EFFICACY OF FUNGICIDES AND *Trichoderma harzianum* AGAINST SEED BORN PATHOGENS CAUSING BOLL ROT DISEASE OF COTTON

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CERTIFICATE

This is to certify that thesis entitled, ***"Efficacy of Fungicides and Trichoderma harzianum Against Seed Born Pathogens Causing Boll Rot Disease of Cotton"*** submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of *MASTER OF SCIENCE* in *PLANT PATHOLOGY*, embodies the result of a piece of bona fide research work carried out by *SAMIA AFRIN, Registration No. 06-01857* under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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

Dated: 31st December, 2013
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ABSTRACT

Experiments were conducted on efficacy of fungicides and *Trichoderma harzianum* against seed born pathogens of cotton. A field experiment was conducted for the prevalence of cotton boll rot disease in Cotton Research Training and Seed Multiplication Farm, Sreepur, Gazipur and laboratory experiments were conducted in the laboratory of Sher-e Bangla Agricultural University, Dhaka, Bangladesh during the period of April 2012 to May 2013. Three cotton varieties CB-9, CB-11 and CB-12 were used in this experiment. Three genera of fungi viz. *Colletotrichum* , *Alternaria* and *Fusarium oxysporum* were identified and isolated from infeted bolls and seeds. The range of incidence of boll rot was 4.9% to 6.32% during the survey period of November 2012 to March 2013. *In vitro* and *in vivo* pathogenicity test showed that the *F. oxysporum was* pathogenic on cotton seedling causing pre and post-emergence seedling mortality. Five fungicides namely Proud 250EC (Propiconazole), Bavistin 50WP (Carbendazim), Provax-200 (Carboxin), Rovral 50WP (Iprodione), Ridomil gold MZ 68WP (Metalaxyl M+Mancozeb) and bio control agent *Trichoderma harzianum* was tested against *Fusarium oxysporum.* Among the tested fungicides Proud 250EC and Bavistin 50WP exhibited significant effect in arresting radial mycelial growth of *F. oxysporum* even at 100 ppm and found effective in controlling pre and post emergence seedling mortality and yielded highest germination. Among the varieties tested CB-9 was found more responsive to the treatments. Study showed that, *Fusarium oxysporum* can be bettter controlled and germination (%) of different cotton varieties were increased by using Bavistin 50WP and Proud 250EC as fungicides and *Trichoderma harzianum* as bio control agent.

LIST OF SYMBOLS AND ABBREVIATIONS

%	=	Percentage
et al.	=	And others
Spp.	=	Species
J.	=	Journal
viz.	=	Namely
&	=	And
Etc.	=	Etcetera
^{o}C	=	Degree Celsius
cm	=	Centimeter
cfu	=	Colony forming unit
NaCl	=	Sodium chloride
Kg	=	Kilogram
g	=	Gram
ml	=	Milliliter
hrs	=	Hours
pv.	=	Pathovar
i.e.	=	That is
SAU	=	Sher-e-Bangla Agricultural University
BAU	=	Bangladesh Agricultural University
BBS	=	Bangladesh Bureau of Statistics
NA	=	Nutrient Agar
PDA	=	Potato Dextrose Agar
LSD	=	Least Significant Difference
CV%	=	Percentage of Co-eficient of Variance
cv.	=	Cultivar
@	=	At the rate of
WP	=	Wettable Power

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CHAPTER I

INTRODUCTION

Cotton, the "King of Fibres", enjoys a pre-eminent status among all cash crops in the country and is the principal raw material for flourishing textile industry. It provides livelihood to about sixty million people and is an important agricultural commodity providing remunerative income to millions of farmers both in developed and developing countries. Inspite of severe competition from synthetic fibres it is occupying the premier position with 70 per cent share in the textile industry (Anon. 2010a).The cotton plant belongs toMalvaceae family.Economically, the most important varieties are *Gossypiumhirsutum* and *Gossypiumbarbadense*. Currently, *Gossypium*includes 50 species, four of which are cultivated, 44 are wilddiploids, and two are wild tetraploids (Percival and Kohel, 1990).

The production potential of the crop has not been fully exploited due toseveral biotic and abiotic factors. Cotton crop remains in the field for nearly six months or more, it is affected by various diseases caused by organisms such as fungi, bacteria and viruses that grow on and with in the plant tissues. These organisms often causestunting of the plants, defoliation, reduced vigour and yield and sometimes death(Blasingame, 1994; Jiskani, 1992and Terrence and Rockroth,2001).Most frequently occurring diseases are caused by bacteria and fungi. The most common and widely distributed bacterial diseases of cotton are bacterial blight (Xanthomonascampestrispv. *malvacearum*), crown gall (*Agrobacterium tumefaciens*) and lint degradation (*Erwiniaherbicola*). Most common fungal diseases are leaf spot and leaf blight caused by *Alternariamacrospora,* *Alternariaalternata,*

CercosporagossypinaCochliobolusspicifera, Curvulariaspicifera, Bipolarisspicifera, Myrotheciumroridum, Rhizoctoniasolani, Stemphyliumsolani(Kamal and Moghal, 1968; Jagirdar and Jagirdar, 1980; Anon, 1982; Hafiz, 1986; Jiskani 1992 and Jiskani, 2001). The spots may occur on leaves at any stage of plant growth starting from seedling to maturity. Anthracnose (*Colletotrichumgossypii*), areolate mildew (*Cercosporellagossypii*), Ascochyta blight (*Ascochytagossypii)* and black root rot (*Thielaviopsisbasicola*) sometimes appear in the cotton fields with high severity. Boll rot is caused by several pathogens including *Ascochytagossypii, Colletotrichumgossypii, Fusarium spp., Lasiodiplodiatheobromae, Rhizoctoniasolani.* Under favourable environmental conditions charcoal rot (*Macrophominaphaseolina*), *Fusarium*wilt (*Fusariumoxysporumf.sp. vasinfectum*) powdery mildew (*Leveillulataurica*), cotton rust (*Pucciniaschedonnardii, Pucciniacacabata*), *Sclerotium* stem and root rot (*Sclerotiumrolfsii*) are threat to cotton cultivation (Silva *et al.* 1995 and Belot andZambiasi, 2007). Pathogen that attack bolls may enter three different ways. Commonly, wounds from insect or mechanical injury allow entry by fungi and bacterial pathogens. Pathogens also may penetrate bolls throw the stomata, nectarines and opening structures between the carpals. Several fungi are capable of direct penetration through the boll walls. (Guthrie *et al.*1994).

Atleast 170 microorganisms are capable of causing boll rot(Belot and Zambiasi, 2007). The bolls of cotton were found to be attacked by *Fusarium spp.,Diplodia spp.,GlomerellaGossypii, Xanthomonas spp.,Rhizoctonia spp.,Alternariaspp.etc.*(Dikson 1956, Hillocks 1992, Anon. 2010b, Anon. 2010c).Boll rot is a problem that continues to frustrate growers. Once a boll is infected, it is too late to control the

disease. Because boll rot can severely undercut even the most intensive earliness management efforts.Boll rot is a generic term referring to a number of diseases whereby bacteria and fungi cause damage to bolls, lint and seed. (Guthrie, 1994; Batson and Borazjani, 1984).

Among different type of causal organism of cotton boll rot Fusarium is a ubiquitous fungus widely distributed in soil, plants and different organic substrates. *Fusarium* species are important as plant pathogens causing different diseases and being responsible for important economic loss (Agrios, 1988).Most *Fusarium*species are soil fungi and have a world-wide distribution. Some are plant pathogens causing root and stem rot, vascular wilt or fruit rot. Other species cause storage rot and are important mycotoxin producers. *F. oxysporum* is considered a warm-weather disease that is generally found in sandy and acidic soil. It can remain in the soil for up to 10 years. The optimal soil and air temperature for the pathogen is about 28°C. If the air temperature is cooler but the soil temperature is optimal, *F. oxysporum* will extend into the lower sections of the plant's stem. This case is very dangerous because the plant will not exhibit any external symptoms. The pathogen's strength is also increased by certain micronutrients found in the soil, as well as ammonium nitrogen and phosphorus (Roncadori*et al.* 1994).

