACATAATATGAAGGCTTCGGCTGGATTATTTATTTCACCCTTGTCTTTTGCGCACTTGTTGTT

Fusarium fujikuroi = AAATTTACCGAAGTTCTAGTTGCCAGCGCTTAACTGCGCGGCGAAAAAGAAAAAAGCAGAGTGTCTTT

F. oxysporum = GGATCGGCGGGGGAATTCCTACCTGCATCGAGGTCAACATTCAGAAGCGGGGGGGGTGTTACGGCGT

F. solani = GAGGGGCCATTAACCCGAAGGTTATACAACTCATCAACCCTGTGAACATACCTATAAAGGAAAGACAG

F. moniliforme = GGTCGGGCGTGCAGCTCCAACCCCTGTGACATACCAATTGTTGCCTCGGCGGATCAGCCCGCTCCCG

Lasiodiplodia theobromae = AAGCATTTCCGAAGTGGCTAGGGCTCCGGTTCGACTCTCCCACCCTTTGAGAAAAAGAACTCTGTTGC

Mucor sp = AAGGATCATTAAATAATTTGATAATTAAACAATTATCTAATTTACTGTGAACTGTTTTAATTATGACAC

Penicillium citrinum = GACATAACCGGAAGTGCGGGCCCTCGCGGCCCAACCTCCCACCCGGGAGGGACCGAAACTATGTTGC

P. aculeatum = CGGGGGGGGGGAAAGGAAATACGGAGGGGCGGGCCCCTCCCGGCCCAACCTCCCGCCCTTAAAACGG

Rhizopus oryzae = CTTCTCCTTGTGGGTATAATATGTAAAGCGCCTTTTATCAGGGTTTCCTGGGGTAAGAGAAGGCTTCTA

Trichoderma viride = AACCAACAGGGATTGCCCCAGTAACGGCGAGTGAAGCGGCAACAGCTCAAATTTGAAATCTGGCCCC

Meyerozyma guilliermondii = CAAAATTAAGTAATTCATTTGCCAGCGCTTAACTGCGCGGCGAAAAAGAAAAAAAGGACAGTGTCT

Fig. 14. DNA sequences of the PCR products of isolated fungi.

Table 15. Identification of fungal isolates using ITS sequence comparison with data from GenBank through BLAST search.

Isolates No.	Length (Base pair)	ITS sequence of test fungi matches with						
		Accession No.	Description	Max score	Total score	Query coverage (%)	E-value	Identity
1	573	MN186997	*Aspergillus aculeatus* isolate KUASN10	985	985	98%	0.0	99.09%
2	595	MN180857	*Aspergillus flavus* isolate	981	981	97%	0.0	97.74%
3	611	MH865314	*Aspergillus toxicarius* strain CBS 129270	942	942	98%	0.0	96.51%
4	589	MH856464	*Aspergillus wentii* strain CBS 131.49	1002	1002	98%	0.0	98.93%
5	583	GU183175	*Aspergillus fumigatus* isolate HF11	1002	1002	98%	0.0	98.93%
6	603	MH562046	*Aspergillus tamarii* isolate MH3	952	952	97%	0.0	96.70%
7	608	FR733823	*Aspergillus subramanianii* strain DTO245E4	1013	1013	95%	0.0	99.11%
8	620	MG571435	*Curvularia lunata* strain AME-83	867	867	79%	0.0	99.38%
9	510	KY425745	*Lasiodiplodia theobromae* strain E42F	917	917	99%	0.0	98.47%
10	621	MK108436	*Rhizopus oryzae* isolate EV62	1033	1033	95%	0.0	98.31%
11	545	KX958025	*Penicillium citrinum* isolate 14R-2-F05	873	873	98%	0.0	94.97%
12	590	HQ392496	*Penicillium aculeatum* strain LP65	454	454	86%	0.0	97.24%
13	568	KY785016	*Fusarium solani* strain GuangX9	942	942	98%	0.0	98.15%
14	585	MK371768	*Mucor* sp. isolate 580816	845	845	100%	0.0	92.86%
15	560	KF439055	*Trichoderma viride* strain TVJ-S-1	942	942	100%	0.0	99.81%
16	598	KU671029	*Fusarium oxysporum* isolate FLS 4	942	942	98%	0.0	98.15%
17	586	MH084746	*Fusarium fujikuroi* isolate EFS3	911	911	96%	0.0	92.60%
18	576	KC895528	*Gibberella moniliforme* isolate CJBB12-18	993	993	96%	0.0	99.74
19	596	MG601177	*Meyerozyma guilliermondii* strain Q2	996	996	96%	0.0	98.25%

Table 16. Comparison between morphological and molecular identification of 19 fungal isolates.

lsolates No.	Morphological identification	Molecular identification
1.	*Aspergillus flavus*	*A. flavus* isolate En14
2.	*A. fumigatus*	*A. fumigatus* isolate HF11
3.	*A. ochraceous*	*Aspergillus subramanianii* strain DTO245E4
4.	*Aspergillus* sp. 1	*Aspergillus aculeatus* isolate KUASN10
5.	*Aspergillus* sp. 2	*Aspergillus tamarii* isolate MH3
6.	*Aspergillus* sp. 3	*Aspergillus wentii* strain CBS 131.49
7.	*Aspergillus* sp. 4	*Aspergillus toxicarius* strain CBS 129270
8.	*Curvularia lunata*	*Curvularia lunata* strain AME-83
9.	*Fusarium fujikuroi*	*Fusarium fujikuroi* isolate EFS3
10.	*Fusarium oxysporum*	*Fusarium oxysporum* isolate FLS 4
11.	*Fusarium* sp. 1	*Fusarium solani* strain GuangX9
12.	*Fusarium* sp. 2	*Fusarium moniliforme i*solate CJBB12-18
13.	*Lasiodiplodia theobromae*	*Lasiodiplodia theobromae* strain E42F
14.	*Mucor* sp.	*Mucor* sp. isolate 580816
15.	*Penicillium* sp. 1	*Penicillium citrinum* isolate 14R-2-F05
16.	*Penicillium* sp. 2	*Penicillium aculeatum* strain LP65
17.	*Rhizopus oryzae*	*Rhizopus oryzae* isolate EV62
18.	*Trichoderma viride*	*Trichoderma viride* strain TVJ-S-1
19.	Unidentified fungus	*Meyerozyma guilliermondii* strain Q2

4.10. Interrelationship between the quality factor through correlation and regression analysis

Some interrelationships between the quality factors included in Table17 through correlation and regression analysis has been estimated in this study, which is very much important in controlling seed quality. Significant relationships has been estimated in all the cases (Figs. 15-16).

Fig. 15A shows the relationship between seedling mortality and purity percentage and here found negative correlation between two variables. It is obvious that the regression line gives a downward slopping curve, which means that the increase of one variable decrease of other variable i.e. when purity percentage of seeds increases then percentage of seedling mortality decreases and vice versa. The correlation co-efficient value was -0.584 between seedling mortality and physical purity of cotton seeds (Table 18).

Positive correlation between two variables was observed between frequency of fungi and seedling mortality (Fig. 15B). Here regression line gives an upward slopping curve, which revealed that both the variable change in the same direction i.e. increase or decrease of one variable increase or decrease the other variable. When the percentage of fungi increases, the mortality of seedling also increases and vice versa. The correlation co-efficient value between percentage of fungi and seedling mortality was +0.055 (Table 18).

Positive correlation was also found between purity percentage and germination rate of the seeds (Fig. 15C). Here regression line gives an upward slopping curve, which indicates that both the variable changes in the same direction. The correlation co-efficient value between physical purity and germination rate was +0.394 (Table 18).

Fig. 15D shows the relationship between germination rate and frequency percentage of fungi. It shows negative correlation between two variables. Here the regression line gives a downward slopping curve that means germination of seeds increases when the percentage of fungi increases or the germination of seed decreases when the percentage of fungi decreases. In the present study, the correlation co-efficient value between percentage of fungi and percentage of germination was -0.273 (Table 18).

The relationship between fungal frequency and purity percentage showed positive correlation between two variables (Fig. 16A). Here regression line gives an upward slopping curve, which indicates that increase or decrease of one variable increase or decrease of the other one. The correlation co-efficient value between physical purity of seeds and percentage of fungi was +0.340 (Table 18). This value was similar with the value (+0.05) reported by Anonymous (1983).

Positive correlation was found between seed moisture percentage and germination rate (Fig. 16B). Here an upward slopping curve was given by the regression line, which indicates that both the variable changes in the same direction. The correlation co-efficient value between germination rate and seed moisture was +0.070 (Table 18). The relationship between seed moisture and percentage of fungus frequency shows positive correlation between two variables (Fig. 16C). The correlation co-efficient value between seed moisture and percentage of fungi was +0.230 (Table 18).

In the present study, the average physical purity of cotton seeds was recorded 98.69% (Table 4). The result matches with the annual report of CDB (Cotton Development Board, 2017). The average germination and moisture of seeds in this investigation was 85.36% and 10.78% respectively (Table 4). The results also agrees with the standard of CDB recommendation (Anonymous 2017).

In storage, the saprophytic fungi secrete toxic metabolites which damages the quality of seed (Christensen 1972). Among the saprophytes *Aspergillus flavus, A. fumigatus, A. niger* and *Curvularia lunata* were reported to affect germination (Fazli and Ahmed 1959).

Table 17. Quality analysis of different varieties of cotton seeds (CB1-14).

Cotton varieties	Seed purity (%)	Seed germination (%)	Seedling mortality (%)	Associated fungi (%)	Seed moisture (%)
CB 1	99.55^{abc}	82^{efg}	25.39^{def}	63^{d}	10.9^{b}
CB 2	98.78^{cde}	83de	27.71^{cdef}	53^{f}	10.6^{cd}
CB 3	99.0^{cde}	85^{cd}	35.29^{bc}	52^{f}	11.2^{a}
CB 4	98.45^{abcde}	80^{g}	31.25^{cde}	72^{b}	10.5^{d}
CB 5	99.91^{ab}	81^{fg}	33.33^{cd}	63^{d}	10.0^{e}
CB 6	97.47^{de}	87^{c}	43.93^{ab}	34^{i}	10.7^{bc}
CB 7	98.77^{abcd}	81^{fg}	16.05^{g}	43^{h}	10.7^{bc}
CB 8	98.41^{abcde}	92^{ab}	23.91^{efg}	24^{j}	10.4^{d}
CB 9	98.39^{abcde}	81^{fg}	45.89^{a}	68^{c}	10.8^{bc}
CB10	99.80^{abc}	93^{a}	26.88^{fg}	78^{a}	10.6^{bcd}
CB11	97.91^{cde}	92^{ab}	50.30^{a}	52^{f}	10.7^{b}
CB12	97.08^{e}	84^{ef}	21.43^{fg}	58^{e}	11.3^{a}
CB13	99.92^{a}	83^{ef}	22.98^{efg}	48^{g}	11.2^{a}
CB14	98.19^{bcde}	91^{b}	47.88^{a}	51^{f}	11.2^{a}
CV (%)	0.5320	1.17	9.70	1.84	0.9297

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

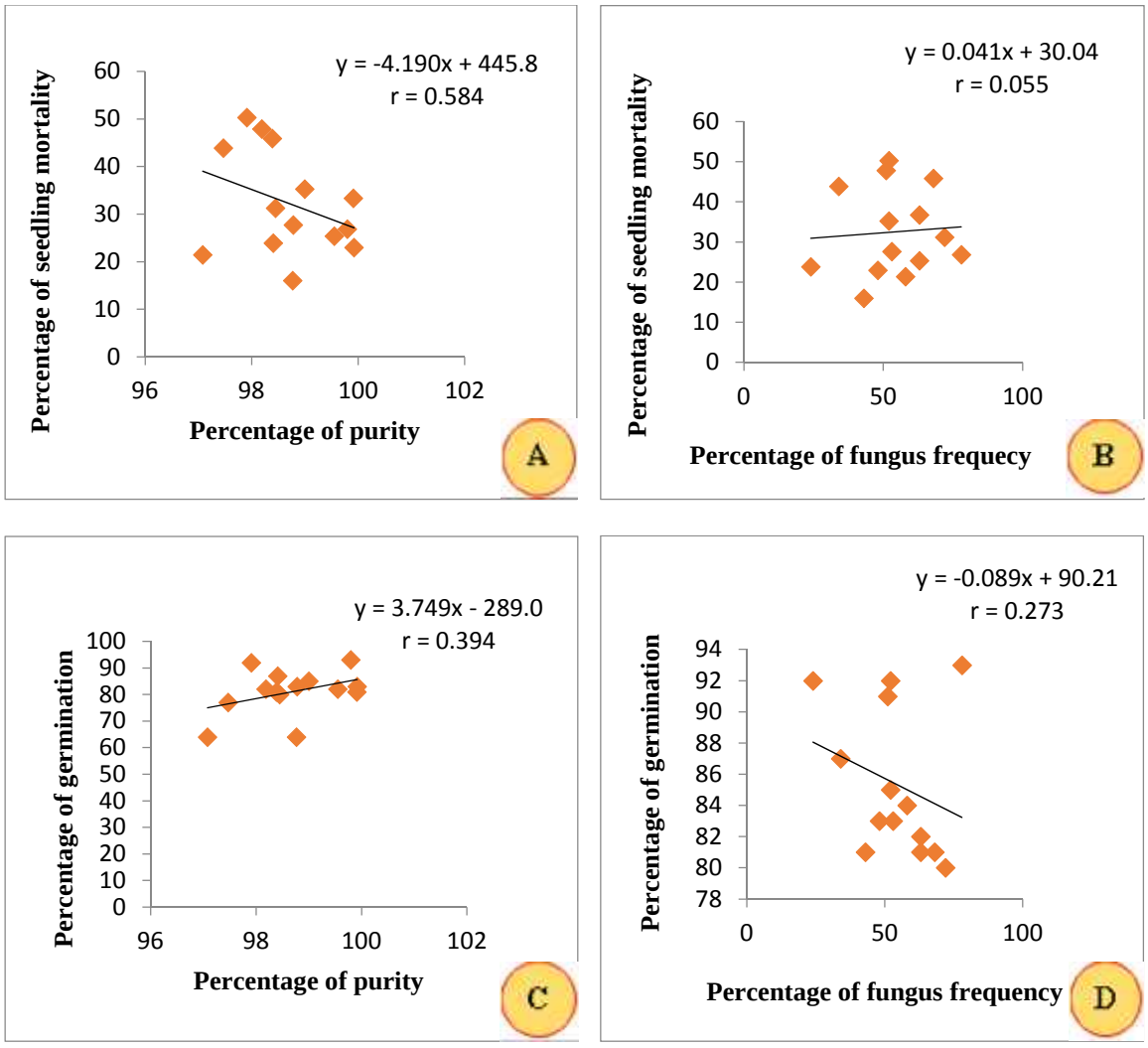

Fig. 15. Correlation co-efficient and regression equation between A. percentage of seedling mortality and percentage of purity, **B.** percentage of seedling mortality and percentage of fungus frequency, **C.** percentage of germination and percentage of purity and **D.** Percentage of germination and percentage of fungus frequency.

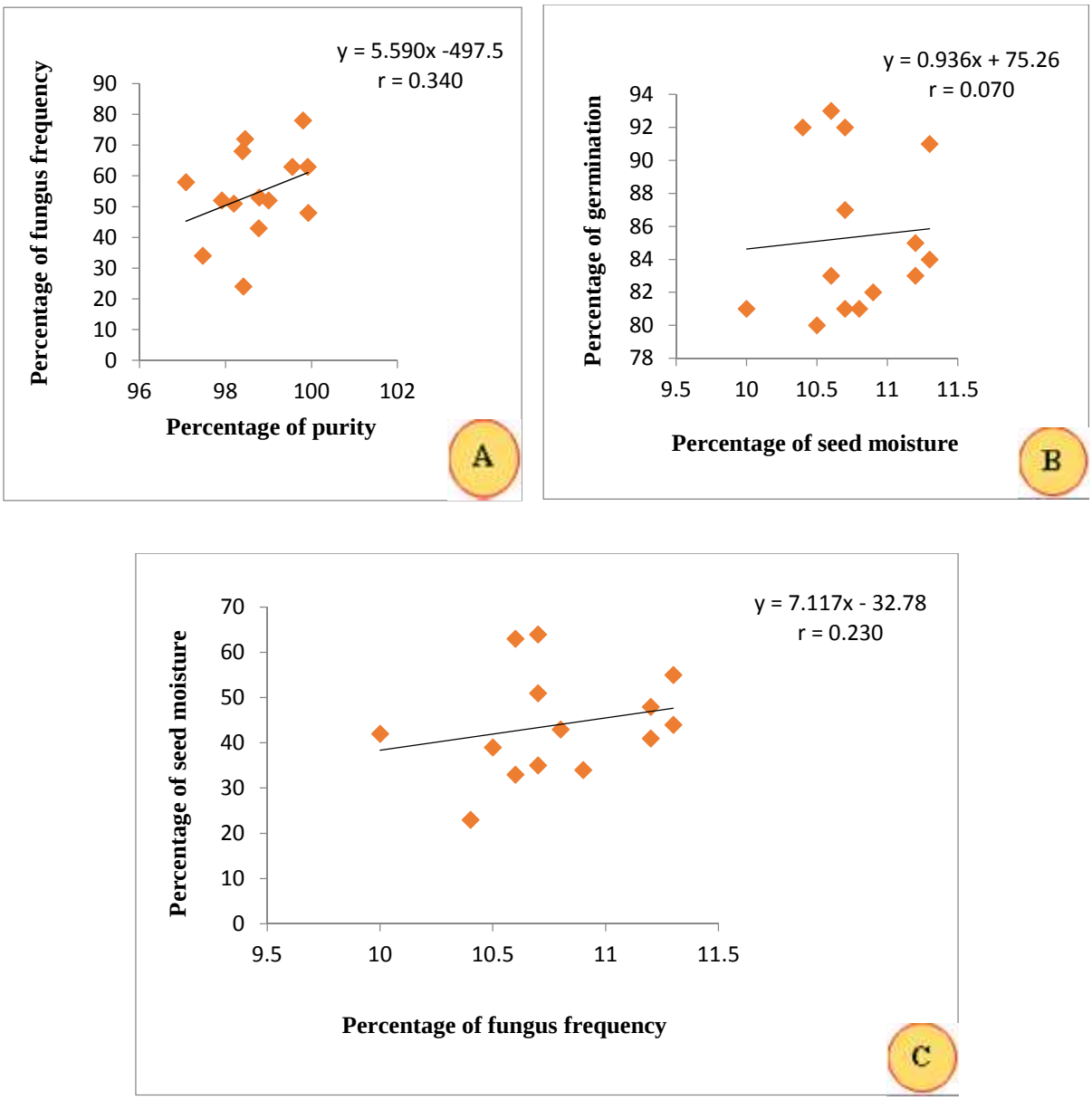

Fig. 16. Correlation co-efficient and regression equation between A. percentage of frequency and percentage of purity, **B.** percentage of germination and percentage of seed moisture and **C.** percentage of seed moisture and percentage of fungus frequency.

Fig. 15D showed that increase of one variable (i.e. germination or frequency of fungi) decrease of the other variable (i.e. germination or frequency of fungi). This result was unlike with the result reported by Khandakar (1987).

Positive correlation was exhibited by Figs 15B, 15C and 16A which indicate that both the variable changes in the same direction. These results are in accordance with the findings of Khatun and Shamsi (2016) and Mamun *et al*. (2016).

Figs 15A and 15D showed negative correlation which means that increase of one variable decrease the other. This result was also resembling with the result of Khatun and Shamsi (2016) and Mamun *et al*. (2016). Fig. 15B showed positive correlation which means that both the variable changes in the same direction. This result was dissimilar with the result observed by Droworth and Christensen (1968).

Table 18. Correlation co-efficient (r) between quality factors of cotton seeds.

Quality factors	Correlation co-efficient (r)
Seedling mortality (%) and physical purity (%)	-0.584
Seedling mortality (%) and fungus frequency (%)	+0.055
Germination rate (%) and physical purity (%)	+0.394
Germination rate (%) and fungus frequency (%)	-0.273
Fungus frequency (%) and physical purity (%)	+0.340
Germination rate (%) and seed moisture (%)	+0.070
Seed moisture (%) and fungus frequency (%)	+0.230

Positive correlation was showed by Fig. 16C which indicates that both the variable changes in the same direction i.e. the fungus frequency become high when the moisture content of seeds become

high. This result was in accordance with the result of Delhom and Rodgers (2016), Droworth and Christensen (1968) and Harman *et al*. (1972).

4.11. Pathogenicity test of the isolated fungi associated with cotton seeds.

A total of twenty nine species of fungi were isolated and identified from the seeds of 14 varieties (CB 1- CB 14) of cotton. All the isolated fungi were selected for pathogenicity test.

4.11.1. Pathogenic potentiality of the fungi

Pathogenicity of the isolated fungi was done following seed inoculation technique described by Chowdhury *et al*. (2015). Seeds of cotton were inoculated by all the isolated fungi. Healthy seeds did not show any fungal growth, but inoculated seeds showed fungal growth on seedlings.

During pathogenicity test, out of 29 isolated fungi, nine showed positive results. The pathogenic fungi were *Aspergillus flavus*, *A. niger*, *A. tamarii*, *Colletotrichum gloeosporioides, Curvularia lunata, Fusarium nivale, F. moniliforme, Mucor* sp. and *Rhizoctonia solani* (Plates 6, 7). The fungi were re-isolated from the artificially inoculated seeds which suggested that they were pathogenic for cotton seeds (Plate 7). Rest of the fungi did not grow in re-isolation plate i.e. they were not pathogenic for cotton seeds.

4.11.2. Effects of pathogenic fungi on cotton seeds

The effect of test fungi on the seeds of cotton are presented in Table 19. Present experiment revealed that, the germination percentage of control seeds was 88 in all the varieties but in the inoculated seeds, owing to the presence of pathogenic fungi, the germination rate showed a considerable reduction (Fig. 17) and it was varied from 20-82%. Among the nine pathogenic fungi, *Rhizoctonia solani* showed highest reduction in seed germination (20%). *Aspergillus flavus*,

Colletotrichum gloeosporioides and *Fusarium moniliforme* also involved for near about 45% reduction of seed germination (Table 19, Fig. 19).

Mortality percentage of control seeds was low (6%) but in inoculated seeds it was high (Fig. 18) and ranged from 7-23%. The highest mortality percentage was 23.0% observed in *Mucor* sp. inoculated seeds and the lowest mortality percentage (7.0%) was observed in *A. flavus* inoculated seeds. All the fungal isolates also contributed in reduction of seedling root and shoot length. Root-shoot ratio of control seeds was high but low in inoculated seeds. The average shoot length of healthy seeds was 10.8 cm whereas the highest shoot length (10.3 cm) was recorded in *Fusarium nivale* inoculated seeds and lowest (4.8 cm) in *Aspergillus flavus* inoculated seeds. In healthy seeds, the average root length was 6.8 cm whereas the highest root length (5.3 cm) was recorded in *Fusarium nivale* inoculated seeds and lowest (2.7 cm) in *Aspergillus flavus* inoculated seeds (Table 19, Fig. 19).

Plants emerged from remaining germinated seeds of these experiment were also studied for vigor index. The vigor index of control plant was 1548.8 but more less in inoculated plants. The maximum vigor index was noticed for *Aspergillus tamarii* (1213.6) and minimum for *Rhizoctonia solani* (202.0) (Table 19, Fig. 20). All the Fungal isolates showed direct influence on reduction of plant height, vigor and root length. Among the isolated pathogenic fungi, *Curvularia lunata* and *Rhizoctonia solani* were aggressive and showed a greater impact in reduction of cotton seed germination and reduced vigor index.

Similar observation was also noticed in the study of Naznin and Shamsi (2018) where *Aspergillus flavus, A. niger* (Type-I), *Curvularia lunata, Fusarium moniliforme* var. *subglutinans, F. sporotrichioides* and *Rhizoctonia solani* were found to be pathogenic for 3 varieties of Hill cotton (*Gossypium arboreum* L.) in Bangladesh.

Table 19. Effect of pathogenic fungi on seed germination, seedling mortality, root-shoot length and vigor index in different varieties of cotton seeds.

Treatments	Seed Germination (%)	Seedling Mortality (%)	Root- shoot length (cm)		Vigor index
			Root	Shoot	
Control seeds	88[a]	6[f]	6.8[a]	10.8[a]	1548.8[a]
Inoculated seeds					
Aspergillus flavus	40[g]	7[ef]	2.7[g]	4.8[i]	300.0[h]
A. niger	73[c]	20[b]	4.1[d]	8.0[f]	883.3[e]
A. tamarii	82[b]	11[d]	5.2[b]	9.5[d]	1213.6[b]
Colletotrichum gloeosporioides	40[g]	9[de]	4.2[d]	8.1[f]	488.0[g]
Curvularia lunata	21[h]	20[b]	3.7[e]	8.7[e]	260.4[i]
Fusarium moniliforme	47[f]	14[c]	3.6[e]	7.4[g]	517.0[f]
F. nivale	69[d]	22[ab]	5.3[b]	10.3[b]	1076.4[c]
Mucor sp.	60[e]	23[a]	4.8[c]	10.0[c]	888.0[d]
Rhizoctonia solani	20[h]	20[b]	3.0[f]	7.1[h]	202.0[j]
CV%	1.85	6.58	2.30	1.18	0.09

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

Palmateer *et al.* (2004) observed that *Fusarium moniliforme, F. semitectum* and *F. solani* were the most pathogenic fungi which causes mortality of cotton plants. They also reported that In Alabama, *Fusarium oxysporum, F. solani* and *F. equiseti* were the most common fungi at the seedling stage of the upland cotton.

In Egypt, *Rhizoctonia solani* is the most important pathogen involved in cotton seedling disease reported by Asran-Amal *et al*. (2005). Pre or post emergence cotton seedling damping-off caused by *R. solani* can be quite serious in the United States and often results in a substantial stand loss (Brown and Carter 1976). These observations were in accordance with the findings of the present study.

F*usarium moniliforme, F. semitectum, F. oxysporum, F. solani, F. equiseti* and *F. compactum* from cotton seedlings and bolls were isolated by Wang *et al.* (1992) and they observed that *Fusarium moniliforme* was the predominant pathogen causing seedling and boll red rot of cotton. *Aspergillus* sp. and *Fusarium* sp. reduced the seed germination by causing seed rot was reported by Kumar *et al*. (1984).

From the above result it can be terminated that all the pathogenic fungi associated with cotton seeds are potential phytopathogens which can lead to severe crop damage and they also showed remarkable effect on the seed germination, seedling mortality, vigor and root-shoot length. This results will be helpful for the cotton producer for designing proper control measure of seed borne fungi and production of healthy seeds of cotton.

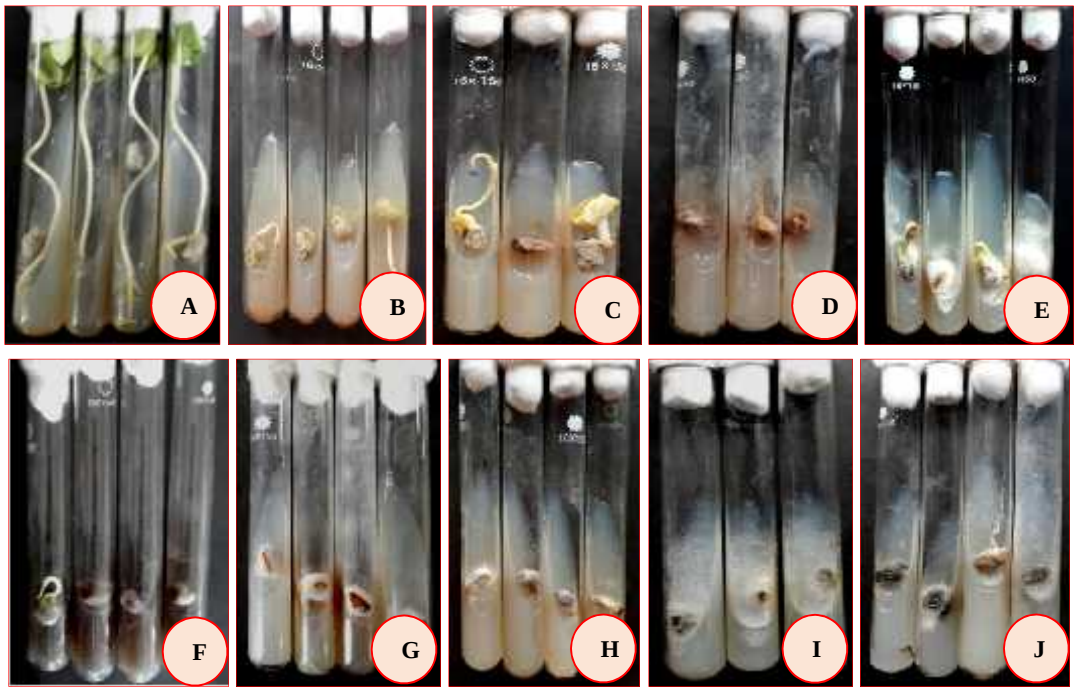

Fig. 17. Pathogenecity test of the isolated fungi. A. Control**, B-J.** Inoculated seeds**. B**. *A. flavus*, **C**. *A. niger*, D. *A. tamarii*, **E**. *C. gloeosporioides*, **F**. *C. lunata*, **G**. *F. nivale*, **H**. *F. moniliforme*, **I.** *Mucor* sp., and **J.** *R. solani*.

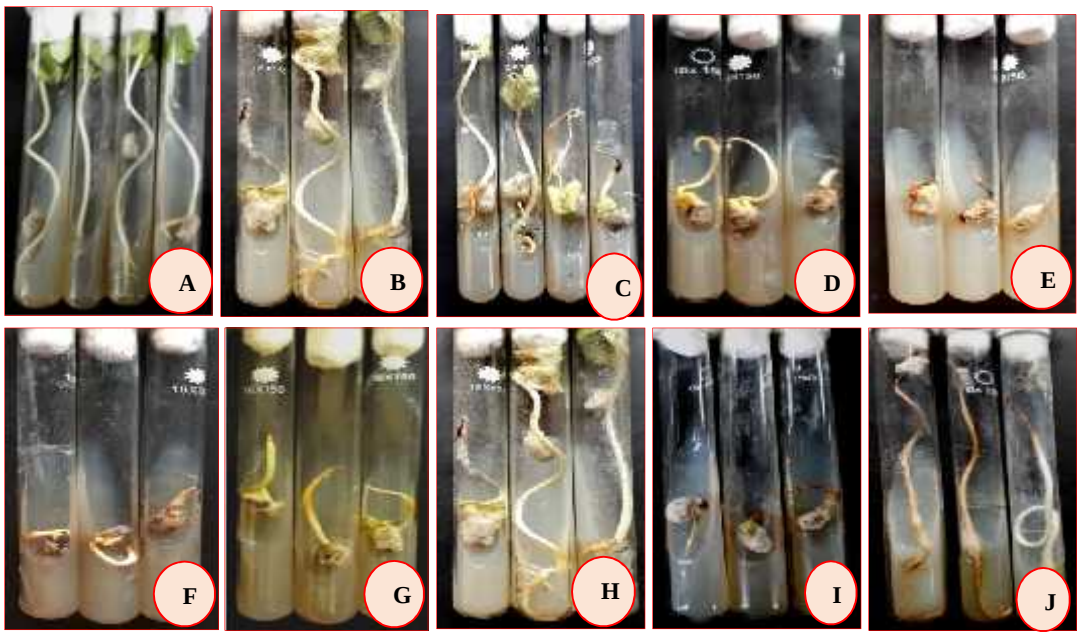

Fig. 18. Mortality of the cotton seedlings due to the presence of pathogenic fungi. A. Control, **B-J.** Inoculated seedlings**. B**. *A. flavus*, **C**. *A. niger*, D. *A. tamarii*, **E**. *C gloeosporioides*, **F**. *C. lunata*, **G.** *F. nivale*, **H**. *F. moniliforme*, **I**. *Mucor* sp., and **J**. *R. solani*.

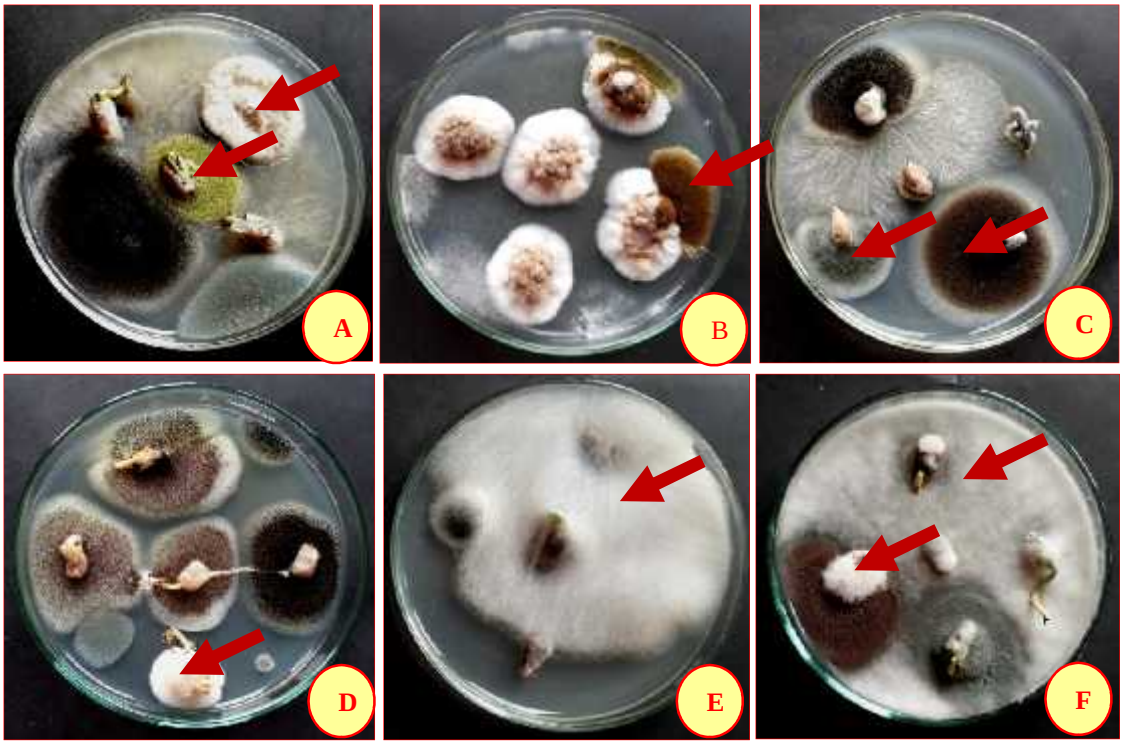

Plate 6. Petri plates showing the growth of pathogenic fungi from the inoculated cotton seeds. A. *A. flavus* and *F. nivale*, **B.** *A. tamarii*, **C.** *A. niger* and *C. lunata*, **D.** *F. moniliforme*, **E.** *Mucor* sp. and **F.** *R. solani* and *C. gloeosporioides*.

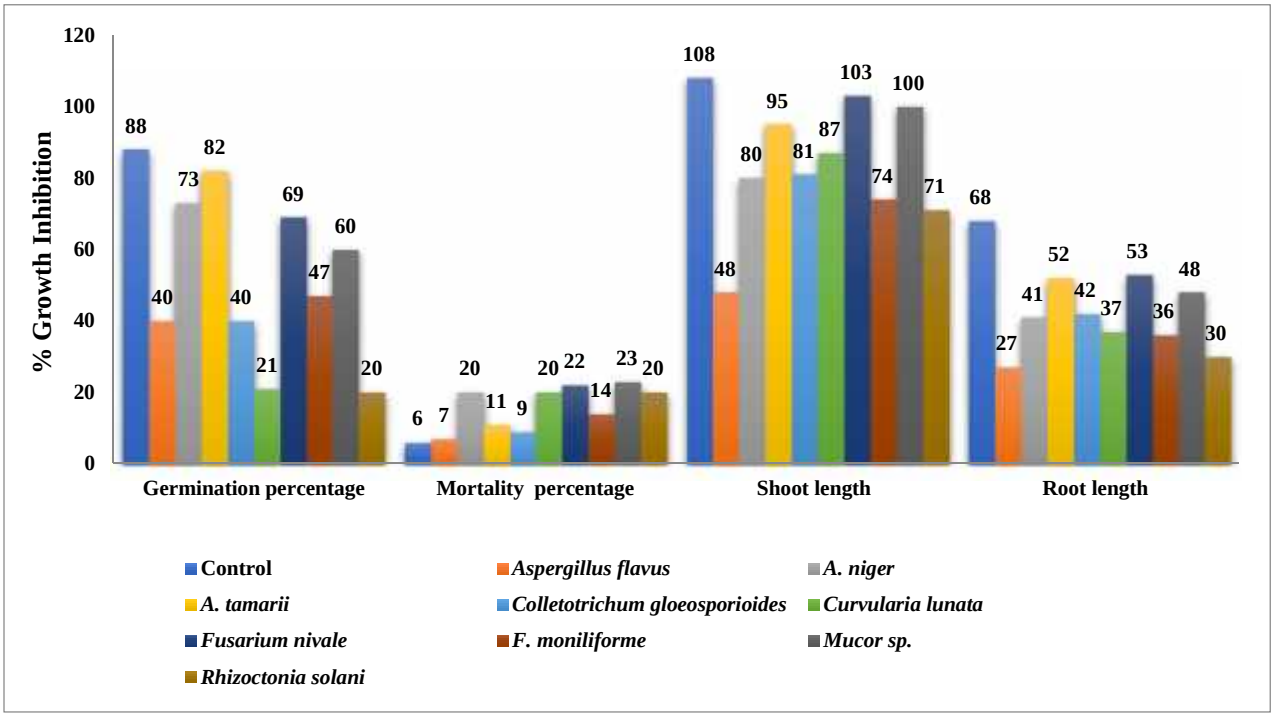

Fig. 19. Effects of pathogenic fungi on different parameters of cotton seeds.

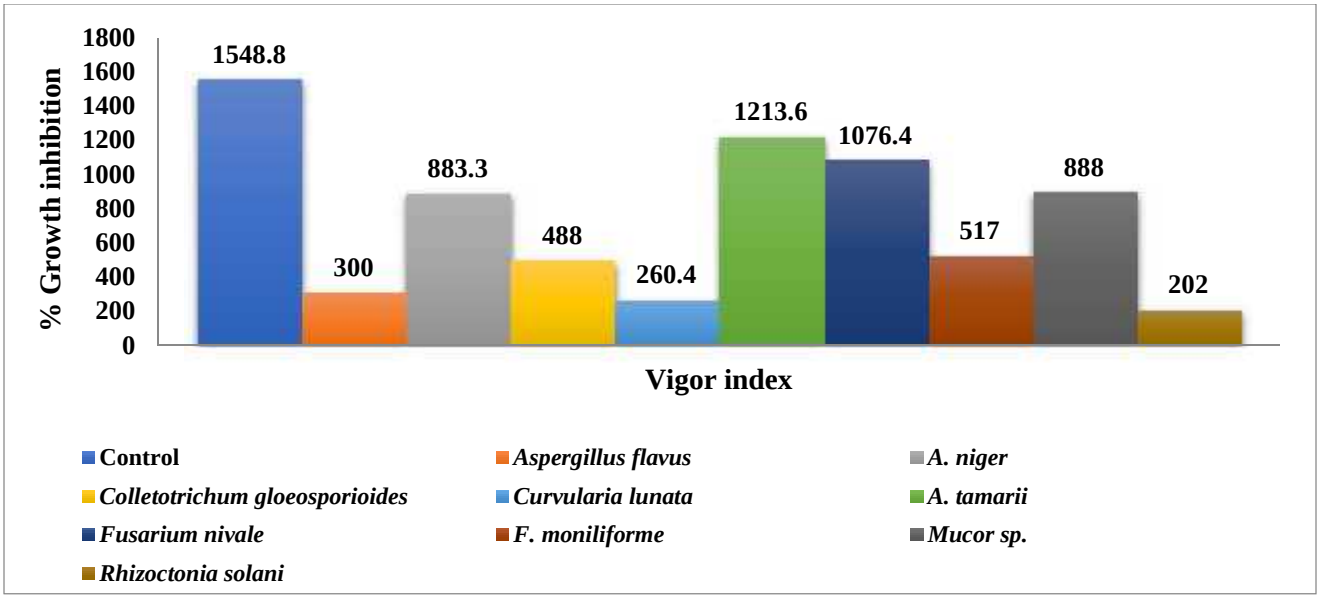

Fig. 20. Effects of pathogenic fungi on vigor index of cotton seeds.

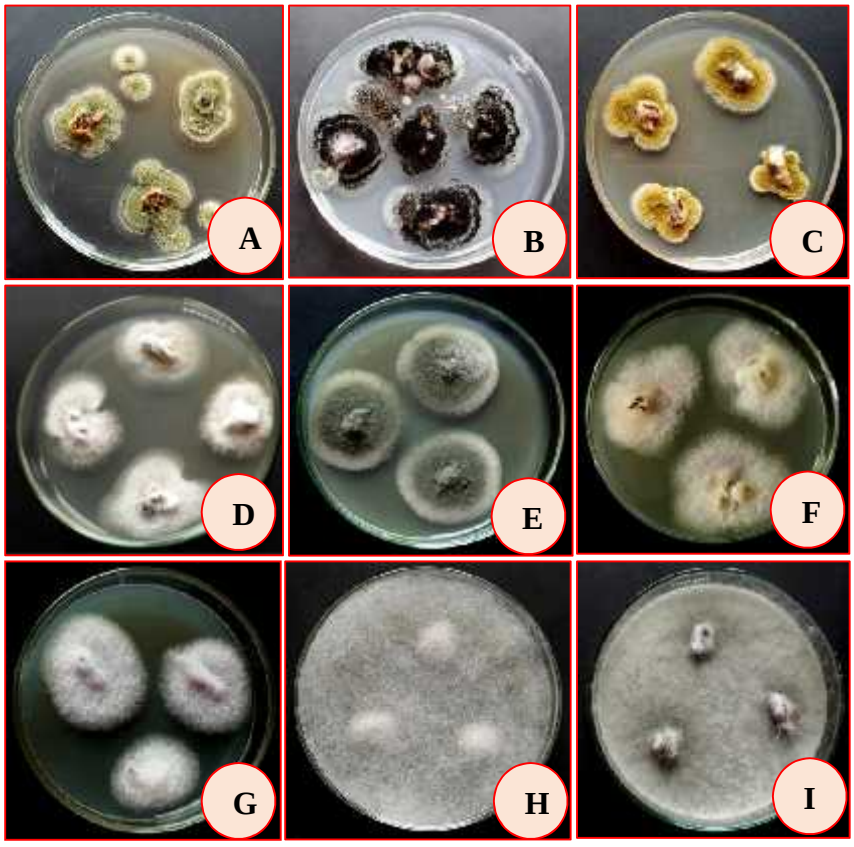

Plate 7. Re- isolated colony of the pathogenic fungi. A. *Aspergillus flavus,* **B.** *A. niger*, C. *A. tamarii*, **D.** *Colletotrichum gloeosporioides,* **E.** *C. lunata,* **F.** *Fusarium nivale,* **G.** *F. moniliforme,* **H.** *Mucor* sp. and **I.** *R. solani*.