Fungicides are a primary component in a program to manage cotton boll rot diseases. Several fungicides have been labeled for control of *Fusariumoxysporum*including flutolanil, propiconazole, fenarimol, iprodione, chlorothalonil, quintozene, mancozeb (Couch, 1995; Ghini, and Kimati, 2000). The fungicide has broad spectrum activity with protectant and acropetal systemic capabilities. It is necessary to ensure to plant only fungicide treated seed. The fungicides, such as captan or thiram, provide surface protection from disease organisms carried on the

seed and from organisms found in nearby soil that cause seed rot. (Mayur*et al.* 2001and Madden, 2007). The fungicides, such as metalaxyl, Ipridion, carboxin (Vitavax) or mefenoxam (Ridomil Gold), are absorbed through the seed coat of the germinating seed and are taken up by the young seedling. Systemic fungicides provide temporary protection from certain types of pre-emergence and post-emergence damping off(Harwick, 2006andRaju*et al.* 2001). In most years, seed treatment fungicides are sufficient for controlling seedling disease, unless the quality of the seed is low or weather conditions are unfavorable for germination (Minton *et al*. 1982, Chambers, 1995, Minton and Garber 1983;Colyer and Vernon 2005and Koenning 2010). When different pathogens are present in the soil requires seed treatment with a combination of fungicides (Howell, 2006).Role of the seed treating chemical incontrolling boll rot disease of cotton in Bangladesh has not yet been studied and available.

In view of the above facts the present piece of research was undertaken with the following objectives:

I. To identify the causes of boll rot disease of cotton.

II. To find out the potentiality of some fungicides and one bio-agent against*Fusariumoxysporum*the causal pathogen ofboll rot of cotton.

CHAPTER II

REVIEW OF LITERATURE

2.1. Cotton diseases

Cotton is a soft, fluffy fiber that grows in a boll around the seeds of the cotton. The cotton plant is a shrub native to tropical and subtropical regions around the world. The crop is a subject to attack by a large number of diseases. The diseases can attack cotton at any stage of plant growth and from the seed through the seedling stage. The diseases are caused by fungi, bacteria, nematode, viruses and phytoplasma. Most frequently occurring known diseases are caused by bacteria and fungi (Terrence and Rockroth 2001).

Dikson (1956) stated that the most common and widely distributed bacterial diseases of cotton are bacterial blight (*Xanthomonas campestris* pv. *malvacearum*), crown gall (*Agrobacterium tumefaciens*) and lint degradation (*Erwinia herbicola*). Most common fungal diseases are leaf spot and leaf blight caused by *Alternaria macrospora, Alternaria alternata, Cercospora gossypina Cochliobolus spicifera, Curvularia spicifera, Bipolaris spicifera, Myrothecium roridum, Rhizoctonia solani, Stemphylium solani*, *Fusarium* wilt (*Fusarium oxysporum* f.sp. *vasinfectum*), seedling mortality caused by *Fusarium* spp., *Rhizoctonia solani*, *Pythium* sp., *Curvularia* sp., *Aspergillus* spp.

Anthracnose (*Colletotrichum gossypii*), areolate mildew (*Cercosporella gossypii*), *Ascochyta* blight (*Ascochyta gossypii*) and black root rot (*Thielaviopsis basicola*) sometimes appear in the cotton fields with high severity. Boll rot is caused by several pathogens including *Ascochyta gossypii, Colletotrichum gossypii, Fusarium* spp., *Lasiodiplodia theobromae, Rhizoctonia solani*. Under favourable environmental

conditions charcoal rot (*Macrophomina phaseolina*), *Fusarium* wilt (*Fusarium oxysporum f.sp. vasinfectum*) powdery mildew (*Leveillula taurica*), cotton rust (*Puccinia schedonnardii, Puccinia cacabata*), *Sclerotium* stem and root rot (*Sclerotium rolfsii*) are threat to cotton cultivation. Seedling disease complex caused by generally *Colletotrichum gossypii, Fusarium* spp., *Pythium* spp., *Rhizoctonia solani, Macrophomina phaseolina*, *Thielaviopsis basicola* are main cause of low plant sand (Hillocks 1992, Terrence *and* Rockroth 2001, Anon. 2010a, Anon. 2010b).

Anon (2010c) and Anon (2010d) reported that diseases cause significant losses in cotton production throughout the cotton growing countries of the world. Clearly cotton diseases are a serious detriment to the cotton farmer's income and the economy of Missouri .

Diseases are major constraints for cotton cultivation in Pakistan, one of the important cotton growing courtiers in the world. One of the most common diseases is boll rot caused by different fungi (*Aspergillus niger, Fusarium oxysporum, Rhizopus oryzae, X. campestis pv. malvacearum*). Angular leaf spot or bacterial blight (*X. campestris pv. malvacearum*) cause angular leaf spot or bacterial blight or boll rot of cotton. (Kamal and Moghal, 1968, Jagirdar and Jagirdar 1980, Anon. 1982, Hafiz, 1986 and Jiskani 1992).

Anon (2006) reported on the occurrence of cotton diseases in Bangladesh is scanty. In the country *Fusarium* wilt, angular leaf spot or bacterial blight, damping off, leaf spots and anthracnose have been identified. Due to death of cotton plants yield is severely reduced. Observations on the field of Comilla cotton indicated that the crop was attacked by seed, soil and airborne plant pathogens causing death of plants, about 68% of

seedling mortality was observed when seeds were sown at farmer's traditional practice.

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 problems of seedlings diseases of cotton from the USA. In 1929, the problems were also demonstrated from Uganda by Hansford (1929).

Miller and Weindling (1939) conducted research work and began to identify the pathogens involved considering cotton boll rot diseases which were threats to the cotton cultivation in 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 million pounds in 2001, 19.6 million pounds in 2002, and 39.7 million pounds in 2003 (Anon. 2010c).

Damping-off diseases of cotton is considered a disease complex, in which several pathogens can be involved. In SW Spain, post-emergence damping-off seems to be mainly associated with *R. solani* and *Thielaviopsis basicola*, posing a serious limitation for crop, especially in cold springs. Ninety-seven commercial plots, where post emergence damping-off of cotton seedlings was observed. However, the primary pathogen of the cotton seedling disease complex was *R. solani* Kühn (Arndt 1943, Huisman 1988, Delgado *et al.* 2006, Rothrock *et al*. 2007).

Several soil-borne fungi are responsible for diseases of cotton. However, cultural and environmental factors that delay seed germination and seedling growth make the problem more severe. In addition to the

prevalence of the seedling disease pathogens, soil environment plays an important role in disease. Soil temperatures and soil water during the first few weeks after planting are important in stand establishment because of effects on both the host and the pathogens (Minton *et al*. 1982, Riley *et al.* 1969).

2.2. Cotton boll rot diseases

Roy and Bourland (1982) and Batson and Borazjani (1984) isolated *Fusarium* spp. which were highly virulent on cotton bolls. Colyer (1988) was initially isolated fungi from diseased boll, which were collected from Louisiana. *Fusarium* spp. represented 42% of all fungi associated, compared with 40% for *Rhizoctonia*. The main species were *Fusarium oxysporum, F. solani, F. equisiti* and *F. graminearum.*

Hillocks (1992) isolated a great number of microorganisms from cotton bolls rot, and these pathogens can be divided into three groups: those capable of penetrating intact bolls; those which are introduced by insects; and those are introduced after the boll are damaged by insects or after the suture of the boll lobes are broken. Most of the agents that cause cotton bolls rot penetrate through wounds from insect or pests and / or rupture of the division through the lobes of the bolls. However, primary infection of boll, when the pathogen penetrates directly into the healthy boll, is common in areas with high humidity or in those where the crop has dense vegetative growth.

The presence of aphids and insects in cotton fields has favored the penetration of various microorganisms mainly due to the damage they cause on the earliest bolls. Moreira *et al.* (1994) found that the main causes of bolls rot in regions of the state of São Paulo were the attacks of

the boll weevil (*Anthonomus grandis*), yellowstriped bugs (*Horcias nobilellus*) and cotton-stainer (*Dysdercus* spp). These insects favor the penetration of fungi and bacteria. The damage related to the attack of these pathogens was between 12.6% and 15.2% of bolls rot.

Silva *et al.* (1995) found fungi associated with cotton bolls rot those were *Alternaria* spp., *Colletotrichum* spp., *Fusarium* spp., *Botryodiplodia theobromae*, *Myrothecium roridum* and *Aspergillus* spp.

Charchar *et al.* (1999) observed that the fungus *Sclerotinia sclerotiorum* was infesting cotton plants in areas under central pivot, with symptoms of wilting, necrosis and wet rot of the stem, the petiole of the leaf and the bolls. Inside the capsule was observed a white, cottony mycelium and black and irregular sclerotia, that is, resistance structures of the pathogen.

Ribeiro *et al.* (2000) found in samples of cotton bolls from Mato Grosso, Mato Grosso do Sul and Bahia showed no visible external symptoms, but all had symptoms of internal decay. Some samples detected external damage caused by stink bugs (migrant populations of *Euschistus heros* and *Piezodorus guildinii* from soybean and striped-bugs from cotton), but internally infected by pathogens. In a study conducted by the same authors, they observed mixed infection among the eleven samples of the variety Ita-90, where the incidence of *Fusarium* sp. was the highest, followed by *Aspergillus* sp. and *Colletotrichum* spp.. With respect to bacteria, *Erwinia* was identified in 2 samples.

Zancan *et al.* (2001) worked with three cotton cultivars BRS Araçá, Delta Opal and FMT 701, using two spaces between the rows of cotton, 0.80 and 0.90 m, two treatments, with and without surface disinfection of cotton bolls with (1%) sodium hypochlorite, and three levels of bolls rot

initial, intermediate and final. Nine fungi associated with the cotton bolls rot were detected in the spacing of 0.80 m and seven, in the spacing of 0.90. Among them, *Alternaria* spp., *Aspergillus* sp, *Botryodiplodia* sp, *Colletotrichum* spp., *Fusarium* spp., *Myrothecium roridum* and *Penicillium* sp. were associated with the cotton bolls rot in different percentages in both row spacings.

Belot and Zambiasi (2007) stated many pathogens that cause boll rot, such as Alternaria spp., Ascochyta gossypii, Aspergillus flavus, Bacillus pumilus, Colletotrichum spp., Diplodia gossypina, Erwinia aroideae, Fusarium spp., Lasiodiplodia theobromae, Myrothecium roridum, Pantoea agglomerans, Phoma exigua, Phomopsis sp., Phytophthora spp., Rhizoctonia solani and Xanthomonas axonopodis pv. malvacearum. Various symptoms may be due to the existence of a complex of pathogens. Commonly, the bolls are soft and blackened, and in some cases, arise from lesions in both the apex and at its base. Fructifications in various colors, from white to purple are also verified.

Iamamoto (2007) reported that Cotton boll rot caused 20-30% losses in productivity. In general, affected plants lost the first bolls positions, where the plants produce the best quality of cotton fiber.

Belot and Zambiasi (2007) observed the symptoms of boll rot and they stated that the first symptoms appeard on the bottom leaves, spreading then to the bracts, bolls, petioles and stems. In the cotton bolls, petioles and stems, the lesions are irregularly shaped and have a dark color, surrounded by a violet and red color.

2.3. Causal pathogen of cotton boll rot (*Fusarium oxysporum*)

Booth (1977) reported that colonies of *Fusarium* are usually fast growing, pale or brightly colored (depending on the species) and may or may not have a cottony aerial mycelium. The color of the thallus varies from whitish to yellow, brownish, pink, reddish or lilac shades. Species of *Fusarium* typically produce both macro- and microconidia from slender phialides. Macroconidia are hyaline, two- to several-celled, fusiform- to sickle-shaped, mostly with an elongated apical cell and pedicellate basal cell. Microconidia are 1- to 2-celled, hyaline, pyriform, fusiform to ovoid, straight or curved. Chlamydoconidia may be present or absent.

Murumkor and Chavan (1985) reported growth of *Fusarium* at 25° C on chickpea meal agar appears cottony white, wrinkled in old culture. The fungus hypha is septate and profusely branched. On simple, short conidiophores micro-conidia are produced. Microconidia and macro conidia appear generally spare on solid medium. These are straight to curved or oval to cylindrical in shape measuring 2.5-3.5 x 5-11 µm.

Gupta *et al.* (1986) reported that at 25° C and 30° C. the funges attained the maximum growth 76.8 and 85.4 mm while at 35° C, it was 59.3 mm after seven days of inoculation. No growth was observed at 5° C.

Saxena and Singh (1987) reported that *F. oxysporum* is septate, profusely branched growing on potato sucrose/dextrose agar at 25°C initially white turning light buff or deep brown later, fluffy or submerged. The growth becomes felted or wrinkled in old cultures. Various types of pigmentation (yellow, brown, crimson) can be observed in culture. In old cultures chlamydospores are formed, which are rough or smooth walled, intercalary or terminal and may be formed singly, in chains or pairs.

Smith *et al.* (1988) showed that *F. oxysporum* is an abundant and active saprophyte in soil and organic matter, with some specific forms that are plant pathogenic. Its saprophytic ability enables it to survive in the soil between crop cycles in infected plant debris. The fungus can survive either as mycelium, or as any of its three different spore types (Agrios, 1988).

Smith *et al.* (1988) found in solid media culture, such as potato dextrose agar (PDA), the different special forms of *F. oxysporum* can have varying appearances. In general, the aerial mycelium first appears white, and then may change to a variety of colors - ranging from violet to dark purple - according to the strain (or special form) of *F. oxysporum.* If sporodochia are abundant, the culture may appear cream or orange in color.

Agrios (1988) studied that *F. oxysporum* produces three types of asexual spores: microconidia, macroconidia, and chlamydospores. [Microconidia] are one or two celled, and are the type of spore most abundantly and freqeuntly produced by the fungus under all conditions. It is also the type of spore most frequently produced within the vessels of infected plants. [Macroconidia] are three to five celled, gradually pointed and curved toward the ends. These spores are commonly found on the surface of plants killed by this pathogen as well as in sporodochialike groups. [Chlamydospores] are round, thick-walled spores, produced either terminally or intercalary on older mycelium or in macroconidia. These spores are either one or two celled.

Guthrie *et al*. (1994) reported that several species of *Fusarium* can invade bolls.These species often gain entrance to the boll through its base. The initial infection is seen on bracts surrounding bolls that are older than 35 days. The infection then spreads across the bracts to their attachment

point at the base of the boll. Growth continues through the peduncle into the boll and progresses to the top. The rot may appear blue-black to brown on the inside of the boll. The outside surface often appears salmon-pink to white. The pink color on surface indicates the presence of conidial spores.

Paiva *et al.*(2001) stated that, In the rot caused by *Fusarium* spp., small necrotic spots ranging in color from dark blue to brown occur in the bracts and in the bolls, and after sporulation of the pathogen, the lesions are covered by a pink color mass. In these cases, the capsule does not open.

Sharma *et al*. (2005) describe that optimum inoculums load of macro conidia for disease development must be 10 4-10 5.

Farooq *et al.* (2005) reported that growth of *F. oxysporum* was maximum at 30° C and reduced below 15° C and above 35° C. No growth was observed at 5° C.

Verweij and Brandt (2007) recordrd that *Fusarium* colonies are usually fast growing on Sabouraud dextrose agar at 25°C maturing within 4 days to pale or brightly colored (depending on the species) and may be felty, cottony or wooly or sparse and wet-looking . The only slow-growing species is *Fusarium dimerum*. The color of the thallus varies from whitish to yellow, brownish, pink, reddish or lilac or blue to blue-green shades. *Fusarium* may be any colour except olivaceous black or black. A sclerotium, which is the organized mass of hyphae that remains dormant during unfavorable conditions, may be observed macroscopically and is usually dark blue in color. On the other hand, sporodochium, the cushion-like mat of hyphae bearing conidiophores over its surface, is usually absent in culture. When present, it may be observed in cream to tan or

orange color, except for *Fusarium solani*, which gives rise to blue-green or blue sporodochia .