4.12. Transmission of test pathogens from seed to seedling

Nine pathogenic seed borne fungi *viz., Aspergillus flavus, A. niger, A. tamarii, Colletotrichum gloeosporioides, Curvularia lunata, Fusarium moniliforme, F. nivale, Mucor* sp. and *Rhizoctonia solani* were used for seedling symptoms test. Seedling symptoms owing to pathogenic fungi were recorded in water agar test and pot experiment according to Dhakar and Ratnoo (2017). Seedlings showed characteristics symptoms after 21 days of incubation except control set. Inoculated pathogens were re-isolated from the symptomatic area of seedlings. All the pathogenic fungi showed seed transmission nature that means they were transferred from seed to seedlings.

Results revealed that, yellowing of leaf followed by blight symptom were observed in seedlings after 21 days of inoculation. The re-isolation of the fungal pathogens was made from symptomatic leaves of seedlings raised from inoculated seeds. In pot experiment, all the pathogens showed yellowing and seedling blight symptoms (Fig. 21). *Aspergillus flavus, A. tamarii, Colletotrichum gloeosporioides, Fusarium moniliforme* and *Rhizoctonia solani* showed wilting symptom. *Fusarium nivale* showed stunted growth symptom. *Curvularia lunata, F. moniliforme, A. flavus* and *Mucor* sp. showed seedling rot symptom (Table 20).

Fungal pathogens were also studied for their effect on seed germination. All the fungal strains showed a considerable reduction of seed germination (Fig. 21). Germination percentage of control seeds was 99% whereas, in inoculated seeds it was varied from 43.37 to 84.34%. In overall seed germination assay pot experiment showed more reduction compared to water agar method. Among all the fungal pathogens *Curvularia lunata* showed maximum reduction (43.37%) in cotton seed germination (Table 20).

The mortality percentage was highest in *F. moniliforme* (48.28%) and lowest (19.64%) in *A. niger*. Seed to seedling transmission of pathogens varied from 17.85 to 46.56%. The maximum seed to seedling transmission was recorded in *F. moniliforme* and minimum in *A. niger*. All the fungal pathogens showed varied reduction in length of root and shoot (Table 20).

The symptoms observed in seedlings raised from inoculated seeds and seedlings grown by seed inoculations with test pathogens in water agar test were more or less similar to that of pot experiment (Fig. 22). *Rhizoctonia solani* showed the higher percentage of seed to seedling transmission i.e., 35.27% which was followed by *F. moniliforme* (31.29%), *C. gloeosporioides* (22.68%), *C. lunata* (21.25%), *Aspergillus flavus* (20.38%), *A. tamarii* (18.98%), *F. nivale* (14.89%), *Mucor* sp. (11.76%) and *A. niger* (8.03%).

All the test pathogens showed seedling rot symptom. Stunting of seedlings is also a common symptom showed for maximum pathogens. *Curvularia lunata* and *Mucor* sp. showed wilting symptom. *Aspergillus flavus, Curvularia lunata* and *Mucor* sp. showed blight symptoms. Healthy control seeds did not show any symptoms on seedlings (Table 21, Fig. 22).

Germination percentage of control seeds were 90.32% whereas, in inoculated seeds it was varied from 58.33 to 89.07%. The mortality percentage was highest in *Rhizoctonia solani* (35.27%) and lowest in *A. niger* (8.03%). Seed to seedling transmission of pathogens varied from 8.03 to 35.27%. The root and shoot length of control seedlings were 6.2% and 9.78%, respectively but low for test pathogens treated seeds (Table 21).

Table 20. Transmission of test pathogens from seed to seedlings in pot experiment.

Sl. No.	Test pathogens	% Germination of seeds	% Mortality	% Transmission of pathogens	Length (cm)		Symptoms
					Root	Shoot	
01.	*Aspergillus flavus*	79.01^{c}	27.59^{g}	25.58^{i}	3.39^{b}	10.93^{b}	Yellowing of leaves, seedling rot, blight, wilting
02.	*A. niger*	84.34^{b}	19.64^{i}	17.85^{g}	3.89^{cd}	11.03^{c}	Yellowing of leaves, blight
03.	*A. tamarii*	71.35^{e}	39.62^{c}	37.74^{b}	2.78^{g}	9.26^{i}	Wilting, blight, yellowing of leaves
04.	*Colletotrichum gloeosporioides*	58.34^{f}	35.56^{e}	33.32^{e}	3.26^{de}	10.73^{e}	Stem rot, leaf rot, yellowing of leaves, wilting, blight
05.	*Curvularia lunata*	43.37^{i}	37.04^{d}	34.33^{d}	4.0^{a}	10.09^{f}	Blight, leaf rot, stem rot
06.	*Fusarium moniliforme*	76.37^{d}	48.28^{a}	46.56^{a}	4.0^{a}	9.96^{g}	Yellowing of leaves, seedling death, blight, wilting
07.	*F. nivale*	50.56^{h}	32.02^{f}	31.20^{f}	3.14^{ef}	8.89^{j}	Stunting, yellowing of leaves, blight
08.	*Mucor* sp.	72.09^{e}	25.93^{h}	24.08^{h}	3.0^{f}	9.71^{h}	Blight, leaf rot, yellowing of leaves
09.	*Rhizoctonia solani*	55.67^{g}	41.86^{b}	36.88^{c}	3.36^{cd}	10.78^{d}	Yellowing of leaves, blight, wilting, seedling death
10.	**Control**	**99.0^{a}**	**0^{j}**	**0^{j}**	**4.14^{a}**	**11.98^{a}**	Healthy seedlings without any symptoms
	CV (%)	0.4591	0.0308	0.1143	1.61	0.0968	

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

Fig. 21. Transmission of pathogenic fungi from seed to seedlings in pot experiment. A. Control: Healthy seedlings and **(B-J).** Infected seedlings caused by **B.** *Aspergillus fiavus*, **C.** *A. tamarii*, **D**. *A. niger*, **E.** *Colletotrichum gloeosporioides*, **F.** *Curvularia lunata*, **G.** *Fusarium moniliforme*, **H**. *F. nivale*, **I.** *Mucor* sp. and **J.** *Rhizoctonia solani*.

Table 21. Transmission of test pathogens from seed to seedlings in water agar test.

Sl. No.	Test pathogens	% Germination of seeds	% Mortality	% Transmission of pathogens	Length (cm)		Symptoms
					Root	Shoot	
01.	*Aspergillus flavus*	83.32^{d}	20.53^{h}	20.38^{e}	4.18^{fg}	5.83^{f}	Yellowing of leaves, blight, stunting, seedling rot
02.	*A. niger*	89.07^{b}	22.05^{g}	8.03^{i}	4.4^{ef}	6.51^{e}	Yellowing of leaves, stunting, root rot, stem rot
03.	*A. tamarii*	58.33^{j}	38.57^{d}	18.98^{f}	5.42^{c}	6.4^{e}	Stunting, wilting, seedling rot, yellowing of leaves
04.	*Colletotrichum gloeosporioides*	64.10^{i}	18.67^{i}	22.68^{c}	4.7^{d}	8.10^{c}	Stem rot, yellowing of leaves, wilting
05.	*Curvularia lunata*	76.78^{e}	41.23^{c}	21.25^{d}	5.8^{b}	8.91^{b}	Wilting, blight, seedling rot
06.	*Fusarium moniliforme*	65.57^{h}	45.32^{b}	31.29^{b}	4.58^{de}	6.79^{e}	Yellowing of leaves, seedling rot, root rot
07.	*F. nivale*	71.67^{f}	29.94^{e}	14.89^{g}	5.1^{c}	7.56^{d}	Stunting, yellowing of leaves, stem rot
08.	*Mucor* sp.	88.35^{c}	25.58^{f}	11.76^{h}	5.7^{b}	8.48^{bc}	Wilting, seedling blight, stunting, root rot.
09.	*Rhizoctonia solani*	67.32^{g}	46.68^{a}	35.27^{a}	4.14^{g}	7.54^{d}	Yellowing of leaves, seedling rot, stunting
10.	**Control**	**90.32^{a}**	**16.67^{j}**	-	**6.2^{a}**	**9.78^{a}**	Healthy seedlings without any symptoms
	CV (%)	0.0132	0.0328	0.0495	1.54	1.95	

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

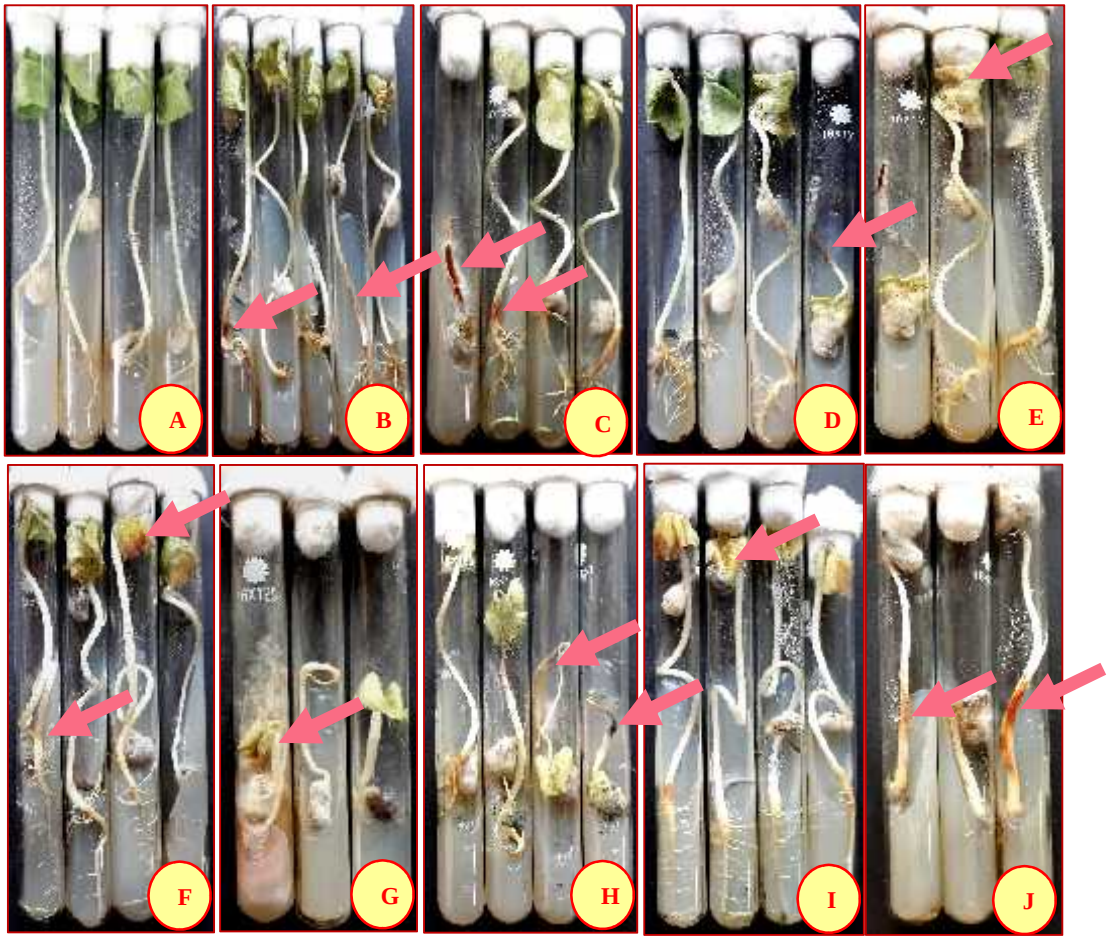

Fig. 22. Transmission of pathogenic fungi from seed to seedlings in water agar test tubes. **A.** Healthy seedlings and **B-J.** Infected seedlings caused by **B.** *Aspergillus flavus*, **C.** *A. niger*, **D.** *A. tamarii*, **E.** *Colletotrichum gloeosporioides*, **F.** *Curvularia lunata*, **G.** *Fusarium nivale*, **H.** *F. moniliforme*, **I.** *Mucor* sp. and **J.** *Rhizoctonia solani*.

All the nine test pathogens were positive and potentially infected the cotton seedlings by inducing various symptoms like root browning, root rot, stem rot, seedling blight, wilting, leaf spots etc. *Fusarium* and *Curvularia* spp. showed direct influence on the reduction of seed germination, seedling height, vigor, and root length (Fig. 22).

Among the other pathogens *C. lunata* and *R. solani* were aggressive and caused different symptoms. Majority of the test pathogen attacked during the seed germination. Among the isolated fungal pathogens, *C. lunata* and *F. nivale* showed a greater impact in reduction of seed germination. The germinated seeds after emergence up to the entire plantlet showed reduced vigor, vitality and overall reduced plant health. Although *A. niger* and *A. flavus* did not show much impact in reduction of seed germination, but the foliar disease symptoms were observed at mature stage and showed moderate effect in reduction of seed germination. From the present findings it can be concluded that all the test pathogens are potential phytopathogens and have the capacity to induce infection to plantlet as well as during germination of cotton seed.

In contrast to the present study, Fakir *et al.* (1976) in their studies on seed transmission of *Macrophomina phaseolina* in sunflower revealed that it caused death of the emerging radical, discoloration of roots, hypocotyls and cotyledons. Cotton seedling diseases are a worldwide problem caused by a complex of microorganisms.

Fungi are the widest pathogens which affect cotton crop especially at the seedling stage causing pre or post emergence damping off (Aly *et al*. 2008). According to Sultana (2021), six pathogenic fungi of rice *viz*., *Bipolaris oryzae, Curvularia lunata, Fusarium equiseti, F. fujikuroi, Microdochium fisheri* and *Nigrospora oryzae* showed seed to seedling transmission nature in both water agar test tube and earthen pot. The present findings are also in agreement with the results of above mentioned workers.

4.13. Colony interaction between the test pathogens and antagonistic fungi.

Assessment of the 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 III which was primarily based on the observation of Dickinson and Boardman (1970). The

colony interaction includes grading, per cent inhibition of growth of the test pathogens owing to antagonists, intermingling zone and the zone of inhibition (Skidmore and Dickinson 1976).

Antagonistic fungi were isolated from rhizosphere soil of the host cotton varieties following serial dilution method. Among the isolated fungi, *Aspergillus flavus, A. fumigatus, A. niger* and *Trichoderma viride* were selected to test their antagonistic potentials against the test pathogens because they exhibit strong antagonistic effect against the pathogens following "Dual culture technique" described by Bashar and Rai (1994). The parameter used for the assessment of the colony interaction and per cent inhibition of radial growth was calculated by the formula of Fokkema (1976).

The results of colony interactions have been summarized in Table 22, Fig. 23 and Plates 8-13. Different antagonistic effects of the soil fungi were noted against the test pathogens. In the test of pathogenicity, six fungal pathogens *viz*., *Colletotrichum gloeosporioides, Curvularia lunata, Fusarium nivale, F. moniliforme, Mucor* sp. and *Rhizoctonia solani* isolated from the 14 varieties of cotton seeds were found virulent. These test pathogens were used in colony interaction.

Antagonistic relationships among the soil fungi and test pathogens were grade 2 and 4 in the colony interactions. But, grade 2 was found to be the most commonly encountered type of colony interaction. 17 interactions were incorporated in grade 2 which was followed by grade 4 (Table 22).

The intermingled zone between the soil fungi and test pathogens was very common. The maximum intermingled zone (0.3 cm) was found in case of *A. fumigatus* and *T. viride* against *Mucor* sp. and *R. solani*. *Trichoderma viride* grew over the colony of the test pathogens but in case of *A. flavus, A. fumigatus* and *A. niger* inhibition zone was found and it was 0.2, 0.2 and

0.1 cm, respectively. The highest inhibition zone was observed in *A. flavus*, *A. fumigatus* and *A. niger* (0.2 cm) (Table 22, Fig. 23 and Plates 8-13).

All the tested soil fungi inhibited the growth of all the test pathogens to different degrees in dual culture experiments on PDA plates. *Aspergillus fumigatus* showed the maximum inhibition on radial growth of *C. gloeosporioides* (84%) followed by *A. niger* (80%), *A. flavus* (73.9%) and *T. viride* (63.1%) (Table 22, Fig. 23 and Plate 8).

In dual culture colony interaction, *T. viride* exhibited the highest (76.4%) growth inhibition on *C. lunata* which was followed by *A. niger* (72.2%), *A. fumigatus* (68.4%) and *A. flavus* (59.0%) (Table 22, Fig.23 and Plate 9). On the other hand, *A. niger* showed the highest growth inhibition (72.27%) against *F. nivale* followed by *A. flavus* (68.1%), *T. viride* (66.6%) and *A. fumigatus* (45.4%) (Table 22, Fig. 23 and Plate 10).

At the same time, *T. viride* showed the highest growth inhibition on *F. moniliforme* (73.7%), which was followed by *A. flavus* (67.6%), *A. niger* (60.0%) and *A. fumigatus* (53.8%) (Table 22, Fig. 23 and Plate 11).

Trichoderma viride exhibited the maximum growth inhibition on *Mucor* sp. (60.0%) which was followed by *A. niger* (52.1%) *A. flavus* (50.0%) and *A. fumigatus* (44.1%). *Trichoderma viride* also responsible for the highest growth inhibition on *R. solani* (46.8%) followed by *A. niger* (30.2%) *A. flavus* (26.6%), and *A. fumigatus* (19.3%) ((Table 22, Fig. 23 and Plate 12-13).

Similar observations was also observed in the study of Akter *et al*. (2014), Bashar and Chakma (2014), Helal and Shamsi (2019) where *A. flavus, A. fumigatus, A. niger* and *T. viride* were responsible for significant growth inhibition against the spp of *Colletotrichum, Curvularia* and *Fusarium*.

In dual culture technique, maximum growth inhibition was recorded for *Trichoderma* spp. by different workers (Tapwal *et al.* 2015, Patel and Joshi 2001, Sunita and Kurundkar 2007, Al-Ameen *et al*. 2017, Goswami and Islam 2002). In the present investigation, the same antagonists also showed different effects on different fungi.

Four tested soil fungi inhibited the growth of all the test pathogens in varied degrees in dual cultures experiments on agar plates. This variation might be due to the selection of different test pathogens.

The biology of *Trichoderma* has reviewed by Papavizas (1985), which is a fast growing and antagonistic fungus to many pathogenic and non-pathogenic fungi. The antagonistic activity of *Trichoderma* sp. is very much important because of the fast growing nature, rapid sporulation and toxic metabolites producing capacity (Garrett 1981). Therefore, high antagonistic activity of the *Trichoderma viride* was found against the test pathogens may be due to the above reasons.

In dual culture technique, significantly maximum inhibition was observed in *T. viride* by Patel and Joshi (2001). Similarly, culture and culture filtrate of *Trichoderma viride* was more effective than *T. harzianum* in inhibiting the mycelial growth of *Botryodiplodia theobromae* and *Colletotrichum gloeosporioides* reported by Shirshikar (2002).

Bhuvaneswari (1999) evaluated *Trichoderma viride* under *in vitro* condition against postharvest pathogens of mango and found that the growth of *Pestalotia* spp., *A. flavus* and *C. gloeosporioides* were inhibited 72.88, 70.74, 62.41 and 56.83% respectively.

According to Sultana (2021), in dual culture colony interaction, out of four antagonistic fungi, *T. viride* showed highest growth inhibition against all the pathogenic fungi of rice. The results of the present investigation are in agreement with the findings of above mentioned researchers.

Table 22. Effects of dual culture between fungal antagonists and cotton pathogens.

Test pathogens		% inhibition of radial growth, intermingled and inhibition zone and type of reactions of the test pathogens			
		Aspergillus flavus	***A. fumigatus***	***A. niger***	***Trichoderma viride***
Colletotrichum gloeosporioides	% inhibition	73.9	84.0	80.0	63.1
	IMZ (cm)	0.1	0.2	0.2	0.1
	IHZ (cm)	-	-	-	-
	Grade	2	2	2	2
	Type	Bii	Bii	Bii	Bii
Curvularia lunata	% inhibition	59.0	68.4	72.2	76.4
	IMZ (cm)	-	-	-	0.2
	IHZ (cm)	0.2	0.2	0.1	-
	Grade	4	4	4	2
	Type	C	C	C	Bii
Fusarium moniliforme	% inhibition	67.6	53.8	60.0	73.7
	IMZ (cm)	-	-	-	0.2
	IHZ (cm)	0.1	0.2	0.2	-
	Grade	4	4	4	2
	Type	C	C	C	Bii
F. nivale	% inhibition	68.1	45.4	72.2	66.6
	IMZ (cm)	0.2	0.1	0.1	0.1
	IHZ (cm)	-	-	-	-
	Grade	2	2	2	2
	Type	Bii	Bii	Bii	Bii
Mucor sp.	% inhibition	50.0	44.1	52.1	60.0
	IMZ (cm)	0.2	0.3	-	0.3
	IHZ (cm)	-	-	0.1	-
	Grade	2	2	4	2
	Type	Bii	Bii	C	Bii
Rhizoctonia solani	% inhibition	26.6	19.3	30.2	46.8
	IMZ (cm)	0.2	0.3	0.2	0.3
	IHZ (cm)	-	-	-	-
	Grade	2	2	2	2
	Type	Bii	Bii	Bii	Bii

IMZ = Intermingling zone, IHZ = Inhibition zone, and '-' = not applicable.

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

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

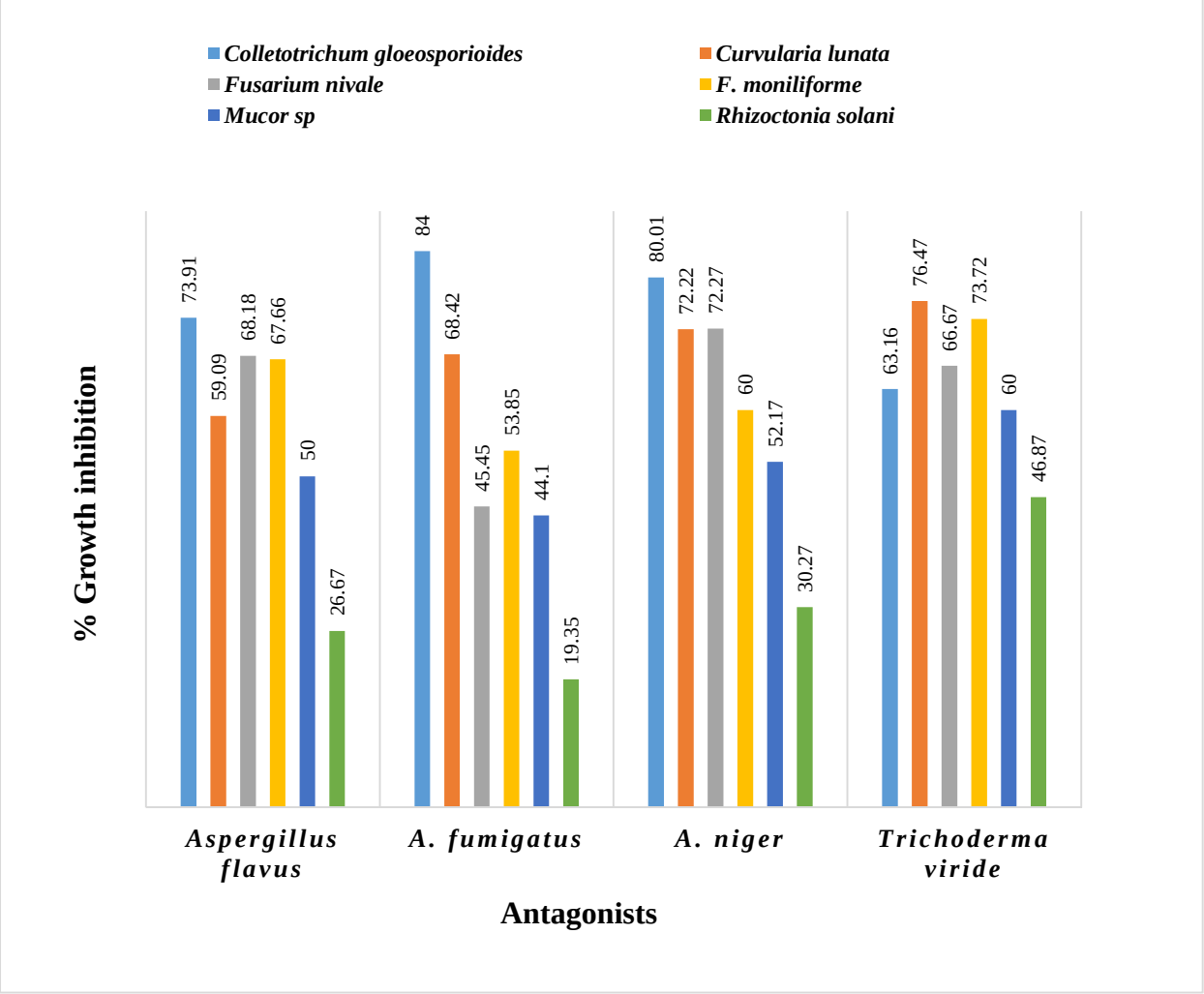

Fig. 23. Colony interaction between the test pathogens and antagonistic fungi.

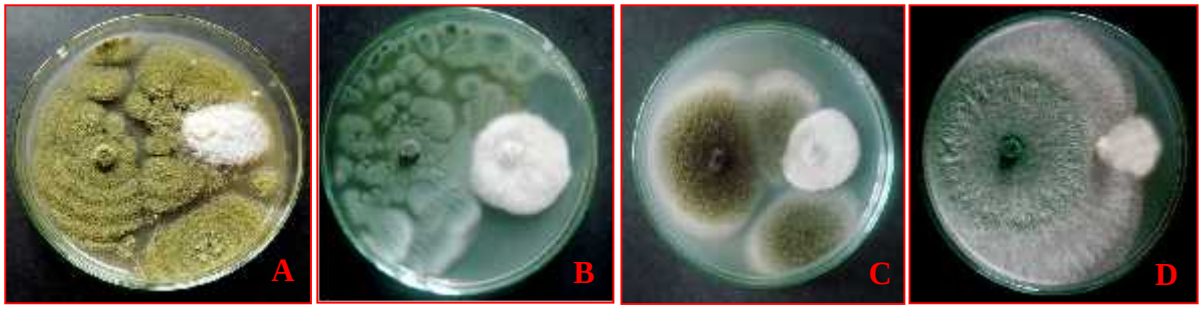

Plate 8. Colony interaction between *Colletotrichum gloeosporioides* and antagonistic fungi.

A. *C. gloeosporioides* and *A. flavus*
B. *C. gloeosporioides* and *A. fumigatus*
C. *C. gloeosporioides* and *A. niger*
D. *C. gloeosporioides* and *T. viride*.

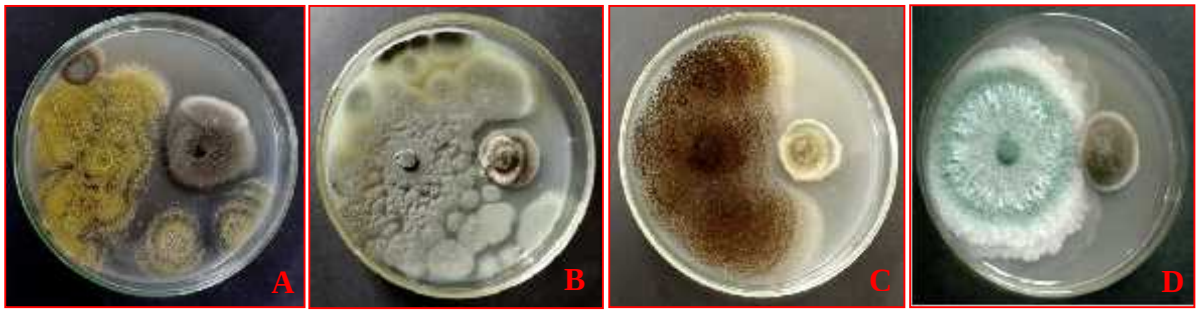

Plate 9. Colony interaction between *Curvularia lunata* and antagonistic fungi.

A. *C. lunata* and *A. flavus*
B. *C. lunata* and *A. fumigatus*
C. *C. lunata* and *A. niger*
D. *C. lunata* and *T. viride*

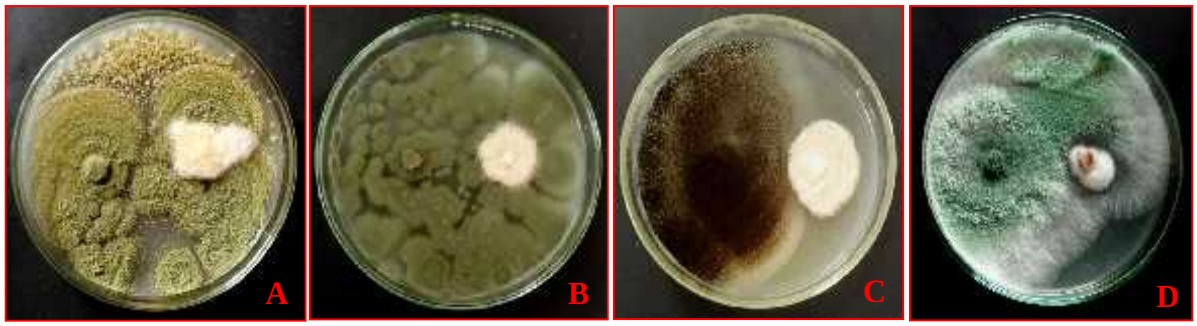

Plate 10. Colony interaction between *Fusarium nivale* and antagonistic fungi.

A. *F. nivale* and *A. flavus*
B. *F. nivale* and *A. fumigatus*
C. *F. nivale* and *A. niger*
D. *F. nivale* and *T. viride*.

Plate 11. Colony interaction between *Fusarium moniliforme* and antagonistic fungi.

A. *F. moniliforme* and *A. flavus*
B. *F. moniliforme* and *A. fumigatus*
C. *F. moniliforme* and *A. niger*
D. *F. moniliforme* and *T. viride*.

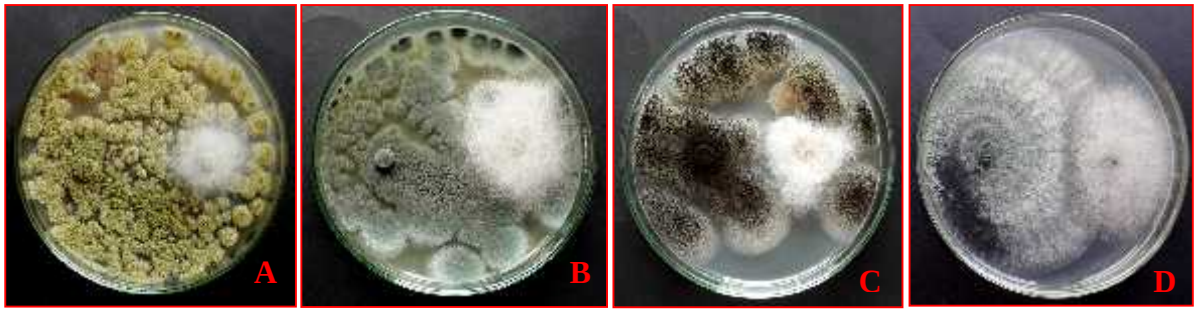

Plate 12. Colony interaction between *Mucor* sp. and antagonistic fungi.

A. *Mucor* sp. and *A. flavus*
B. *Mucor* sp. and *A. fumigatus*
C. *Mucor* sp. and *A. niger*
D. *Mucor* sp. and *T. viride*.

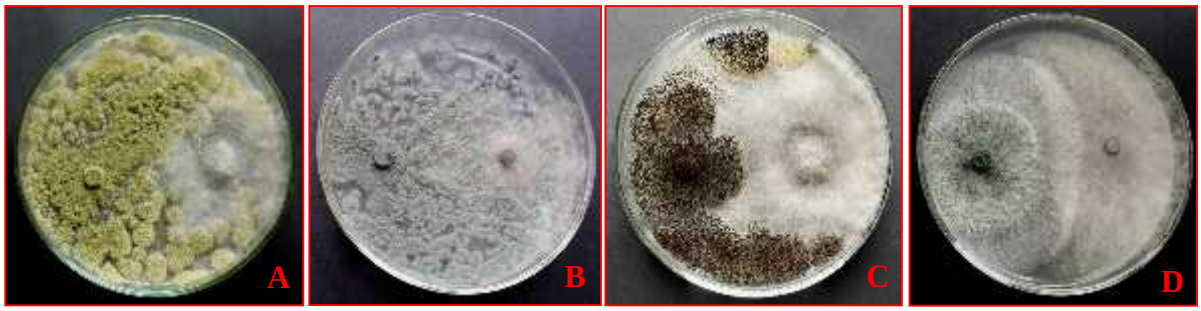

Plate 13. Colony interaction between *Rhizoctonia solani* and antagonistic fungi.

A. *R. solani* and *A. flavus*
B. *R. solani* and *A. fumigatus*
C. *R. solani* and *A. niger*
D. *R. solani* and *T. viride*.

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

The effect of volatile substance emanating from the antagonistic fungi against the test pathogens of cotton seeds are presented in Table 23, Fig. 24 and Plates 14-19. Six test pathogens *viz. Colletotrichum gloeosporioides*, *Curvularia lunata*, *Fusarium moniliforme*, *Fusarium nivale*, *Mucor* sp. and *Rhizoctonia solani* were used in this experiment. It is obvious from the result that the volatile substances produced from the cultures of *Aspergillus flavus, A. fumigatus, A. niger* and *Trichoderma viride* inhibited the radial growth of the test pathogens to different degrees.

The maximum inhibition of radial growth of *Colletotrichum gloeosporioides* was found in case of *A. flavus* (59%), which was followed by *A. niger* (48.7%), *A. fumigatus* (38.2%) and *T. viride* (35.9%) owing to the volatiles after 6 days of incubation at 25±2˚C (Table 23, Fig. 24 and Plate 14). The highest inhibition of the radial growth of *Curvularia lunata* was observed in case of *T. viride* (71.4%) followed by *A. flavus* (38.1%), *A. fumigatus* (47.6%) and *A. niger* (33.3%) due to volatile metabolites (Plate 15).

The maximum inhibition of radial growth of *Fusarium nivale* was also recorded by *T. viride* (64.2%) which was followed by *A. flavus* (40.4%), *A. niger* (54.7%) and *A. fumigatus* (47.6%) (Plate 16). Highest inhibition of the radial growth of *F. moniliforme* was noticed in case of *T. viride* (51.1%) followed by *A. fumigatus* (42.2%), *A. flavus* (35.5%) and *A. niger* (40.0%) (Plate 17). *Aspergillus fumigatus* showed 58.2% inhibition of the growth of *Mucor* sp. which was followed by *A. flavus* (47.2%), *A. niger* (36.3%) and *T. viride* (55.5%) (Plate 18). Finally, maximum inhibition of the radial growth of *Rhizoctonia solani* was observed by *A. fumigatus* (72.4%) which was followed by *T. viride* (55.5%), *A. niger* (53.3%) and *A. flavus* (52.2%) (Table 23, Fig. 24 and Plate 19).

These observations were similar with the findings of Akter *et al*. (2014), Bashar and Chakma (2014), Helal and Shamsi (2019); where *A. flavus, A. fumigatus, A. niger* and *T. viride* showed significant growth inhibition against *Colletotrichum* spp., *Curvularia lunata* and *Fusarium* spp. Differences in per cent inhibition with the present investigation might be due to the difference in organism involved in the interaction. The present findings also in agreement with the observation of Hosen and Shamsi (2019) who observed that the volatile metabolites produced from *T. viride, A. niger, A. flavus* and *A. fumigatus* inhibited the radial growth of *F. merismoides* by 67.69, 64.62, 61.54 and 56.57%, respectively.

Similarly, some volatile metabolites released from *T. viride* cultures might be responsible for extending the inhibitory activity against some pathogens such as *Colletotrichum* and *Fusarium* species isolated from banana (Al- Ameen *et al*. 2017). Thakur and Harsh (2014) also found that volatile metabolites emanating from the culture of *Aspergillus niger* showed 42.43% inhibition of mycelial growth of *C. gloeosporioides. Trichoderma viride* showed maximum growth inhibition against the pathogens of rice owing to the effects of volatile metabolites.

The growth inhibition of the test pathogens may be attributed due 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 depends on the interactions between the volatile factors of two fungi as some kind of chemical reaction may occur there, which may include the nullification of the metabolites by each other.

The present investigation suggests that there were qualitative and quantitative differences in the volatile substances produced by the antagonistic soil fungi. So, they exhibited different amounts of growth inhibition of the test pathogens. Dennis and Webster (1971b) 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 one of the volatiles. Some protective compounds recently isolated from endophytes are taxol, oocydin A, cryptocin, ambuic acid and jesteron (Li *et al*. 2000, Li and Strobel 2001). However, Hutchinson (1973) gave direct evidence by quantitative analysis of these volatiles. Fries (1973) discussed the mode of action of volatile compounds in the following manner:

I. by the activation of enzymes
II. by removal or neutralization of the inhibitors
III. by influence of nutrient uptake from the medium
IV. by stimulation of a limiting factor in intermediary metabolites.

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

Table 23. Per cent inhibition of radial growth of test pathogens owing to volatile metabolites of antagonistic fungi.

Antagonists	**% inhibition of radial growth of the test pathogens**					
	Colletotrichum gloeosporioides	***Curvularia lunata***	***Fusarium nivale***	***F. moniliforme***	***Mucor* sp.**	***Rhizoctonia solani***
Aspergillus flavus	58.98[a]	38.10[c]	40.48[d]	35.56[d]	47.27[c]	52.22[d]
A. fumigatus	38.46[c]	47.62[b]	47.63[c]	42.23[b]	58.19[a]	72.44[a]
A. niger	48.72[b]	33.34[d]	54.76[b]	40.0[c]	36.37[d]	53.33[c]
Trichoderma viride	35.90[d]	71.42[a]	64.29[a]	51.12[a]	55.56[b]	55.57[b]
CV%	0.0220	0.0210	0.0193	0.0237	0.0234	0.0171

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

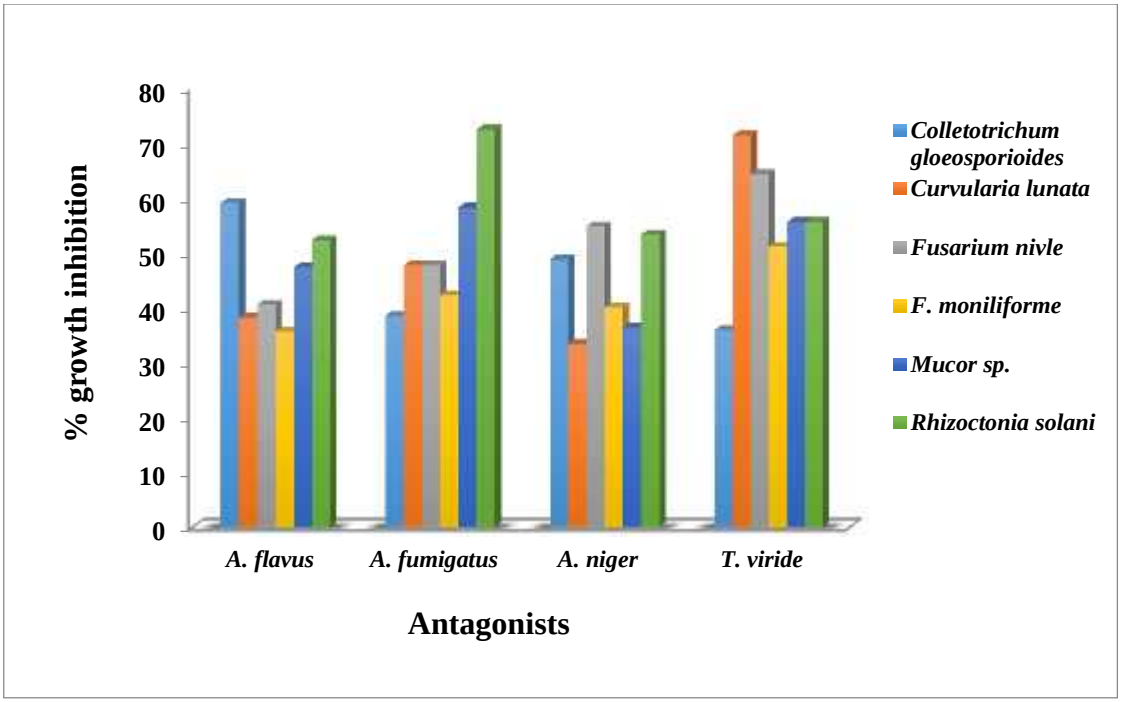

Fig. 24. Per cent growth inhibition of radial growth of test pathogens owing to volatile metabolites of antagonistic fungi.