Kulkarni (2006) stated that micro conidia were abundant, hyaline, continuous or one septate,ovoid and ovate and measured 3.2 – 5.4 x 1.1 - 2.4µm (Average 4.3 x 1.75 µm). Macro conidia were scarce, often lacking and variable, 3- septate measuring 19 – 21.0 x 3.1 – 4.2 µm (Average 20.0 x 3.65 µm). Chlamydospores were hyaline, usually vacuolated, spherical and 1- celled, produced either singly or in chain and 7- 10 µm in diameter (average 8.5 µm diameter)

Nucci and Anaissie (2007) noted that hyaline septate hyphae, conidiophores, phialides, macroconidia, and microconidia are observed microscopically. *Fusarium* have septate hyphae with two types of conidiation: unbranched or branched conidiophores with phialides that produce large (2-6 x 14-80 µm) sickle- or canoe-shaped macroconidia, and long or short simple conidiophores bearing small (2-4 x 4-8 µm) oval, 1 or 2 celled conidia singly or in clusters. Chlamydospores are sparse, in pairs, clumps or chains, thick-walled, hyaline, intercalary or terminal and are produced by *Fusarium chlamydosporum*, *Fusarium napiforme*, *Fusarium oxysporum*, *Fusarium semitectum*, *Fusarium solani*, and *Fusarium sporotrichoides* .

2.4. Epidemiology

Ranney *et al.* (1971) classified four factors that favor infection of cotton bolls rot: long wet periods (5 to 7 days), long periods with relative humidity above 75%, low light intensity, i.e., long overcast periods and high temperatures.

Ranney *et al.* (1971) observed yield losses in the order of 1.5% caused by cotton bolls rot, in a particularly dry year, while in the next year, these losses increased to 14%, due to higher humidity and temperature. The same authors concluded that with the cotton plant has vigorous vegetative growth, the moisture retained in the canopy may be sufficient to promote boll rot, even if the relative humidity is lower. This effect was observed in the United States during the 70s, where cotton farmers experienced great productivity losses related to the bolls rot due to the use of high doses of nitrogen fertilize, that favored plant growth and provided a favorable environment conditions for the disease.

Hillocks (1992) stated that long periods with high atmospheric humidity is the main factor favoring the development of an epidemic of cotton bolls rot.

Jiskani (1992) stated that the main predisposing factor for bolls rot is an excess of moisture. In the rainy season or under conditions of high relative humidity, the bolls remain with moisture on their surface for long periods, causing the gradual tissue flooding, and, in this case, promoting the penetration of the primary agents that cause the disease followed by secondary action of saprophytes. The excessive vegetative growth, high planting density and unbalanced fertilization are also factors that can increase the incidence of cotton bolls rot.

Guthrie *et al*. (1994) reported that all bacterial and fungal pathogens, three conditions must be met for disease to occur. First, the microorganism responsible for a given disease must be present. Most fungal causal organisms produce air borne spores or cells that infect the bolls. The second condition necessary for disease occurrence is the presence of a susceptible host. The causal organism must be able to grow

and develop on a prospective host. Finally, environmental conditions must fall within a certain range for the pathogen to germinate, grow and develop within the host. The optimum temperature and relative humidity for growth range from 60- 900P and 95-1000/0 RH. Outside of these ranges, growth slows or stops completely. The environmental requirement for heat plus humidity explains why the rainbelt is more likely to suffer substantial yield loss from boll rot.

Guthrie *et al*. (1994) observed that the primary factor controlling the prevalence and severity of boll rot is moisture. Microorganisms capable of producing boll rot can be found in virtually every cotton field. Ideal temperatures for boll maturation and opening also favor the growth and spread of boll rot. Persistent moisture and / or relative humidity predisposes a crop to the full range of boll rot organisms. It should not come as any surprise that the Southeast and Mid-South are the regions most impacted by boll rot. These areas receive more frequent late summer rainfall with accompanying higher humidities. This predisposition can be further aggravated by a dense canopy that restricts air flow and drying potential.

Navas-Cortes *et al.* (1998) stated that the disease control was dependent on a number of factors including virulence, concentration of the pathogen and the susceptibility of cultivar used.

Paiva *et al.* (2001) found that the fungus *D. gossypina* forms small brown lesions on the bolls and cotton bracts, and in high humidity conditions, the spots may expand, affecting the whole cotton boll. With the evolution of lesions, sporulation of the pathogen occurs, and a black coloration to the boll is observed. Under these conditions, the boll dries and opens prematurely, exposing blackened fibers and seeds.

2.5. Evaluation of disease management strategy

2.5.1. Chemical management

Minton *et al.* (1982) showed that in relation to organostannic compounds or group 30 fungicides, a common example is the fentin hydroxide based fungicide, that when used in controlling bolls rot, observed a reduction in the number of bolls rot in the cultivars NUOPAL (0.90 m), BRS Araça (0.80 m) and FMT701 (0.80 m). This fungicide acts as multi-site inhibitors, prevents spore germination and inhibits the metabolism of fungi, particularly respiration, inhibits oxidative phosphorylation in mitochondria and induces lipid peroxidation. Low concentrations of organostannic compounds inhibit the translocation of H + bound to the membrane, H +ATPase and ions such as Na + and K +.

Dhrub and Singh (1988) found that management of Fusarium wilt through fungicides in Kagazi lime. An isolate of *F. solani* from wilted seedlings of Kagazi lime was completely inhibited in vitro by carbendazim; Topsin M [thiophanate-methyl] was almost as effective. Thiophanate-methyl was the most effective fungicide in reducing mortality of infected seedlings, followed by carbendazim, both at 0.1%.

Champawat (1990) found that efficacy of fungicides against *Fusarium oxysporum* f.sp. *cumini*. Among 10 fungicides tested *in vivo* and *in vitro* against this pathogen of *Cuminum cyminum*, Bavistin [carbendazim] and RH 893 were the most effective. Both also enhanced seed germination and seedling vigour.

Kapoorand and Usman (1996) found that the relative efficacy of systemic (benomyl, carbendazim and thiophanate-methyl) fungicides against 2 isolates each of *F. oxysporum* and *F. solani* from infected tomatoes, was studied using the poison food technique. Carbendazim and benomyl were

most toxic. *Fusarium solani* isolate KHF-41 was most sensitive and *F. oxysporum* isolate DF0-13 was least sensitive to these fungicides. The decreasing order of overall efficacy of the fungicides was carbendazim, benomyl, captafol, thiram, thiophanate-methyl and captan.

Hillocks (1992) reported that the use of fungicides to control boll rot does not prove effective because it is difficult to reach the bolls, due to the dense foliage of cotton, rainy periods are responsible for washing the fungicides from the leaves and the bolls of cotton.

Roncadori *et al.* (1994) proposed that the fungicides applied at the flowering stage may be important in controlling *Fusarium*, one of the pathogens of bolls rot. The protection of flowers with fungicides more efficiently reduced bolls rot in relation to fungicide application at the opening bolls. These authors analyzed different methods to control the cotton bolls rot in Georgia, among these, the protectant fungicides captan and none, and found that they were ineffective in controlling this disease, becoming necessary to associate management combinations, such as reduce nitrogen rates and larger plant spacing.

Reis *et al.* (2010) found that,In relation to the fungicides, there were variations in the number of cotton bolls rot. The fungicide Azoxystrobin (strobilurin) + cyproconazole (triazole) reduced the cotton bolls rot in the cultivar NUOPAL (0.80 m) and BRS Araça (0.90 m). Both act differently on the fungi: strobilurins, with mesostemic action, inhibit mitochondrial respiration, while triazoles, with systemic action are related to the inhibition of sterol biosynthesis, and their function is related to the maintenance of the membrane integrity, which is preset in all eukaryotes.