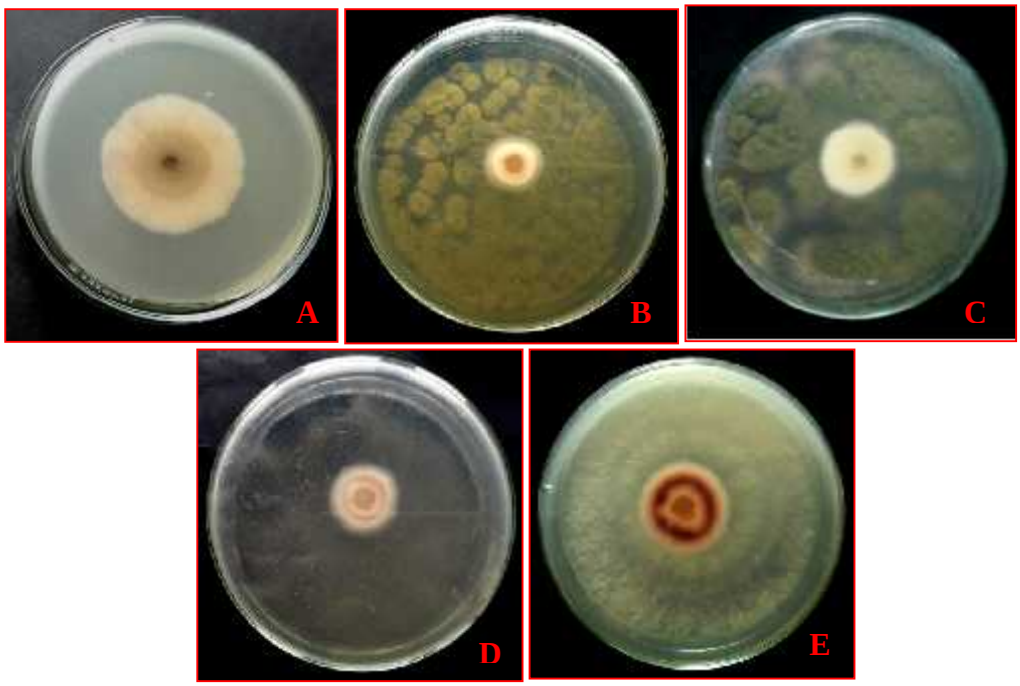

Plate 14. Growth inhibition of *Colletotrichum gloeosporioides* due to volatile metabolites of antagonists.

A. Control **B.** *A. flavus*: *C. gloeosporioides* **C.** *A. fumigatus*: *C. gloeosporioides*
D. *A. niger*: *C. gloeosporioides* **E.** *T. viride*: *C. gloeosporioides*

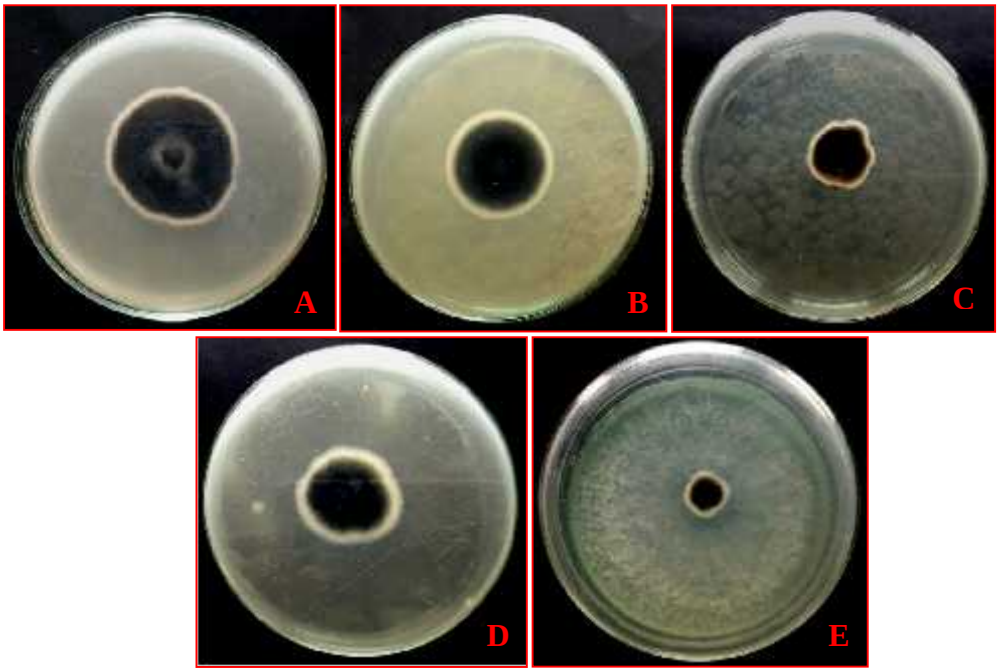

Plate 15. Growth inhibition of *Curvularia lunata* owing to volatile metabolites of antagonists.

A. Control **B.** *A. flavus*: *C. lunata* **C**. *A. fumigatus*: *C. lunata*
D. *A. niger*: *C. lunata* **E.** *T. viride*: *C. lunata*

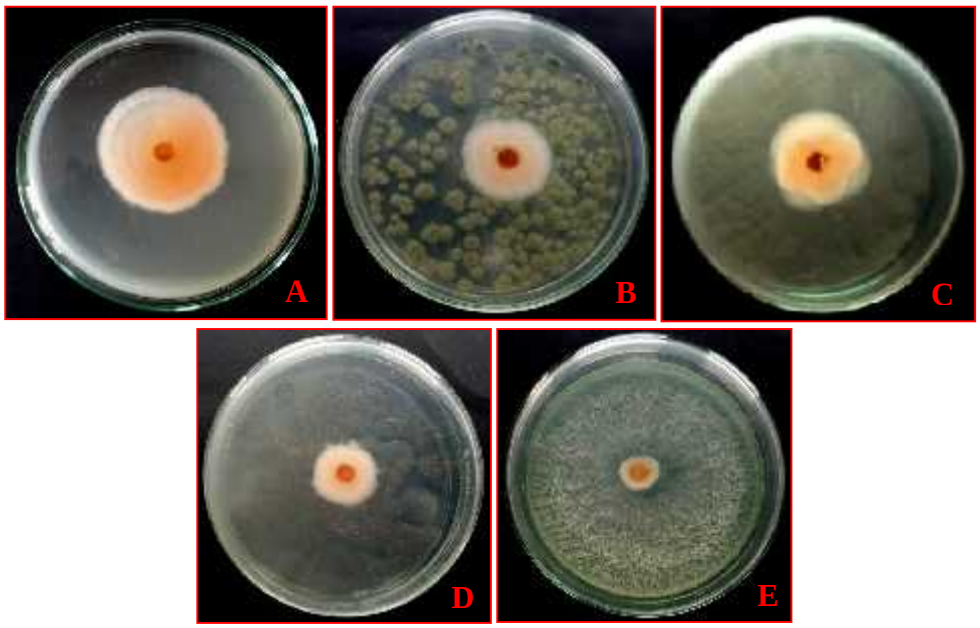

Plate 16. Growth inhibition of *Fusarium nivale due* to volatile metabolites of antagonists.

A. Control **B.** *A. flavus*: *F. nivale* **C.** *A. fumigatus*: *F. nivale*
D. *A. niger*: *F. nivale* **E.** *T. viride*: *F. nivale*

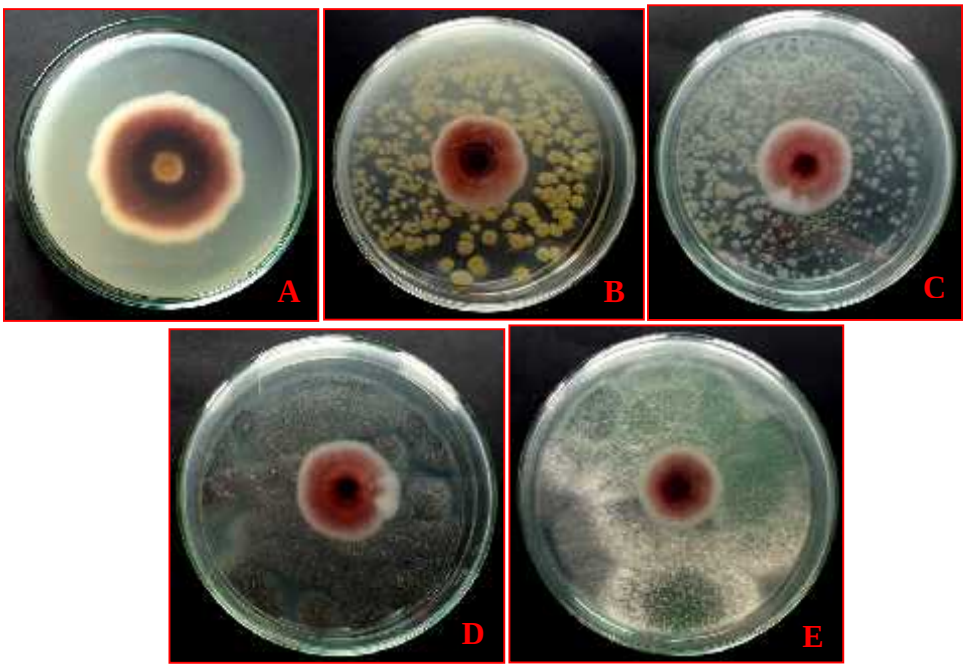

Plate 17. Growth inhibition of *Fusarium moniliforme* owing to volatile metabolites of antagonists.

A. Control **B.** *A. flavus*: *F. moniliforme* **C.** *A. fumigatus*: *F. moniliforme*
D. *A. niger*: *F. moniliforme* **E.** *T. viride*: *F. moniliforme*

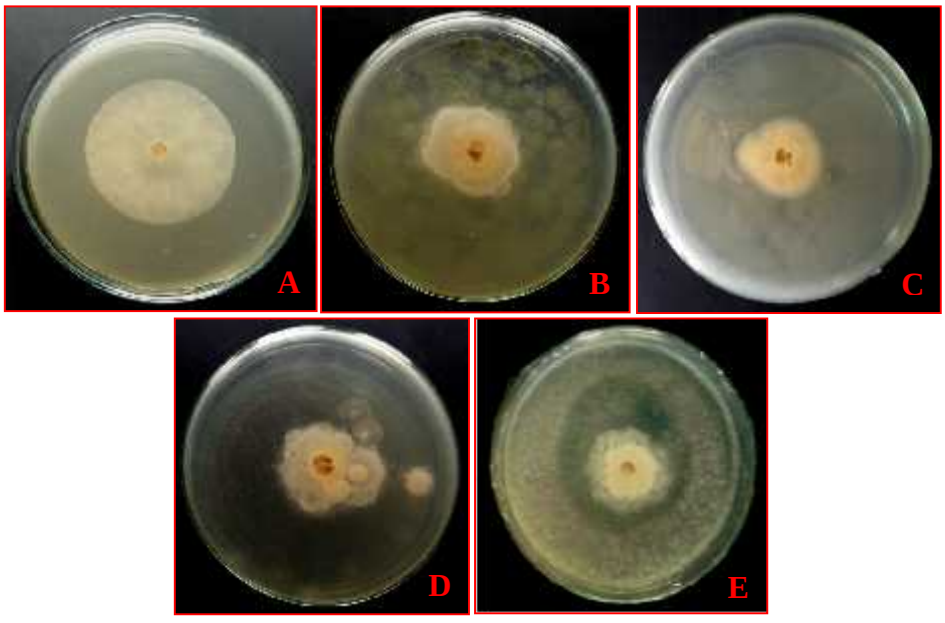

Plate 18. Growth inhibition of *Mucor* sp. owing to volatile metabolites of antagonists.

A. Control **B.** *A. flavus*: *Mucor* sp. **C.** *A. fumigatus*: *Mucor* sp.
D. *A. niger*: *Mucor* sp. **E.** *T. viride*: *Mucor* sp.

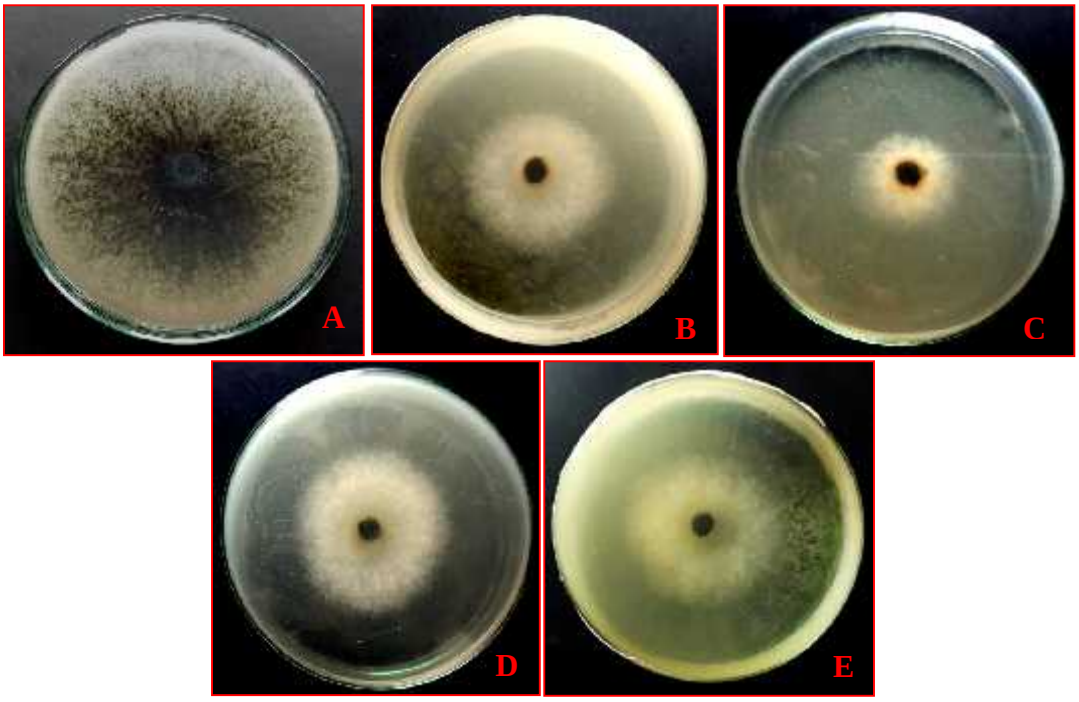

Plate 19. Growth inhibition of *Rhizoctonia solani* due to volatile metabolites of antagonists.

A. Control **B.** *A. flavus*: *R. solani* **C.** *A. fumigatus*: *R. solani*
D. *A. niger*: *R. solani* **E.** *T. viride*: *R. solani*

4.15. Effect of Non-volatile metabolites of the antagonistic fungi on the growth of the test pathogens.

The Table 24, Figs 25-30 and Plates 20-25 showed the effect of non-volatile metabolites of antagonistic fungi on the radial growth of *Colletotrichum gloeosporioides, C. lunata, F. nivale, F. moniliforme, Mucor* sp. and *R. solani*. The selected antagonists showed different degrees of growth inhibition of the test pathogens at different concentrations.

The highest inhibition of radial growth of *C. gloeosporioides* was recorded with the non-volatile metabolites of *A. niger* (76.3%), which was followed by *T. viride* (70.9%), *A. fumigatus* (58.4%) and *A. flavus* (57.7%) at 20% concentration (Table 24, Fig. 25 and Plate 20) after 6 days of incubation at 25±2˚C. The inhibition of the pathogen increases with the increase of the concentration of the culture filtrates in culture medium. At 20% concentration,

the order of effectiveness against *Colletotrichum gloeosporioides* was *Aspergillus niger >T. viride > A. fumigatus > A. flavus.*

The highest inhibition of the radial growth of *Curvularia lunata* was observed with the non-volatile metabolites of *T. viride* (78.2%) which was followed by *A. niger* (73.1%), *A. flavus* (72.3%) and *A. fumigatus* (57.4%) at 20% concentration (Table 24, Fig. 26 and Plate 21) after 6 days of incubation at 25±2˚C. In culture medium, the inhibition of the pathogen increases with the increase of the concentration of the culture filtrates. The order of effectiveness against *Curvularia lunata* at 20% concentration was *Trichoderma viride >A. niger > A. flavus > A. fumigatus.*

The maximum inhibition of radial growth of *Fusarium moniliforme* was noticed by the non-volatile metabolites of *T. viride* (76.3%) followed by *A. niger* (74.0%), *A. fumigatus* (67.2%) and *A. flavus* (56.3%) at 20% concentration (Table 24, Fig. 27 and Plate 22) after 6 days of incubation at 25±2˚C. The inhibition of the pathogen increases with the increase of the concentration of the culture filtrates in culture medium. The order of effectiveness against *Fusarium moniliforme* at 20% concentration was *Trichoderma viride >A. niger > A. fumigatus > A. flavus.*

The highest inhibition of the radial growth of *F. nivale* was recorded with the non- volatile metabolites of *A. niger* (81.8%), which was followed by *A. fumigatus* (71.4%), *A. flavus* (64.2%) and *T. viride* (74.0%) at 20% concentration (Table 24, Fig. 28 and Plate 23). In the culture medium, with the increase of the concentration of the culture filtrates the inhibition of the pathogen increases. The order of effectiveness against *F. nivale* at 20% concentration was *A. niger > T. viride > A. fumigatus > A. flavus.*

The maximum inhibition of radial growth of *Mucor* sp. was noticed with the non- volatile metabolites of *A. fumigatus* (64.7%) which was followed by *T. viride* (60.0%), *A. niger*

(57.5%) and *A. flavus* (47.0%) at 20% concentration (Table 24, Fig. 29 and Plate 24) after 6 days of incubation. The inhibition of the pathogen increases with the increase of the concentration of the culture filtrates. The order of effectiveness against *Mucor* sp. at 20% concentration was *A. fumigatus* >*Trichoderma viride* > *A. niger* > *A. flavus.*

The highest inhibition of radial growth of *Rhizoctonia solani* was noticed by the non- volatile metabolites of *A. fumigatus* (72.2%) which was followed by *T. viride* (62.8%), *A. niger* (58.8%) and *A. flavus* (55.5%) at 20% concentration (Table 24, Fig. 30 and Plate 25) after 6 days of incubation at 25±2˚C. The order of effectiveness against *Rhizoctonia solani* at 20% concentration was *A. fumigatus* > *T. viride* > *A. niger* > *A. flavus.* Differences in per cent inhibition with the present study might be due to the difference in organism strains involved in the interaction.

Similar results was observed in the study of Akter *et al*. (2014), Bashar and Chakma (2014), Helal and Shamsi (2019), where *A. flavus, A. fumigatus, A. niger* and *T. viride* showed significant growth inhibition against *Colletotrichum* spp., *Curvularia lunata* and *Fusarium* spp.

The non-volatile metabolites emanating from the culture filtrates of *T. viride* and *A. niger* were responsible for the highest inhibition against different pathogenic species of *Colletotrichum* and *Fusarium* in accordance with Al- Ameen *et al*. (2017), Madhanraj *et al*. (2010) and Tran (2010), who used *T. viride* to control *Sclerotium rolfsii* and found effective result.

Similarly, the maximum inhibition of radial growth of *C. lunata, F. fujikuroi* and *M. fisheri* was observed owing to non- volatile metabolites of *T. viride* whereas, *A. niger* was responsible for the highest inhibition of the radial growth of *B. oryzae, F. equiseti* and *A. fumigatus* which showed maximum inhibition of radial growth of *N. oryzae* according to Sultana (2021).

Culture filtrates of *T. viride* showed major growth inhibition on *C. gloeosporioides* reported by Tapwal *et al.* (2015). A number of *Trichoderma* species are effective agents for the control of some plant pathogenic fungi, such as *Fusarium* spp. (Sivan and Chet 1986), *Pythium* spp. (Naseby *et al.* 2000) and *Rhizoctonia* spp. (Lewis and Papavizas 1987).

The inhibition of radial growth of the test pathogens due to non-volatile metabolites have been attributed to the production of toxic substances in the culture filtrates (Brian 1957, 1960, Gottlieb and Shaw 1970, Dennis and Webster 1971a, Singh and Webster 1978, Skidmore and Dickinson 1976, Kexiang *et al.* 2002, Krupke *et al.* 2003), nutrient impoverishment (Fokkema 1976 and Skidmore 1976) and alteration of pH of the culture medium resulting from staling growth products (Bhatt and Vaughan 1962, Bier 1966).

The per cent inhibition of the test pathogens against the soil fungi also varied due to differences in nature, quality and quantity of the inhibitory substances produced by the soil fungi. Different antibiotics and toxins such as geodin, terricin, terric acid, aspergillic acid, hadacidine, gliotoxin, viridian, trichodermin, dermadin etc. are known to be produced by the species of *Aspergillus* and *Trichoderma* (Brian 1949, Boosalis 1956, Ooka *et al.* 1966, Meyer and Reusser 1967).

The results also show that the test pathogens have the ability to tolerate the effect of the culture filtrates of soil 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). Park and Robinson (1967) recorded marked differences in the sensitivity of hyphae to tolerate the effect of fungal growth products. Thus, the growth of a fungus in culture filtrates depends upon the level and balance of metabolites and the nutrient status of the culture filtrate according to Robinson (1969).

The present study suggests that the potential isolates of *Aspergillus* and *Trichoderma* may be further exploited as bio control agents against the fungal pathogens of cotton in field trial.

Table 24. Per cent inhibition of radial growth of test pathogens by non-volatile metabolites of antagonistic fungi.

Test pathogens	Concentration (%)	% inhibition of radial growth of test pathogens				CV (%)
		Aspergillus flavus	*A. fumigatus*	*A. niger*	*Trichoderma viride*	
Colletotrichum gloeosporioides	5	28.9^{c}	27.6^{d}	47.2^{a}	41.8^{b}	0.0275
	10	48.8^{c}	42.5^{d}	58.1^{a}	49.0^{b}	0.0201
	15	55.5^{c}	51.0^{d}	66.0^{a}	65.4^{b}	0.0168
	20	57.7^{d}	58.4^{c}	76.3^{a}	70.9^{b}	0.0152
Curvularia lunata	5	36.1^{d}	38.1^{c}	56.3^{a}	45.6^{b}	0.0227
	10	48.9^{c}	44.6^{d}	65.4^{a}	65.2^{b}	0.0178
	15	63.8^{c}	53.1^{d}	72.7^{a}	69.5^{b}	0.0154
	20	72.3^{c}	57.4^{c}	73.1^{b}	78.2^{a}	0.0142
Fusarium moniliforme	5	30.9^{d}	38.1^{b}	33.3^{c}	43.6^{a}	0.0685
	10	43.6^{d}	52.7^{c}	55.5^{a}	54.5^{b}	0.0224
	15	50.9^{d}	58.1^{c}	62.9^{b}	65.4^{a}	0.0168
	20	56.3^{d}	67.2^{c}	74.0^{b}	76.3^{a}	0.0146
F. nivale	5	33.3^{b}	30.9^{d}	32.7^{c}	50.0^{a}	0.0272
	10	40.4^{c}	38.1^{d}	50.9^{b}	64.0^{a}	0.0207
	15	47.6^{d}	50.0^{c}	67.2^{b}	68.1^{a}	0.0871
	20	64.2^{d}	71.4^{c}	81.8^{a}	74.0^{b}	0.0137
Mucor sp.	5	27.5^{d}	36.4^{b}	35.0^{c}	38.6^{a}	0.0290
	10	38.8^{d}	44.7^{a}	43.7^{c}	44.0^{b}	0.0234
	15	42.3^{d}	55.3^{a}	53.7^{c}	54.6^{b}	0.0194
	20	47.0^{d}	64.7^{a}	57.5^{c}	60.0^{b}	0.0581
Rhizoctonia solani	5	20.0^{a}	10.0^{d}	18.8^{b}	14.1^{c}	0.0634
	10	37.7^{b}	22.2d	31.1^{c}	54.1^{a}	0.0275
	15	45.5^{b}	30.0^{d}	37.7^{c}	58.8^{a}	0.0232
	20	55.5^{d}	72.2^{c}	58.8^{b}	62.8^{a}	0.0211

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

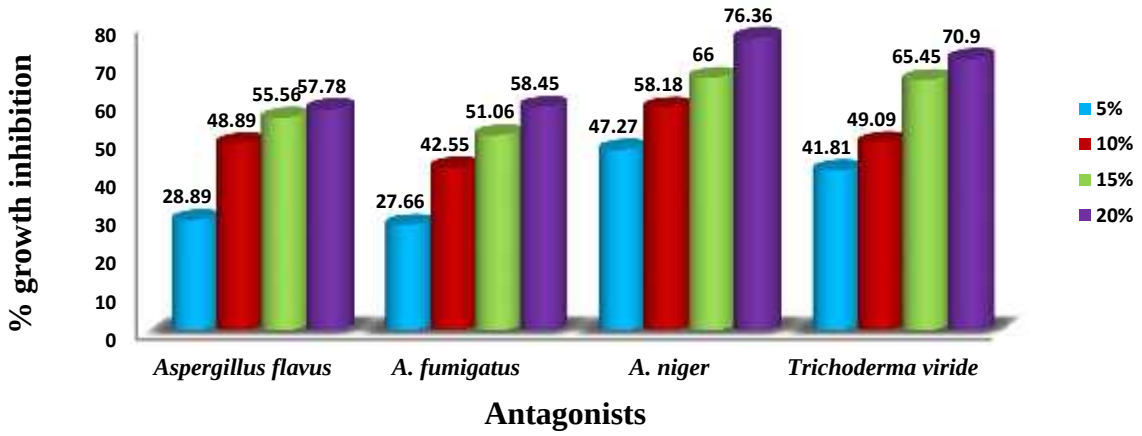

Fig. 25. Growth inhibition of *Colletotrichum gloeosporioides* owing to non-volatile metabolites of antagonists.

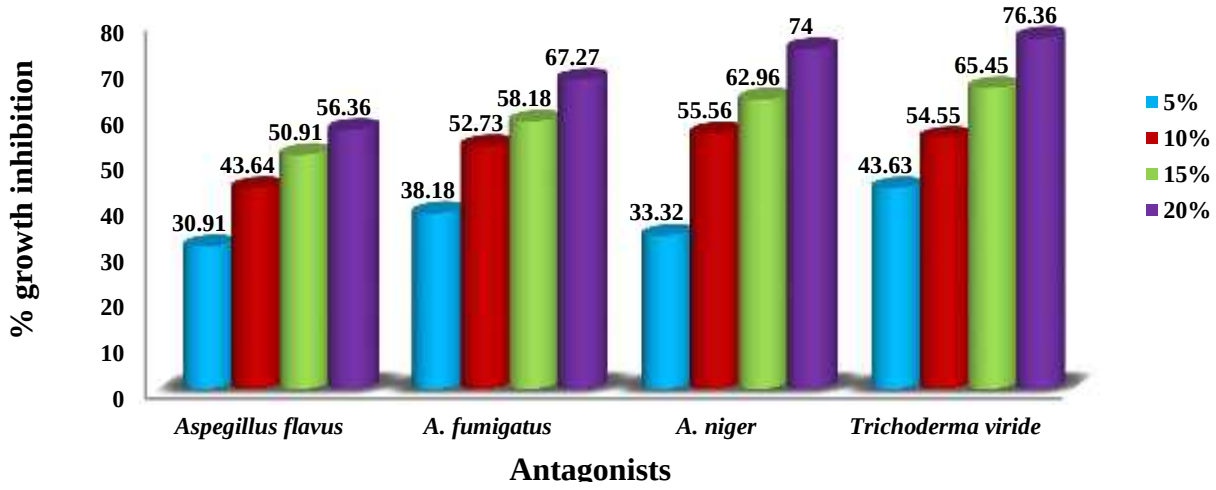

Fig. 26. Growth inhibition of *Curvularia lunata* owing to non-volatile metabolites of antagonists.

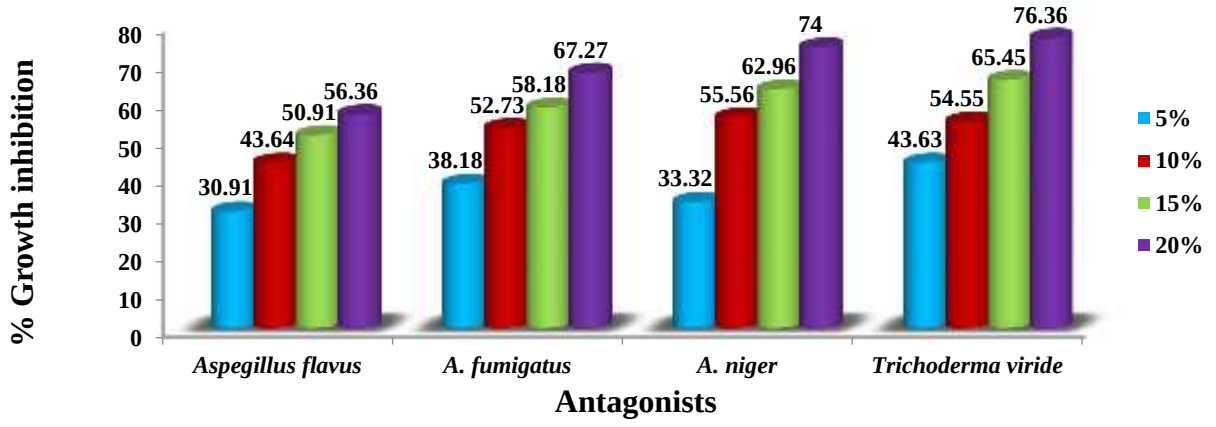

Fig. 27. Growth inhibition of *Fusarium moniliforme* owing to non-volatile metabolites of antagonists.

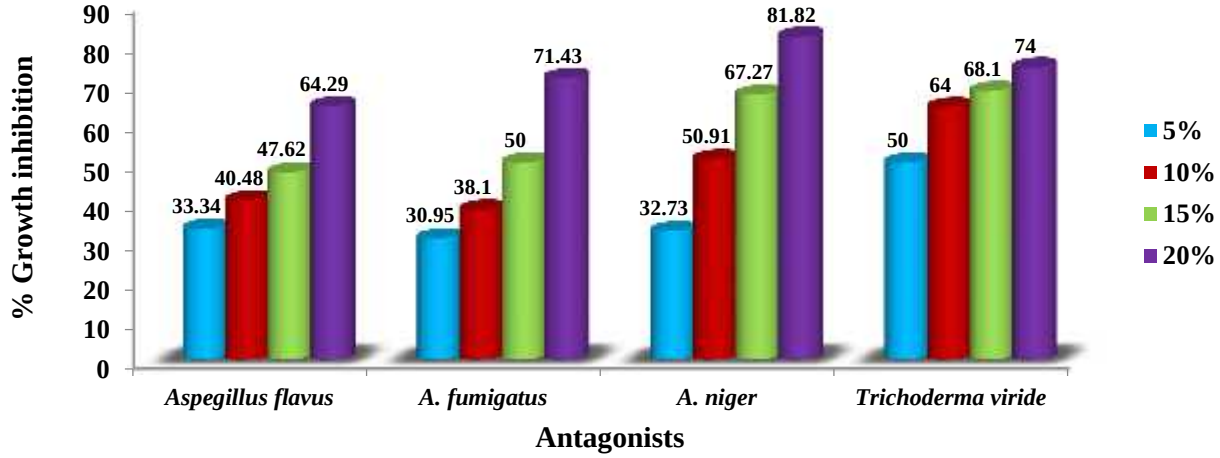

Fig. 28. Growth inhibition of *Fusarium nivale* owing to non-volatile metabolites of antagonists.

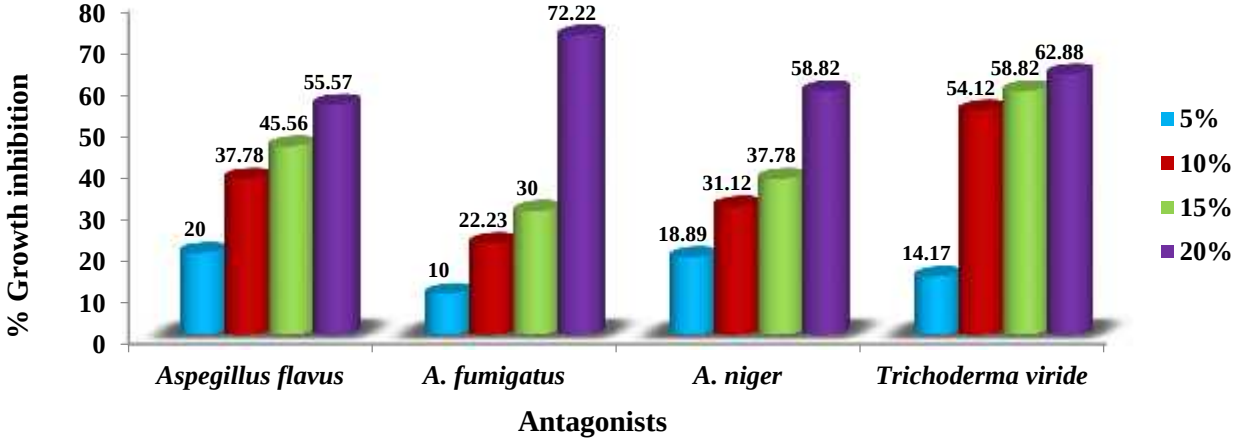

Fig. 29. Growth inhibition of *Rhizoctonia solani* owing to non-volatile metabolites of antagonistic fungi.

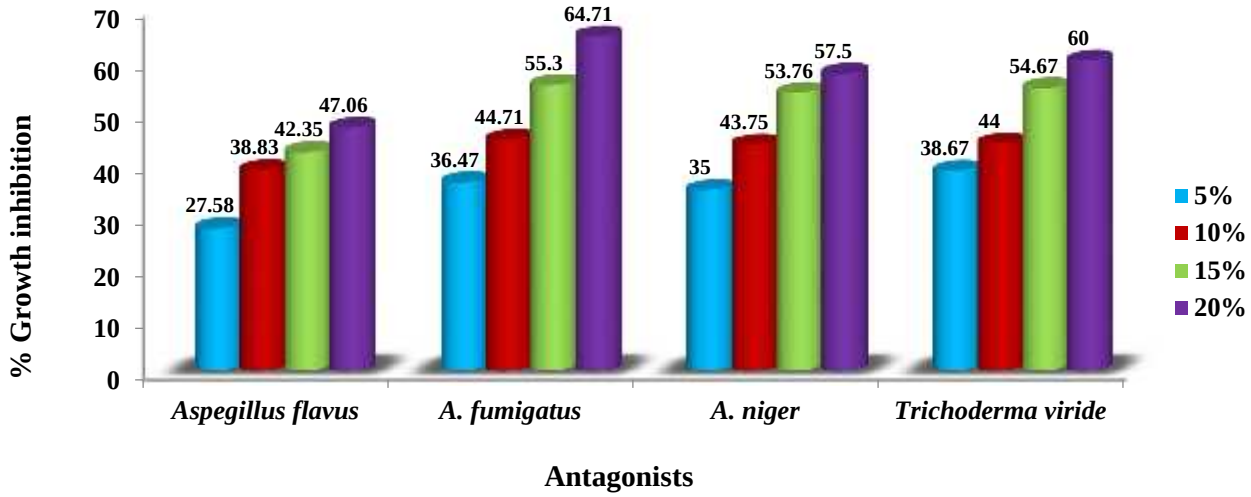

Fig. 30. Growth inhibition of *Mucor* sp. owing to non-volatile metabolites of antagonists.

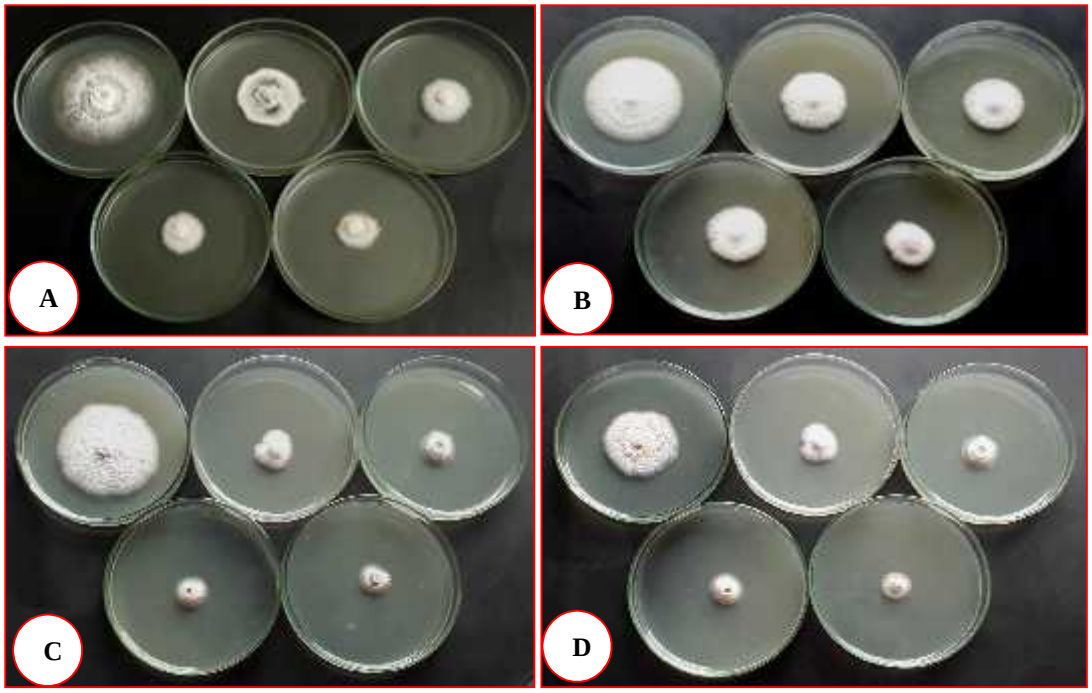

Plate 20. Growth inhibition of *Colletotrichum gloeosporioides* due to non-volatile metabolites of four antagonists at 5, 10, 15 and 20% concentrations.

A. *C. gloeosporioides*: *A. flavus*
B. *C. gloeosporioides*: *A. fumigatus*
C. *C. gloeosporioides: A. niger*
D. *C. gloeosporioides: T. viride*

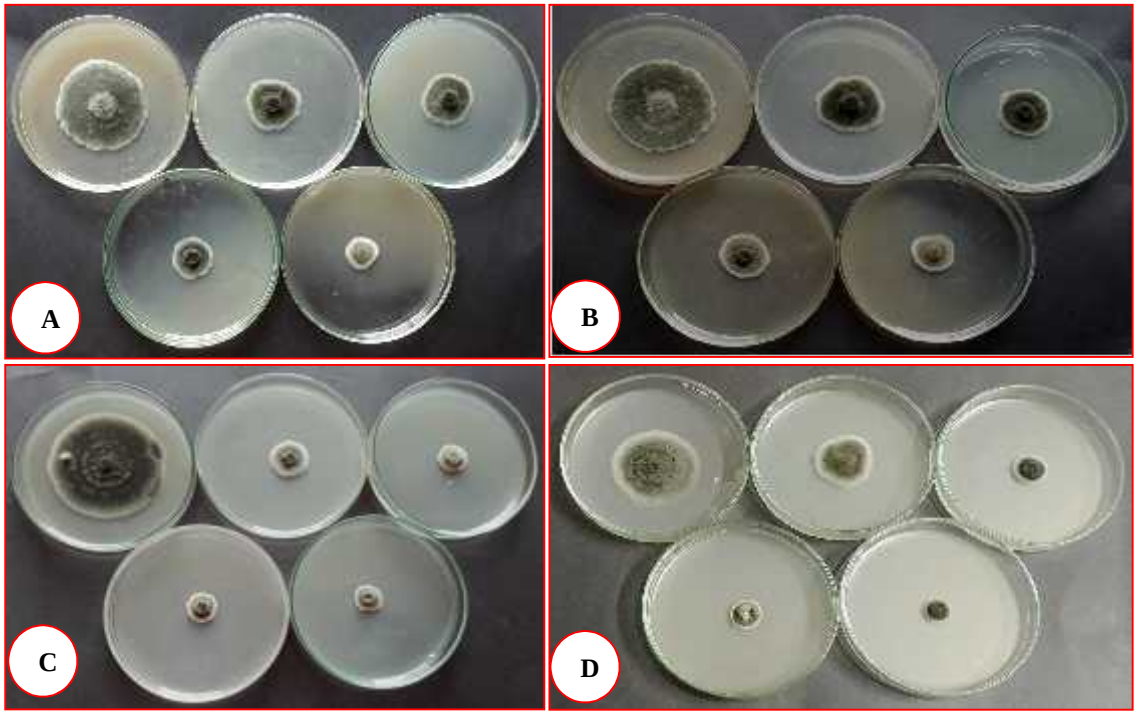

Plate 21. Growth inhibition of *Curvularia lunata* owing to non-volatile metabolites of four antagonists at 5, 10, 15 and 20% concentrations.

A. *C. lunata: A. flavus*
B. *C. lunata: A. fumigatus*
C. *C. lunata: A. niger*
D. *C. lunata: T. viride.*

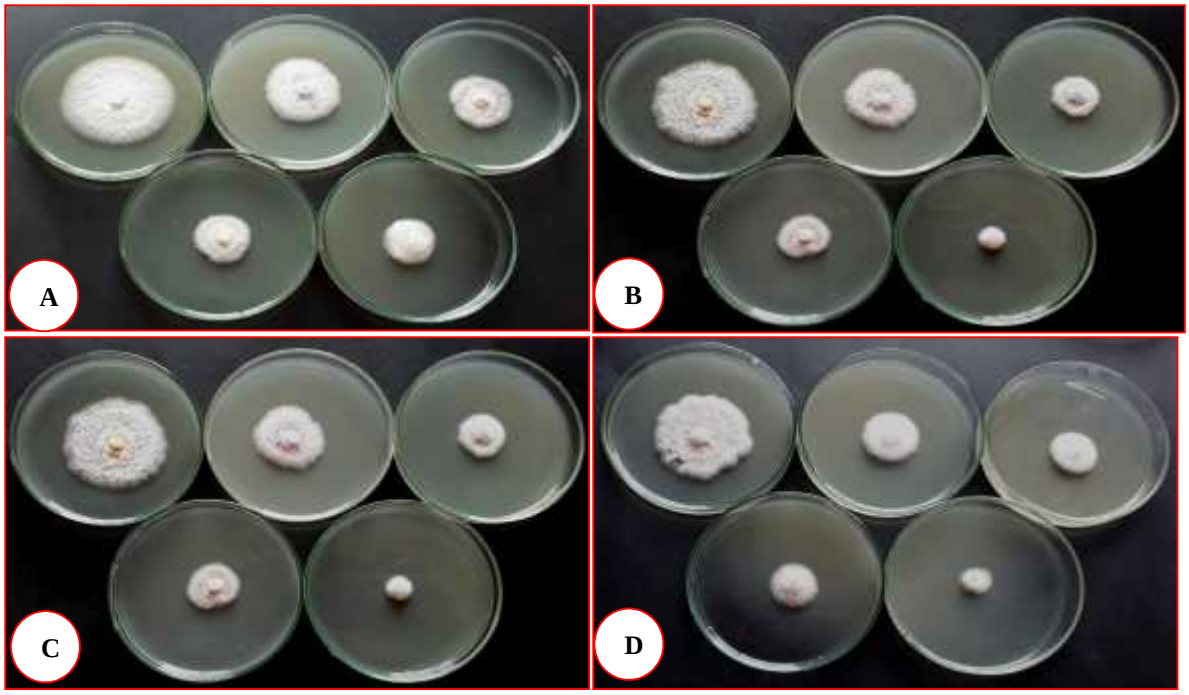

Plate 22. Growth inhibition of *Fusarium moniliforme* owing to non-volatile metabolites of four antagonists at 5, 10, 15 and 20% concentrations.

A. *F. moniliforme: A. flavus*
B. *F. moniliforme: A. fumigatus*
C. *F. moniliforme: A. niger*
D. *F. moniliforme: T. viride*

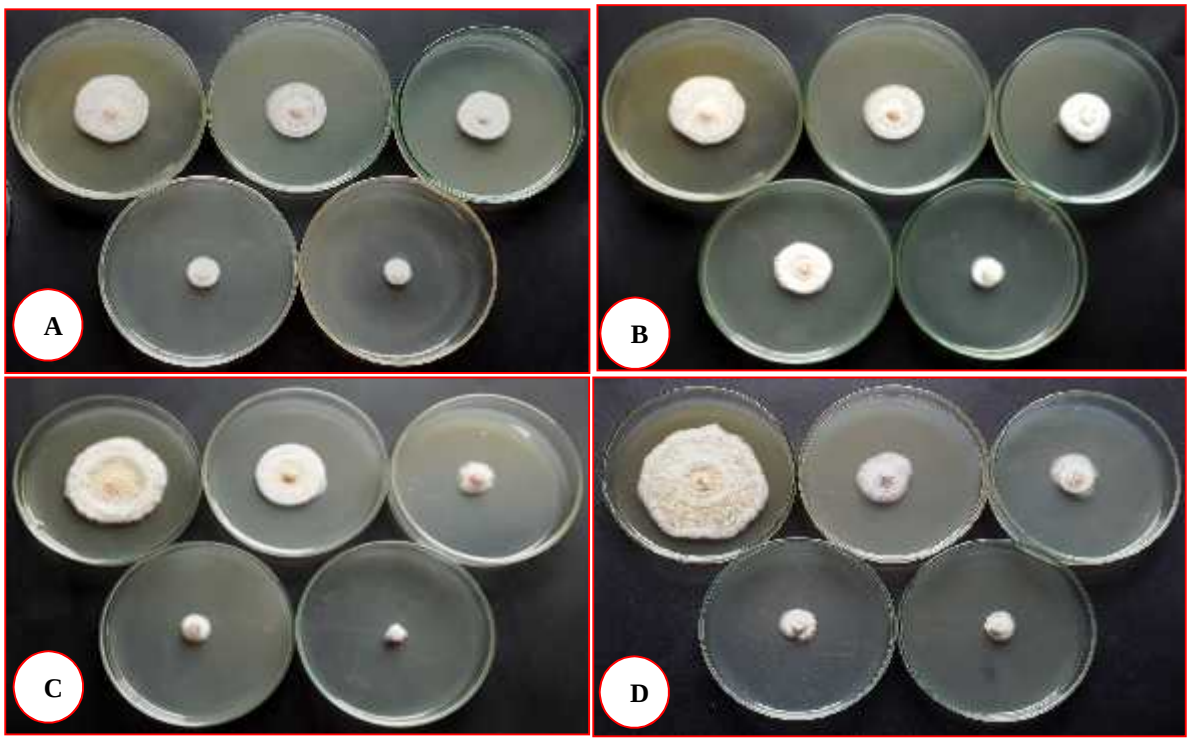

Plate 23. Growth inhibition of *Fusarium nivale* due to non-volatile metabolites of four antagonists at 5, 10, 15 and 20% concentrations.

A. *F. nivale*: *A. flavus*
B. *F. nivale*: *A. fumigatus*
C. *F. nivale*: *A. niger*
D. *F. nivale*: *T. viride.*

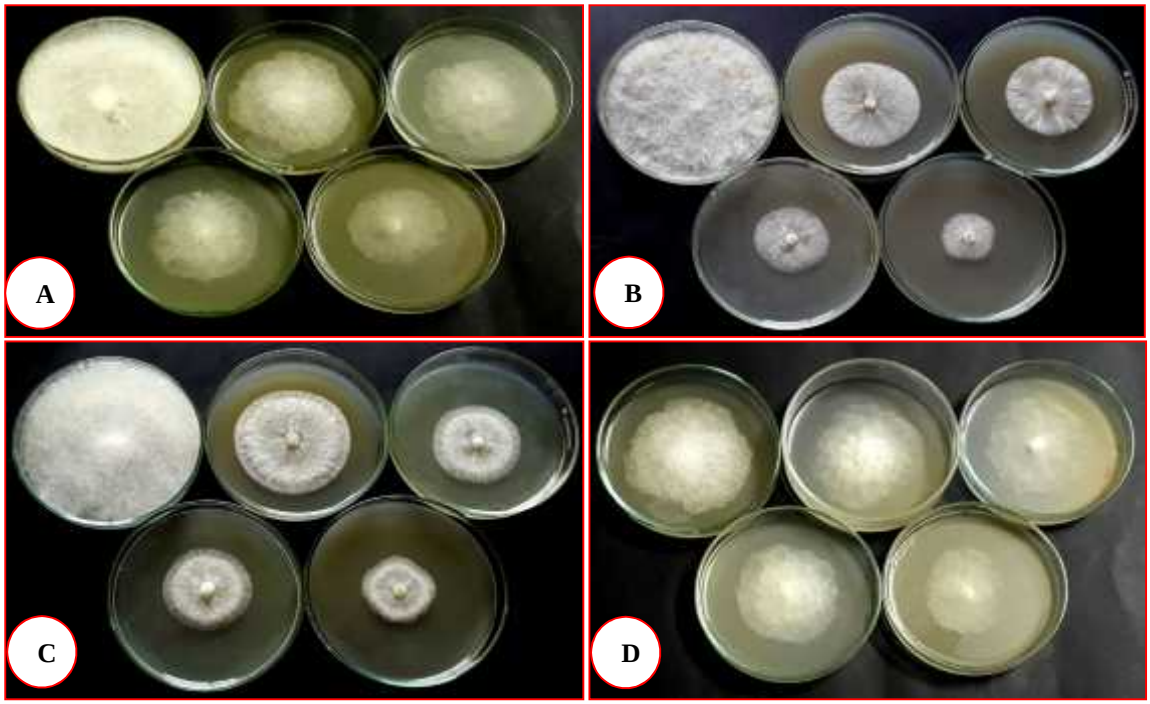

Plate 24. Growth inhibition of *Mucor* sp. owing to non-volatile metabolites of four antagonists at 5, 10, 15 and 20% concentrations.

A. *Mucor* sp.: *A. flavus*
B. *Mucor* sp.: *A. fumigatus*
C. *Mucor* sp.: *A. niger*
D. *Mucor* sp.: *T. viride*

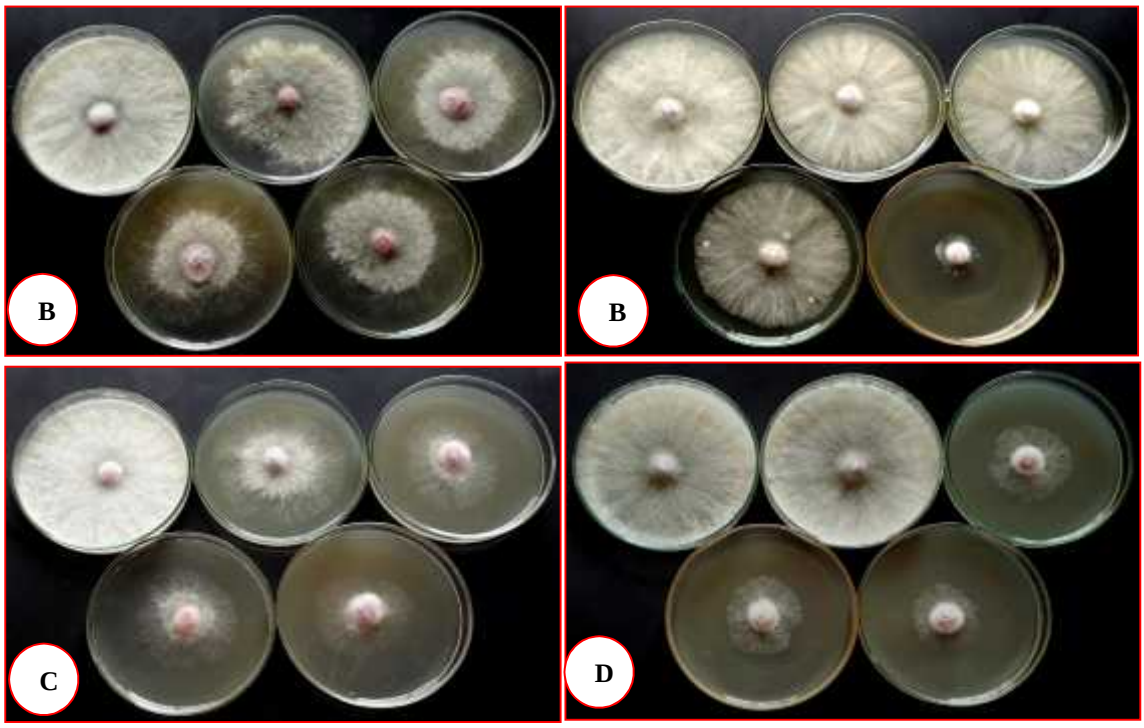

Plate 25. Growth inhibition of *Rhizoctonia solani* owing to non-volatile metabolites of four antagonists at 5, 10, 15 and 20% concentrations.

A. *R. solani*: *A. flavus*
B. *R. solani*: *A. fumigatus*
C. *R. solani*: *A. niger*
D. *R. solani*: *T. viride*

Management of Test Pathogens Associated with Seeds of Different Varieties of Cotton with Fungicides and Botanicals

4.16. Effects of fungicides against test pathogens

A total of twenty nine species of fungi were isolated and identified from the seeds of 14 varieties (CB 1- CB 14) of cotton. Out of 29 isolated fungi, nine showed positive results during pathogenicity test. They were *Aspergillus flavus*, *A. niger*, *A. tamarii*, *Colletotrichum gloeosporioides, Curvularia lunata, Fusarium nivale, F. moniliforme, Mucor* sp. and *Rhizoctonia solani.* These fungi were tried to control with selected fungicides hand plant extracts.

Amongst the 10 fungicides used in the present investigation, Contaf 5 EC, Knowin 50 WP, Dithane M-45, Score 250 EC and Silica 80 WG were systemic while Capvit 50 WP, Secure and Tilt 250 EC were protectant fungicides. Nativo 75 WP and Ridomil MZ Gold are both systemic as well as protectant fungicides. Complete inhibition of the test pathogens were observed with Contaf 5 EC and Tilt 250 EC at all the concentrations used. Their performance were best and other fungicides inhibited the radial growth of the test pathogens at different grades.

The details of these fungicides are given in Table 1 and the data with regard to their effect on the radial growth inhibition of *Aspergillus flavus, A. niger, Aspergillus tamarii, Colletotrichum gloeosporioides, Curvularia lunata, Fusarium nivale, F. moniliforme, Mucor* sp. and *Rhizoctonia solani* owing to 100, 200, 300, 400 and 500 ppm concentrations are presented in Tables 25-33, Figs 31-39 and Plates 26-27. All the fungicides inhibited the radial growth of the nine test pathogens. The extent of growth inhibition, however, varied amongst the test pathogens (Figs 31-39)

4.16.1. Effects of fungicides against *Aspergillus flavus*

Contaf 5 EC, Knowin 50 WP, Score 250 EC and Tilt 250 EC were responsible for complete inhibition of the radial growth of *Aspergillus flavus* at all the concentrations used. Capvit 50 WP and Nativo 75 WP also showed 100% inhibition at 500 ppm whereas Secure, Silica 80 WG, Dithane M 45 and Ridomil MZ Gold showed 86.67, 84.45, 73.34 and 73.33% inhibition, respectively at the same concentration. Capvit 50 WP and Nativo 75 WP at 400 ppm concentration also showed 100% growth inhibition (Table 25, Fig. 31 and Plates 26-27).

At 300 ppm concentration Capvit 50 WP, Contaf 5 EC, Knowin 50 WP, Score 250 EC and Tilt 250 EC showed 100% radial growth inhibition of *Aspergillus flavus* which was followed by Nativo 75 WP (77.78%), Silica 80 WG (75.56%), Secure (74.44%), Ridomil MZ Gold (68.89%) and Dithane M 45 (66.67%). Contaf 5 EC, Knowin 50 WP, Score 250 EC and Tilt 250 EC showed complete growth inhibition of the test pathogen at 200 ppm concentration that was followed by Nativo 75 WP (72.23%), Secure (72.22%), Silica 80 WG (71.11%), Dithane M 45 64.44%), Ridomil 68 WG (54.45%) and Capvit 50 WP (11.12%).

Contaf 5 EC, Knowin 50 WP, Score 250 EC and Tilt 250 EC showed complete inhibition of mycelial growth of *A. flavus* even at the lowest concentration (100 ppm) and it was followed by Nativo 75 WP (71.11%), Secure (61.11%), Dithane M 45 (52.23%), Ridomil MZ Gold (52.22%), Silica 80 WG (38.89%) and Capvit 50 WP (0.0%) (Table 25, Fig. 31 and Plates 26-27).

The toxicity of these fungicides against *A. flavus* at 100 ppm concentration in descending order was Contaf 5 EC / Knowin 50 WP / Score 250 EC / Tilt 250 EC > Nativo 75 WP > Secure > Dithane M 45 > Ridomil MZ Gold > Silica 80 WG > Capvit 50 WP (Table 25).

The present result is in accordance with the findings of Khatun and Shamsi (2016), who found that Bavistin 50 WP showed complete radial growth inhibition of *Aspergillus flavus* at all the tested concentrations. Similarly, Nahar and Shamsi (2020) also reported that the complete inhibition of the radial growth of *Aspergillus flavus* was observed with Autostin 50 WDG and Nativo 75 WP at all the treated concentrations. Rathod and Pawar (2013) also reported that the combination of Mancozeb and Cupravit 50 WP both at 0.4% significantly reduced the mycelial growth of *A. flavus* after seven days of observation.

4.16.2. Effects of fungicides against *Aspergillus niger*

Amongst the 10 fungicides, Contaf 5 EC, Dithane M 45, Knowin 50 WP, Nativo 75 WP and Tilt 250 EC at different concentrations showed 100% inhibition of the radial growth of *A. niger*. Ridomil MZ Gold, Score 250 EC and Secure also showed 100% inhibition whereas, Silica 80 WG, Capvit 50 WP showed 72.23% and 66.67% inhibition, respectively at 500 ppm concentration (Table 26, Fig. 32 and Plates 26-27).

Ridomil MZ Gold and Score 250 EC also inhibited the fungal growth completely (100%) at 400 ppm concentration which was followed by Secure (91.12%), Silica 80 WG (66.67%) and Capvit 50 WP (61.11%). Ridomil MZ Gold and Score 250 EC showed complete inhibition of mycelial growth of *A. niger* at 300 ppm concentration which was followed by Secure (80.0%), Silica 80 WG (61.11%) and Capvit 50 WP (44.46%).

At 200 ppm concentration, Score 250 EC showed complete growth inhibition (100%) which was followed by Secure (77.78%), Ridomil MZ Gold (55.56%), Silica 80 WG (44.45%), and Capvit 50 WP (22.23%). At 100 ppm concentration, Score 250 EC showed highest (97.23%) growth inhibition which was followed by Secure (66.67%), Ridomil MZ Gold (42.22%), Capvit 50 WP (11.14%) and Silica 80 WG (11.12%) (Table 26, Fig. 32 and Plates 26-27).

The toxicity of these fungicides against *A. niger* at 100 ppm concentration in descending order was Contaf / Dithane M 45 / Knowin 50 WP / Nativo 75 WP / Tilt 250 EC > Score 250 EC > Secure > Ridomil MZ Gold > Capvit 50 WP > Silica 80 WG (Table 26).

The results of the present study are in agreement with the result of Hosen and Shamsi (2017), where they reported Bavistin DF and Tilt 250 EC found to be the best inhibiting fungicides against *A. niger*. Similarly, Nahar and Shamsi (2020) found that the complete inhibition of the radial growth of *Aspergillus niger* was observed with Autostin 50 WDG and Nativo 75 WP at all the tested concentrations. Rathod and Pawar (2013) also reported that the combination of Mancozeb and Cupravit 50 WP both at 0.4% significantly reduced the mycelial growth of *A. niger* after seven days of observation.

4.16.3. Effects of fungicides against *Aspergillus tamarii*

Contaf 5 EC, Knowin 50 WP, Score 250 EC and Tilt 250 EC completely inhibited the radial growth of *Aspergillus tamarii* at all the concentrations used in the present study. Capvit 50 WP and Secure also inhibited the radial growth of the fungus completely at 500 ppm whereas Silica 80 WG, Nativo 75 WP, Dithane M 45 and Ridomil MZ Gold showed 91.11, 89.90, 76.68 and 76.67% growth inhibition at the same concentration (Table 27, Fig. 33 and Plates 26-27).

At 400 ppm concentration Capvit 50 WP showed 100% radial growth inhibition of *A. tamarii* which was followed by Secure (93.34%), Silica 80 WG (88.89%), Nativo 75 WP (83.33%), Ridomil MZ Gold (70.0%) and Dithane M 45 (66.67%). Capvit 50 WP inhibited the radial growth of the fungus completely at 300 ppm concentration whereas secure, Nativo 75 WP, Dithane M 45, Ridomil MZ Gold and Silica 80 WG showed 88.89, 77.78, 63.33, 57.78 and 55.56% growth inhibition at the same concentration.

Capvit 50 WP showed 91.10% growth inhibition of the tested pathogen at 200 ppm concentration that was followed by Secure (85.56%), Nativo 75 WP (74.44%), Dithane M 45

(61.11%), Ridomil MZ Gold (55.56%) and Silica 80 WG (11.11%). Contaf 5 EC, Knowin 50 WP, Score 250 EC. Tilt 250 EC showed complete inhibition of mycelial growth of *A. tamarii* even at the lowest concentration (100 ppm) and it was followed by Capvit 50 WP (89.67%), Secure (83.33), Nativo 75 WP (66.67%), Dithane M 45 (55.56%), Ridomil MZ Gold (33.34%) (Table 27, Fig. 33 and Plates 26-27).

The toxicity of these fungicides against *A. tamarii* at 100 ppm concentration in descending order was Contaf 5 EC / Score 250 EC / Tilt 250 EC / Knowin 50 WP > Capvit 50 WP > Secure > Nativo 75 WP > Dithane M 45 > Ridomil MZ Gold > Silica 80 WG (Table 27).

4.16.4. Effects of fungicides against *Colletotrichum gloeosporioides*

The complete inhibition of radial growth of *Colletotrichum gloeosporioides* was observed with Contaf 5 EC, Knowin 50 WP, Nativo 75 WP and Tilt 250 EC at all the tested concentrations. Capvit 50 WP and Score 250 EC also showed 100% inhibition of growth at 400 and 500 ppm concentrations whereas Dithane M-45, Ridomil MZ Gold and Secure showed 72.5, 65.0 and 77.58% inhibition at 500 ppm concentration, respectively (Table 28, Fig. 34 and Plates 26-27).

At 400 ppm concentration, Capvit 50 WP, Score 250 EC and Contaf 5 EC also inhibited the fungal growth completely (100%) which was followed by Silica 80 WG (68.57%), Secure (67.93%), Dithane M 45 (67.50%) and Ridomil MZ Gold (62.50%). Score 250 EC showed highest inhibition (84.89%) of mycelial growth of *C. gloeosporioides* at 300 ppm concentration which was followed by Capvit 50 WP (68.29%), Secure (64.15%), Silica 80 WG (62.87%), Dithane M 45 (62.50%) and Ridomil MZ Gold (50.0%).

At 200 ppm concentration, Score 250 EC showed highest growth inhibition (83.02%) which was followed by Capvit 50 WP (65.85%), Secure (60.38%), Dithane M 45 (50.0%), Silica 80 WG (42.86%) and Ridomil MZ Gold (42.50%). At 100 ppm concentration, Score 250 EC showed highest (81.13%) growth inhibition which was followed by Capvit 50 WP (51.22%),

Dithane M 45 (45.0%), Secure (43.40%), Ridomil MZ Gold (37.50%) and Silica 80 WG (34.29%) (Table 28, Fig. 34 and Plates 26-27).

The toxicity of these fungicides against *C. gloeosporioides* at 100 ppm concentration in descending order was Contaf 5 EC / Knowin 50 WP / Nativo 75 WP / Tilt 250 EC > Score 250 EC > Capvit 50 WP > Dithane M 45 > Secure > Ridomil MZ Gold > Silica 80 WG (Table 28).

The present findings are in accordance with the results reported by Helal and Shamsi (2018) where they found complete inhibition of the radial growth of *C. gloeosporioides* was observed with Autostin 50 WDG and Tilt 250EC at all the tested concentrations. Dithane M 45 and Ridomil inhibited the conidial germination of *C. gloeosporioides* reported by Imtiaj *et al.* (2005). This findings also supported the results obtained in the present investigation.

Shamsi *et al.* (2014) observed that the radial growth of *C. gloeosporioides* isolated from *Senna alata* completely inhibited by Tilt 25 EC at all the concentrations examined. Bavistin checked the growth of *C. gloeosporioides*, the causal agent of anthracnose of *Mangifera indica* L., completely at 100 ppm according to Sharma and Verma (2007).

Hosen *et al.* (2016) found complete inhibition of the radial growth of *C. gloeosporioides* with Bavistin DF, Greengel 72 WP and Tilt 250 EC at 100, 200 and 400 ppm, respectively. The present results are in agreement with the findings of the above mentioned workers.

Table 25. Effects of fungicides against *Aspergillus flavus* at different concentrations.

Fungicides	% growth inhibition at different concentrations (ppm)				
	100	**200**	**300**	**400**	**500**
Capvit 50 WP	0^{f}	11.12^{f}	100^{a}	100^{a}	100^{a}
Contaf 5 EC	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Dithane M 45	52.23^{d}	64.44^{d}	66.67^{f}	70.0^{d}	73.34^{d}
Knowin 50 WP	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Nativo 75 WP	71.11^{b}	72.23^{b}	77.78^{b}	100^{a}	100^{a}
Ridomil MZ Gold	52.22^{d}	54.45^{e}	68.89^{e}	70.0^{d}	73.33^{d}
Score 250 EC	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Secure	61.11^{c}	72.22^{b}	74.44^{d}	77.78^{c}	86.67^{b}
Silica 80 WG	38.89^{e}	71.11^{c}	75.56^{c}	82.22^{b}	84.45^{c}
Tilt 250 EC	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
CV (%)	0.0942	0.0855	0.0823	0.0913	0.0847

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

Table 26. Effects of fungicides against *Aspergillus niger* at different concentrations.

Fungicides	% growth inhibition at different concentrations (ppm)				
	100	**200**	**300**	**400**	**500**
Capvit 50 WP	11.14^{e}	22.23^{e}	44.46^{d}	61.11^{d}	66.67^{c}
Contaf 5 EC	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Dithane M 45	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Knowin 50 WP	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Nativo 75 WP	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Ridomil MZ Gtold	42.22^{d}	55.56^{c}	100^{a}	100^{a}	100^{a}
Score 250 EC	97.23^{b}	100^{a}	100^{a}	100^{a}	100^{a}
Secure	66.67^{c}	77.78^{b}	80.0^{b}	91.12^{b}	100^{a}
Silica 80 WG	11.12^{e}	44.45^{d}	61.11^{c}	66.67^{c}	72.23^{b}
Tilt 250 EC	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
CV (%)	0.0976	0.0971	0.0971	0.0912	0.0954

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

Table 27. Effects of fungicides against *Aspergillus tamarii* at different concentrations.

Fungicides	% growth inhibition at different concentrations (ppm)				
	100	**200**	**300**	**400**	**500**
Capvit 50 WP	89.67[b]	91.10[b]	100[a]	100[a]	100[a]
Contaf 5 EC	100[a]	100[a]	100[a]	100a	100[a]
Dithane M 45	55.56[e]	61.11[e]	63.33[d]	66.67[f]	76.68[d]
Knowin 50 WP	100[a]	100[a]	100[a]	100[a]	100[a]
Nativo 75 WP	66.67[d]	74.44[d]	77.78[c]	83.33[d]	89.90[c]
Ridomil MZ Gold	33.34[f]	55.56[f]	57.78[e]	70.0[e]	76.67[d]
Score 250 EC	100[a]	100[a]	100[a]	100[a]	100[a]
Secure	83.33[c]	85.56[c]	88.89[b]	93.34[b]	100[a]
Silica 80 WG	0[g]	11.11[g]	55.56[f]	88.89[c]	91.11[b]
Tilt 250 EC	100[a]	100[a]	100[a]	100[a]	100[a]
CV (%)	0.0873	0.2034	0.0843	0.0815	0.0832

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

Table 28. Effects of fungicides against *Colletotrichum gloeosporioides* at different concentrations.

Fungicides	% growth inhibition at different concentrations (ppm)				
	100	**200**	**300**	**400**	**500**
Capvit 50 WP	51.22[c]	65.85[c]	68.29[c]	100[a]	100[a]
Contaf 5 EC	100[a]	100[a]	100[a]	100[a]	100[a]
Dithane M 45	45.0[d]	50.0[e]	62.5[f]	67.5[d]	72.5[c]
Knowin 50 WP	100[a]	100[a]	100[a]	100[a]	100[a]
Nativo 75 WP	100[a]	100[a]	100[a]	100[a]	100[a]
Ridomil MZ Gold	37.50[f]	42.50[g]	50.0[g]	62.50[e]	65.0[d]
Score 250 EC	81.13[b]	83.02[b]	84.91[b]	100[a]	100[a]
Secure	43.40[e]	60.38[d]	64.15[d]	67.93[c]	77.58[b]
Silica 80 WG	34.29[g]	42.86[f]	62.87[e]	68.57[b]	100[a]
Tilt 250 EC	100[a]	100[a]	100[a]	100[a]	100[a]
CV (%)	0.1062	0.0893	0.0839	0.0897	0.0979

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

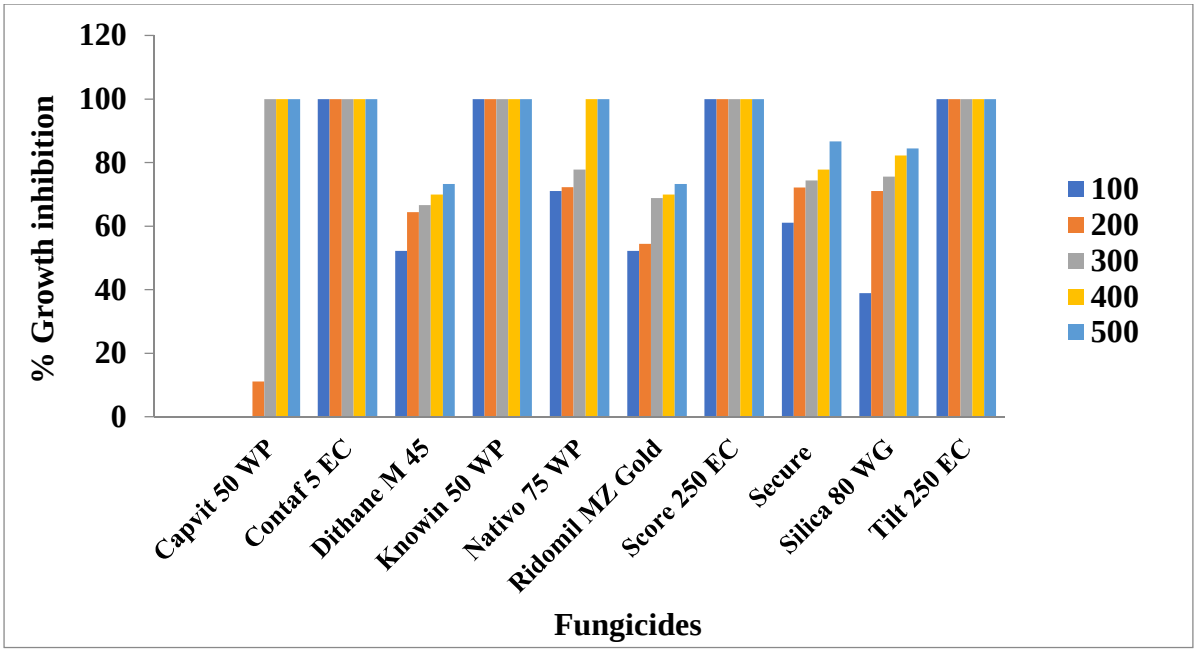

Fig. 31. Per cent growth inhibition of *Aspergillus flavus* at different concentrations of fungicides.

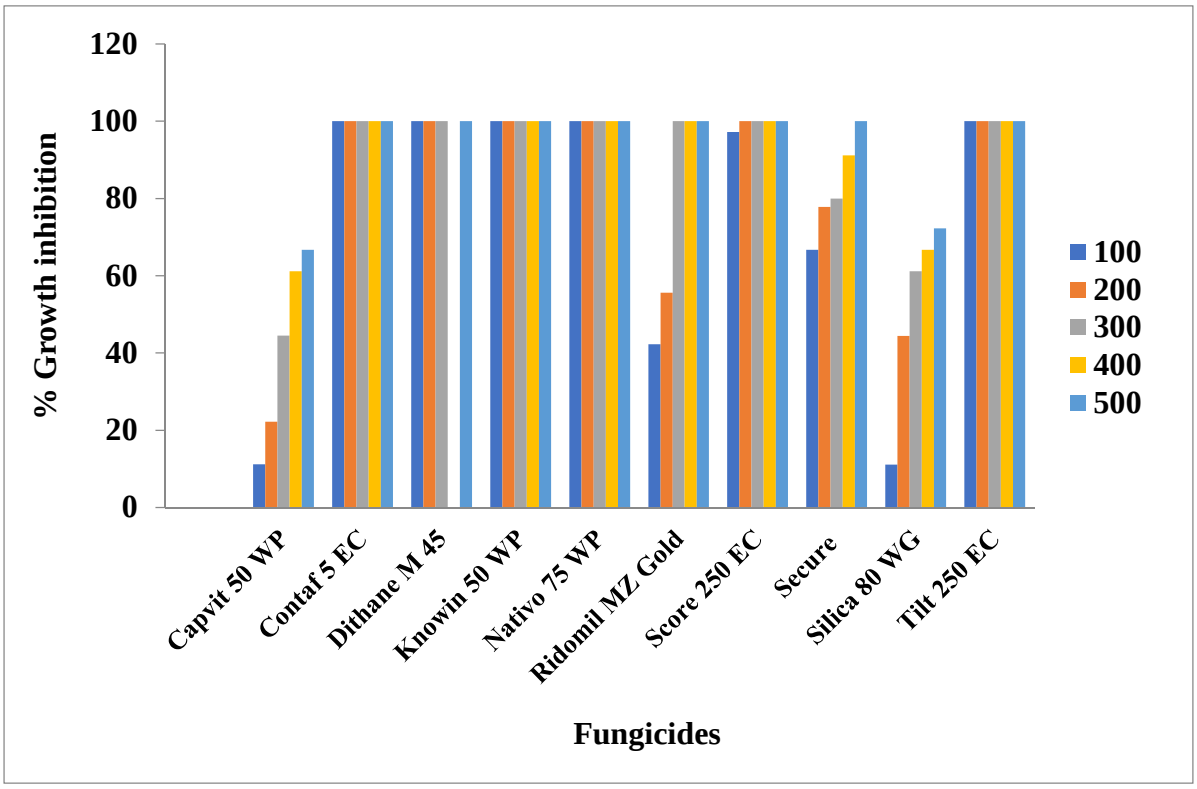

Fig. 32. Per cent growth inhibition of *Aspergillus niger* at different concentrations of fungicides.

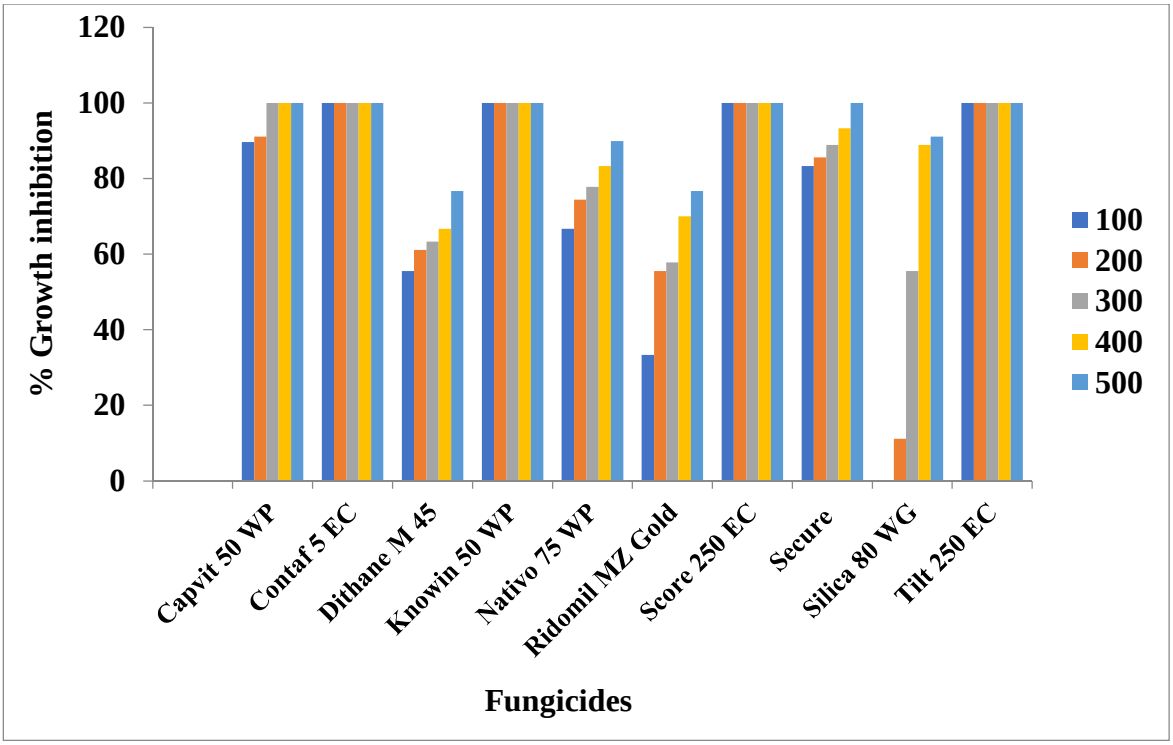

Fig. 33. Per cent growth inhibition of *Aspergillus tamarii* at different concentrations of fungicides.

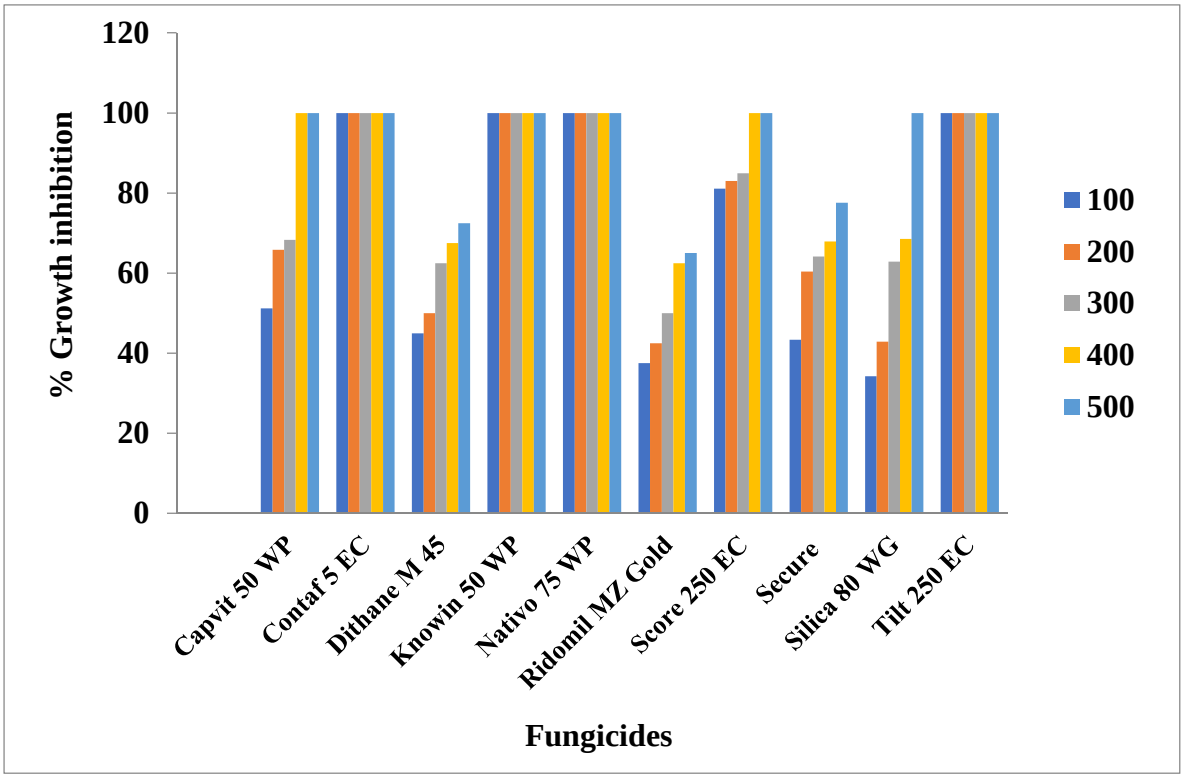

Fig. 34. Per cent growth inhibition of *Colletotrichum gloeosporioides* at different concentrations of fungicides.

4.16.5. Effects of fungicides against *Curvularia lunata*

Growth of *Curvularia lunata* was completely inhibited with Contaf 5 EC, Dithane M-45, Nativo 75 WP, Score 250 EC, Silica 80 WG and Tilt 250 EC at all the concentrations used. Ridomil MZ Gold and Secure showed 100% inhibition whereas, Capvit 50 WP and Knowin 50 WP inhibited 88.57 and 85.72% growth of the fungus at 500 ppm concentration (Table 29, Fig. 35 and Plates 26-27).

Ridomil MZ Gold also showed complete inhibition at 400 ppm concentration which was followed by Capvit 50 WP (85.72%), Secure (85.46%) and Knowin 50 WP (82.86%). Capvit 50 WP showed highest inhibition (84.26%) against the radial growth of *C. lunata* at 300 ppm concentrations which was followed by Secure (81.82%), Knowin 50 WP (81.43% and Ridomil MZ Gold (60.0%). At 200 ppm concentration, Capvit 50 WP showed maximum (82.86%) inhibition of the fungus followed by Secure (80.0%), Knowin 50 WP (75.71%) and Ridomil MZ Gold (56.0%). The lowest activity (36.0%) was shown by Ridomil MZ Gold at 100 ppm concentration (Table 29, Fig. 35 and Plates 26-27).

The toxicity of these fungicides against *C. lunata* at 100 ppm concentration in descending order was Contaf 5 EC / Dithane M 45 / Nativo 75 WP / Score 250 EC / Silica 80 WG / Tilt 250 EC > Capvit 50 WP > Knowin 50 WP > Secure > Ridomil MZ Gold (Table 29).

Chowdhury *et al*. (2015) and Sultana (2021) observed higher mycelial growth inhibition of *Curvularia lunata* due to Bavistin 50 WP and Tilt 250EC which is in agreement with the present findings. Nahar and Shamsi (2020) observed that the radial growth of *Curvularia lunata* was completely inhibited by Nativo 75 WP at all the treated concentrations which is fully supported the results obtained in the present investigation.

Similarly, Khatun and Shamsi (2016) observed that Bavistin 50 WP showed complete radial growth inhibition of *Curvularia lunata* at 400 and 500 ppm concentrations. 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. Besides, Mamun *et al.* (2016) reported that Dithane and Tilt completely inhibited the growth of *C. lunata* at 100, 200, 400 and 500 ppm concentrations. But Dithane showed complete inhibition of *C. lunata* at all the treated concentration in the present research work.

4.16.6. Fungitoxicity of fungicides against *Fusarium nivale*

The complete inhibition of radial growth of *Fusarium nivale* was observed with Contaf 5 EC, Dithane M 45, Knowin 50 WP, Nativo 75 WP, Score 250 EC and Tilt 250 EC at all the concentrations tested. Here, all the fungicides showed 100% inhibition of growth at 500 ppm concentrations except Secure which showed 82.61% inhibition (Table 30, Fig. 36 and Plates 26-27).

All the fungicides also showed complete growth inhibition at 400 ppm concentration except Capvit 50 WP (84.62%), Secure (80.43%) and Silica 80 WG (75.10%). At 300 ppm concentration, Capvit 50 WP showed maximum inhibition (81.54%) followed by Ridomil MZ Gold (75.0%), Secure (73.91%) and Silica 80 WG (67.50%). Capvit 50 WP showed maximum inhibition (80.0%) at 200 ppm concentration followed by Ridomil MZ Gold (72.5%), Secure (65.22%) and Silica 80 WG (62.50%). Lowest activity was shown by Ridomil MZ Gold at 100 ppm and that was 42.50% (Table 30, Fig. 36 and Plates 26-27).