Baird *et al.* (1998) states that due to difficulty in controlling bolls rot, the efficiency of fungicides in Georgia has not justified the cost of control, with no increase in production. Numerous tests have been conducted and the results obtained are not accurate or verify no effective control.

Ghini and Kimati (2000) reported that the fungicide fluazinam or group 29, acts as a potent uncoupler of oxidative phosphorylation and this proved to be an intermediary between other fungicides in the control of bolls rot, along with thiophanate methyl. The latter specifically affects cell division, as it has selective activity for tubulin fungi, and binds to the protein, preventing the occurrence of polymerization of microtubules forming the mitotic spindle.

Juliatti (2001) evaluated the effectiveness of the inductor resistance acilbenzolar-S-methyl (Bion) at doses of 5, 15 and 25 g of commercial product, in combination with the fungicide azoxystrobin and copper oxychloride to control Ramularia spot, rust and boll rot. The authors observed no difference among treatments in relation to disease severity, but there was a reduced incidence of boll rot, and they acknowledged the potential use of induced resistance as a strategy for controlling this disease.

Mayur *et al.* (2001) conducted an experiment to determine the effect of fungicides against *Fusarium oxysporium* f.sp. *ciceri* a chickpea wilt pathogen. The following fungicides were tested against this isolate: systemic (carbendazim, difenoconazole, thiophanate-methyl, propiconazole, hexaconazole and ridomil [metalaxyl]) and non-systemic (thiram, ziram, mancozeb, captan and chlorothalonil) at 50, 100 and 200ppm of the recommended dose. Among the systemic fungicides, carbendazim was the most effective, inhibiting the radial mycelial growth

by 100ppm even at the lowest concentration, and with the maximum toxicity index of 300.

Pant and Mukhopadhayay (2001) utilized three fungicides viz. Carboxin (Vitavax) 75 WP, Thiram 75 WP and carbendazim (Bavistin) 50 WP for the soybean seed treatment against the seed and seedling rot complex of soybean caused by *Rhizoctonia solani*, *Sclerotium rolfsii*. *Macrophomina phaseolina* and *Fusarium* spp. Carboxin gave 100% inhibition of *S. rolfsii* at all the three concentrations, while 82% inhibition of *R. solani*, 66% of *M. Phaseolina* and 52% inhibition of *Fusarium* spp. was obsrved at 50 µg concentration of carboxin. Thiram inhibited all the four test fungi with inhibition ranging from 53% in Fusarium sp. to 78% in *S. rolfsii* at 75 µg a.i./ml Carbendazim and very little inhibition on S rolfsii and only 7.40% inhibition was recorded at 10 µg a.i., while in other test fungi inhibition ranged from 68% in Fusarium spp. to 100% in *R. solant* at 10 µg a.i.

Raju *et al.* (2001) observed that the persistence and efficacy of carbendazim, chlorothalonil and mancozeb against *F. solani* were studied. Carbendazim (0.1%), chlorothalonil (0.2%) and mancozeb (0.2%) suspensions were incorporated into the soil columns until the soil was compeletely saturated. All fungicides were less mobile and less effective in the inhibition of fungal growth in clay loam soil. Carbendazim was the most persistent among the fungicides (up to 30 days) at a soil depth of 22.5 cm

Bhaskar *et al.* (2003) reported that copper oxychloride (500, 1000, 2000, 2500 and 3000 ppm), mancozeb (500, 1000, 2000, 2500 and 3000 ppm), carbendazim (500, 750, 1000 and 2000 ppm) and metalaxyl (500, 750, 1000 and 2000 ppm) were used against *Fusarium solani* causing dry corm rot disease in *Colocasia*. Disease incidence and weight loss in the crops decreased with increasing concentrations of the fungicides, with

carbendazim treatment resulting in the lowest disease incidence and crop weight loss.

Barbosa *et al.* (2007) found a reduction of bolls rot with the use of two applications of copper tribasic (1.5 kg/ha), obtaining an increase of 330kg of raw cotton by hectare when compared to the control.

Alviter and Nita (2011) states that the abusive uses of fungicides can cost not only growers budget, but it also can cost the society and the environment. Therefore, fungicide usages need to be carefully planned with a good understanding of plant disease epidemics, their components (host, environment and pathogen), fungicide mode of action (biochemical, biological, physical), risk of resistance development, and host physiology, among others aspects.

2.5.2. Management with biocontrol

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*.

Monaco *et al.* (1991) reported that *Trichoderma* spp. a biocontrol agent of *Fusarium* spp. and *Sclerotium rolfsii* by seed treatment. *T. harzianum*, *T. koningii* and *T. aureoviride* were isolated from tomato fields in the horticultural area. Three species were effective against *Fusarium spp*. and *S. rolfsii*, *in vitro*, *Trichoderma koningii* was most effective against *S. rolfsii*, *T. aureoviride* against *F. oxysporum* and *T. harzianum* against *F. equiseti*.

Liu (1992) found that biological control of the cowpea diseases *R. solani* and *F. oxysporum* with *Trichoderma*. A brief account is given about the control of these diseases using *T. harzianum*.

Abouzaid *et al*. (1993) found that the effect of *F. oxysporum* f.sp. *cepae* and *T. harzianum* and their combined effect on the anatomical structure of onion roots. Mycelial growth of *T. harzianum* surrounded the epidermal tissues of the root without penetration. No anatomical changes were noticed in roots inoculated with *Fusarium* up to 24 h after inoculation. After 10 days, tissues were completely destroyed and filled with chlamydospores.

Elias *et al*. (1993) observed that the control of *F. oxysporum* f.sp. *cucumerinum* and *R. solani* using antagonistic *T. harzianum* species was studied *in vitro* and on artificially inoculated plants of cucumber . *Trichoderma harzianum* reduced mycelial growth of *F. oxysporum* f. sp. *cucumerinum* and *R. solani*.

Ercole and Gennari (1993) found that the use of *T. harzianum* for the biological control of *F. oxysporum* f.sp. *melonis* on melon . There was no significant difference in the wt of the fruits between treated and untreated plants, but the incidence of the disease at 35-40 d after transplanting was slightly lower in treated plants.

Reis *et al.* (2010) found that the potential of 41 *Trichoderma* isolates applied as a biological powder to naturally infected soil was evaluated for the control of *Phaseolus vulgaris* wilt caused by *F. oxysporum f. sp. phaseoli*. Among the isolates, 15 were selected and tested for disease control effectively.

Datnoff *et al.* (1995) found that the evaluation of commercial formulations of 2 beneficial fungi, *T. harzianum* and *G. intraradices* for

the control of *Fusarium* crown and root rot of tomato. Significant decreases in disease incidence were obtained with treatments of *T. harzianum* in 1993. It is suggested that commercial biological control agents may be effective in reducing Fusarium crown and root rot and that further evaluation of these agents is justified.

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 the mechanism of antagonism between the mycoparasite *T. harzianum* and the cotton wilt pathogen *F. oxysporum f. sp. vasinfectum* was determined. *T. harzianum* potentially reduced the radial growth *F. oxysporum* f. sp. *vasinfectum.*

Cheetham *et al*. (1997) found that the interactions between *F. culmorum* and *T. harzianum* were investigated. *Fusarium culmorum* was the dominant organism at the beginning of the experiment and was gradually replaced by *T. harzianum* .The population of *T. harzianum* had a direct effect on that of *F. culmorum*.

Charati *et al.* (1998) conducted pot experiments during kharif 1997 to study the effect of seed treatment with *Trichoderma* spp. on the incidence of Fusarium wilt (*F. oxysporum* f.sp. *vasinfectum*) of cotton (cv. DH-2). The seed was treated with talc-formulated *T. viride* and *T. harzianum* at 2, 4, and 6 g/kg seed. The results showed that all treatments were significant.

Ellil *et al.* (1998) reported that *T. harzianum* reduced root rot infection by 6.7-45.0% in bean. *Trichoderma* spp. obviously antagonized the effects caused by the pathogen, *S. rolfsii* and *F. solani*.

Kumar *et al.* (1998) found that *T. harzianum* hyper-parasitized *F. solani* by forming appressoria-like structures over the pathogenic hyphae, and by tightly coiling around it, within 96 h of contact. Within 6 days, the fungus was completely inhibited while *T. harzianum* multiplied extensively by conidiogenesis.

Bernal *et al*. (2001) observed that the antagonistic effect between *Trichoderma* spp. and *Fusarium oxysporum* f. sp. *cubense* was studied using dual culture on potato-dextrose-agar medium. The competitive capacity and inhibition of radial growth of *F. oxysporum* due to *Trichoderma* spp. were evaluated. An inhibition zone of 70% was observed, followed by total invasion of the fungal surface.

Goswami and Islam (2002) reported that antagonists such as *Trichoderma* spp. showed greater inhibition of tomato wilt pathogen (*F. oxysporum* f. sp. *lycopersici*). Dual culture of *Trichoderma* spp. on PDA along with the test fungus *F. oxysporum* f. sp. *lycopersici* was found to have antagonistic effect on the later.

CHAPTER Ш

MATERIALS AND METHODS

Experiments were carried out both in the field and in the laboratory. The materials used and the methodology and procedures followed in the experiments have been described in this chapter.

3.1. Experimental site

Field experiment was carried out in Cotton research, Training and Seed Multiplication Farm, Sreepur, Gazipur. *In-vitro* experiments were conducted in the Seed Pathology laboratory (SPL) and Laboratory of the Department of Plant Pathology of Sher-e Bangla Agricultural University, Dhaka.

3.2. Experimental period

The experiments were carried during the period of April 2012 to May 2013.

3.3. Varieties used in experiment:

Three cotton varieties CB-9, CB-11 and CB-12 were used in this experiment.

3.4. Experimental plot

In the field experiment, size of each plot was 6m×3.6m, row to row distance was 90cm and plant to plant distance was 45cm. From the experimental field five plots were randomly selected for each variety. From each selected plot total numbers of cotton plant and number of boll rot infected plants were recorded. Moreover five plants per plot were randomly selected and then infected bolls per plant were also recorded to determine the disease incidence.

3.5. Observation of disease incidence of cotton boll rot

3.5.1. Percent infected plant per plot

Number of plants infected per plot for CB-9, CB-11 and CB-12 cotton varieties were recorded and used for calculation of percent plant infection.The plant in which boll with characteristic brown or black colored or rot or blighted were observed and noted as diseasesd plant.

3.5.2. Percent infected boll per plant

Number of bolls infected per plant for CB-9, B-11 and CB-12 cotton varieties were recorded and used for calculation of percent boll infection.

3.5.3. Determination of disease incidence

Assessment of the disease incidence of cotton boll rot was determined by the following formula:

$$\text{Percent plant infection} = \frac{\text{Number of diseased plant}}{\text{Number of total plants observed}} \times 100$$

Percent disease incidence (PDI) was determined by the following formula (Rai and Mamatha, 2005):

$$\text{Percent disease incidence (boll)} = \frac{\text{Number of diseased bolls on each plant}}{\text{Number of total bolls on each plant}} \times 100$$

3.6. Isolation of causal organism(s) and purification

3.6.1. Collection of diseased cotton boll

Infected boll of cotton showing typical symptoms were collected from experimental field. The Varieties were CB-9, CB-11 and CB-12. Visible symptoms of the disease was recorded and the disease was identified based on symptoms (Watkinson 1981, Hillocks 1992). Collected samples were put in polyethylene bags immediately after collection to protect them from drying. Then the samples were taken to the Plant Pathology Laboratory, Sher-e Bangla Agricultural University. Collected samples were wrapped with brown paper packet and kept at refrizerator at 4°C until isolation of the fungus.

3.6.2. Isolation of causal organisms

The pathogens associated with the diseased boll of cotton were isolated following tissue planting method and agar plate method (Tuite, 1969 and Mian, 1995).

a) Tissue planting method

The pathogen associated with the diseased plant parts (boll) were cut into several pieces by scissors and surface sterilized with 0.1% chlorox (NaOCl) for 5 minutes and three times washed in sterile water then placed on the moist filter (Whatman no.1). Three pieces of filter paper were moistened by dipping in sterile water. The petridishes with the diseased specimens were incubated in the incubation room of the Seed Pathology Lab for three days. After incubation the plates were examined under stereomicroscope for primary identification of the organisms (fungi). The fungi were transferred to PDA plates for proper sporulation and purification.

b) Agar plate method

Diseased plant parts showing characteristic symptoms along with a small part of healthy tissues were cut into small spices (0.5 mm), surface sterilized with 1% chlorox (NaOCl) for 5 minutes, and rinsed with sterilized tap water for 3 times. Surface sterilized plant pieces were plated on PDA plates and incubated at 25±2°C for several days and examined daily for any fungal growth. After incubation period the inoculated plates were observed to identify the causal organism.

The PDA medium was prepared by mixing of 200g peeled potato in 500 ml distilled water, 20g dextrose and 17g agar. Distilled water added to the mixture to make the volume 1000 ml. The medium was cooked properly and poured into conical flasks at 250 ml/flask. The cooked medium was sterilized in an autoclave at 121° C under 1.1 kg/cm^2 pressure for 20 minutes (Mian 1995, Tuite 1969). Pure culture of each fungus was made following hyphal tip culture methods (Tuite 1969, Mian 1995).

Temporary mounts of each fungus was prepared and observed under a compound microscope. Each of them was first identified up to genus level on the basis of colony characters and morphology of mycelium, hyphae and reproductive structures using appropriate key books (Bernett and Hynter 1972). Pure culture of isolated pathogen transferred to fresh PDA slants in test tubes and stored in refrizerator for future use.

3.6.3. Seed health study (Blotter test)

Seeds of three cotton variety viz. CB-9, CB-11 and CB-12 were collected from Cotton research, Training and Seed Multiplication Farm, Sreepur, Gazipur. Seeds were sterilized with 1% chlorox (NaOCl) for 5 minutes,

and rinsed with sterilized tap water for 3 times. Seed germination was determined by the blotter method according to international Rules for Seed Health Testing Agency (ISTA 1996). Ten seeds were plated on 3 layer moist blotter paper in 90 mm petri dishes maintaining uniform distance. The blotter paper and petri dishes were sterilized properly before plating. The plates were incubated at 25 ±4°C temperature for 5 to 7 days. After incubation the plates were examined under stereomicroscope for primary identification of the organisms (fungi). The fungi were transferred to PDA plates for proper sporulation and purification. Pure culture of each fungus was made following hyphal tip culture methods (Tuite 1969, Mian 1995).

Temporary mounts of each fungus was prepared and observed under a compound microscope and data on the seed germination recorded.

The germination percentage was recorded using the following formula-

$$\%\text{Germination} = \frac{\text{No.of germinated seed}}{\text{No. of total seed}} \times 100$$

3. 7. *In-vitro* pathogenicity test of of *Fusarium oxysporum* on cotton seedlings

In-vitro test was conducted to observe the pathogenicity of *Fusarium oxysporum* associated with diseased cotton seedlings. Direct inoculation technique was followed as recommended by Yang *et al.* (1996). Water agar (2%) was prepared (Tuite 1969). After autoclaving the water agar was poured into 9 cm Petri dishes @ 20 ml/dish. Five millimeter diameter

mycelial discs were cut from 3 days old PDA culture of selected isolates in Petri dish for inoculation. One disc was placed at the centre of each agar plate.

Apparently healthy and delinted seeds of three cotton varieties CB-9,CB-11 and CB-12 were sorted out and surface sterilized with 1% NaOCl for 3 minutes and rinsed in sterilized water for 3 times. Five surfaces sterilized cotton seeds were placed around the inoculum of *F. oxysporum* in each inoculated plate. Three replicated plates were used for each variety. The plates kept in an incubator at 25^0C for 15 days. The pathogen was selected based on colony, cultural and morphological characters. At the end of incubation period data on germination, pre-emergence mortality, post emergence mortality and seedling infection on agar media were recorded.