The toxicity of these fungicides against *F. nivale* at 100 ppm concentration in descending order was Contaf 5 EC / Dithane M 45 / Knowin 50 WP / Nativo 75 WP / Score 250 EC / Tilt 250 EC > Capvit 50 WP > Secure > Silica 80 WG > Ridomil MZ Gold (Table 30).

The results of the present investigation are in agreement with the result of Helal and Shamsi (2018) where they found that Tilt 250 EC and Autostin 50 WDG (50% Carbendazim) was responsible for complete inhibition of *F. nivale* at all the concentrations tested. In contrast to

present study Phillip *et al*. (2003) reported that combination of Mancozeb (Dithane M 45) up to ten days prior to planting can control *Fusarium* decay of seeds. Fravel *et al*. (2005) also reported the activity of Mancozeb (Dithane M 45) and Cupravit against the mycelial growth of *Fusarium*. Similarly, Hosen and Shamsi (2017) observed that Bavistin DF and Tilt 250 EC found to be the best inhibiting fungicides against *Fusarium merismoides* at all the tested concentrations which is fully supported the present result.

4.16.7. Effects of fungicides against *Fusarium moniliforme*

Contaf 5 EC, Nativo 75 WP and Tilt 250 EC completely inhibited the radial growth of *Fusarium moniliforme* at all the concentrations used in the present study. Capvit 50 WP, Dithane M 45, Ridomil MZ Gold, Score 250 EC and Silica 80 WG also inhibited the radial growth of the fungus completely at 500 ppm whereas Secure and Knowin 50 WP showed 84.62 and 80.0% growth inhibition in the same concentration (Table 31, Fig. 37 and Plates 26-27).

At 400 ppm concentration Score 250 EC showed highest inhibition (86.67%) against the radial growth of *Fusarium moniliforme* which was followed by **S**ecure (81.54%), Silica 80 WG (78.0%), Dithane M 45 (72.97%), Knowin 50 WP (72.86%), Ridomil MZ Gold (72.10%) and Capvit 50 WP (68.57%). Score 250 EC showed maximum radial growth inhibition (85.0%) at 300 ppm concentration followed by Secure (72.31%), Silica 80 WG (71.88%), Dithane M 45 (70.27%), Knowin 50 WP (70.0%), Capvit 50 WP (64.29%) and Ridomil MZ Gold (60.47%). At 100 ppm Silica 80 WG showed the lowest inhibition and it was 43.75% (Table 31, Fig. 37 and Plates 26-27).

The toxicity of these fungicides against *F. moniliforme* at 100 ppm concentration in descending order was Contaf 5 EC / Nativo 75 WP / Tilt 250 EC > Score 250 EC > Knowin 50 WP > Capvit 50 WP > Dithane M 45 > Secure > Ridomil MZ Gold > Silica 80 WG (Table 31).

The results of the present study are in accordance with the findings of Nahar and Shamsi (2020) who observed the complete inhibition of *Fusarium moniliforme* var. *subglutinans* with Autostin 50 WDG and Nativo 75 WP at all the treated concentrations. Similarly, Chowdhury *et al.* (2015) observed the complete inhibition of *Fusarium moniliforme* associated with rice grains *in vitro* with different concentrations of Tall 25 EC or Tilt 250 EC.

Helal and Shamsi (2018) also reported that Tilt 250 EC showed complete growth inhibition of *Fusarium* sp. at 300, 400 and 500 ppm. Previously it was reported that Bavistin, Dithane, Contaf, Cupravit and Benlate inhibited the radial growth of *Fusarium* spp. (Fravel *et al*. 2005, Iqbal *et al*. 2010 and Mamun *et al*. 2016). Rathod and Pawar (2013) found that the combination of Dithane and Cupravit at 0.4% significantly reduce the mycelial growth of *Fusarium* spp.

Mohana *et al*. (2011) mentioned that Bavistin / Knowin perform better mycelial growth inhibition of *F*. *moniliforme.* Here we found 80% growth inhibition of *F*. *moniliforme* by Knowin at 500 ppm concentration. Muthomi *et al*. (2007) reported that Copper oxychloride (Capvit) completely inhibit the growth of *Fusarium graminearum* in *in vitro* condition. But in present investigation, capvit show complete growth inhibition of *F*. *moniliforme* at 500 ppm concentration only.

Hossain *et al.* (2015) noticed the efficacy of Bavistin, Sunphanate, Nativo and Carzeb which completely inhibited *Fusarium moniliforme in vitro.* 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. All these findings are very much similar with the findings of the present investigation.

4.16.8. Effects of fungicides against *Mucor* sp.

Contaf 5 EC, Nativo 75 WP and Tilt 250 EC were responsible for complete inhibition of the radial growth of *Mucor* sp. at all the tested concentrations. Capvit 50 WP, Knowin 50 WP and Score 250 EC also showed 100% inhibition at both 400 ppm and 500 ppm concentration whereas Silica 80 WG and Ridomil MZ Gold showed only 50.0 and 33.34% inhibition, respectively at 500 ppm concentration.

At 400 ppm concentration, Secure showed 82.86%, Silica 80 WG showed 41.11%, Dithane M 45 showed 38.89% and Ridomil MZ Gold showed 27.78% growth inhibition. At 300 ppm concentration, Knowin 50 WP showed highest inhibition (93.34%) which was followed by Score 250 EC (91.11%), Capvit 50 WP (74.45%), Secure (64.29%), Silica 80 WG (38.89%) and Dithane M 45 (33.34%). Lowest activity was shown by Silica 80 WG (24.45%) whereas, Dithane M 45 and Ridomil MZ Gold showed no inhibition at 100 ppm concentration (Table 32, Fig. 38 and Plates 26-27).

The toxicity of these fungicides against *Mucor* sp. at 100 ppm concentration in descending order was Contaf 5 EC / Nativo 75 WP / Tilt 250 EC > Score 250 EC > Capvit 50 WP > Knowin 50 WP > Secure > Silica 80 WG > Dithane M 45 / Ridomil MZ Gold (Table 32).

4.16.9. Effects of fungicides against *Rhizoctonia solani*

Amongst the 10 fungicides, Contaf 5 EC, Dithane M 45, Knowin 50 WP, Nativo 75 WP, Ridomil MZ GOLD and Tilt 250 EC at different concentrations showed 100% inhibition of the radial growth of *Rhizoctonia solani*. Capvit 50 WP, Score 250 EC, Secure and Silica 80 WG showed 100% inhibition at 400 and 500 ppm concentration. Capvit 50 WP also inhibited the fungal growth completely at 300 ppm concentration.

At 300 ppm concentration Score 250 EC, Secure and Silica 80 WG showed 84.29, 72.22 and 78.89 radial growth inhibitions, respectively. At 100 ppm concentration Secure showed lowest

inhibion 53.50% whereas Silica 80 WG showed no inhibition at 100 ppm concentration (Table 33, Fig. 39 and Plates 26-27).

The toxicity of these fungicides against *R. solani* at 100 ppm concentration in descending order was Contaf 5 EC / Dithane M 45 / Knowin 50 WP / Nativo 75 WP / Ridomil MZ Gold / Tilt 250 EC > Score 250 EC > Capvit 50 WP > Secure > Silica 80 WG (Table 33).

Nahar and Shamsi (2020) reported that, in *Rhizoctonia solani* the complete inhibition was observed with Autostin 50 WDG / Knowin 50 WP and Nativo 75 WP at all the treated concentrations which is in accordance with the present study. Similarly, 15gm Nativo per acre land was enough to complete control of *Rhizoctonia solani* according to Shahid *et al.* (2014). The present investigation was similar with aforementioned experiment.

Table 29. Effects of fungicides against *Curvularia lunata* at different concentrations.

Fungicides	% growth inhibition at different concentrations (ppm)				
	100	**200**	**300**	**400**	**500**
Capvit 50 WP	81.43[b]	82.86[b]	84.26[b]	85.72[b]	88.57[b]
Contaf 5 EC	100[a]	100[a]	100[a]	100[a]	100[a]
Dithane M 45	100[a]	100[a]	100[a]	100[a]	100[a]
Knowin 50 WP	64.26[c]	75.71[d]	81.43[d]	82.86[d]	85.72[c]
Nativo 75 WP	100[a]	100[a]	100[a]	100[a]	100[a]
Ridomil MZ Gold	36.0[e]	56.0[e]	60.0[e]	100[a]	100[a]
Score 250 EC	100[a]	100[a]	100[a]	100[a]	100[a]
Secure	54.55[d]	80.0[c]	81.82[c]	85.46[c]	100[a]
Silica 80 WG	100[a]	100[a]	100[a]	100[a]	100[a]
Tilt 250 EC	100[a]	100[a]	100[a]	100[a]	100[a]
CV (%)	0.1002	0.0961	0.0925	0.0879	0.0919

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

Table 30. Effects of fungicides against *Fusarium nivale* at different concentrations.

Fungicides	% growth inhibition at different concentrations (ppm)				
	100	**200**	**300**	**400**	**500**
Capvit 50 WP	76.92[b]	80.0[b]	81.54[b]	84.62[b]	100[a]
Contaf 5 EC	100[a]	100[a]	100[a]	100[a]	100[a]
Dithane M 45	100[a]	100[a]	100[a]	100[a]	100[a]
Knowin 50 WP	100[a]	100[a]	100[a]	100[a]	100[a]
Nativo 75 WP	100[a]	100[a]	100[a]	100[a]	100[a]
Ridomil MZ Gold	42.5[e]	72.5[c]	75.0[c]	100[a]	100[a]
Score 250 EC	100[a]	100[a]	100[a]	100[a]	100[a]
Secure	60.87[c]	65.22[d]	73.91[d]	80.43[c]	82.61[b]
Silica 80 WG	45.0[d]	62.50[e]	67.50[e]	75.10[d]	100[a]
Tilt 250 EC	100[a]	100[a]	100[a]	100[a]	100[a]
CV (%)	0.0942	0.0883	0.0865	0.0892	7.53

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

Table 31. Effects of fungicides against *Fusarium moniliforme* at different concentrations.

Fungicides	% growth inhibition at different concentrations (ppm)				
	100	**200**	**300**	**400**	**500**
Capvit 50 WP	57.14[d]	62.86[f]	64.29[g]	68.57[g]	100[a]
Contaf 5 EC	100[a]	100[a]	100[a]	100[a]	100[a]
Dithane M 45	56.76[e]	64.86[d]	70.27[e]	72.97[e]	100[a]
Knowin 50 WP	64.29[c]	68.57[c]	70.0[f]	72.86[e]	80.0[c]
Nativo 75 WP	100[a]	100[a]	100[a]	100[a]	100[a]
Ridomil MZ Gold	48.84[g]	58.14[g]	60.47[h]	72.10[f]	100[a]
Score 250 EC	80.0[b]	83.33[b]	85.0[b]	86.67[b]	100[a]
Secure	53.85[f]	64.62[e]	72.31[c]	81.54[c]	84.62[b]
Silica 80 WG	37.50[h]	43.75[h]	71.88[d]	78.0[d]	100[a]
Tilt 250 EC	100[a]	100[a]	100[a]	100[a]	100[a]
CV (%)	0.0793	0.0743	0.0698	0.0665	0.0928

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

Table 32. Effects of fungicides against *Mucor* sp. at different concentrations.

Fungicides	% growth inhibition at different concentrations (ppm)				
	100	**200**	**300**	**400**	**500**
Capvit 50 WP	61.13^{c}	66.67^{d}	74.45^{d}	100^{a}	100^{a}
Contaf 5 EC	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Dithane M 45	0^{f}	22.22^{g}	33.34^{g}	38.89^{d}	100^{a}
Knowin 50 WP	61.11^{c}	68.89^{c}	93.34^{b}	100^{a}	100^{a}
Nativo 75 WP	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Ridomil MZ Gold	0^{f}	20.05^{h}	22.23^{h}	27.78^{e}	33.34^{c}
Score 250 EC	84.44^{b}	88.89^{b}	91.11^{c}	100^{a}	100^{a}
Secure	44.29^{d}	57.14^{e}	64.29^{e}	82.86^{b}	100^{a}
Silica 80 WG	24.45^{e}	33.34^{f}	38.89^{f}	41.11^{c}	50.0^{b}
Tilt 250 EC	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
CV (%)	0.0960	0.0843	0.0772	0.0983	0.1014

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

Table 33. Effects of fungicides against *Rhizoctonia solani* at different concentrations.

Fungicides	% growth inhibition at different concentrations (ppm)				
	100	**200**	**300**	**400**	**500**
Capvit 50 WP	55.56^{c}	60.02^{c}	100^{a}	100^{a}	100^{a}
Contaf 5 EC	100^{a}	100^{b}	100^{a}	100^{a}	100^{a}
Dithane M 45	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Knowin 50 WP	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Nativo 75 WP	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Ridomil MZ Gold	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Score 250 EC	80.0^{b}	82.86^{b}	84.29^{b}	100^{a}	100^{a}
Secure	53.50^{d}	60.11^{c}	72.22^{d}	100^{a}	100^{a}
Silica 80 WG	0^{e}	38.89^{d}	78.89^{c}	100^{a}	100^{a}
Tilt 250 EC	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
CV (%)	0.0984	0.0923	0.0896	0.1000	0.1000

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

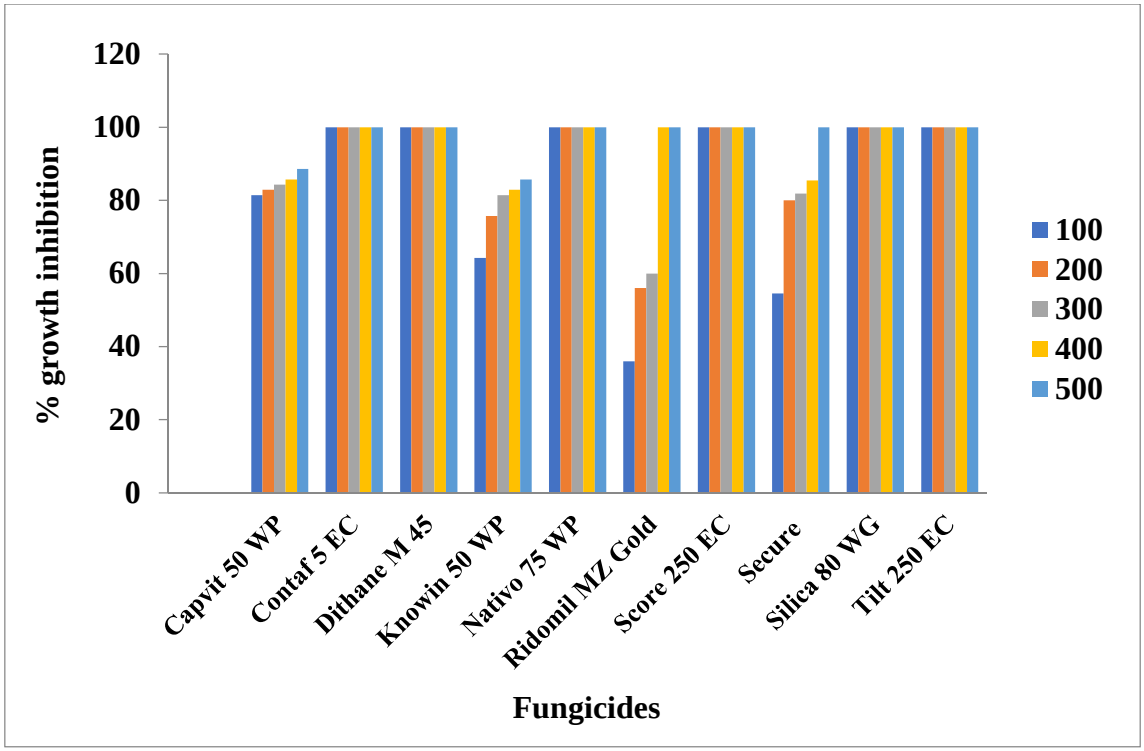

Fig. 35. Per cent growth inhibition of *Curvularia lunata* at different concentrations of fungicides.

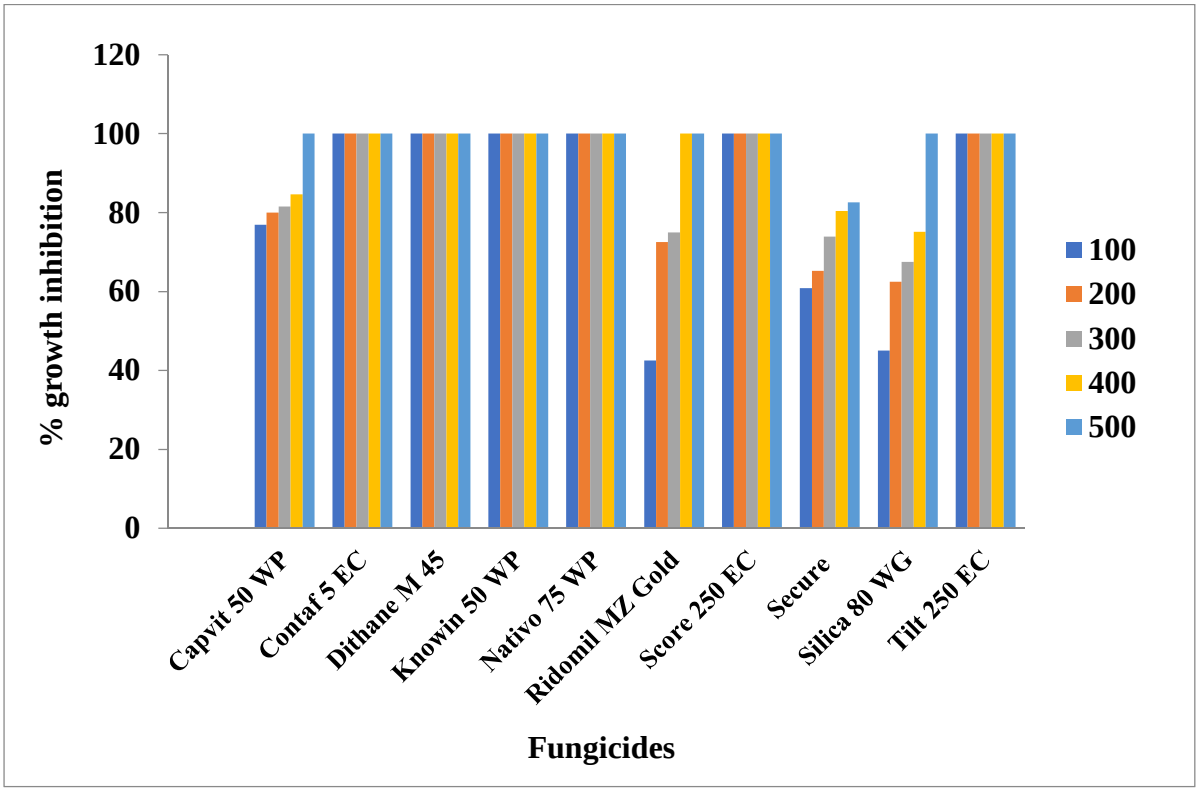

Fig. 36. Per cent growth inhibition of *Fusarium nivale* at different concentrations of fungicides.

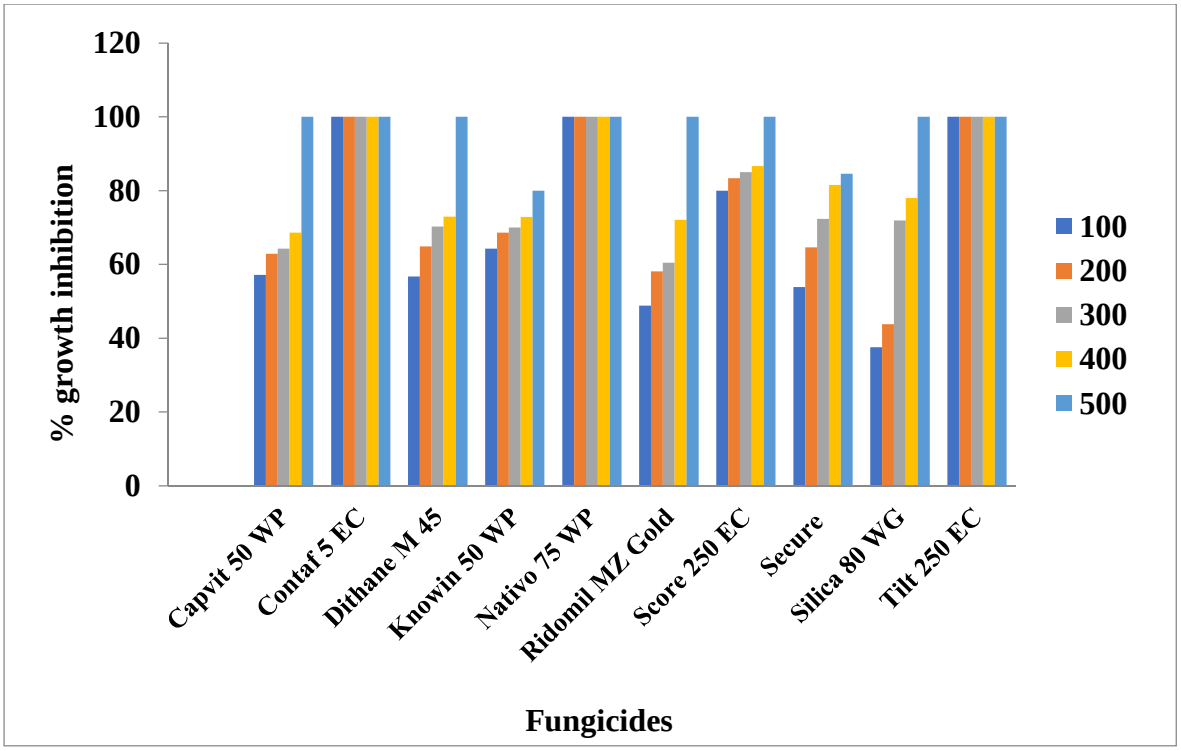

Fig. 37. Per cent growth inhibition of *Fusarium moniliforme* at different concentrations of fungicides.

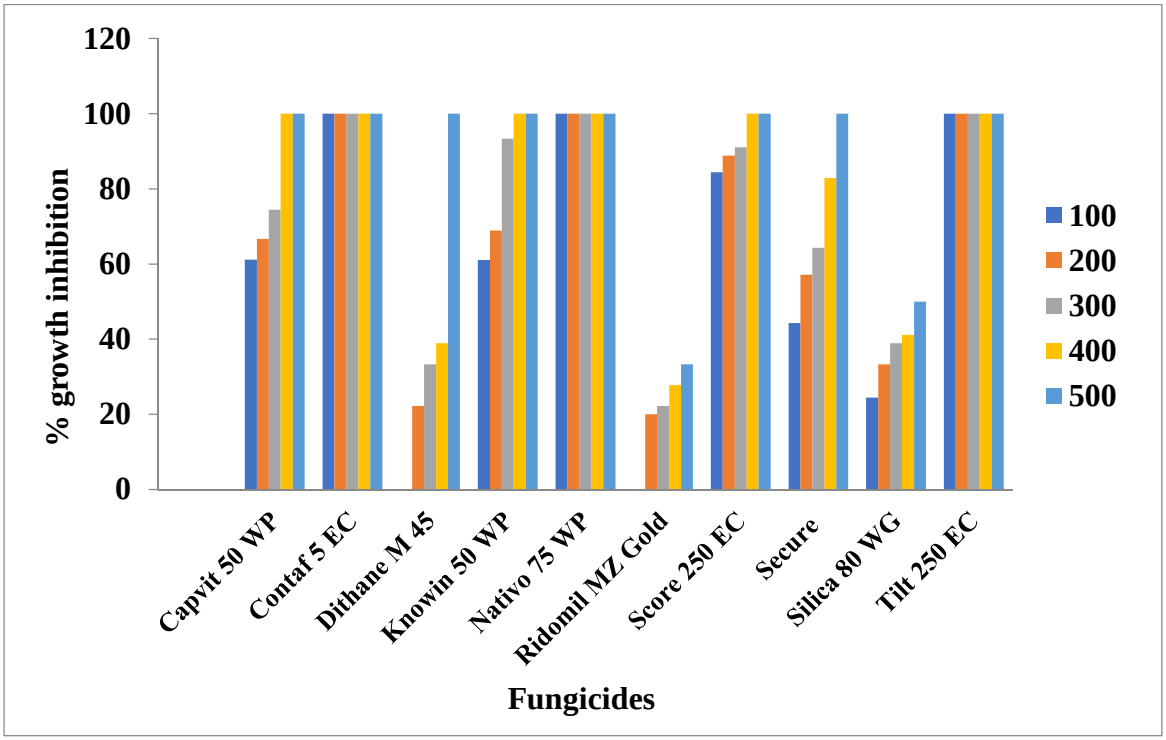

Fig. 38. Per cent growth inhibition of *Mucor* sp. at different concentrations of fungicides.

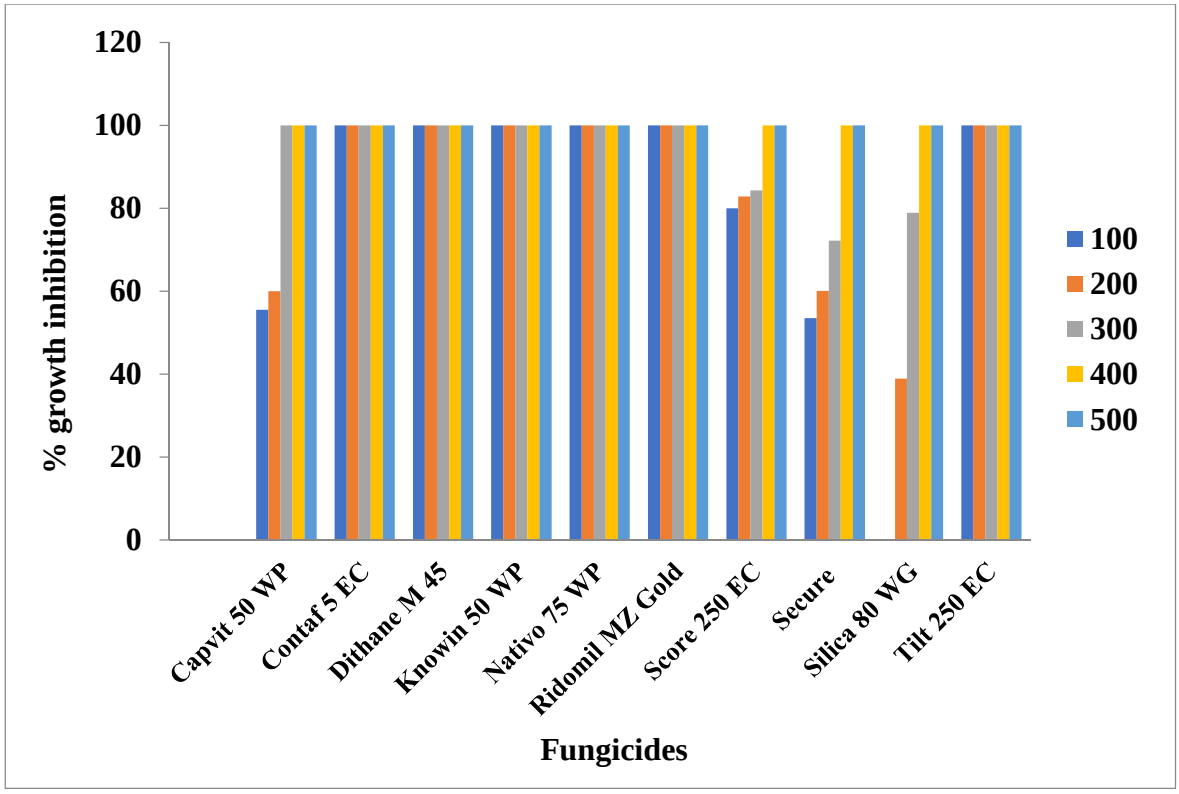

Fig. 39: Per cent growth inhibition of *Rhizoctonia solani* at different concentrations of fungicides.

Amongst the ten fungicides used in this present study, Contaf 5 EC and Tilt 250 EC showed best result which completely inhibited the radial growth of all the test pathogens at all the treated concentrations. On the other hand, Ridomil MZ Gold and Silica 80 WG showed least percentage of inhibition. It is also clear from the results that the per cent growth inhibition of the test pathogens gradually increased with the increase in concentration of the fungicides.

Plate 26. Per cent inhibition of radial growth of A. *Aspergillus flavus*, **B.** *A. niger*, **C.** *A. tamarii*, D. *Colletotrichum gloeosporioides*, **E.** *Curvularia lunata*, **F.** *Fusarium nivale*, **G.** *F. moniliforme*, **H.** *Mucor* sp. and **I.** *Rhizoctonia solani* at 100, 200, 300, 400 and 500 ppm concentrations of **Tilt 250 EC**.

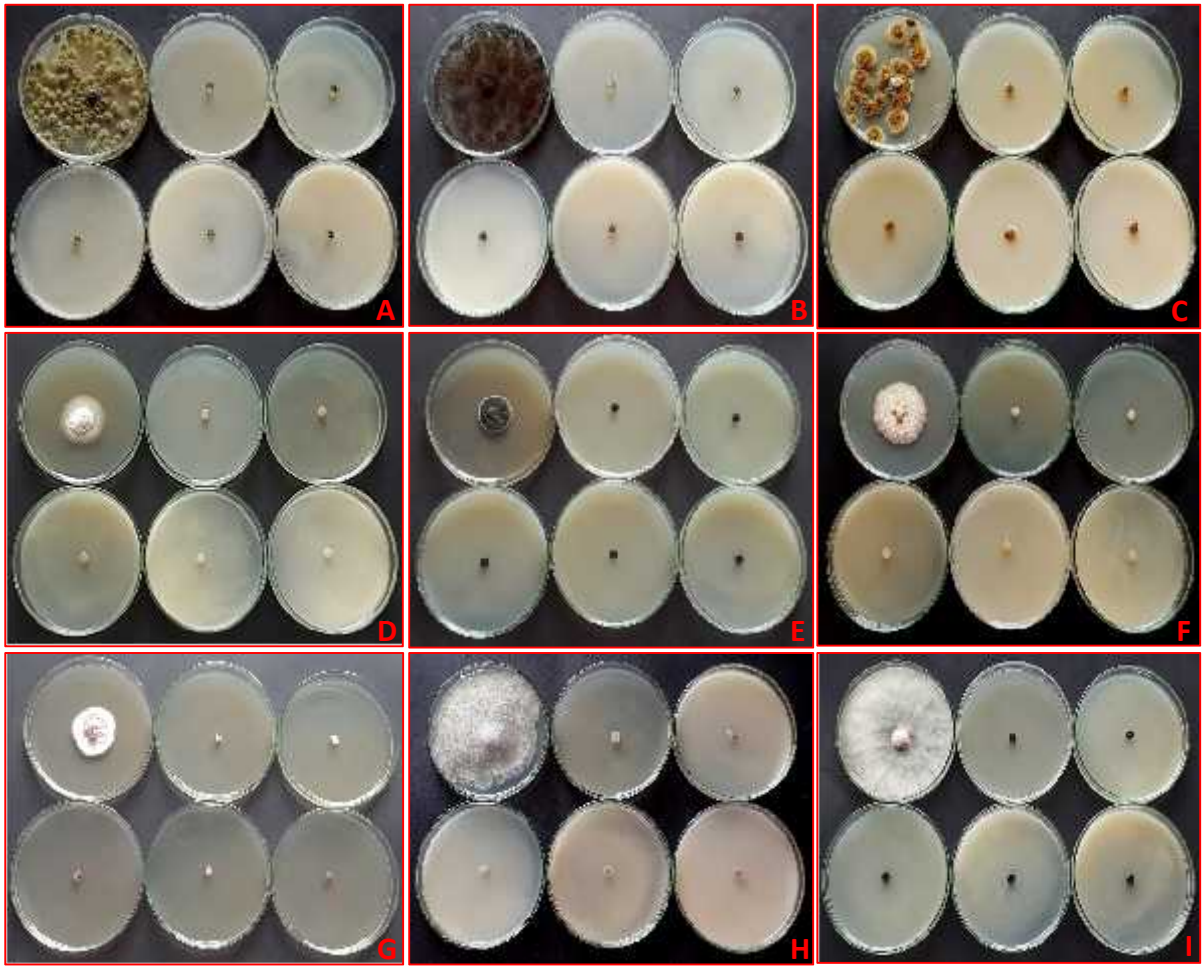

Plate 27. Per cent inhibition of radial growth of A. *Aspergillus flavus*, **B.** *A. niger*, **C.** *A. tamarii*, D. *Colletotrichum gloeosporioides*, **E.** *Curvularia lunata*, **F.** *Fusarium nivale*, **G.** *F. moniliforme*, **H.** *Mucor* sp. and **I.** *Rhizoctonia solani* at 100, 200, 300, 400 and 500 ppm concentrations of **Contaf 5 EC**.

4.17. Effects of plant extracts on the growth of the test pathogens

Antifungal properties of ethanol extracts of ten plant parts *viz*., *Adhatoda vasica* L., *Aegle mermelos* L., *Azadirachta indica* A. Juss., *Citrus lemon* L., *Datura metel* L., *Mangifera indica* L., *Moringa oleifera* Lam., *Ocimum sanctum* L., *Psidium guajava* L. and *Tagetes patula* L. at 5, 10, 15 and 20% concentrations were evaluated on the test pathogens. All the plant extracts showed varied degree of growth inhibition of the pathogens at different concentrations. *Psidium guajava* and *Azadirachta indica* was found most efficient inhibitor of the test pathogens followed by *C. lemon, A. vasica, D. metel*, *A. mermelos, T. patula*, *M. oleifera, O. sanctum* and *M. indica*. Results of plant extracts on the radial growth of *Aspergillus flavus, A. niger, A. tamarii, Colletotrichum gloeosporioides, Curvularia lunata, Fusarium nivale, F. moniliforme, Mucor* sp. and *Rhizoctonia solani* are presented in Tables 34-42, Figs 40-48 and Plates 28-30.

4.17.1. Effect of plant extracts against *Aspergillus flavus*

Amongst 10 plant extracts used in this experiment ethanol extract of *Psidium guajava* showed highest (84.89%) inhibition of radial growth of *Aspergillus flavus* at 20% concentration whereas *Citrus lemon* showed 83.34% inhibition at the same concentration. *Adhatoda vasica, Azadirachta indica, Tagetes patula, Ocimum sanctum, Mangifera indica, Moringa oleifera, Datura metel* and *Aegle mermelos* showed 77.78, 76.67, 75.56, 67.89, 65.09, 21.34, 16.68 and 11.14% growth inhibition, respectively at this concentration. The inhibition of the pathogen increases with the increase of the concentration of plant extracts in culture medium (Table 34, Fig. 40 and Plates 28-30).

The order of effectiveness of plant extracts against *Aspergillus flavus* at 20% concentration was *P. guajava > C. lemon > A. vasica > A. indica > T. patula > O. sanctum > M. indica > M. oleifera > D. metel > A. mermelos* (Table 34).

Azadirachta indica, D. metel and *P. guajava* showed 77.05, 86.0 and 83.5% growth inhibition of *A. flavus* at 20% concentration, respectively according to Khatun and Shamsi (2016) which is in agreement with the present study. Similarly, *Psidium guajava* was most active against the growth of *A. flavus* (81.29%) among five plant parts extract *viz*., *Adhatoda vasica*, *Aegle mermelos*, *Azadirachta indica*, *Datura metel* and *Psidium guajava* in *Gossypium arboreum* L. (Nahar and Shamsi 2020).

Mondall *et al*. (2009) also observed that the crude aqueous and alcoholic leaf extracts of *A. indica* was more effective against the growth of the fungi *Aspergillus* in the artificial culture medium. Mohana *et al*. (2011) reported that methanol extracts of *Acacia nilotica, Caesalpinia coriaria, Decalepis hamiltonii, Emblica officinalis* and *Mimosops elengi* showed significant antifungal activity at 3500 µg/ml concentration on *Aspergillus flavus.*

4.17.2. Effect of plant extract against *Aspergillus niger*

Twenty percent ethanol extracts of *Tagetes patula* was responsible for the highest inhibition (60.11%) of radial growth of *Aspergillus niger* whereas ethanol extracts of *O. sanctum, A.indica, A. vasica* and *C. lemon* showed 60.00, 51.11, 50.0 and 44.45% inhibition of the fungal growth, respectively at the same concentration. The ethanol plant extracts of *A. mermelos, M. indica, D. metel* and *P. guajava* showed less than 30.0% inhibition at 20% concentration. There was no growth inhibition of *Aspergillus niger* was owing to *Moringa oleifera*. The inhibition of the test pathogen increase with the increase of the concentration of the plant extracts in the culture medium (Table 35, Fig. 41 and Plates 28-30).

The order of effectiveness of plant extracts against *Aspergillus niger* at 20% concentration was *T. patula > O. sanctum > A. indica > A. vasica > C. lemon > A. mermelos > M. indica > D. metel > P. guajava > M. oleifera* (Table 35).

The findings of the present study are in accordance with the result of Nahar and Shamsi (2020) who found that *Azadirachta indica* was most active to inhibit the growth of *Aspergillus niger* (Type-I) (65.56%) among the five plant parts extract namely *Adhatoda vasica*, *Aegle mermelos*, *Azadirachta indica*, *Datura metel* and *Psidium guajava* against *Gossypium arboreum* L.

Similarly, Suwanmanee *et al*. (2014) tested the effect of *P. guajava* aqueous extract (leaf) against fungi and found that 2.67 to 16 mg/ml plant extract can successfully inhibit the growth of *Aspergillus niger*. Effects of plant extracts on the conidial germination of *A. niger* were also studied by Locke (1995), William (2008) and they found that *A. niger* was mostly inhibited by *Azadirachta indica*. According to Hosen and Shamsi (2017), out of five plants extract, *Allium sativum* showed complete growth inhibition of the test pathogen at all the concentrations tested against *A. niger*.

4.17.3. Effect of plant extract against *Aspergillus tamarii*

Twenty per cent ethanol extracts of *Adhatoda vasica* and *Psidium guajava* were responsible for 100% inhibition of the radial growth of *Aspergillus tamarii* whereas *C. lemon*, *O. sanctum* and *A. mermelos* showed 91.14, 66.68 and 56.68% inhibition of the fungus at the same concentration. The ethanol extracts of *A. mermelos*, *M. indica*, *D. metel*, *A. indica* and *M, oleifera* showed 55.59, 48.09, 44.45, 35.56 and 34.45% inhibition at 20% concentration, respectively (Table 36, Fig. 42 and Plates 28-30).

The order of effectiveness of plant extracts against *Aspergillus niger* at 20% concentration was *A. vasica* / *P. guajava* > *T. patula* > *C. lemon* > *O. sanctum* > *A. mermelos* > *M. indica* > *D. metel* > *A. indica* > *M. oleifera* (Table 36).

4.17.4. Effect of plant extract against *Colletotrichum gloeosporioides*

Twenty percent ethanol extracts of *Adhatoda vasica* and *Datura metel* were responsible for complete inhibition of radial growth of *Colletotrichum gloeosporioides* whereas *P. guajava*,

C. lemon, A. mermelos, T. patula and *A. indica* showed 97.87, 89.90, 77.51, 71.14 and 70.79% inhibition of the fungal growth, respectively at the same concentration (Table 37, Fig. 43 and Plates 28-30).

The order of effectiveness of plant extracts against *Aspergillus niger* at 20% concentration was *A. vasica* / *D. metel* > *P. guajava* > *C. lemon* > *A. mermelos* > *T. patula* > *A. indica* > *M. oleifera* > *O. sanctum* > *M. indica* (Table 37).

The findings of the present investigation are in accordance with the results of Yasmin and Shamsi (2019) who observed that ethanol plant extract of *A. indica, C. lemon, M. oleifera* and *P. guajava* exhibited promising result in controlling the radial growth of *C. gloeosporioides*, the causal agents of anthracnose of *Rauwolfia serpentina*. Similarly, Helal and Shamsi (2018) reported that *Ocimum sanctum* showed highest (88.57%) radial growth inhibition of *C. gloeosporioides* at 20% concentration. The effectiveness of neem is also well established by Rahejha and Thakore (2002).

Chavan (1996) reported that, the water extracts of 10% concentration of *Azadirachta indica, Ocimum sanctum* and *vitex negundo* suppressed the mycelial growth of *Colletotrichum gloeosporioides* causing anthracnose by 76.22, 71.19 and 61.25%, respectively. In the present study, *A. indica* and *O. sanctum* showed 70.79 and 39.78% inhibition of *C. gloeosporioides* respectively.

Ashoka (2005) recorded that neem was found effective in inhibiting mycelial growth of *C. gloeosporioides*. Plant extracts such as *Curcuma longa, Tagetes erecta* and *Zingeber officinale* were also most effective against *Colletotrichum gloeosporioides* according to Imtiaj *et al* (2005). Here *Tagetes patula* showed 71.14% inhibition against *C. gloeosporioides* which is in agreement with the result of Imtiaj *et al.* (2005).

Table 34. Effects of plant extracts on the radial growth of *Aspergillus flavus* at different concentrations.

Plants	% growth inhibition at different concentrations			
	5	**10**	**15**	**20**
Adhatoda vasica L.	0^{f}	11.12^{f}	23.33^{g}	77.78^{c}
Aegle mermelos L.	0^{g}	0^{f}	0^{i}	11.14^{j}
Azadirachta indica A. Juss.	2.34^{g}	16.67^{e}	38.89^{f}	76.67^{d}
Citrus lemon L.	33.34^{a}	64.44^{d}	75.56^{a}	83.34^{b}
Datura metel L.	0^{g}	0^{f}	5.56^{h}	16.68^{i}
Mangifera indica L.	0^{e}	12.3^{f}	58.34^{e}	65.09^{g}
Moringa oleifera Lam.	0^{g}	0^{f}	0^{i}	21.34^{h}
Ocimum sanctum L.	16.05^{d}	57.76^{b}	64.45^{d}	67.89^{f}
Psidium guajava L.	58.89^{b}	67.78^{a}	70.09^{b}	84.89^{a}
Tagetes patula L.	16.68^{c}	24.21^{c}	67.66^{c}	75.56^{e}
CV (%)	0.1659	0.0425	0.0221	0.0218

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

Remarks of efficiency gradient of plant extracts at 20% concentration against *Aspergillus flavus*: *Psidium guajava > Citrus lemon > Adhatoda vasica > Azadirachta indica > Tagetes patula > Ocimum sanctum > Mangifera indica > Moringa oleifera > Datura metel > Aegle mermelos.*

Table 35. Effects of plant extracts on the radial growth of *Aspergillus niger* at different concentrations.