3.8. Effect of inoculum levels of *Fusarium oxysporum* on seedling mortality of three cotton varieties

A pot experiment was conducted to test inoculums level of *Fusarium oxysporum* on seedlings of three cotton varieties viz. CB-9, CB-11 and CB-12 following soil inoculation technique (Tuite 1969).

Wheat grains colonized with of *F. oxysporum* were used as inocula. To prepare inocula, wheat grains were soaked in sterilized tap water for 12 hours. After soaking excess water was drained off and grains were poured into 500 ml Erlenmeyer flask keeping two-third of each flask empty from the mouth. The water soaked wheat grains in the flasks were sterilized in an autoclave at 121°C under 1.0 kg/cm^2 pressure for 15 minutes. Wheat grains were inoculated with 5 mycelial discs of each isolate cut from 3 days old PDA culture. After inoculation the flasks were incubated at 25°C for 21 days. The flasks were shaken by hand at 2-3 days interval for even colonization of the wheat grains by *F. oxysporum.* The colonized wheat

grains were air dried for 2 weeks under sterile conditions and stored at 4° C for the use as inocula. The colonized wheat grains were used as inocula (Kundu 2010).

F. oxysporum was evaluated by soil infestation method for its inoculums level in a pot culture experiment under the shade condition in front of the Plant Pathology laboratory at SAU. Experimental soil was sandy loam and was sterilized with 1.0% formalin solution @ 1 ml/kg soil. Formalin solution was thoroughly mixed with the soil, placed on a concrete floor and covered with a polyethylene sheet. After 2 days the soil was spread on the floor to escape formalin vapor. Each earthen pot was filled with 3 kg sterilized soil. Inocula of the *F. oxysporum* were thoroughly mixed with soil in each pot @ 10, 20 and 30g/kg soil (Goswamy and Islam 2002). Four pots were used for each inoculum level. Pots under control did not receive any inocula.

Cotton seeds of each selected variety were delinted with concentrated H_2SO_4, surface sterilized with 1% NaOCl for 5 minutes and planted in the pot soil at 10 seed per pot. The experiment was laid out following randomized complete block design with four replications. Data on pre-emergence and post-emergence mortality of seedling were recorded at 10 and 20 days after sowing,respectively. Reisolation of the pathogen from ungerminated seeds and also from post emergence infected and dead seedling was done to identify the causal agent of seedling infection.

3.9. Efficacy of fungicide against *Fusarium oxysporum* at different incubation periods

3.9.1. Selection of fungicides

Five fungicides namely Proud 250 EC, Bavistin 50 WP, Provax 200 WP, Rovral 50WP and, Rydomil gold at three different concentrations viz.,

100, 200, and 400 ppm were tested *in-vitro* to evaluate their effect on colony growth of *Fusarium oxysporum* following poison food technique (Dhingra and Sinclair 1985). The details of the fungicides are presented in the Table 1.

Table 1. Detailed particular of fungicides used in the laboratory evaluation against *F. oxysporum*

Trade name	Common name	Chemical name	Active ingredient (%)
Proud 250 EC	Propiconazole	Propiconazole	25
Bavistin 50 WP	Carbendazim	Mythyl-2-benzimidazole carbamate	50
Provax-200 WP	Carboxin	5,6-dihydro-2-methyl-1,4-oxathin-3-carboxanilide	75
Rovral-50WP	Iprodione	(3-(3,5-dichlorophenyl)-N-(1-(methyl)-2,4-dioxo-1-imidazolidine-carboxamide	50
Ridomil gold MZ 68 WP	Metalaxyl M + Mancozeb	Metalaxyl M + Mancozeb	72

3.9.2. Effect of fungicides on the radial growth of *Fusarium oxysporum*

The effect of fungicides on radial growth of *Fusarium oxysporum* were determined on PDA medium. PDA was prepared by mixing infusion of 200g peeled potato, 20g dextrose and 17g agar in 1000 ml distilled water. The medium was cooked properly and poured into conical flasks at 100 ml per flask. Before solidification, requisite quantity of individual

fungicides was added to the medium to have concentration of 100, 200, and 300 ppm. After thorough mixing fungicides, the medium was autoclaved at 121° C under 1.1 kg/cm^2 pressure for 20 minutes. Approximately 15 ml melted PDA mixed with fungicides was poured into each 90 mm petridish. After solidification, the plates were inoculated by 5 mm discs of 3 days old PDA cultures of *F. oxysporum.* The discs were cut of the test plates using a flame sterilized needle at one disc per plate inside a clean bench. Three replicated plates were used for each dose of every fungicide.

Three replicated PDA plates received no fungicides were also inoculated as control. The inoculated plates were incubated at 27°C and data on radial growth was taken after 2-3 days of incubation.

Diameter of the colonies on PDA with and without fungicides was measured from the bottom side of the petridishes. Inhibition of radial growth was computed based on colony diameter on control plate using the following formula shown below (Sunder *et al.* 1995).

$$\%\text{inhibition} = \frac{X-Y}{X} \times 100$$

X= Radial growth of control plates.

Y= Radial growth of fungicides treated plates.

3.9.3. Data collection and Statistical analysis

In this experiment, six different treatments were evaluated namely T1=Proud, T2=Provax, T3=Bavistin, T4=Rovral, T5=Ridomil gold and T6=Untreated control. Fungicide solutions were prepared separately by taking requisite amount of fungicides for each dose.

The experiment had six treatments with three replications following Complete Randomized Design (CRD). Data were analyzed for ANOVA using MSTAT-C program (Steel and Torrie 1980). Duncan s Multiple Range Test (DMRT) and Least Significant difference (LSD) test were performed to determine the level of significant differences and to separate the means within the parameters.

3.10. Evaluation of *Trichoderma* harzianum against *Fusarium oxysporum* in dual culture

Trichoderma harzianum were collected from the stock culture of the Plant Pathology laboratory of BSMRAU. An experiment was conducted to find out the antagonistic effect of the *T. harzianum* against *F. oxysporum* by dual culture technique using Potato Dextrose Agar (PDA) medium (Tuite 1969).

The medium was prepared by mixing infusion of 200 g potato slices, 20 g dextrose and 20 g agar in 1000 ml water. After preparation it was sterilized in an autoclaved at 121° C with 1.1kg/cm^2 pressure for 15 minutes. The medium was poured into petridishes (9 cm) at the rate of 20 ml per plate. After solidification, young mycelial disc of *T. harzianum* and *F. oxysporum* of 5 mm in diameter were cut from the peripheries of expanding colonies grown on PDA of three days old culture with a cork borer. One mycelial disc of individual *T. harzianum* and one mycelial disc of *F. oxysporum* was placed simultaneously on the edge of the each PDA petridish at opposite direction. In the same way antagonistic effect of *T. harzianum* was tested against *F. oxysporum.* The plates were incubated in the laboratory having ambient temperature of 25±3 °C.

In this Dual culture method, pathogen was grown with the antagonist in a same PDA plate. After 5 days when the control plate was full grown,the growth of the pathogen in presence of antagonist was measured and thus calculates the inhibition of pathogen.

Inhibition percentage of the radial growth of *F. oxysporum* were calculated after 7 days of incubation following the formula as suggested by Sunder *et al.* (1995).

$$\% \text{ inhibition} = \frac{X-Y}{X} \times 100$$

Where,

X= Mycelial growth of pathogen *F. oxysporum* isolates in absence of *Trichoderma*

Y= Mycelial growth of pathogen *F. oxysporum isolates* in the presence of *Trichoderma*.

3.11. Effect of seed treatment with fungicides against *Fusarium oxysporum* the causal agent of cotton boll rot under pot culture condition

A pot experiment was conducted to find out the effect of seed treatment with five fungicides on seedling mortality of cotton caused by *Fusarium oxysporum* under inoculated condition. The details of the fungicides are presented in the Table 1.