Plants	% growth inhibition at different concentrations			
	5	**10**	**15**	**20**
Adhatoda vasica L.	0	0^{d}	44.45^{b}	50.0^{b}
Aegle mermelos L.	0	0^{d}	3.36^{g}	28.24^{d}
Azadirachta indica A. Juss.	0	5.58^{c}	11.13^{d}	51.11^{b}
Citrus lemon L.	0	11.12^{b}	33.23^{c}	44.45^{c}
Datura metel L.	0	0^{d}	5.56^{f}	17.98^{e}
Mangifera indica L.	0	0^{d}	9.89^{e}	18.16^{e}
Moringa oleifera Lam.	0	0^{d}	0^{h}	0^{g}
Ocimum sanctum L.	0	48.78^{a}	51.45^{a}	60.00^{a}
Psidium guajava L.	0	0^{d}	5.57^{f}	12.34^{f}
Tagetes patula L.	0	0^{d}	11.13^{d}	60.11^{a}
CV (%)	Constant	0.0836	0.0540	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 plant extracts at 20% concentration against *Aspergillus niger*: *Tagetes patula > Ocimum sanctum > Azadirachta indica > Adhatoda vasica > Citrus lemon > Aegle mermelos > Mangifera indica > Datura metel > Psidium guajava > Moringa oleifera.*

Table 36. Effects of plant extracts on the radial growth of *Aspergillus tamarii* at different concentrations.

Plants	% growth inhibition at different concentrations			
	5	**10**	**15**	**20**
Adhatoda vasica L.	0^{c}	0^{h}	10.12^{i}	100^{a}
Aegle mermelos L.	0^{c}	5.67^{f}	34.56^{d}	55.59^{d}
Azadirachta indica A. Juss.	0^{c}	6.69^{e}	13.15^{g}	35.56^{g}
Citrus lemon L.	0^{c}	12.34^{c}	55.57^{c}	66.68^{c}
Datura metel L.	0^{c}	0^{h}	4.98^{j}	44.45^{f}
Mangifera indica L.	0^{c}	4.48^{g}	32.34^{e}	48.09^{e}
Moringa oleifera Lam.	0^{c}	0^{h}	12.24^{h}	34.45^{g}
Ocimum sanctum L.	5.57^{b}	9.67^{d}	24.45^{f}	56.68^{d}
Psidium guajava L.	84.44^{a}	87.78^{a}	92.23^{a}	100^{a}
Tagetes patula L.	0^{c}	16.68^{b}	71.14^{b}	91.14^{b}
CV (%)	0.0994	0.0584	0.0285	0.7071

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

Remarks of efficiency gradient of plant extracts at 20% concentration against *Aspergillus tamarii*: *Adhatoda vasica* / *Psidium guajava* > *Tagetes patula* > *Citrus lemon* > *Ocimum sanctum* > *Aegle mermelos* > *Mangifera indica* > *Datura metel* > *Azadirachta indica* > *Moringa oleifera.*

Table 37. Effects of plant extracts on the radial growth of *Colletotrichum gloeosporioides* at different concentrations.

Plants	% growth inhibition at different concentrations			
	5	**10**	**15**	**20**
Adhatoda vasica L.	29.63^{h}	33.45^{h}	59.26^{g}	100^{a}
Aegle mermelos L.	37.53^{f}	51.54^{e}	75.56^{b}	77.51^{d}
Azadirachta indica A. Juss.	42.30^{c}	55.53^{d}	61.65^{e}	70.79^{e}
Citrus lemon L.	62.23^{a}	71.13^{a}	78.67^{a}	89.90^{c}
Datura metel L.	39.03^{e}	60.98^{b}	68.98^{c}	100^{ab}
Mangifera indica L.	22.23^{i}	27.02^{i}	35.56^{j}	37.90^{h}
Moringa oleifera Lam.	44.0^{b}	46.78^{f}	53.0^{h}	57.56^{f}
Ocimum sanctum L.	21.12^{j}	23.34^{j}	37.89^{i}	39.78^{g}
Psidium guajava L.	36.67^{g}	60.09^{c}	66.69^{d}	97.87^{b}
Tagetes patula L.	40.08^{d}	44.46^{g}	60.12^{f}	71.14^{e}
CV (%)	0.0860	0.0149	0.0110	0.6048

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

Remarks of efficiency gradient of plant extracts at 20% concentration against *Colletotrichum gloeosporioides*: *Adhatoda vasica* / *Datura metel* > *Psidium guajava* > *Citrus lemon* > *Aegle mermelos* > *Tagetes patula* > *Azadirachta indica* > *Moringa oleifera* > *Ocimum sanctum* > *Mangifera indica.*

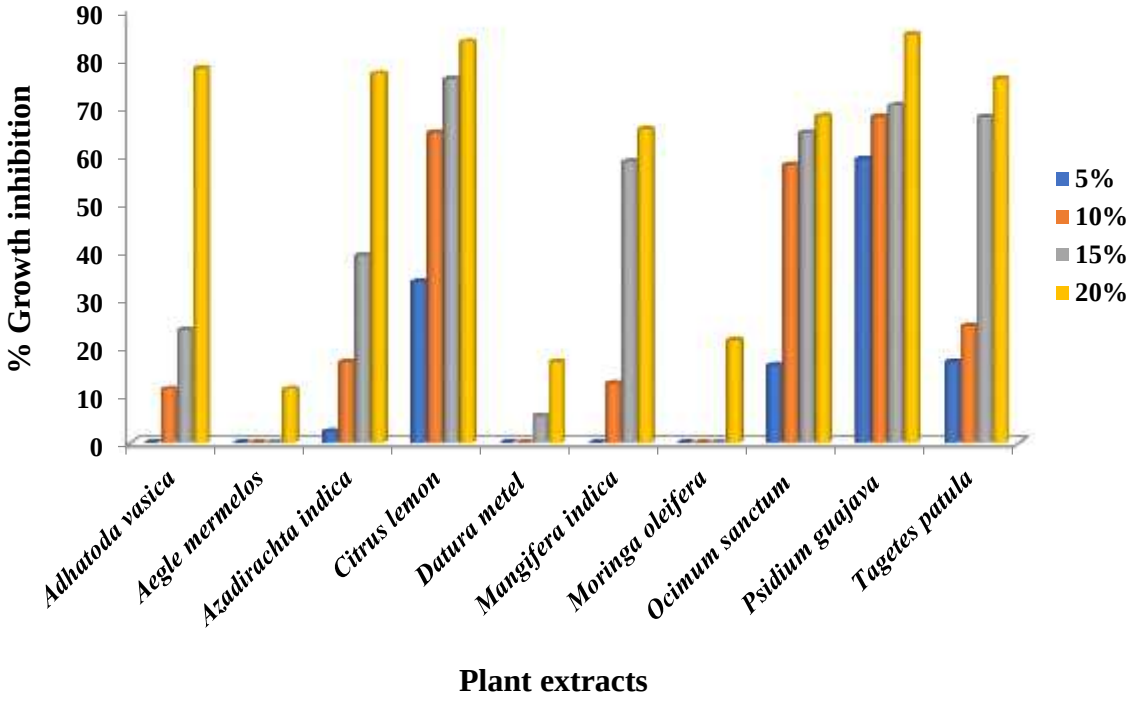

Fig. 40. Effects of plant extracts on the radial growth of *Aspergillus flavus* at different concentrations.

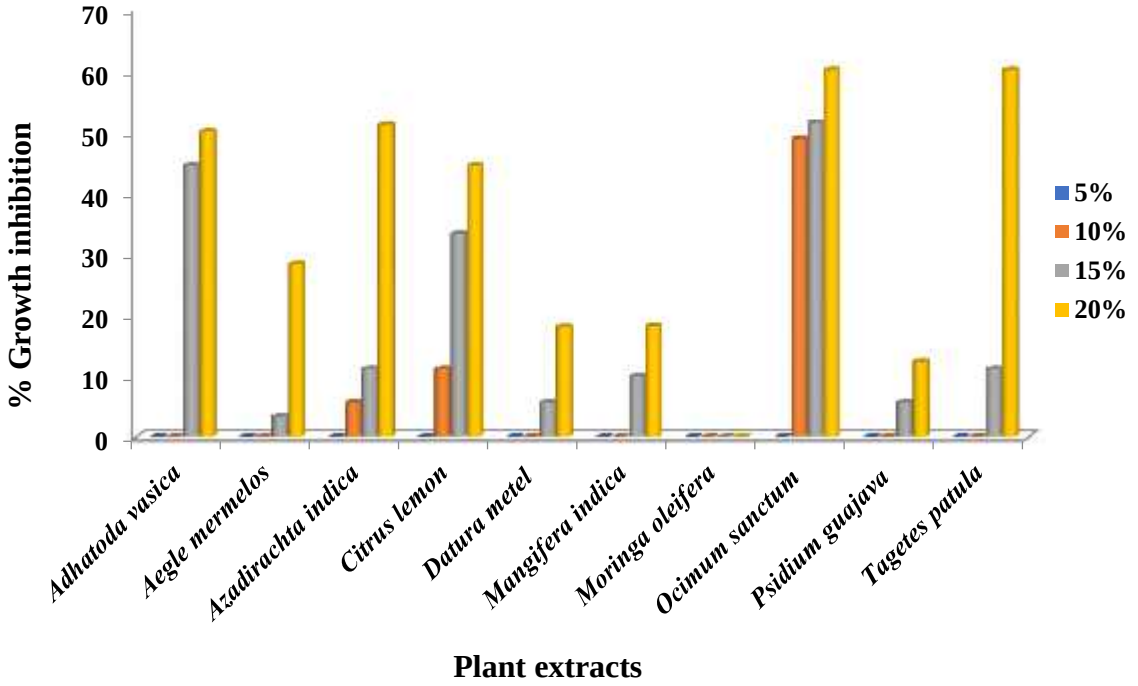

Fig. 41. Effects of plant extracts on the radial growth of *Aspergillus niger* at different concentrations.

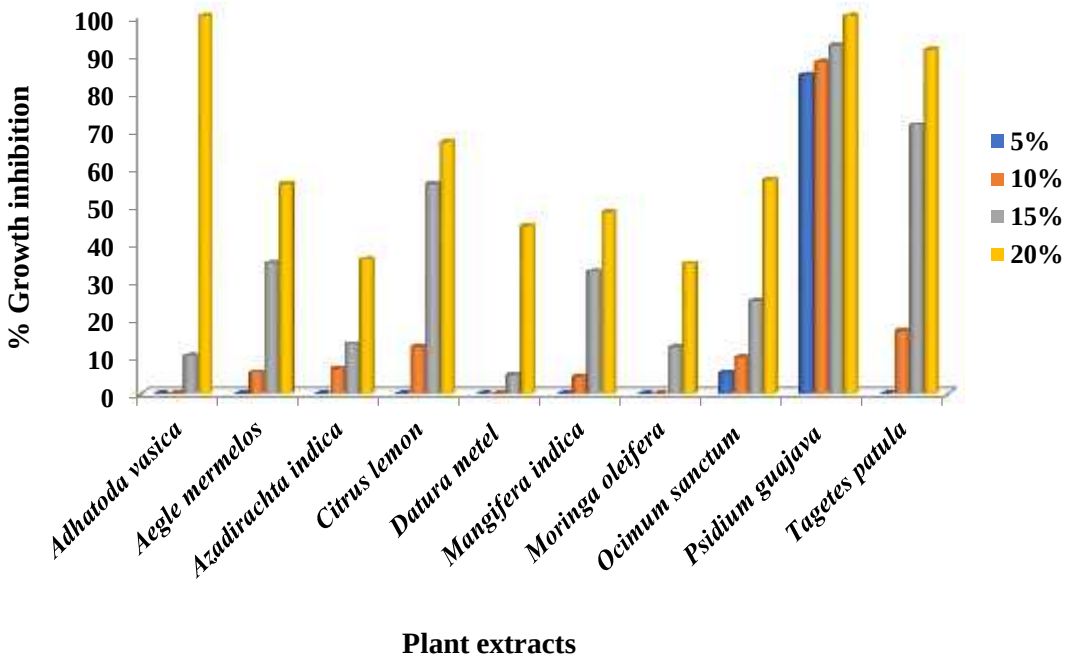

Fig. 42. Effects of plant extracts on the radial growth of *Aspergillus tamarii* at different concentrations.

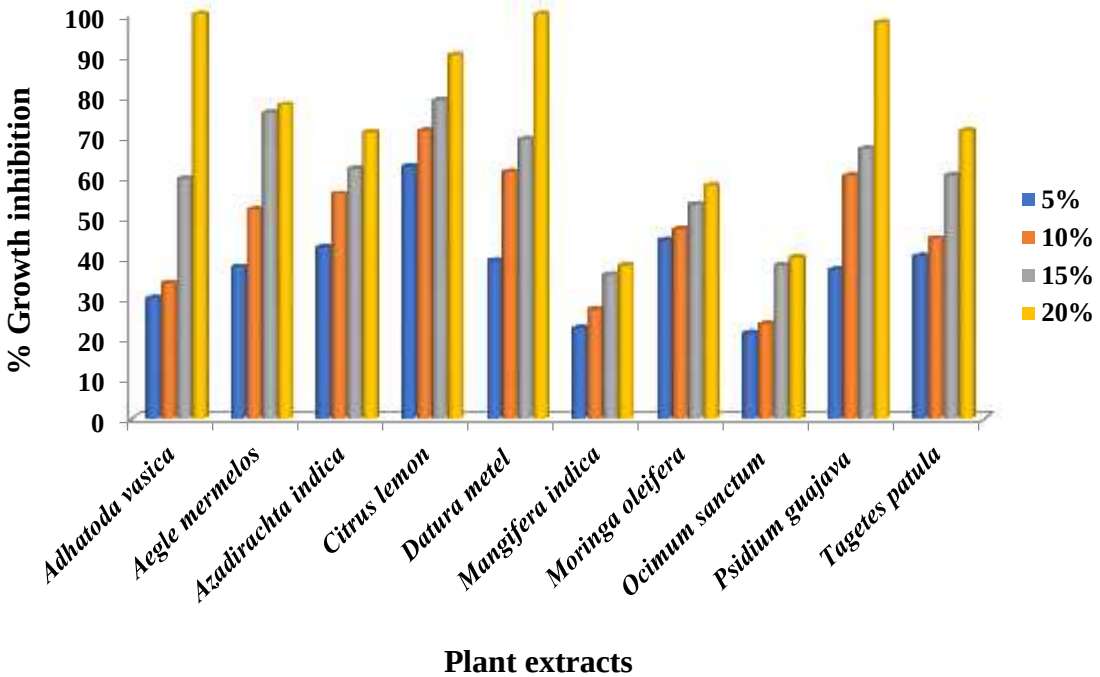

Fig. 43. Effects of plant extracts on the radial growth of *Colletotrichum gloeosporioides* at different concentrations.

4.17.5. Effect of plant extracts against *Curvularia lunata*

Ethanol extract of *Azadirachta indica, Citrus lemon, Datura metel, Moringa oleifera, Psidium guajava* and *Tagetes patula* showed 100% inhibition of radial growth of *C. lunata* at 20% concentration whereas, *A. mermelos, O. sanctum, A. vasica* and *M. indica* showed 64.02, 59.67, 57.14 and 50.08% inhibition at the same concentration. 15% concentration of *A. indica, C. lemon, D. metel, M. oleifera* and *P. guajava* also showed complete inhibition of the pathogenic fungus. The inhibition of the pathogen increases with the increase of the concentration of the plant extracts in culture medium (Table 38, Fig. 44 and Plates 28-30).

The order of effectiveness of plant extracts against *Curvularia lunata* at 20% concentration was *A. indica / C. lemon / D.metel / M. oleifera / P. guajava / T. patula >A. mermelos > O. sanctum > A. vasica > M. indica* (Table 38).

The complete inhibition of radial growth of *Curvularia lunata* was observed with plant extract of *A. indica* and *D. metel* at 20% concentration according to Khatun and Shamsi (2016). *Psidium guajava* was most active against the growth of *Curvularia lunata* (72.23%) among the five plant parts extract *viz*., *Adhatoda vasica*, *Aegle mermelos*, *Azadirachta indica*, *Datura metel* and *Psidium guajava* on *Gossypium arboreum* L. (Nahar and Shamsi 2020). The results of the present investigation are in agreement with the above mentioned workers.

Chowdhury *et al.* (2015) observed that 20% ethanol extract of *Tagetes erecta, Datura metel, Azadirachta indica, Citrus medica* and *Mangifera indica* completely inhibited the radial growth of *Curvularia lunata*. In the present study *Datura metel, Azadirachta indica* and *Citrus* lemon also showed complete inhibition against *C. lunata* at 20% concentration. Sultana (2021) reported that *Azadirachta indica* and *Psidium guajava* showed the complete growth inhibition of *C. lunata* at 20% concentration which is fully support the result of 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. Mohana *et al*. (2011) reported that methanol extracts of *Acacia nilotica, Caesalpinia coriaria, Decalepis hamiltonii, Emblica officinalis* and *Mimosops elengi* showed significant antifungal activity at 3500 µg/ml concentration on *C.* lunata.

4.17.6. Effects of plant extracts against *Fusarium moniliforme*

Ethanol extract of *Aegle mermelos, Azadirachta indica, Datura metel* and *Psidium guajava* showed 100% inhibition of the radial growth of *Fusarium moniliforme* at 20% concentration which was followed by *A. vasica* (99.30%), *C. lemon* (98.90%), *T. patula* (75.57%), *O. sanctum* (63.34%), *M. oleifera* (62.45%) and *M. indica* (54.89%). Fifteen percent concentration of *D. metel* and *P. guajava* also showed complete inhibition of this test pathogen (Fig. 2f). The inhibition of the pathogen increases with the increase of the concentration of the plant extracts in culture medium (Table 39, Fig.45 and Plates 28-30).

The order of effectiveness of plant extracts against *Fusarium moniliforme* at 20% concentration *A. mermelos* / *A. indica* / *D. metel* / *P. guajava* > *A. vasica* > *C. lemon* > *T. patula* > *O. sanctum*> *M. oleifera* > *M. indica* (Table 39).

In contrast to the present study, Bashar and Chakma (2014) reported that the plant extract of *Azadirachta indica* showed 62.03% growth inhibition of *F. oxysporum* at 20% concentration. The result of the present investigation are in accordance with the results of Nahar and Shamsi (2020) who observed that *Azadirachta indica* and *Datura metel* was most active to inhibit the growth of *Fusarium moniliforme* var. *subglutinans* among the five plant parts extract *viz*., *Adhatoda vasica*, *Aegle mermelos*, *Azadirachta indica*, *Datura metel* and *Psidium guajava* on *Gossypium arboreum* L.

Chowdhury *et al.* (2015) observed that 10 and 20% ethanol extract of *Tagetes erecta, Datura metel, Azadirachta indica, Citrus medica* and *Mangifera indica* completely inhibited the radial growth of *Fusarium moniliforme. Azadirachta indica* and *Citrus medica* also showed complete inhibition of *F. moniliforme* at 5% concentration. Helal and Shamsi (2018) reported that *Adhatoda vasica* showed highest (80.02%) radial growth inhibition of *Fusarium* sp. at 20% concentration. These findings are also in agreement with the present investigation.

Sultana (2021) reported that *Azadirachta indica, Moringa oleifera* and *Cassia alata* showed highest radial growth inhibition of *F. equiseti* and *F. fujikuroi* at 20% concentration. Mohana *et al*. (2011) reported that methanol extracts of *Acacia nilotica, Caesalpinia coriaria, Decalepis hamiltonii, Emblica officinalis* and *Mimosops elengi* showed significant antifungal activity at 3500 µg/ml concentration on *Fusarium moniliforme* by poisoned food technique. The plant extracts of *Azadirachta indica* also showed different effects on different test pathogens in the present investigation. This variation might be due to the selection of different test pathogens.

4.17.7. Effects of plant extracts against *Fusarium nivale*

Twenty percent ethanol extracts of *Adhatoda vasica, Azadirachta indica, Citrus lemon, Datura metel* and *Psidium guajava* were completely inhibited the radial growth of *Fusarium nivale* whereas *Aegle mermelos, M. oleifera, T. patula, M. indica* and *O. sanctum* showed 77.89%, 65.78%, 60.10%, 50.87% and 48.89% inhibition of the fungal growth, respectively at the same concentration. The inhibition of the pathogen increases with the increase of the concentration of the plant extracts in culture medium (Table 40, Fig.46 and Plates 28-30).

The order of effectiveness of plant extracts against *Fusarium sporotrichioides* at 20% concentration was *A. vasica / A. indica / C. lemon / D. metel / P. guajava > A. mermelos > M. oleifera > T. patula > M. indica > O. sanctum* (Table 40).

Azadirachta indica and *Datura metel* showed promising activity against *Fusarium* spp. according to Mamun *et al*. (2016) and Hossain *et al*. (2013). The present result also exhibited similar activity. Ethanol extracts of *Allium sativum* showed complete growth inhibition of *F. merismoides* at all the tested concentration described by Hosen and Shamsi (2017).

Dwivedi and Shukla (2000) reported that 100% aqueous neem leaf extract caused complete inhibition of spore germination of *Fusarium* spp. Mycelial growth of various *Fusarium* species were inhibited by plant extracts of *Adhatoda vasica, Azadirachta indica, Cinnamomum camphora* and *Ocimum sanctum* (Prasad and Ojha 1986).

Helal and Shamsi (2018) reported that *Curcuma longa* showed highest (88.55%) radial growth inhibition of *F. nivale*. 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.

4.17.8. Effects of plant extracts against *Mucor* sp.

The highest inhibition of the growth of *Mucor* sp. was observed with *Psidium guajava* (100%) at 20% concentration which was followed by *T. patula* (88.30%), *D. metel* (87.76%), *A. vasica* (86.78%), *A. mermelos* (83.33%), *C. lemon* (48.67%), *A. indica* (44.89%), *O. sanctum* (38.34%), *M. oleifera* (37.78%) and *M. indica* (28.23%). The inhibition of the pathogen increases with the increase of the concentration of the plant extracts in culture medium (Table 41, Fig.47 and Plates 28-30).

The order of effectiveness of plant extracts against *Fusarium moniliforme* at 20% concentration *P. guajava > T. patula > D. metel > A. vasica > A. mermelos > C. lemon > A. indica > O. sanctum > M. oleifera> M. indica* (Table 41).

4.17.9. Effects of plant extracts against *Rhizoctonia solani*

Amongst 10 plant extracts used in this experiment ethanol extract of *Citrus lemon* showed 93.33% inhibition of radial growth of *Rhizoctonia solani* at 20% concentration whereas *Psidium guajava* showed 90.11% inhibition at the same concentration. *A. vasica, M. oleifera, D. metel, T. patula, A. indica, A. mermelos, O. sanctum* and *M. indica* showed 87.79, 83.33, 80.0, 74.44, 72.23, 65.55, 63.26 and 29.78% growth inhibition, respectively at this concentration (Table 42, Fig.48 and Plates 28-30).

The order of effectiveness of plant extracts against *Fusarium moniliforme* at 20% concentration *C. lemon > P. guajava > A. vasica > M. oleifera > D. metel > T. patula > A. indica > A. mermelos> O. sanctum > M. indica* (Table 42).

Shahnaz *et al*. (2010) reported the inhibitory effect of *Datura alba* against *Rhizoctonia solani* which is in agreement with the present study.

Datura metel was most active against the growth of *Rhizoctonia solani* (42.44%) among the five plant parts extract *viz. Adhatoda vasica*, *Aegle mermelos*, *Azadirachta indica*, *Datura metel* and *Psidium guajava* on *Gossypium arboreum* L. (Nahar and Shamsi 2020). In the present investigation, *D. metel* showed 80% growth inhibition of *R. solani*.

Table 38. Effects of plant extracts on the radial growth of *Curvularia lunata* at different concentrations.

Plants	% growth inhibition at different concentrations			
	5	**10**	**15**	**20**
Adhatoda vasica L.	25.15^{i}	36.07^{i}	45.44^{cd}	57.14^{d}
Aegle mermelos L.	30.09^{g}	36.34^{h}	43.34^{d}	64.02^{b}
Azadirachta indica A. Juss.	76.44^{a}	79.07^{c}	100^{a}	100^{a}
Citrus lemon L.	66.67^{e}	70.09^{e}	100^{a}	100^{a}
Datura metel L.	46.67^{f}	67.74^{f}	100^{a}	100^{a}
Mangifera indica L.	27.08^{h}	33.34^{j}	35.42^{e}	50.08^{a}
Moringa oleifera Lam.	72.5^{c}	75.09^{d}	100^{a}	100^{a}
Ocimum sanctum L.	21.34^{j}	36.67^{g}	46.78^{c}	59.67^{c}
Psidium guajava L.	67.78^{d}	80.87^{a}	100^{a}	100^{a}
Tagetes patula L.	73.34^{b}	80.02^{b}	95.0^{b}	100^{a}
CV (%)	0.0651	0.0162	1.00	0.9256

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

Remarks of efficiency gradient of plant extracts at 20% concentration against *Curvularia lunata*: *Azadirachta indica / Citrus lemon / Datura metel > Moringa oleifera / Psidium guajava / Tagetes patula >Aegle mermelos > Ocimum sanctum > Adhatoda vasica > Mangifera indica.*

Table 39. Effects of plant extracts on the radial growth of *Fusarium moniliforme* at different concentrations.

Plants	% growth inhibition at different concentrations			
	5	**10**	**15**	**20**
Adhatoda vasica L.	31.43^{g}	37.15^{i}	68.57^{c}	99.30^{ab}
Aegle mermelos L.	47.51^{c}	67.60^{b}	75.0^{b}	100^{a}
Azadirachta indica A. Juss.	48.50^{b}	50.09^{e}	55.79^{f}	100^{a}
Citrus lemon L.	27.50^{h}	48.80^{f}	63.60^{d}	98.90^{b}
Datura metel L.	34.21^{f}	57.89^{d}	100^{a}	100^{a}
Mangifera indica L.	40.0^{d}	45.67^{g}	46.89^{g}	54.89^{e}
Moringa oleifera Lam.	38.18^{e}	58.18^{c}	60.78^{e}	62.45^{d}
Ocimum sanctum L.	37.4^{e}	41.56^{h}	46.34^{g}	63.34^{d}
Psidium guajava L.	72.34^{a}	78.79^{a}	100^{a}	100^{a}
Tagetes patula L.	37.78^{e}	46.68^{a}	62.23^{de}	75.57^{c}
CV (%)	0.7714	0.0664	0.8042	0.7386

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

Remarks of efficiency gradient of plant extracts at 20% concentration against *Fusarium moniliforme*: *Aegle mermelos / Azadirachta indica / Datura metel / Psidium guajava > Adhatoda vasica > Citrus lemon > Tagetes patula > Ocimum sanctum > Moringa oleifera> Mangifera indica.*

Table 40. Effects of plant extracts on the radial growth of *Fusarium nivale* at different concentrations.

Plants	% growth inhibition at different concentrations			
	5	10	15	20
Adhatoda vasica L.	64.29^{c}	77.56^{b}	83.50^{c}	100^{a}
Aegle mermelos L.	44.47^{f}	54.45^{g}	56.67^{f}	77.89^{b}
Azadirachta indica A. Juss.	99.0^{a}	100^{a}	100^{a}	100^{a}
Citrus lemon L.	40.02^{g}	56.78^{f}	64.45^{e}	100^{a}
Datura metel L.	65.38^{b}	74.09^{c}	98.67^{b}	100^{a}
Mangifera indica L.	33.34^{h}	43.87^{i}	49.12^{g}	50.87^{e}
Moringa oleifera Lam.	56.36^{d}	61.82^{e}	63.49^{e}	65.78^{c}
Ocimum sanctum L.	32.75^{h}	41.37^{j}	45.56^{h}	48.89^{f}
Psidium guajava L.	65.67^{b}	71.87^{d}	81.26^{d}	100^{a}
Tagetes patula L.	47.57^{e}	52.50^{h}	55.0^{f}	60.10^{d}
CV (%)	0.5763	0.4979	0.6407	0.7841

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

Remarks of efficiency gradient of plant extracts at 20% concentration against *Fusarium nivale*: *Adhatoda vasica / Azadirachta indica / Citrus lemon / Datura metel / Psidium guajava > Aegle mermelos > Moringa oleifera > Tagetes patula > Mangifera indica > Ocimum sanctum.*

Table 41. Effects of plant extracts on the radial growth of *Mucor* sp. at different concentrations.

Plants	% growth inhibition at different concentrations			
	5	10	15	20
Adhatoda vasica L.	0^{h}	12.01^{g}	44.49^{e}	86.78^{c}
Aegle mermelos L.	23.34^{c}	32.24^{d}	77.78^{c}	83.33^{d}
Azadirachta indica A. Juss.	15.45^{e}	24.67^{f}	35.57^{g}	44.89^{f}
Citrus lemon L.	21.09^{d}	28.88^{e}	37.77^{f}	48.67^{e}
Datura metel L.	11.34^{f}	34.44^{c}	61.11^{d}	87.76^{b}
Mangifera indica L.	4.89^{g}	8.56^{i}	11.45^{i}	28.23^{h}
Moringa oleifera Lam.	0^{h}	11.12^{h}	33.34^{h}	37.78^{g}
Ocimum sanctum L.	0^{h}	0^{j}	6.78^{j}	38.34^{g}
Psidium guajava L.	72.22^{a}	77.79^{a}	87.78^{a}	100^{a}
Tagetes patula L.	66.67^{b}	76.65^{b}	80.0^{b}	88.30^{b}
CV (%)	0.0389	0.0298	0.6630	0.4927

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

Remarks of efficiency gradient of plant extracts at 20% concentration against *Mucor* sp: *Psidium guajava > Tagetes patula > Datura metel > Adhatoda vasica > Aegle mermelos > Citrus lemon > Azadirachta indica > Ocimum sanctum > Moringa oleifera > Mangifera indica.*

Table 42. Effects of plant extracts on the radial growth of *Rhizoctonia solani* at different concentrations.

Plants	% growth inhibition at different concentrations			
	5	**10**	**15**	**20**
Adhatoda vasica L.	5.11^{h}	27.78^{g}	83.33^{b}	87.79^{c}
Aegle mermelos L.	44.45^{b}	55.56^{c}	61.12^{e}	65.55^{h}
Azadirachta indica A. Juss.	0^{i}	27.78^{g}	50.0^{g}	72.23^{g}
Citrus lemon L.	72.23^{a}	83.44^{a}	84.78^{a}	93.33^{a}
Datura metel L.	33.35^{e}	55.56^{c}	72.23^{d}	80.0^{e}
Mangifera indica L.	0^{i}	9.90^{h}	22.67^{h}	29.78^{j}
Moringa oleifera Lam.	25.47^{f}	50.0^{d}	81.11^{c}	83.33^{d}
Ocimum sanctum L.	34.12^{c}	49.67^{e}	56.23^{f}	63.26^{i}
Psidium guajava L.	34.02^{d}	66.68^{b}	71.12^{d}	90.11^{b}
Tagetes patula L.	18.89^{g}	44.47^{f}	60.09^{e}	74.44^{f}
CV (%)	0.0334	0.6715	0.7497	0.4270

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

Remarks of efficiency gradient of plant extracts at 20% concentration against *Rhizoctonia solani*: *Citrus lemon > Psidium guajava > Adhatoda vasica > Moringa oleifera > Datura metel > Tagetes patula > Azadirachta indica > Aegle mermelos> Ocimum sanctum > Mangifera indica.*

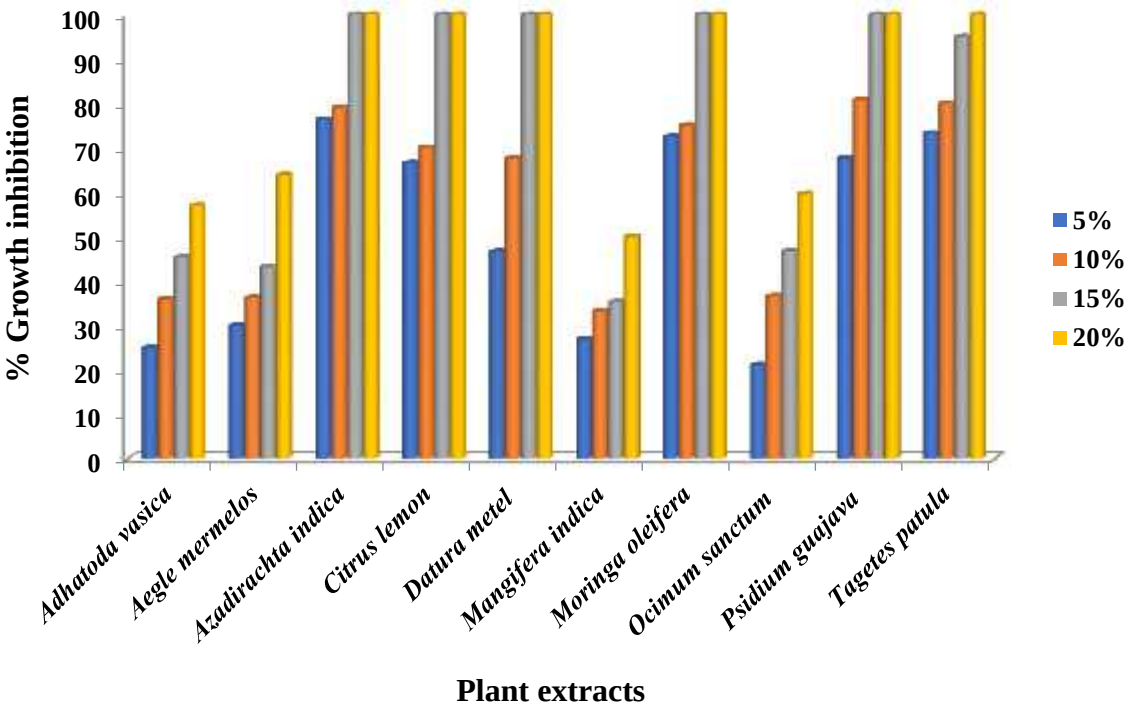

Fig. 44. Effects of plant extracts on the radial growth of *Curvularia lunata* at different concentrations.

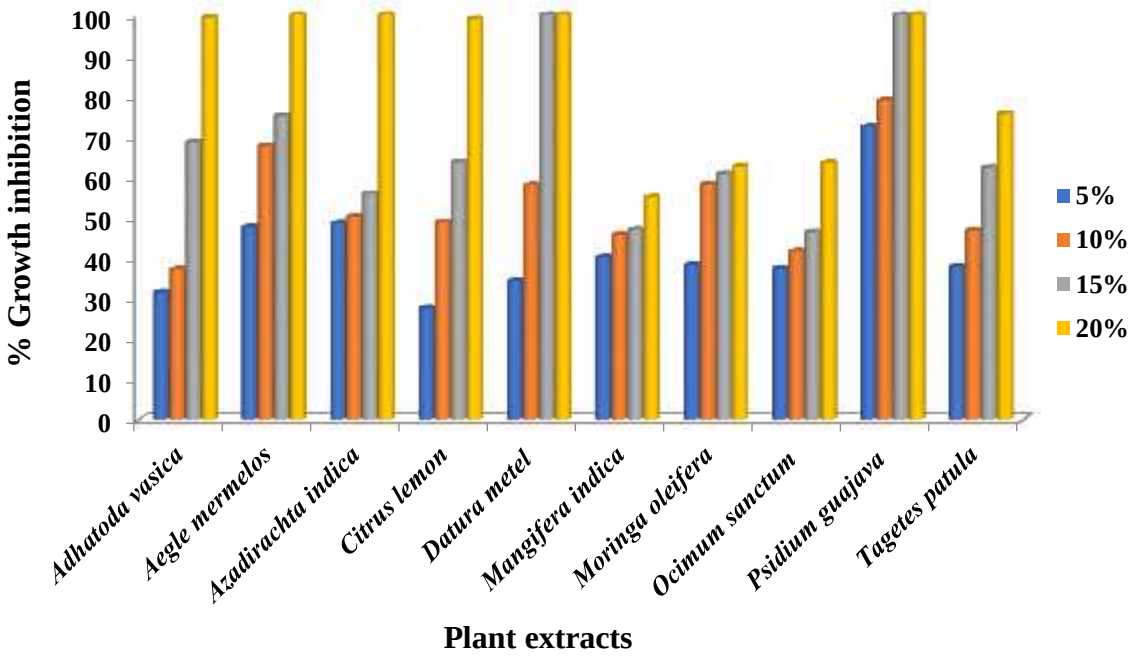

Fig. 45. Effects of plant extracts on the radial growth of *Fusarium moniliforme* at different concentrations.

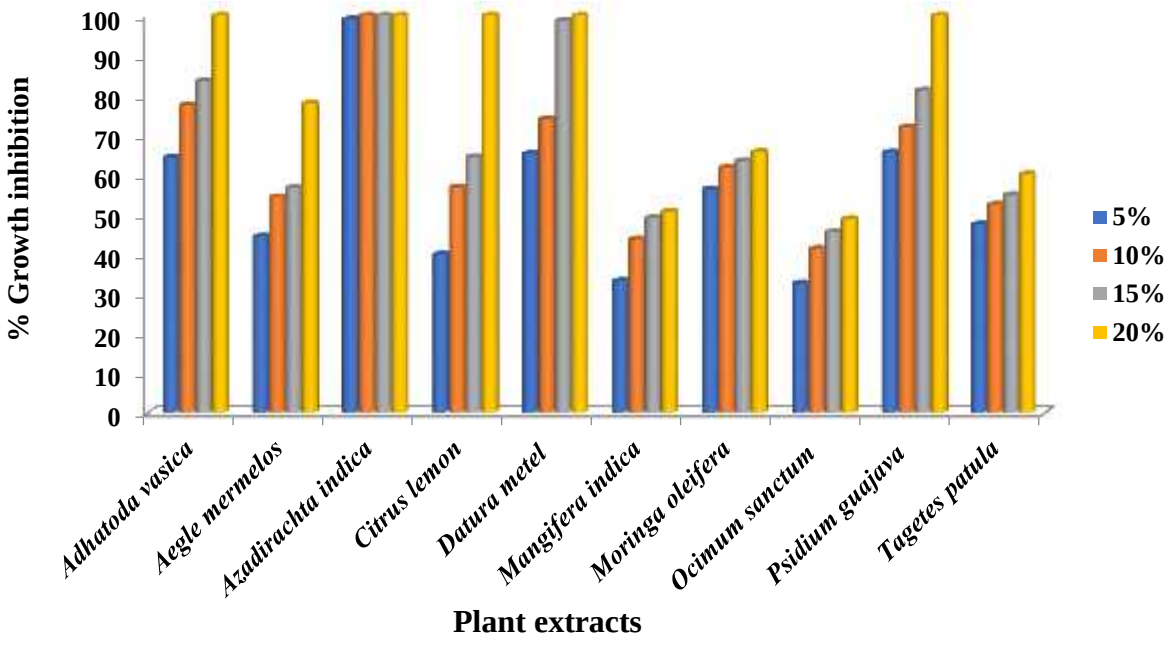

Fig. 46. Effects of plant extracts on the radial growth of *Fusarium nivale* at different concentrations.

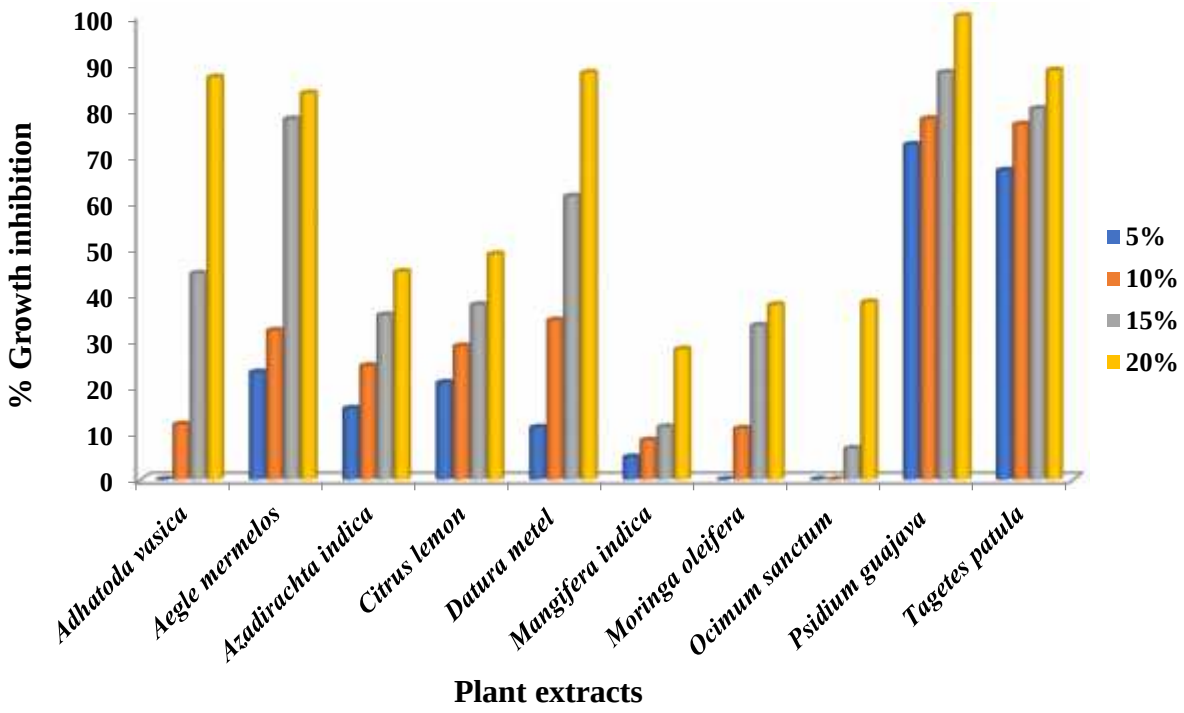

Fig. 47. Effects of plant extracts on the radial growth of *Mucor* sp. at different concentrations.

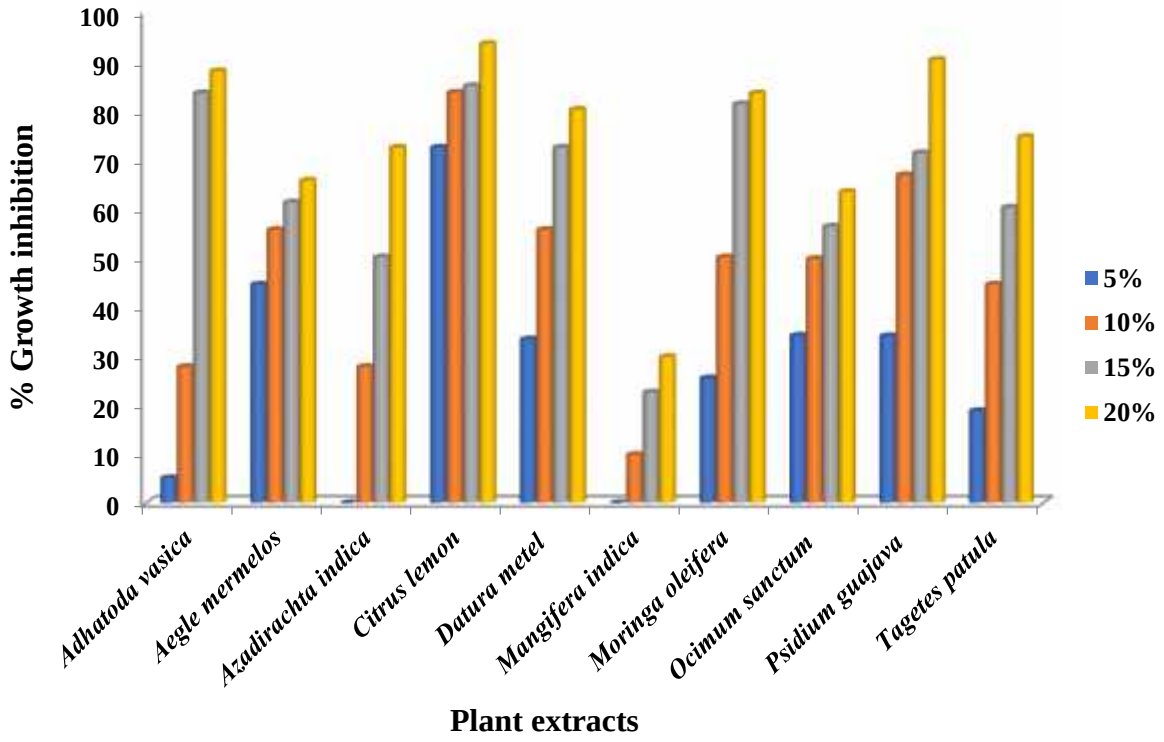

Fig. 48. Effects of plant extracts on the radial growth of *Rhizoctonia solani* at different concentrations.

Plate 28. Fungitoxicity of leaf extracts of *Azadirachta indica* on the radial growth of A. *Colletotrichum gloeosporioides*, **B.** *Curvularia lunata*, **C.** *Fusarium moniliforme* and **D.** *F. nivale* at 5, 10, 15 and 20% concentrations.

Plate 29. Fungitoxicity of leaf extracts of *Adhatoda vasica* on the radial growth of A. *Aspergillus tamarii*, **B.** *Colletotrichum gloeosporioides*, **C.** *Curvularia lunata*, **D.** *Fusarium moniliforme* and **E.** *F. nivale* at 5, 10, 15 and 20% concentrations.

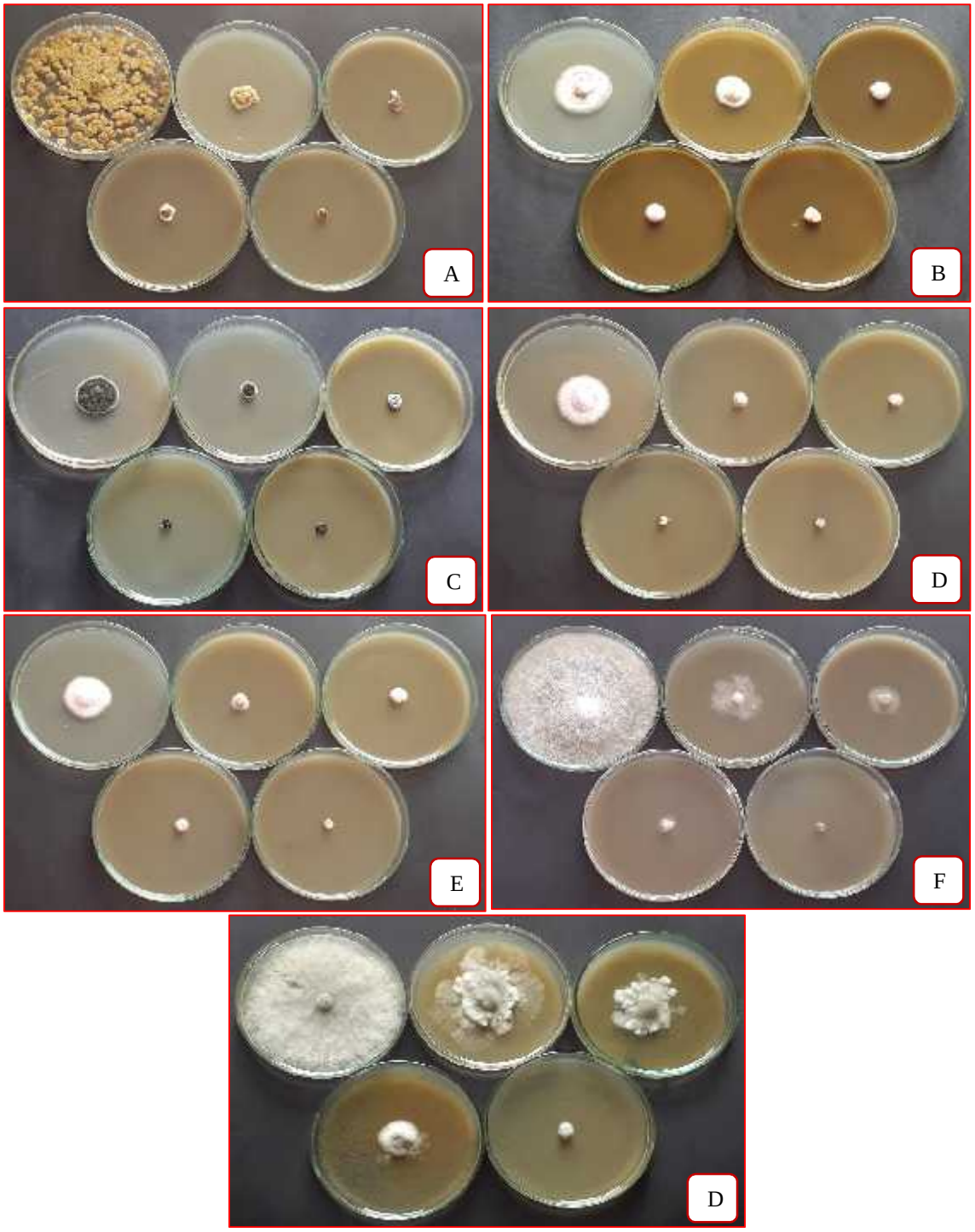

Plate 30. Fungitoxicity of leaf extracts of *Psidium guajava* on the radial growth of A. *Aspergillus tamarii*, **B.** *Colletotrichum gloeosporioides*, **C.** *Curvularia lunata*, **D.** *Fusarium moniliforme*, **E.** *F. nivale*, **F.** *Mucor* sp. and **G.** *Rhizoctonia solani* at different concentrations.

Some researches on the fungitoxicity of various higher plants extracts have indicated the possibility of their exploitation as natural fungitoxicants for controlling plant diseases (Bashar and Rai 1991, Hossain 1993, Anwar *et al.* 1994, Salma 1995). Ahmed and Sultana (1984) and Miah *et al.* (1990) also observed resistant effect of plant extract to the disease without any phytotoxicity to the host. The inhibitory effect of the plant extracts might be attributed to the presence of antifungal compounds.

Antifungal activity of different plant extracts have been reported earlier by several investigators against a number of plant pathogens (Ankita and Dwivedi 2012, Babu *et al*. 2008, Kuri *et al.* 2011). *Ocimum* spp. leaf extracts was found to check spore germination, growth, total proteins and pectolytic and cellulotytic enymes of various rot pathogens (Patel 1991, Patil *et al.* 1992, Vyas 1993 and Godara and Pathak 1995).

Chakraborty *et al.* (2009) reported the efficacy of various cell free extracts of the plants against the growth of pathogens. The effectiveness of extracts varied significantly with dosage, where 100% inhibition of the pathogen was achieved both with neem and garlic extracts.

In the present study, depending on the pathogenic fungi *Azadirachta indica, Datura metel* and *Psidium guajava* were the best botanicals for the inhibition of seedling diseases of cotton.

4.18. Combined effects of fungicides, plant extracts and antagonists against the growth of cotton pathogens.

An investigation was undertaken to screen out the seed treatment method with fungicides, plant extracts and bioagents against the 9 pathogenic fungi *viz. Aspergillus flavus*, *A. niger*, *A. tamarii, Colletotrichum gloeosporioides, Curvularia lunata, Fusarium nivale, F. moniliforme, Mucor* sp. and *Rhizoctonia solani* of cotton. This study revealed that all the treatments could significantly reduce the seed borne fungi and improve the quality of 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 43, Figs 49-52).

4.18.1. Effects of different treatments on *Aspergillus flavus*

Seed inoculated with *Aspergillus flavus* showed 39.44-88.23% germination. Out of 17 treatments, T1 showed the highest (88.23%) germination percentage whereas the lowest (39.44%) was recorded by T12. Six treatments *viz.* T1, T5, T10, T14, T11 and T13 showed promising results compared to control. Highest seedling vigor index (811.72) was observed in T1 and lowest was found in T12 (410.17). Seedling mortality was also counted after 21 days of germination. The maximum seedling mortality (60%) was found in T17 and minimum (20%) in T3 treatments. In this experiment, maximum shoot length (7.2cm) was recorded in T11 and minimum (5.6cm) in T9 whereas highest root length (3.6cm) was noticed in T15 and lowest (1.9cm) in T9 (Table 43).

4.18.2. Effects of different treatments on *Aspergillus niger*

Out of 17 treatments, 5 treatments *viz.* T1, T5, T10, T14 and T11 exhibited best results compared to control. Among these treatments, T1 showed the highest (87.21%) germination percentage whereas the lowest (43.67%) was recorded in T6. Highest seedling vigor index (898.26) was observed in T1 and lowest in T6 (384.23). The maximum seedling mortality (60.2%) was found in T17 and minimum (21.20%) in T15 treatments after 21 days of germination. In the present investigation, maximum shoot length (7.6cm) was recorded by T11 and minimum (5.6cm) in T15 whereas highest root length (3.6cm) was noticed in T10 and lowest (1.9cm) in T9 (Table 43).

4.18.3. Effects of different treatments on *Aspergillus tamarii*

Out of 17 treatments, 3 treatments *viz.*T5, T10 and T14 exhibited best results compared to control. Among these treatments, T11 showed the highest (86.34%) germination percentage whereas the lowest (37.78%) was recorded in T12. Highest seedling vigor index (923.84) was

observed in T11 and lowest was observed in T12 (374.02). The maximum seedling mortality (61.23%) was found in T17 and minimum (27.58%) in T3 treatments after 21 days of germination. In the present experiment, maximum shoot length (7.3cm) was recorded by T11 and minimum (5.4cm) in T16 whereas highest root length (3.8cm) was noticed in T17 and lowest (1.3cm) in T9 (Table 43).

4.18.4. Effects of different treatments on *Colletotrichum gloeosporioides*

Out of 17 treatments, 4 treatments *viz.* T1, T5, T10 and T13 exhibited best results compared to control. Among these treatments, T11 showed the highest (93.35%) germination percentage whereas the lowest (37.44%) was recorded in T12. Highest seedling vigor index (980.18) was observed in T11 and lowest was found in T12 (400.61). The maximum seedling mortality (57.14%) was found in T4 and minimum (20.0%) in T3 treatments after 21 days of germination. In the present study, maximum shoot length (7.4cm) was recorded by T6 and minimum (5.3cm) was noticed in T1 whereas highest root length (3.9cm) was noticed in T12 and lowest (1.9cm) in T1 (Table 43).

4.18.5. Effects of different treatments on *Curvularia lunata*

Out of 17 treatments, 4 treatments *viz.* T5, T10, T1 and T14 exhibited best results compared to control. Among these treatments, T1 showed the highest (85.21%) germination percentage whereas the lowest (42.34%) was recorded in T6. Highest seedling vigor index (979.92) was observed in T1 and lowest in T12 (406.46). The highest seedling mortality (50.56%) was recorded in T6 and lowest (21.20%) in T15 treatments after 21 days of germination. In the present investigation, maximum shoot length (8.1cm) was found in T1 and minimum (6.4cm) in T6 whereas highest root length (3.7cm) was noticed in T13 and lowest (2.8cm) in T3 (Table 43).

4.18.6. Effects of different treatments on *Fusarium nivale*

Out of 17 treatments, 4 treatments *viz.*T5, T10, T13 and T14 exhibited best results compared to control. Among these treatments, T11 showed the highest (88.09%) germination percentage whereas the lowest (40.87%) was recorded in T12. Highest seedling vigor index (960.18) was observed in T11 and lowest in T12 (437.31). The maximum seedling mortality (58.89%) was found in T4 and minimum (21.49%) in T3 treatments after 21 days of germination. In the present experiment, maximum shoot length (8.1cm) was recorded by T11 and minimum (6.6cm) by T2 whereas highest root length (3.7cm) was noticed in T14 and lowest (2.0cm) in T9 (Table 43).

4.18.7. Effects of different treatments on *Fusarium moniliforme*

Out of 17 treatments, 4 treatments *viz.* T5, T10, T13 and T14 exhibited best results compared to control. Among these treatments, T1 showed the highest (82.23%) germination percentage whereas the lowest (37.44%) was recorded in T12. Highest seedling vigor index (920.97) was observed in T1 and lowest was in T12 (348.19). The highest seedling mortality (57.14%) was recorded in T4 and lowest (20.0%) in T3 treatments after 21 days of germination. In the present study, maximum shoot length (7.4cm) was found in T11 and minimum (5.6cm) in T7 whereas highest root length (4.9cm) was noticed in T11 and lowest (2.0cm) in T6 (Table 43).

4.18.8. Effects of different treatments on *Mucor* sp.

Among the 17 treatments, 4 treatments *viz.* T5, T10, T13 and T14 exhibited best results compared to control. Among these treatments, T1 showed the maximum (85.21%) germination whereas the minimum (40.09%) was recorded in T12. Highest seedling vigor index (1013.99) was observed in T1 and lowest in T12 (428.96). The maximum seedling mortality (60.2%) was found in T17 and minimum (21.20%) in T15 treatments after 21 days of germination. In the present investigation, maximum shoot length (8.7cm) was recorded by T1 and minimum

(6.4cm) was recorded by T16 whereas highest root length (3.8cm) was noticed in T14 and lowest (2.2cm) in T16 (Table 43).

4.18.9. Effects of different treatments on *Rhizoctonia solani*

Out of 17 treatments, 4 treatments *viz.*T5, T10, T13 and T14 exhibited best results compared to control. Among these treatments, T11 showed the highest (76.66%) germination percentage whereas the lowest (50.93%) was recorded in T8. Highest seedling vigor index (958.25) was observed in T11 and lowest in T8 (458.37). The maximum seedling mortality (55.78%) was found in T4 and minimum (30.0%) in T3 treatments after 21 days of germination. In the present experiment, maximum shoot length (7.5cm) was recorded in T14 and minimum (4.4cm) in T4 whereas highest root length (3.7cm) was noticed in T14 and lowest (2.2cm) in T6 (Table 48).

The findings of the present investigation are in agreement with the findings of Chowdhury *et al.* (2022), who reported that amongst 13 treatments only T6 (Bavistin + *Azadirachta indica* + *Trichoderma harzianum*) showed highest percentage of seed germination and seedling vigor index of seeds inoculated with *Alternaria alternata, Aspergillus flavus, Curvularia lunata* and *Pestalotiopsis guepinii*. T10 (Bavistin + Tall + *Azadirachta indica* + *Citrus medica*) exhibited promising result against *Drechslera oryzae, Fusarium moniliforme, Microdochium oryzae* and *Sarocladium oryzae.* Treatment T3 (*Azadirachta indica*) was responsible for maximum percentage of seed germination and seedling vigor index with *Fusarium solani* inoculated seeds. Out of all treatments the integrated use of Bavistin, *A. indica* and *T. harzianum* showed the best results for growth reduction of test pathogens and increased the germination of seeds.

Sultana (2021) observed that, in field experiment out of twelve treatments T10 (Tilt + *Azadirachta indica* + *Trichoderma viride*), T3 (Bavistin + Tilt) and T7 (*Trichoderma viride*) was responsible for highest germination percentage and highest seedling vigor index against *Bipolaris oryzae*, *Curvularia lunata* and *Fusarium fujikuroi*. On the other hand, T3 (Bavistin

+ Tilt), T7 (*Trichoderma viride*) and T1 (Bavistin) showed promising germination percentage and seedling vigor index. Phillip *et al*. (2003) reported that combination of Mancozeb and Fludioxonil up to ten days prior to planting can control Fusarium decay of seeds. This is also in accordance with the result of present research work.

Similarly, Mahmood *et al.* (2015) observed that the combined effect of Mancozeb and Cupravit 50 WP (0.4%) significantly reduced the mycelial growth of *Fusarium* spp, *Alternaria* spp, *Sclerotium* spp, *Aspergillus flavus* and *A. niger.* Fravel *et al.* (2005) also find out the efficacy of Mancozeb and Cupravit against the mycelial growth of *Fusarium oxysporum* and observed that Mancozeb and Cupravit both reduced the colony growth of *Fusarium* spp.

Jeyalakshmi *et al*. (1999) observed that application of bioagents and Bavistin increased the percentage of seed germination. Seed treatment with bioagents and fungicides reduced root rot incidence (Patil *et al.* 2003). The present investigation also supports the results of above mentioned researchers.

Table 43. Effect of different treatments with fungicides, leaf extracts and antagonists on seed quality parameters of cotton.

Treatments	Seed quality parameters against test pathogens														
	Aspergillus flavus					*Aspergillus niger*					*Aspergillus tamari*				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
T1	88.23^{a}	22.23^{j}	2.9^{bcd}	6.3^{abcd}	811.72^{a}	87.21^{a}	45.56^{j}	3.2^{bcd}	7.1^{abcd}	898.26^{a}	70.34^{f}	55.56^{abc}	2.3^{cde}	6.7^{ab}	633.06^{j}
T2	49.92^{bcdef}	40.23^{f}	2.9^{bcd}	5.9^{d}	439.23^{c}	68.45^{bcdef}	38.40^{f}	3.1^{bcd}	6.2^{d}	636.59^{c}	61.56^{l}	44.90^{cdef}	2.4^{bcde}	6.3^{bcde}	535.57^{m}
T3	58.78^{abcdef}	20.0^{j}	3.0^{a}	6.4^{abcd}	552.53^{k}	55.65^{abcdef}	25.05^{j}	2.7^{a}	6.3^{abcd}	500.85^{k}	51.67^{c}	27.58^{hi}	2.5^{bcde}	5.7^{def}	423.70^{p}
T4	70.60^{abcdef}	57.14^{b}	2.8^{bcd}	6.2^{abcd}	635.40^{h}	71.78^{abcdef}	35.8^{b}	2.8^{bcd}	6.4^{abcd}	660.38^{h}	68.45^{h}	56.89^{ab}	2.9^{abcd}	6.8^{abcd}	663.97^{f}
T5	84.12^{abc}	33.18^{h}	3.3^{ab}	6.3^{abcd}	807.55^{b}	80.23^{abcdef}	24.43^{h}	2.8^{ab}	6.0^{abcd}	706.12^{b}	77.44^{c}	38.45^{efgh}	3.0^{abcd}	6.1^{bcdef}	704.70^{d}
T6	40.23^{ef}	49.56^{c}	2.2^{cd}	5.9^{d}	325.86^{r}	43.67^{ef}	50.56^{c}	2.7^{cd}	6.1^{d}	384.23^{r}	41.78^{r}	53.77^{abcd}	3.1^{abcd}	6.8^{ab}	413.62^{q}
T7	67.45^{abcdef}	37.40^{g}	3.2^{abc}	6.5^{abcd}	654.27^{g}	69.90^{abcdef}	42.78^{g}	3.3^{abc}	6.7^{abcd}	699.0^{g}	63.88^{k}	47.56^{bcdef}	3.6^{ab}	6.6^{abc}	651.56^{h}
T8	47.16^{cdef}	38.55^{g}	2.7^{bcd}	5.7^{d}	396.14^{q}	53.67^{cdef}	36.17^{g}	2.9^{bcd}	5.6^{d}	461.56^{q}	46.48^{p}	35.55^{fghi}	2.2^{de}	5.8^{cdef}	611.84^{k}
T9	41.28^{def}	37.89^{g}	1.9^{d}	5.6^{d}	313.73^{s}	45.90^{def}	35.58^{g}	1.9^{d}	5.9^{d}	358.02^{s}	43.44^{q}	35.74^{i}	1.3^{e}	5.6^{ef}	299.74^{s}
T10	75.30^{abc}	45.0^{e}	3.5^{ab}	7.0^{abc}	791.18^{c}	78.55^{abc}	25.18^{e}	3.6^{ab}	6.5^{abc}	793.36^{c}	79.49^{b}	37.77^{efghi}	3.5^{abc}	6.8^{ab}	818.75^{b}
T11	73.35^{abcd}	33.12^{h}	2.8^{bcd}	7.2^{a}	733.50^{e}	76.78^{abcd}	25.78^{h}	3.4^{bcd}	7.6^{a}	844.58^{e}	86.34^{a}	39.90^{efh}	3.4^{abcd}	7.3^{a}	923.84^{a}
T12	39.44^{f}	29.74^{i}	3.3^{ab}	7.1^{ab}	410.17^{p}	49.90^{f}	58.23^{i}	3.0^{ab}	7.2^{ab}	508.98^{p}	37.78^{s}	30.45^{ghi}	3.2^{abcd}	6.7^{ab}	374.02^{r}
T13	75.23^{abc}	38.67^{g}	3.2^{abc}	6.5^{abcd}	729.73^{f}	74.80^{abc}	37.89^{g}	3.2^{abc}	6.7^{abcd}	740.52^{f}	66.89^{j}	38.09^{efgh}	3.0^{abcd}	6.8^{ab}	655.23^{g}
T14	80.0^{ab}	49.5^{c}	3.2^{abc}	6.4^{abcd}	768.0^{d}	84.0^{ab}	39.90^{c}	2.9^{abc}	5.9^{abcd}	739.2^{d}	74.56^{e}	48.56^{bcde}	3.0^{abcd}	6.7^{ab}	668.67^{e}
T15	52.2^{bcdef}	30.0^{i}	3.6^{ab}	5.9^{d}	495.90^{m}	56.67^{bcdef}	21.20^{i}	3.2^{ab}	5.6^{d}	498.70^{m}	53.44^{m}	32.33^{ghi}	3.1^{abcd}	6.1^{bcdef}	491.65^{n}
T16	50.1^{bcdef}	47.66^{d}	3.3^{ab}	6.0^{cd}	465.93^{n}	51.23^{bcdef}	48.09^{d}	3.0^{ab}	7.3^{cd}	527.67^{n}	52.39^{n}	46.44^{bcdef}	3.2^{abcd}	5.4^{f}	450.55^{c}
T17	65.3^{abcdef}	60.0^{a}	2.7^{bcd}	6.1^{bcd}	568.11^{j}	66.55^{abcdef}	60.2^{a}	2.4^{bcd}	7.5^{bcd}	658.85^{j}	67.34^{i}	61.23^{a}	3.6^{ab}	6.0^{bcdef}	646.46^{i}
T18	72.5^{abcd}	44.02^{e}	1.9^{d}	6.0^{cd}	572.75^{i}	80.34^{abcde}	32.09^{e}	2.8^{d}	7.1cd	795.37^{i}	76.45^{d}	45.67^{bcdef}	3.8^{a}	5.8^{cdef}	733.92^{c}
Control	66.0^{abcdef}	61.12^{a}	2.0^{d}	5.8^{d}	514.80^{l}	69.09^{abcdef}	36.88^{a}	2.3^{d}	5.9^{d}	566.54^{l}	68.89^{g}	42.45^{defg}	2.4^{bcde}	5.7^{def}	558.09^{l}
CV%	17.21	1.15	11.47	5.33	0.04	17.21	1.15	11.47	5.33	0.04	0.0151	9.34	13.95	4.29	49.0

Contd. of Table 43

Treatments	Seed quality parameters against test pathogens														
	Colletotrichum gloeosporioides					*Curvularia lunata*					*Fusarium moniliforme*				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
T1	78.23^{c}	22.23^{fg}	1.9^{f}	5.3^{g}	563.26^{k}	85.21^{a}	45.56^{e}	3.5^{a}	8.1^{ab}	979.92^{a}	70.34^{h}	55.56^{b}	2.3^{de}	6.7^{cde}	633.06^{k}
T2	49.92^{gh}	40.23^{bcd}	2.9^{cde}	6.9^{abcd}	489.22^{c}	68.45^{h}	38.40^{h}	3.1^{a}	8.2^{a}	773.49^{h}	61.56^{k}	44.90^{h}	2.4^{de}	6.6^{de}	554.04^{n}
T3	58.78^{f}	20.0^{g}	3.0^{bcde}	6.4^{cdef}	552.53^{l}	57.68^{k}	25.05^{p}	2.8^{a}	7.5^{abcd}	605.64^{m}	59.34^{l}	21.49^{s}	2.3^{de}	6.8^{cde}	539.99^{o}
T4	70.70^{d}	57.14^{a}	2.8^{de}	6.6^{bcde}	678.72^{g}	67.78^{i}	35.8^{l}	3.1^{a}	7.8^{abc}	738.80^{i}	71.23^{g}	58.89^{a}	2.8^{bcde}	7.0^{bcde}	698.05^{i}
T5	84.12^{b}	33.18^{cdefg}	3.1^{abcde}	7.1^{abc}	858.02^{c}	78.90^{e}	24.43^{q}	3.4^{a}	8.0^{ab}	899.46^{b}	75.43^{f}	34.45^{p}	2.6^{cde}	7.3^{abcde}	746.76^{g}
T6	40.23^{ij}	49.56^{ab}	3.5^{abcd}	7.4^{a}	438.50^{q}	42.34^{c}	50.56^{c}	3.2^{a}	6.4^{d}	406.46^{r}	42.43^{q}	48.67^{d}	3.0^{abcde}	7.4^{abcde}	441.27^{r}
T7	67.45^{de}	37.40^{bcdef}	3.3^{abcde}	6.7^{abcd}	674.50^{h}	66.89^{j}	42.78^{f}	3.0^{a}	6.8^{bcd}	655.52^{l}	68.9^{i}	39.63^{l}	3.2^{abcd}	6.9^{cde}	695.89^{j}
T8	47.16^{h}	38.55^{bcdef}	3.8^{ab}	6.4^{cdef}	481.03^{p}	48.80^{n}	36.17^{k}	3.1^{a}	6.7^{cd}	353.47^{s}	50.67^{o}	37.44^{m}	3.5^{abc}	6.5^{e}	506.7^{p}
T9	41.28^{i}	37.89^{bcdef}	3. 5^{abcd}	6.3^{def}	404.54^{r}	42.34^{c}	35.58^{m}	2.9^{a}	7.1^{abcd}	423.40^{p}	49.29^{p}	39.82^{k}	2.0^{e}	7.4^{abcde}	463.33^{q}
T10	75.30^{c}	50.0^{ab}	3.1^{abcde}	7.1^{abc}	768.06^{d}	79.65^{d}	22.18^{r}	3.6^{a}	6.9^{bcd}	844.29^{c}	76.90^{e}	46.61^{g}	2.9^{abcde}	8.0^{ab}	838.21^{d}
T11	93.35^{a}	33.12^{cdefq}	3.2^{abcde}	7.3^{ab}	980.18^{a}	75.77^{g}	25.78^{c}	3.5^{a}	7.5^{abcd}	833.47^{e}	88. 09^{a}	31.37^{q}	2.8^{bcde}	8.1^{a}	960.18^{a}
T12	37.44^{j}	29.74^{defq}	3.9^{a}	6.9^{abcd}	400.61^{s}	38.90^{p}	58.23^{b}	3.5^{a}	7.0^{abcd}	408.45^{q}	40.87^{r}	48.52^{e}	3.1^{abcd}	7.6^{abcd}	437.31^{s}
T13	75.23^{c}	38.67^{bcde}	3.6^{abc}	5.9^{efg}	714.69^{f}	76.45^{f}	37.89^{i}	3.7^{a}	7.2^{abcd}	833.31^{f}	77.32^{d}	36.51^{n}	3.5^{abc}	7.7^{abc}	865.98^{c}
T14	85.0^{b}	49.5^{efg}	3.2^{abcde}	7.2^{ab}	884.0^{b}	81.87^{b}	39.90^{g}	3.0^{a}	7.3^{abcd}	843.26^{d}	79.45^{b}	42.28^{i}	3.7^{ab}	6.8^{cde}	834.23^{e}
T15	52.45^{g}	30.0^{defq}	3.5^{abcd}	7.0^{abcd}	550.73^{m}	51.45^{l}	21.20^{s}	3.2^{a}	6.9^{bcd}	519.65^{n}	55.34^{m}	27.44^{r}	3.6^{abc}	7.3^{abcde}	603.21^{l}
T16	50.67^{gh}	45.33^{abcd}	3.7^{ab}	6.8^{abcd}	532.04^{n}	50.89^{m}	48.09^{d}	3.3^{a}	6.7^{cd}	508.90^{c}	54.87^{n}	47.49^{f}	3.3^{abcd}	7.2a^{bcde}	576.14^{m}
T17	65.51^{e}	35.57^{bcdefq}	3.1^{abcde}	5.8^{fg}	583.05^{j}	66.45^{j}	60.2^{a}	3.4^{a}	6.9^{bcd}	684.44^{k}	67.38^{j}	5.48^{c}	3.5^{abc}	7.7^{abc}	754.66^{f}
T18	84.0^{b}	44.02^{abcd}	2.7^{e}	6.3^{def}	756.0^{e}	80.89^{c}	32.09^{n}	2.8^{a}	7.5^{abcd}	833.17^{g}	78.49^{c}	35.5^{o}	3.8^{ab}	7.4^{abcde}	879.09^{b}
Control	67.89^{d}	47.8^{abc}	2.8^{de}	6.8^{abcd}	665.32^{i}	68.78^{h}	36.88^{j}	2.7^{a}	7.4^{abcd}	694.68^{j}	70.37^{h}	40.45^{j}	3.9^{a}	6.6^{de}	745.92^{h}
CV%	1.91	14.15	7.78	3.72	0.05	0.22	0.125	10.50	5.70	0.0054	0.0322	0.0679	11.01	4.68	0.0037

Contd. of Table 43

Treatments	Seed quality parameters against test pathogens														
	Fusarium nivale					*Mucor* sp.					*Rhizoctonia solani*				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
T1	82. 23^{a}	22.23^{k}	3.9^{ab}	7.3^{ab}	920.97^{a}	85.21^{a}	45.56^{e}	3.2^{abcd}	8.7^{a}	1013.99^{a}	75.34^{d}	55.56^{a}	2.3^{e}	6.7^{bcde}	678.06^{h}
T2	49.92^{k}	40.23^{f}	2.9^{bcd}	6.9^{abcde}	489.22^{m}	58.45^{j}	38.40^{h}	3.1^{abcd}	8.2^{ab}	660.49^{k}	61.56^{l}	44.90^{cd}	2.4^{e}	6.6^{bcde}	554.04^{o}
T3	58.78^{i}	20.0^{l}	3.0^{bcd}	6.4^{abcdefgh}	552.53^{l}	67.82^{g}	25.05^{p}	3.4^{abc}	7.6^{bcde}	746.02^{g}	60.55^{n}	30.0^{k}	3.0^{abcde}	6.8^{bcde}	593.39^{m}
T4	70.70^{f}	57.14^{a}	3.2^{bcd}	6.0^{defgh}	650.44^{h}	60.91^{h}	35.8^{l}	3.6^{ab}	7.4^{bcdef}	670.01^{j}	67.77^{j}	55.78^{a}	2.8^{cde}	6.2^{de}	609.93^{k}
T5	81.12^{ab}	33.18^{i}	3.5^{abc}	7.3^{ab}	876.10^{c}	80.06^{c}	24.43^{q}	3.7^{ab}	7.9^{abcd}	928.69^{c}	74.78^{e}	35.72^{h}	2.7^{de}	6.3^{de}	673.02^{i}
T6	40.23^{m}	49.56^{c}	2.0^{d}	5.9^{efgh}	317.82^{r}	48.61^{m}	50.56^{c}	2.8^{abcd}	7.2^{bcdefq}	480.61^{q}	68.76^{i}	35.55^{hi}	2.2^{e}	5.0^{fg}	495.07^{p}
T7	67.45^{g}	37.40^{h}	2.9^{bcd}	5.6^{gh}	580.07^{j}	67.52^{g}	42.78^{f}	2.5^{cd}	7.0^{cdefq}	641.44^{m}	60.88^{m}	39.3^{fg}	2.3^{e}	4.4^{g}	407.90^{r}
T8	47.16^{l}	38.55^{h}	3.1^{bcd}	5.8^{fgh}	419.72^{o}	53.62^{l}	36.17^{k}	3.0^{abcd}	6.9^{defq}	530.84^{o}	50.93^{q}	34.67^{i}	3.1^{abcde}	5.9^{ef}	458.37^{q}
T9	41.28^{m}	37.89^{h}	2.8^{bcd}	6.2^{cdefgh}	371.52^{p}	45.88^{n}	35.58^{m}	3.3^{abc}	6.7^{efq}	450.88^{r}	70.90^{h}	40.0^{f}	3.9^{ab}	6.4^{cde}	730.27^{f}
T10	75.30^{d}	50.0^{c}	3.9^{ab}	6.8^{abcdef}	805.71^{f}	79.51^{c}	22.18^{r}	3.5^{abc}	7.8^{abcd}	898.46^{d}	73.88^{g}	38.44^{g}	4.0^{a}	6.6^{bcde}	783.13^{d}
T11	73.35^{e}	33.12^{i}	4.9^{a}	7.4^{a}	902.21^{b}	75.34^{e}	25.78^{o}	2.9^{abcd}	8.6^{a}	873.94^{e}	76. 66^{b}	41.23^{e}	3.6^{abcd}	8.9^{a}	958.25^{a}
T12	37.44^{n}	29.74^{i}	2.3^{cd}	7.0^{abcd}	348.19^{q}	40.09^{o}	58.23^{b}	2.8^{abcd}	7.9^{abcd}	428.96^{s}	36.66^{r}	40.09^{f}	3.5^{abcd}	6.8^{bcde}	377.598^{s}
T13	75.90^{d}	38.67^{j}	3.8^{ab}	7.2^{abc}	834.9^{e}	77.76^{d}	37.89^{i}	3.7^{ab}	7.2^{bcdefq}	847.58^{f}	74.55^{f}	39.92^{f}	3.6^{abcd}	7.0^{bcd}	790.23^{c}
T14	80.0^{bc}	49.5^{gh}	4.0^{ab}	6.5^{abcdefgh}	840.0^{d}	83.61^{b}	39.90^{g}	3.8^{a}	8.0^{abc}	986.59^{b}	75.77^{c}	45.63^{c}	3.8^{abc}	7.5^{b}	856.20^{b}
T15	52.0^{j}	30.0^{c}	2.8^{bcd}	6.6^{abcdefg}	488.80^{m}	55.57^{k}	21.20^{s}	2.5^{cd}	7.1^{cdefq}	533.47^{n}	54.60^{o}	31.12^{j}	3.5^{abcd}	7.4^{bc}	595.14^{l}
T16	50.89^{jk}	47.9^{jd}	3.2^{bcd}	6.3^{bcdefgh}	483.45^{n}	59.53^{i}	48.09^{d}	2.2^{d}	6.4^{fq}	510.24^{p}	52.73^{p}	49.73^{b}	3.7^{abcd}	7.1^{bcd}	569.48^{n}
T17	65.09^{h}	40.0^{fg}	3.6^{abc}	5.9^{efgh}	624.86^{i}	69.0^{f}	60.2^{a}	2.7^{bcd}	6.6^{efq}	641.70^{l}	66.74^{k}	50.45^{b}	3.9^{ab}	6.9^{bcde}	720.79^{g}
T18	79.88^{c}	44.02^{e}	3.0^{bcd}	5.8^{fgh}	702.94^{g}	80.06^{c}	32.09^{n}	3.0^{abcd}	6.3^{q}	744.56^{h}	77.61^{a}	44.43^{d}	2.9^{bcde}	6.6^{bcde}	737.29^{e}
Control	66.0^{h}	55.56^{b}	3.2^{bcd}	5.5^{h}	574.20^{k}	68.54^{f}	36.88^{j}	3.1^{abcd}	6.9^{defq}	685.40^{i}	67.72^{j}	50.32^{b}	2.8^{dce}	6.7^{bcde}	643.34^{j}
CV%	0.6345	1.16	14.30	5.23	0.0375	0.3471	0.1285	10.91	4.55	0.0048	0.0428	0.7707	10.67	5.08	0.0044

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

T1: Tilt , T2: Contaf, T3: *Psidium guajava*, T4: *Azadirachta indica*, T5: *Trichoderma viride*, T6: Contaf + Tilt, T7: *P. guajava* + *A. indica*, T8: Contaf + *P. guajava* + *T. viride*, T9: Contaf + *A. indica* + *T. viride*, T10: Tilt + *P. guajava* + *T. viride*, T11: Tilt + *A. indica* + *T. viride*, T12: Contaf + *A. indica*, T13: Contaf + *P. guajava*, T14: Tilt + *A. indica*, T15: Tilt + *P. guajava*, T16: Contaf + Tilt + *P. guajava*+ *A. indica*, T17: Contaf + Tilt + *P. guajava*+ *A. indica*+ *T. viride*, T18: Inoculated without treatment, Control: Uninoculated healthy seeds. A: % germination, B: % mortality, C: Root length (cm), D: Shoot length (cm) and E: Seedling vigor index.

Fig. 49. Combined effect of seed treatment with fungicides, leaf extracts and antagonists on seed quality parameters of cotton varieties. T1. Tilt, **T2.** Contaf, **T3.** *Psidium guajava*, **T4.** *Azadirachta indica* and **T5.** *Trichoderma viride*.

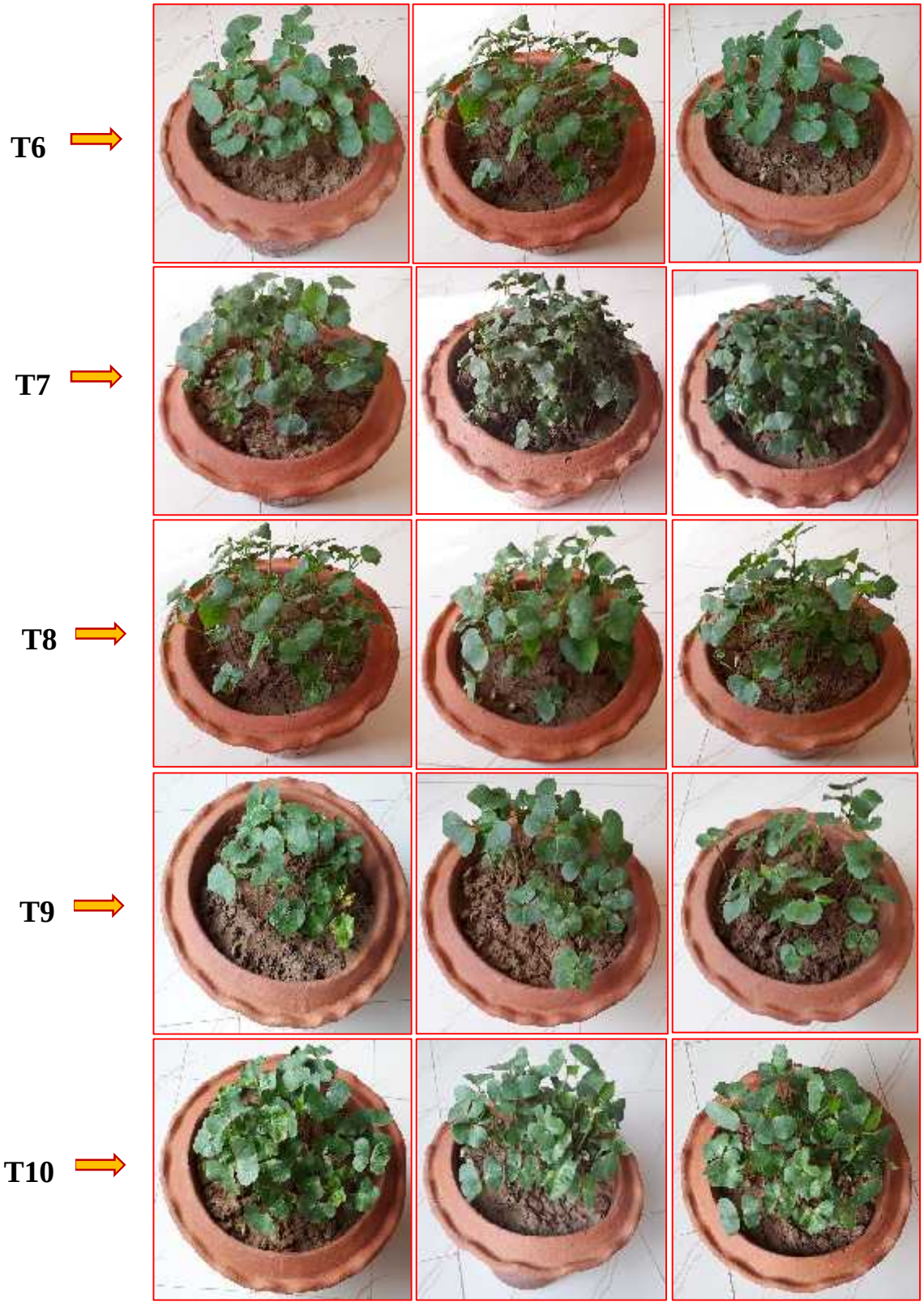

Fig. 50. Combined effect of seed treatment with fungicides, leaf extracts and antagonists on seed quality parameters of cotton varieties. T6. Contaf + Tilt, **T7.** *Psidium guajava* + *Azadirachta indica*, **T8.** Contaf + *P. guajava* + *Trichoderma viride*, **T9.** Contaf + *A. indica* + *T. viride* and **T10.** Tilt + *P. guajava* + *T. viride*.

Fig. 51. Combined effect of seed treatment with fungicides, leaf extracts and antagonists on seed quality parameters of cotton varieties. T11. Tilt + *A. indica* + *T. viride*, **T12.** Contaf + *A. indica*, **T13.** Contaf + *Psidium guajava*, **T14.** Tilt + *Azadirachta indica* and **T15.** Tilt + *P. guajava*.

Fig. 52. Combined effect of seed treatment with fungicides, leaf extracts and antagonists on seed quality parameters of cotton varieties. T16. Contaf + Tilt + *P. guajava* + *A. indica*, **T17.** Contaf + Tilt + *P. guajava* + *A. indica* + *Trichoderma viride*, **T18.** Inoculated without treatment and **T19.** Control or Uninoculated healthy seeds.

CHAPTER: 5
CONCLUSION AND RECOMMENDATIONS

CONCLUSION

Based on the findings of the present istudy, following conclusions were drawn:

- Seeds of cotton in storage condition were more susceptible to fungal infection resulting in the reduction of seed germination and yield.
- A total of 29 species of fungi were isolated from 14 varieties of cotton seeds. Highest fungal infection was observed in CB10 and lowest in CB 8.
- Association of *Aspergillus subramanianii, A. toxicarius, A. wentii, Penicillium aculeatum, P. citrinum, Rhizomucor* sp. and *Meyerozyma guilliermondii* were new records in cotton seeds for Bangladesh.
- Out of 14 cotton varieties CB 5, CB 10 and CB 13 showed best results on the basis of purity percentage of seeds. CB10 showed the highest vigor index value and maximum germination percentage.
- Molecular identification of 19 fungi was conducted by using ITS sequence based analysis.
- *Aspergillus flavus, A. fumigatus, A. niger, Chaetomium globosum, Penicillium citrinum* and *Rhizopus stolonifer* were the most predominant fungi in terms of prevalence and their frequency were also gradually increased with the increase of storage period.
- *Chaetomium globosum* was the most predominant fungi noticed in blotter method and Paper Towel method.
- Fourteen species of fungi were isolated from cotton seed coat, embryo and endosperm. Maximum number of fungi were isolated from seed coat.

- Positive correlations were noted between fungal frequency and physical purity, seedling mortality and fungal frequency, seed moisture and fungal frequency, germination and purity percentage of seeds, fungal frequency and purity, germination and seed moisture.
- Negative correlations were observed with seedling mortality and purity percentage, moisture content and germination percentage of seeds.
- Out of 29 fungi, 9 were found to be pathogenic to cotton seeds. These were *Aspergillus flavus, A. niger, A. tamarii, Colletotrichum gloeosporioides, Curvularia lunata, Fusarium nivale, F. moniliforme, Mucor* sp. and *Rhizoctonia solani.*
- Among the pathogenic fungi, *Curvularia lunata* and *Rhizoctonia solani* showed a greater impact in reduction of cotton seed germination and reduced vigor index.
- All the pathogenic fungi showed seeds to seedlings transmission nature in both the earthen pot experiment and water agar test tube experiment.
- Among the 10 fungicides, Contaf and Tilt were found to be the most efficient fungicides.
- *Azadirachta indica* and *Psidium guajava* were found to be most effective plants against the test pathogens.
- Out of 19 treatments, T1 (Tilt) and T11 (Tilt + *A. indica* + *T. viride*) showed highest seed germination, seedling vigor index against *A. flavus, A. niger, C. lunata, F. nivale, Mucor* sp. and *A. tamarii, Colletotrichum gloeosporioides, F. moniliforme, Rhizoctonia solani*, respectively.
- T5 (*T. viride*), T10 (Tilt + *P. guajava* + *T. viride*) and T14 (Tilt + *A. indica*) showed promising germination percentage and seedling vigor index against the test pathogens.

RECOMMENDATIONS

Seed borne pathogenic fungi are known to cause significant crop losses by reducing cotton yield. Since cotton is a major textile fiber, better seed health management is a prerequisite for successful cotton cultivation. In connection to the main findings from this experiment the following recommendations are drawn.

- Contaf 5 EC and Tilt 250 EC at 100 ppm concentration may be exploited commercially for managing the cotton pathogens. For more confirmation the fungicides also need some trials in field condition and nursery level.
- *Azadirachta indica* and *Psidium guajava* at 20% concentration can be used for controlling diseases and production of healthy cotton seeds in small scale.
- Molecular identification of the fungal species using ITS sequence based analysis may be the best method then conventional detection method.
- Combined application of Contaf, Tilt, *Azadirachta indica, Psidium guajava* and *Trichoderma viride* in seed treatments may be used commercially to control the cotton pathogens. These treatments also need few years trials in field and nursery level for more confirmation.
- Findings of this research work will be helpful for the farmers or cotton producers to design a proper management system as well as for production of healthy, disease free cotton seeds.

CHAPTER: 6
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REFERENCES

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CHAPTER: 7
APPENDICES

APPENDICES

Appendix-I

(a). Preparation of Lacto Phenol

Lacto phenol solution used as mounting medium consisted of the following composition (Anisworth 1963).

Substances	Amount
Phenol crystals	20 gm
Lactic acid	20 ml
Glycerol	40 ml
Distilled water	20 ml

After weighting the constituents were taken in a conical flask to which distilled water added. The flask was taken well till a homogenous solution was obtained.

(b). Preparation of Cotton Blue Stain

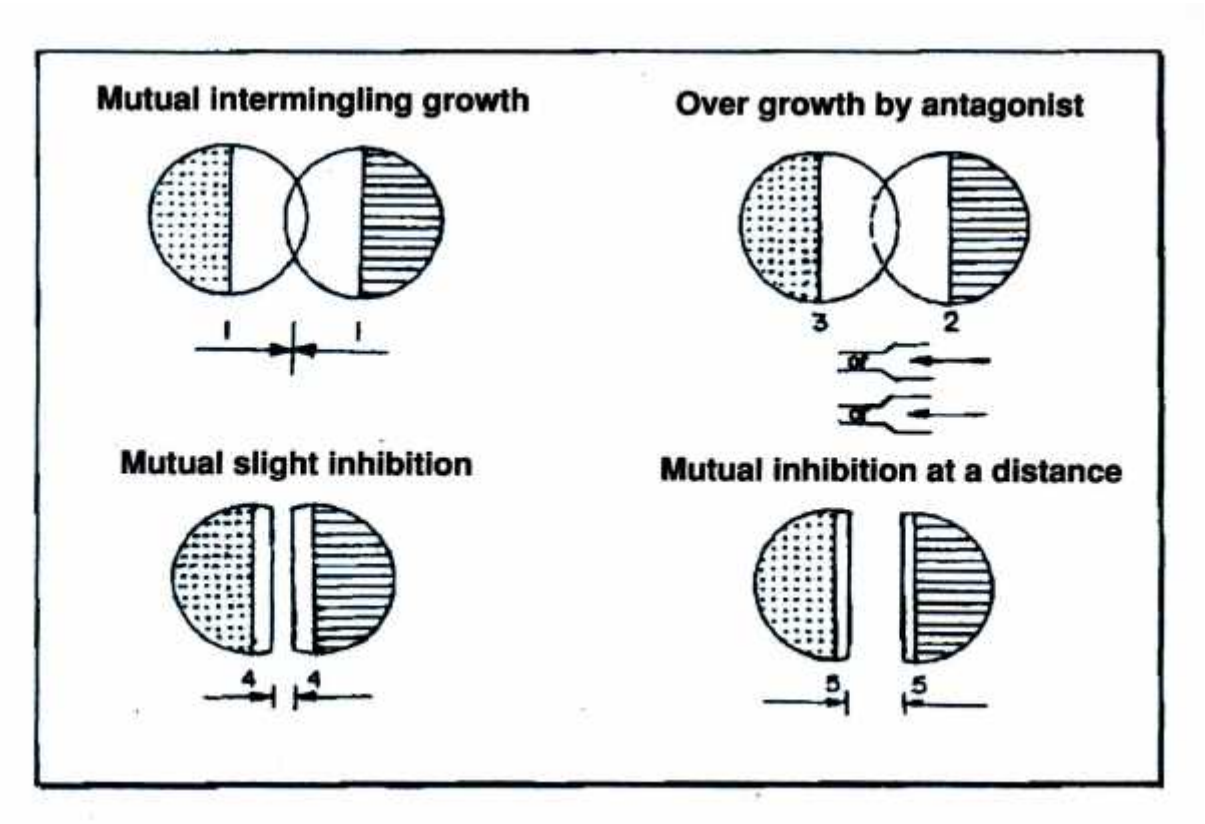
One gram of cotton blue was added to 100 ml of lacto phenol and shaken well it was dissolved. The solution of lacto phenol and cotton blue were stored in cool dark place and it was stored in an amber colored bottle.

(c). Potato Dextrose Agar (PDA) Medium

Substances	Amount
Peeled and sliced potato	200.00 gm
Dextrose	20.00 gm
Agar	15.00 gm
Distilled water	1000.00 ml
pH	6.0

Appendix-II

The colony interaction model of Skidmore and Dickinson (1976)



* = Grades from 1 (mutually intermingling growth) to 5 (mutual inhibition at a distance), based on Skidmore and Dickinson (1976).

**** A =** Mutually intermingling growth were both fungi grew into one another without any microscopic sign of interaction (1).

Bi = Intermingling growth where the test fungus being observed into the opposed fungus either above or below its colony (3).

Bii = Intermingling growth where the fungus under observation has ceased the growth and is being overgrown by another colony (2).

C = Slight inhibition with a narrow demarcation line, 1-2 mm (4).

D = Mutual inhibition at a distance more than 2 mm (5).

Appendix-III

Analysis of variance of the percentage of pure seeds, other seeds, abio or non-seeds, moisture, germination, mortality and associated fungi of different varieties of cotton seeds

For pure seeds

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Variety.name	13	24.8015	1.9078	6.95	0.0000
Error	28	7.6821	0.2744		
Total	41	32.4836			

For other seeds

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Variety.name	13	2.0611	0.1585	15.81	0.0000
Error	28	0.2808	0.0100		
Total	41	2.3419			

For abio or non-seeds

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Variety.name	13	2.8527	0.2194	271.87	0.0000
Error	28	0.0226	0.0008		
Total	41	2.8753			

For moisture

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Variety.name	13	5.0237	0.3864	38.63	0.0000
Error	28	0.2801	0.0100		
Total	41	5.3038			

For germination

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Variety.name	13	769.0714	59.1593	59.16	0.0000
Error	28	28.0000	1.0000		
Total	41	797.0714			

For mortality

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Variety.name	13	4947.6630	380.5895	39.96	0.0000
Error	28	266.6697	9.5239		
Total	41	5214.3328			

For associated fungi

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Variety.name	13	8245.0714	634.2363	634.24	0.0000
Error	28	28.0000	1.0000		
Total	41	8273.0714			

Analysis of variance of the per cent frequency of fungi associated with different parts of cotton seeds (randomly selected seeds of 14 cotton varieties).

For seed coat

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Name.of.fungi	9	15358.8000	1706.5333	1896.15	0.0000
Error	20	18.0000	0.9000		
Total	29	15376.8000			

For embryo

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Name.of.fungi	9	17449.2000	1938.8000	3877.60	0.0000
Error	20	10.0000	0.5000		
Total	29	17459.2000			

For endosperm

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Name.of.fungi	9	36994.8000	4110.5333	10276.33	0.0000
Error	20	8.0000	0.4000		
Total	29	37002.8000			

Analysis of variance of the per cent frequency of fungi associated with different parts of cotton seeds with highest fungal frequency (variety CB10)

For seed coat

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Name.of.fungi	8	3744.0000	468.0000	526.50	0.0000
Error	18	16.0000	0.8889		
Total	26	3760.0000			

For embryo

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Name.of.fungi	8	2362.6667	295.3333	1329.00	0.0000
Error	18	4.0000	0.2222		
Total	26	2366.6667			

For endosperm

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Name.of.fungi	8	5294.6667	661.8333	1985.50	0.0000
Error	18	6.0000	0.3333		
Total	26	5300.6667			

Analysis of variance of the per cent frequency of fungi associated with different parts of cotton seeds with lowest fungal frequency (variety CB8).

For seed coat

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Name.of.fungi	10	9643.6364	964.3636	1060.80	0.0000
Error	22	20.0000	0.9091		
Total	32	9663.6364			

For embryo

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Name.of.fungi	10	7813.6364	781.3636	1719.00	0.0000
Error	22	10.0000	0.4545		
Total	32	7823.6364			

For endosperm

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Name.of.fungi	10	13568.1818	1356.8182	4975.00	0.0000
Error	22	6.0000	0.2727		
Total	32	13574.1818			

Analysis of variance of the per cent frequency of fungi associated with different parts of cotton seeds with moderate fungal frequency (variety CB3).

For seed coat

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Name.of.fungi	6	4844.5714	807.4286	942.00	0.0000
Error	14	12.0000	0.8571		
Total	20	4856.5714			

For embryo

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Name.of.fungi	6	11318.5714	1886.4286	4401.67	0.0000
Error	14	6.0000	0.4286		
Total	20	11324.5714			

For endosperm

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Name.of.fungi	6	7043.1429	1173.8571	2054.25	0.0000
Error	14	8.0000	0.5714		
Total	20	7051.1429			

Analysis of variance of the per cent frequency of fungi associated with different parts of cotton seeds

For seed coat

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Name.of.fungi	13	19067.1429	1466.7033	2281.54	0.0000
Error	28	18.0000	0.6429		
Total	41	19085.1429			

For embryo

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Name.of.fungi	13	19276.2857	1482.7912	4151.82	0.0000
Error	28	10.0000	0.3571		
Total	41	19286.2857			

For endosperm

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Name.of.fungi	13	41219.1429	3170.7033	11097.46	0.0000
Error	28	8.0000	0.2857		
Total	41	41227.1429			

Analysis of variance of the effect of pathogenic fungi on the seeds and seedling growth of cotton

For germination

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatments	9	15744.0000	1749.3333	1749.33	0.0000
Error	20	20.0000	1.0000		
Total	29	15764.0000			

For mortality

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatments	9	1156.8000	128.5333	128.53	0.0000
Error	20	20.0000	1.0000		
Total	29	1176.8000			

For shoot length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatments	9	87.2430	9.6937	969.37	0.0000
Error	20	0.2000	0.0100		
Total	29	87.4430			

For root length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatments	9	40.3320	4.4813	448.13	0.0000
Error	20	0.2000	0.0100		
Total	29	40.5320			

For vigor index

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatments	9	5580902.3550	620100.2617	1527340.55	0.0000
Error	20	8.1200	0.4060		
Total	29	5580910.4750			

Analysis of variance of the transmission of test pathogens from seed to seedlings in pot experiment
For germination

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatments	9	7603.3860	844.8207	8440.61	0.0000
Error	20	2.0018	0.1001		
Total	29	7605.3878			

For mortality

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatments	9	5028.3386	558.7043	6207825.37	0.0000
Error	20	0.0018	0.0001		
Total	29	5028.3404			

For root length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatments	9	5.1569	0.5730	186.64	0.0000
Error	20	0.0614	0.0031		
Total	29	5.2183			

For shoot length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatments	9	23.2339	2.5815	25815.50	0.0000
Error	20	0.0020	0.0001		
Total	29	23.2359			

For Seed to seedling transmission of the pathogens

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatments	9	4499.5957	499.9551	462921.37	0.0000
Error	20	0.0216	0.0011		
Total	29	4499.6173			

For Germination

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatments	9	3709.0385	412.1154	4121153.87	0.0000
Error	20	0.0020	0.0001		
Total	29	3709.0405			

For mortality

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatments	9	3564.6541	396.0727	3960726.80	0.0000
Error	20	0.0020	0.0001		
Total	29	3564.6561			

For shoot length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatments	9	42.8155	4.7573	214.78	0.0000
Error	20	0.4430	0.0222		
Total	29	43.2585			

For root length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatments	9	14.4437	1.6049	265.70	0.0000
Error	20	0.1208	0.0060		
Total	29	14.5645			

For Seed to seedling transmission of the pathogens

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatments	9	2951.2218	327.9135	3934962.44	0.0000
Error	20	0.0017	0.0001		
Total	29	2951.2235			

Analysis of variance of per cent inhibition of radial growth of the test pathogens owing to volatile metabolites of antagonistic fungi.

For *Colletotrichum.gloeosporioides*

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatments	3	1001.3985	333.7995	3337995.00	0.0000
Error	8	0.0008	0.0001		
Total	11	1001.3993			

For *Curvularia lunata*

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatments	3	2582.9664	860.9888	8609888.00	0.0000
Error	8	0.0008	0.0001		
Total	11	2582.9672			

For *Fusarium nivale*

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatments	3	931.1276	310.3759	3103758.75	0.0000
Error	8	0.0008	0.0001		
Total	11	931.1284			

For *Fusarium moniliforme*

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatments	3	385.3482	128.4494	1284494.00	0.0000
Error	8	0.0008	0.0001		
Total	11	385.3490			

For *Mucor* sp.

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatments	3	868.6737	289.5579	2171684.25	0.0000
Error	8	0.0011	0.0001		
Total	11	868.6748			

For *Rhizoctonia solani*

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatments	3	807.0822	269.0274	2690274.00	0.0000
Error	8	0.0008	0.0001		
Total	11	807.0830			

Analysis of variance of per cent inhibition of radial growth of *Colletotrichum gloeosporioides* by non-volatile metabolites of antagonistic fungi.

For 5% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	840.6374	280.2125	2802124.75	0.0000
Error	8	0.0008	0.0001		
Total	11	840.6382			

For 10% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	371.7498	123.9166	1239166.00	0.0000
Error	8	0.0008	0.0001		
Total	11	371.7506			

For 15% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	493.1072	164.3691	1643690.75	0.0000
Error	8	0.0008	0.0001		
Total	11	493.1080			

For 20% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	767.8383	255.9461	2559461.00	0.0000
Error	8	0.0008	0.0001		
Total	11	767.8391			

Analysis of variance of per cent inhibition of radial growth of *Curvularia lunata* by non-volatile metabolites of antagonistic fungi.

For 5% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	752.2778	250.7593	2507592.75	0.0000
Error	8	0.0008	0.0001		
Total	11	752.2786			

For 10% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	1057.3905	352.4635	3524635.00	0.0000
Error	8	0.0008	0.0001		
Total	11	1057.3913			

For 15% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	663.6278	221.2093	2212092. 75	0.0000
Error	8	0.0008	0.0001		
Total	11	663.6286			

For 20% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	722.8196	240.9399	2409398. 75	0.0000
Error	8	0.0008	0.0001		
Total	11	722.8204			

Analysis of variance of per cent inhibition of radial growth of *Fusarium moniliforme* by non-volatile metabolites of antagonistic fungi.

For 5% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	285.0890	95.0297	152047.48	0.0000
Error	8	0.0050	0.0006		
Total	11	285.0940			

For 10% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	266.6673	88.8891	666668.25	0.0000
Error	8	0.0011	0.0001		
Total	11	266.6684			

For 15% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	368.7416	122.9139	1229138.75	0.0000
Error	8	0.0008	0.0001		
Total	11	368.7424			

For 20% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	722.6991	240.8997	2408997.00	0.0000
Error	8	0.0008	0.0001		
Total	11	722.6999			

Analysis of variance of per cent inhibition of radial growth of *Fusarium nivale* by non-volatile metabolites of antagonistic fungi.

For 5% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	711.7676	237.2559	2372558.75	0.0000
Error	8	0.0008	0.0001		
Total	11	711.7684			

For 10% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	1256.5106	418.8369	4188368.75	0.0000
Error	8	0.0008	0.0001		
Total	11	1256.5114			

For 15% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	1073.8914	357.9638	139015.07	0.0000
Error	8	0.0206	0.0026		
Total	11	1073.9120			

For 20% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	471.7214	157.2405	1572404.75	0.0000
Error	8	0.0008	0.0001		
Total	11	471.7222			

Analysis of variance of per cent inhibition of radial growth of *Mucor* sp. by non-volatile metabolites of antagonistic fungi.

For 5% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	208.4487	69.4829	694829.00	0.0000
Error	8	0.0008	0.0001		
Total	11	208.4495			

For 10% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	65.3193	21.7731	217731.00	0.0000
Error	8	0.0008	0.0001		
Total	11	65.3201			

For 15% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	339.7634	113.2545	1132544.75	0.0000
Error	8	0.0008	0.0001		
Total	11	339.7642			

For 20% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	502.9284	167.6428	151256.67	0.0000
Error	8	0.0089	0.0011		
Total	11	502.9373			

Analysis of variance of per cent inhibition of radial growth of *Mucor* sp. by non-volatile metabolites of antagonistic fungi

For 5% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	190.3488	63.4496	634496.00	0.0000
Error	8	0.0008	0.0001		
Total	11	190.3496			

For 10% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	1633.3071	544.4357	5444357.00	0.0000
Error	8	0.0008	0.0001		
Total	11	1633.3079			

For 15% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	1358.4218	452.8073	4528072.75	0.0000
Error	8	0.0008	0.0001		
Total	11	1358.4226			

For 20% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	3	4558.2002	1519.4001	15194000.75	0.0000
Error	8	0.0008	0.0001		
Total	11	4558.2010			

Analysis of variance of the effects of Fungicides on *Aspergillus niger* at different concentrations.

For 100 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	38625.3053	4291.7006	849841.70	0.0000
Error	20	0.1010	0.0050		
Total	29	38625.4063			

For 200 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	22810.2521	2534.4725	419614.64	0.0000
Error	20	0.1208	0.0060		
Total	29	22810.3729			

For 300 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	11061.5658	1229.0629	166314.33	0.0000
Error	20	0.1478	0.0074		
Total	29	11061.7136			

For 400 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	6133.3632	681.4848	96939.52	0.0000
Error	20	0.1406	0.0070		
Total	29	6133.5038			

For 500 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	4526.2224	502.9136	62707.43	0.0000
Error	20	0.1604	0.0080		
Total	29	4526.3828			

Analysis of variance of the effects of Fungicides on *Aspergillus flavus* at different concentrations.

For 100 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr> F)
Treatment	9	30360.7987	3373.4221	832943.72	0.0000
Error	20	0.0810	0.0040		
Total	29	30360.8797			

For 200 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	21429.1242	2381.0138	586456.60	0.0000
Error	20	0.0812	0.0041		
Total	29	21429.2054			

For 300 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	5866.4431	651.8270	129074.66	0.0000
Error	20	0.1010	0.0050		
Total	29	5866.5441			

For 400 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	4821.9078	535.7675	79255.55	0.0000
Error	20	0.1352	0.0068		
Total	29	4822.0430			

For 500 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	3498.1691	388.6855	64351.90	0.0000
Error	20	0.1208	0.0060		
Total	29	3498.2899			

Analysis of variance for the effects of Fungicides on *Aspergillus tamarii* at different concentrations.

For 100 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	31639.5942	3515.5105	868027.28	0.0000
Error	20	0.0810	0.0040		
Total	29	31639.6752			

For 200 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	22342.8289	2482.5365	98827.09	0.0000
Error	20	0.5024	0.0251		
Total	29	22343.3313			

For 300 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	9795.1675	1088.3519	215515.24	0.0000
Error	20	0.1010	0.0050		
Total	29	9795.2685			

For 400 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	4498.3669	499.8185	92387.90	0.0000
Error	20	0.1082	0.0054		
Total	29	4498.4751			

For 500 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	2515.8829	279.5425	46281.88	0.0000
Error	20	0.1208	0.0060		
Total	29	2516.0037			

Analysis of variance for the effects of Fungicides on *Colletotrichum gloeosporioides* at different concentrations.

For 100 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	23200.6794	2577.8533	476497.83	0.0000
Error	20	0.1082	0.0054		
Total	29	23200.7876			

For 200 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	16714.9345	1857.2149	420184.38	0.0000
Error	20	0.0884	0.0044		
Total	29	16715.0229			

For 300 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	10515.2588	1168.3621	264335.31	0.0000
Error	20	0.0884	0.0044		
Total	29	10515.3472			

For 400 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	8090.9379	898.9931	148839.92	0.0000
Error	20	0.1208	0.0060		
Total	29	8091.0587			

For 500 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	5272.4195	585.8244	73045.43	0.0000
Error	20	0.1604	0.0080		
Total	29	5272.5799			

Analysis of variance for fungitoxicity of fungicides against *Curvularia lunata*.

For 100 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	15276.7731	1697.4192	241682.85	0.0000
Error	20	0.1405	0.0070		
Total	29	15276.9135			

For 200 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	6302.9568	700.3285	94767.05	0.0000
Error	20	0.1478	0.0074		
Total	29	6303.1046			

For 300 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	4984.7499	553.8611	78636.22	0.0000
Error	20	0.1409	0.0070		
Total	29	4984.8908			

For 400 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	1493.6323	165.9591	23607.28	0.0000
Error	20	0.1406	0.0070		
Total	29	1493.7729			

For 500 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	805.3887	89.4876	11158.06	0.0000
Error	20	0.1604	0.0080		
Total	29	805.5491			

Analysis of variance of the effects of Fungicides on *Fusarium moniliforme* at different concentrations.

For 100 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	14814.1209	1646.0134	536160.73	0.0000
Error	20	0.0614	0.0031		
Total	29	14814.1823			

For 200 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	10809.0054	1201.0006	391205.41	0.0000
Error	20	0.0614	0.0031		
Total	29	10809.0668			

For 300 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	6508.6525	723.1836	235564.69	0.0000
Error	20	0.0614	0.0031		
Total	29	6508.7139			

For 400 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	4311.6485	479.0721	156049.53	0.0000
Error	20	0.0614	0.0031		
Total	29	4311.7099			

For 500 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	1533.1224	170.3469	21240.27	0.0000
Error	20	0.1604	0.0080		
Total	29	1533.2828			

Analysis of variance for fungitoxicity of fungicides against *Fusarium nivale*.

For 100 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	16025.9532	1780.6615	294811.50	0.0000
Error	20	0.1208	0.0060		
Total	29	16026.0740			

For 200 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	7010.2585	778.9176	128959.87	0.0000
Error	20	0.1208	0.0060		
Total	29	7010.3793			

For 300 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	4982.9809	553.6645	91666.32	0.0000
Error	20	0.1208	0.0060		
Total	29	4983.1017			

For 400 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	2642.8765	293.6529	41771.40	0.0000
Error	20	0.1406	0.0070		
Total	29	2643.0171			

For 500 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	2548.5927	283.1770	5.31	0.0009
Error	20	1066.0469	53.3023		
Total	29	3614.6395			

Analysis of variance for fungitoxicity of fungicides against *Mucor* sp.

For 100 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	42150.0347	4683.3372	1535520.39	0.0000
Error	20	0.0610	0.0030		
Total	29	42150.0957			

For 200 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	27519.7056	3057.7451	996008.17	0.0000
Error	20	0.0614	0.0031		
Total	29	27519.7670			

For 300 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	24916.2080	2768.4676	901780.96	0.0000
Error	20	0.0614	0.0031		
Total	29	24916.2694			

For 400 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	24986.4337	2776.2704	459647.42	0.0000
Error	20	0.1208	0.0060		
Total	29	24986.5545			

For 500 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	16745.5004	1860.6112	231996.40	0.0000
Error	20	0.1604	0.0080		
Total	29	16745.6608			

Analysis of variance for fungitoxicity of fungicides against *Rhizoctonia solani*.

For 100 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	30262.8516	3362.5391	557635.00	0.0000
Error	20	0.1206	0.0060		
Total	29	30262.9722			

For 200 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	14152.8923	1572.5436	260354.90	0.0000
Error	20	0.1208	0.0060		
Total	29	14153.0131			

For 300 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	3140.5458	348.9495	49637.20	0.0000
Error	20	0.1406	0.0070		
Total	29	3140.6864			

For 400 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr (> F)
Treatment	9	0.0000	0.0000	0.00	1.0000
Error	20	0.2000	0.0100		
Total	29	0.2000			

For 500 ppm concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	0.0000	0.0000	0.00	1.0000
Error	20	0.2000	0.0100		
Total	29	0.2000			

Analysis of variance for antifungal activity of plant extracts against *Aspergillus flavus*

For 5% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	14580.2929	1620.0325	1528332.59	0.0000
Error	20	0.0212	0.0011		
Total	29	14580.3141			

For 10% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	17705.0232	1967.2248	39344496.07	0.0000
Error	20	0.0010	0.0001		
Total	29	17705.0242			

For 15% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	25599.7410	2844.4157	35555195.83	0.0000
Error	20	0.0016	0.0001		
Total	29	25599.7426			

For 20% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	23424.2280	2602.6920	16266825.02	0.0000
Error	20	0.0032	0.0002		
Total	29	23424.2312			

Analysis of variance for antifungal activity of plant extracts against on *Aspergillus niger*.

For 5% concentration

The data for the response variable 'X5.' is constant.

For 10% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	6316.5485	701.8387	23394624.00	0.0000
Error	20	0.0006	0.0000		
Total	29	6316.5491			

For 15% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	9169.2678	1018.8075	11320083.74	0.0000
Error	20	0.0018	0.0001		
Total	29	9169.2696			

For 20% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	12542.9841	1393.6649	6965.89	0.0000
Error	20	4.0014	0.2001		
Total	29	12546.9855			

Analysis of variance for antifungal activity of plant extracts against *Aspergillus tamarii.*

For 5% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	19048.3494	2116.4833	26456040.83	0.0000
Error	20	0.0016	0.0001		
Total	29	19048.3510			

For 10% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	18817.6053	2090.8450	29869214.71	0.0000
Error	20	0.0014	0.0001		
Total	29	18817.6067			

For 15% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	22915.9895	2546.2211	25462210.53	0.0000
Error	20	0.0020	0.0001		
Total	29	22915.9915			

For 20% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	17316.6430	1924.0714	9615.87	0.0000
Error	20	4.0019	0.2001		
Total	29	17320.6449			

Analysis of variance for antifungal activity of plant extracts against *Colletotrichum gloeosporioides*.

For 5% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	3754.3777	417.1531	401108.73	0.0000
Error	20	0.0208	0.0010		
Total	29	3754.3985			

For 10% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	6570.8587	730.0954	14601908.27	0.0000
Error	20	0.0010	0.0001		
Total	29	6570.8597			

For 15% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	5562.0130	618.0014	14261571.87	0.0000
Error	20	0.0009	0.0000		
Total	29	5562.0139			

For 20% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	14686.7941	1631.8660	8117.93	0.0000
Error	20	4.0204	0.2010		
Total	29	14690.8145			

Analysis of variance for antifungal activity of plant extracts against *Curvularia lunata*.

For 5% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	14149.5611	1572.1735	1442360.97	0.0000
Error	20	0.0218	0.0011		
Total	29	14149.5829			

For 10% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	11923.6960	1324.8551	14194876.19	0.0000
Error	20	0.0019	0.0001		
Total	29	11923.6979			

For 15% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	24035.3459	2670.5940	4450.92	0.0000
Error	20	12.0002	0.6000		
Total	29	24047.3461			

For 20% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	13785.6276	1531.7364	2552.72	0.0000
Error	20	12.0008	0.6000		
Total	29	13797.6284			

Analysis of variance for antifungal activity of plant extracts against *Fusarium moniliforme*.

For 5% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	4280.2623	475.5847	4614.64	0.0000
Error	20	2.0612	0.1031		
Total	29	4282.3235			

For 10% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	5811.3868	645.7096	459035.29	0.0000
Error	20	0.0281	0.0014		
Total	29	5811.4149			

For 15% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	10227.8507	1136.4279	3774.84	0.0000
Error	20	6.0211	0.3011		
Total	29	10233.8718			

For 20% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	10160.8139	1128.9793	2808.17	0.0000
Error	20	8.0407	0.4020		
Total	29	10168.8545			

Analysis of variance for antifungal activity of plant extracts against *Fusarium nivale*.

For 5% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	10797.1169	1199.6797	11986.01	0.0000
Error	20	2.0018	0.1001		
Total	29	10799.1187			

For 10% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	8732.9695	970.3299	9695.54	0.0000
Error	20	2.0016	0.1001		
Total	29	8734.9711			

For 15% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	10723.2993	1191.4777	5925.29	0.0000
Error	20	4.0217	0.2011		
Total	29	10727.3209			

For 20% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	13718.7051	1524.3006	3801.00	0.0000
Error	20	8.0205	0.4010		
Total	29	13726.7257			

Analysis of variance for antifungal activity of plant extracts against *Mucor* sp.

For 5% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	19261.5056	2140.1673	30573818.33	0.0000
Error	20	0.0014	0.0001		
Total	29	19261.5070			

For 10% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	19650.7306	2183.4145	26200974.13	0.0000
Error	20	0.0017	0.0001		
Total	29	19650.7323			

For 15% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	21749.0775	2416.5642	24145.52	0.0000
Error	20	2.0017	0.1001		
Total	29	21751.0791			

For 20% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	19930.4294	2214.4922	21911.20	0.0000
Error	20	2.0213	0.1011		
Total	29	19932.4507			

Analysis of variance for antifungal activity of plant extracts against *Rhizoctonia solani*.

For 5% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	13489.5273	1498.8364	18735454.54	0.0000
Error	20	0.0016	0.0001		
Total	29	13489.5289			

For 10% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	12029.7193	1336.6355	13315.31	0.0000
Error	20	2.0077	0.1004		
Total	29	12031.7269			

For 15% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	9477.0163	1053.0018	4511.12	0.0000
Error	20	4.6685	0.2334		
Total	29	9481.6848			

For 20% concentration

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	9	9312.3765	1034.7085	10337.78	0.0000
Error	20	2.0018	0.1001		
Total	29	9314.3783			

Integrated control of pathogenic fungi associated with cotton seeds

Analysis of variance of the effects of integrated control on *Aspergillus flavus* at different concentrations.

For germination

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	11326.1230	629.2291	5.60	0.0000
Error	38	4270.7889	112.3892		
Total	56	15596.9118			

For mortality

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	7241.1104	402.2839	1821.40	0.0000
Error	38	8.3929	0.2209		
Total	56	7249.5033			

For root length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	18.8891	1.0494	9.38	0.0000
Error	38	4.2533	0.1119		
Total	56	23.1425			

For shoot length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	11.6737	0.6485	5.83	0.0000
Error	38	4.2267	0.1112		
Total	56	15.9004			

For Seedling vigor index

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	1449455.5450	80525.3081	1488211.71	0.0000
Error	38	2.0561	0.0541		
Total	56	1449457.6011			

Analysis of variance of the effects of integrated control on *Aspergillus niger* at different concentrations.

For germination

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	11326.1230	629.2291	5.60	0.0000
Error	38	4270.7889	112.3892		
Total	56	15596.9118			

For mortality

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	7241.1104	402.2839	1821.40	0.0000
Error	38	8.3929	0.2209		
Total	56	7249.5033			

For root length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	18.8891	1.0494	9.38	0.0000
Error	38	4.2533	0.1119		
Total	56	23.1425			

For shoot length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	11.6737	0.6485	5.83	0.0000
Error	38	4.2267	0.1112		
Total	56	15.9004			

For Seedling vigor index

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	1449455.5450	80525.3081	1488211.71	0.0000
Error	38	2.0561	0.0541		
Total	56	1449457.6011			

Analysis of variance of the effects of integrated control on *Aspergillus tamarii* at different concentrations.

For germination

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	10695.5837	594.1991	6641048.73	0.0000
Error	38	0.0034	0.0001		
Total	56	10695.5871			

For mortality

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	5442.5173	302.3621	19.14	0.0000
Error	38	600.3947	15.7999		
Total	56	6042.9120			

For root length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	20.2621	1.1257	6.78	0.0000
Error	38	6.3067	0.1660		
Total	56	26.5688			

For shoot length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	15.1239	0.8402	11.51	0.0000
Error	38	2.7733	0.0730		
Total	56	17.8972			

For Seedling vigor index

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	1336521.5340	74251.1963	64615544.90	0.0000
Error	38	0.0437	0.0011		
Total	56	1336521.5777			

Analysis of variance of the effects of integrated control on *Colletotrichum gloeosporioides* at different concentrations.

For germination

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	15809.0311	878.2795	576.79	0.0000
Error	38	57.8630	1.5227		
Total	56	15866.8941			

For mortality

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	5459.8777	303.3265	10.74	0.0000
Error	38	1072.7291	28.2297		
Total	56	6532.6068			

For root length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	11.5604	0.6422	10.52	0.0000
Error	38	2.3200	0.0611		
Total	56	13.8804			

For shoot length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	16.4526	0.9140	14.97	0.0000
Error	38	2.3200	0.0611		
Total	56	18.7726			

For Seedling vigor index

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	1492570.7546	82920.5975	787076.66	0.0000
Error	38	4.0034	0.1054		
Total	56	1492574.7580			

Analysis of variance of the effects of integrated control on *Curvularia lunata* at different concentrations.

For germination

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	12157.4048	675.4114	32770.21	0.0000
Error	38	0.7832	0.0206		
Total	56	12158.1880			

For mortality

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	6983.6535	387.9808	165283.28	0.0000
Error	38	0.0892	0.0023		
Total	56	6983.7427			

For root length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	4.5193	0.2511	2.22	0.0192
Error	38	4.3000	0.1132		
Total	56	8.8193			

For shoot length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	13.6505	0.7584	4.46	0.0001
Error	38	6.4667	0.1702		
Total	56	20.1172			

For Seedling vigor index

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	1969584.5645	109421.3647	83382590.74	0.0000
Error	38	0.0499	0.0013		
Total	56	1969584.6144			

Analysis of variance of the effects of integrated control on *Fusarium nivale* at different concentrations.

For germination

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	12417.4382	689.8577	4333.52	0.0000
Error	38	6.0493	0.1592		
Total	56	12423.4874			

For mortality

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	5772.3079	320.6838	1513.96	0.0000
Error	38	8.0491	0.2118		
Total	56	5780.3570			

For root length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	23.2281	1.2904	5.96	0.0000
Error	38	8.2333	0.2167		
Total	56	31.4614			

For shoot length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	20.9126	1.1618	10.27	0.0000
Error	38	4.3000	0.1132		
Total	56	25.2126			

For Seedling vigor index

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	2102905.5354	116828.0853	2159221.44	0.0000
Error	38	2.0560	0.0541		
Total	56	2102907.5915			

Analysis of variance of the effects of integrated control on *Fusarium moniliforme* at different concentrations.

For germination

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	9675.5194	537.5289	1220683.05	0.0000
Error	38	0.0167	0.0004		
Total	56	9675.5361			

For mortality

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	4767.9996	264.8889	334781.94	0.0000
Error	38	0.0301	0.0008		
Total	56	4768.0297			

For root length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	16.9839	0.9435	8.31	0.0000
Error	38	4.3133	0.1135		
Total	56	21.2972			

For shoot length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	12.5389	0.6966	6.12	0.0000
Error	38	4.3267	0.1139		
Total	56	16.8656			

For Seedling vigor index

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	1387807.4514	77100.4140	124850102.16	0.0000
Error	38	0.0235	0.0006		
Total	56	1387807.4749			

Analysis of variance of the effects of integrated control on *Mucor* sp. at different concentrations.

For germination

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	9805.5119	544.7507	10331.67	0.0000
Error	38	2.0036	0.0527		
Total	56	9807.5155			

For mortality

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	6982.7615	387.9312	164985.96	0.0000
Error	38	0.0893	0.0024		
Total	56	6982.8509			

For root length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	11.1105	0.6173	5.42	0.0000
Error	38	4.3267	0.1139		
Total	56	15.4372			

For shoot length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	27.7547	1.5419	13.67	0.0000
Error	38	4.2867	0.1128		
Total	56	32.0414			

For Seedling vigor index

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	1842077.3612	102337.6312	89880508.12	0.0000
Error	38	0.0433	0.0011		
Total	56	1842077.4044			

Analysis of variance of the effects of integrated control on *Rhizoctonia solani* at different concentrations.

For germination

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	6441.5319	357.8629	452287.90	0.0000
Error	38	0.0301	0.0008		
Total	56	6441.5620			

For mortality

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	3089.6957	171.6498	1618.67	0.0000
Error	38	4.0297	0.1060		
Total	56	3093.7254			

For root length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	19.7621	1.0979	9.70	0.0000
Error	38	4.3000	0.1132		
Total	56	24.0621			

For shoot length

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	46.1758	2.5653	22.67	0.0000
Error	38	4.3000	0.1132		
Total	56	50.4758			

For Seedling vigor index

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Treatment	18	1235080.3686	68615.5760	86720351.08	0.0000
Error	38	0.0301	0.0008		
Total	56	1235080.3987			

Appendix–III

Some commonly used Abbreviation and Symbols:

Abbreviation	Full word
%	= Per cent
°C	= Degree Celsius
CDB	= Cotton Development Board
cm	= Centimeter
DMRT	= Duncan's Multiple Range Test
et al	= And others
etc	= Etcetra
g	= Gram (s)
j.	= Journal
m^2	= Square meter
mm	= Millimeter
MOA	= Ministry of Agriculture
Mt.	= Megatons
No.	= Number
PDA	= Potato Dextrose Agar
ppm	= Parts Per Million
Res.	= Research
Sci.	= Science
Sl.	= Serial
Univ.	= University
USDA	= United States Department of Agriculture
Var.	= Variety
Viz.	= Namely
Vol.	= Volume

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