Seeds of three cotton variety (CB-9,CB-11 and CB-12) were delinted with concentrated H_2SO_4 and treated with selected fungicides. Provax-200 WP, Bavistin 50WP, Ridomil gold MZ 68 WP and Rovral 50WP mixed with cotton seeds @ 2.5gm per kilogram of seeds in plastic pot

with lid and was jarking for 2min. For each Fungicide separate pot was used. After that seeds was prepared for sowing. In case of Proud 25 EC, fungicide solution was prepared @ 2.5ml per litre water and soaking seed @ 1kg per litre solution (Kundu 2010).

Soil of the experiment was sandy loam, sterilized with 1% formalin and poured into earthen pots at 3 kg/pot. The sterilized soil was inoculated with inoculum of *F. oxysporum* at 20 g inocula (colonized wheat grains) per kilogram of soil. The inocula were thoroughly mixed with soil before planting seeds.

Ten treated seeds were sown in each pot and 3 replicated pots were used for each treatment. Two additional treatments were maintained in this experiment, where untreated seeds were sown in sterilized soil which received only autoclaved wheat grains and untreated seed were sown in sterilized soil inoculated with the isolate of *F. oxysporum*. The last treatment served as control. The pots were placed in an open space on a concrete floor. The pots were arranged following completely randomized design. Data on pre-emergence mortality was recorded at 10 days after sowing and post-emergence seedling mortality was recorded at 30 days of sowing. Data were analyzed statistically following standard methods (Steel and Torrie 1980). MSTAT-C computer program was used to determine analysis of variance and to compare means LSD test was performed.

CHAPTER IV

RESULT

4.1. Symptoms of cotton boll rot

At first the symptoms appeared as small spots depressed reddish-brown or brown or dark blue to brown occur in the bracts and in the bolls. Diseased bolls become dark brown or black with a white to salmon-pink overgrowth of pathogen. With the evolution of lesions, a black coloration to the boll was observed. Under these conditions, the boll dries and opens prematurely, exposing blackened fibers and seeds.

Plate 1. Cotton bolls with symptoms at different stages of decay. A. an B. Early stage, C. and D. Intermediate stage, E. and F. Final stage.

4.2. Effect of different cotton varieties and growing periods on the disease incidence of boll rot

Effect of cotton varieties on incidence of boll rot disease of at different time of interval showed insignificant variation (Table 2). In November, the maximum incidence of boll rot disease was found in variety CB-12 (5.50) and the minimum incidence of boll rot was found in variety CB-9 (4.99%). In January 2013, the maximum incidence of boll rot disease was found in variety CB-12 (5.79) and the minimum incidence of boll rot was found in variety at CB-9 (5.20). The maximum incidence of boll rot disease was found in variety CB-12 (6.32) at March 2013. The minimum incidence of boll rot was found in variety CB-9 (5.70) at November 2012.

Table 2. Effect of different cotton varieties and growing periods on the disease incidence of boll rot

Variety	% Disease incidence		
	November 2012	**January 2013**	**March 2013**
CB-9	4.99 a	5.19 a	5.70 a
CB-11	5.32 a	5.61 a	6.28 a
CB-12	5.49 a	5.78 a	6.32 a
LSD $_{(0.05)}$	0.95	1.25	1.01
CV (%)	7.97	10.01	7.32

Values in a column with same letter(s) do not differ significantly (p=0.01). Each data represents the mean value of three raplicates.

4.3. Isolation and identification of causal pathogen of cotton boll rot

Following pathogens were identified.

Colletotrichum

Coloniesof *Colletotrichum* on potato dextrose agar were grayish white. Acervulus was irregular in shape and setae were absent. The conidia of *Colletotrichum* was hyaline, thin walled, cylindrical or oblong, one celled, slightly curved. They formed on faintly brown conidiophores in acervuli (Plate 2. A and B).

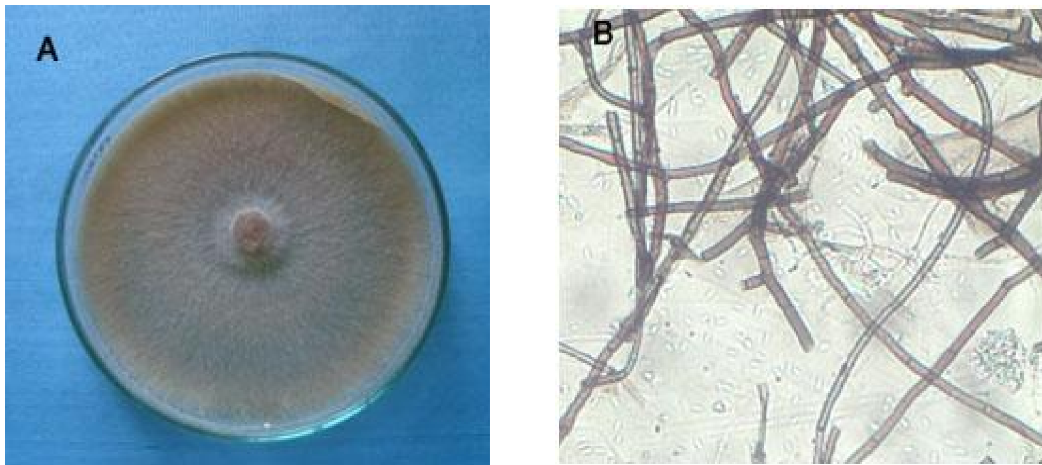


Plate 2.A. Pure culture of *Colletotrichum* sp isolated from diseased cotton boll. B. Conidia of *Colletotrichum* sp under compound microscope (100x).

Fusarium oxysporum

Colonies of *Fusarium oxysporum* on potato dextrose agar were pale orange. Mycelia were white to cream color. The conidia of *Fusarium* is hyaline, thin walled, multicellular, slighty curved or bent at the pointed ends (Plate 3 A and B).

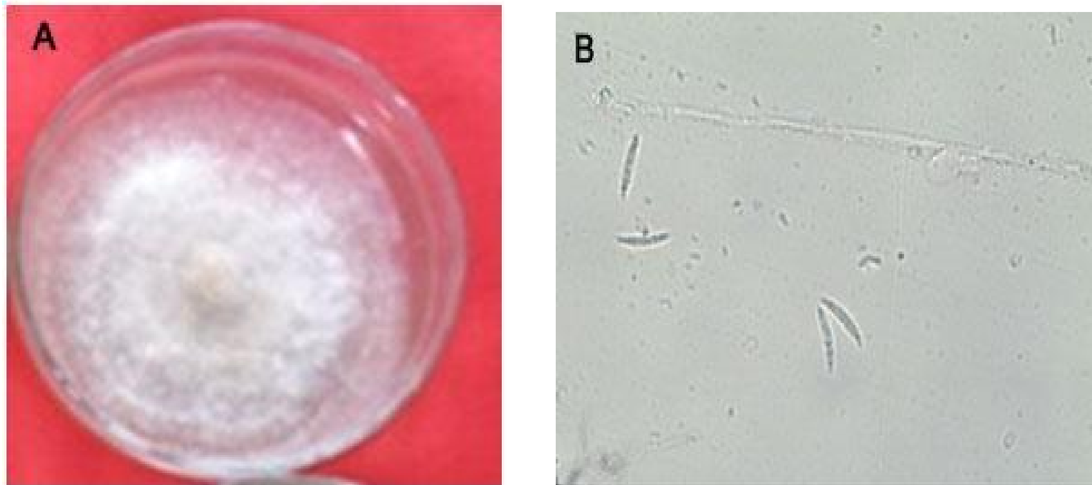


Plate 3. A. Pure culture of *Fusarium oxysporum* isolated from diseased cotton boll. B. Conidia of *F. oxysporum* under compound microscope (40x).

Alternaria

Colonies of *Alternaria* sp on potato dextrose agar were dark brown. Mycelia were light brown to brown in color. The conidia of *Alternaria* are oblong to ovoid, dark brown in color, muriform, several celled, beaked.

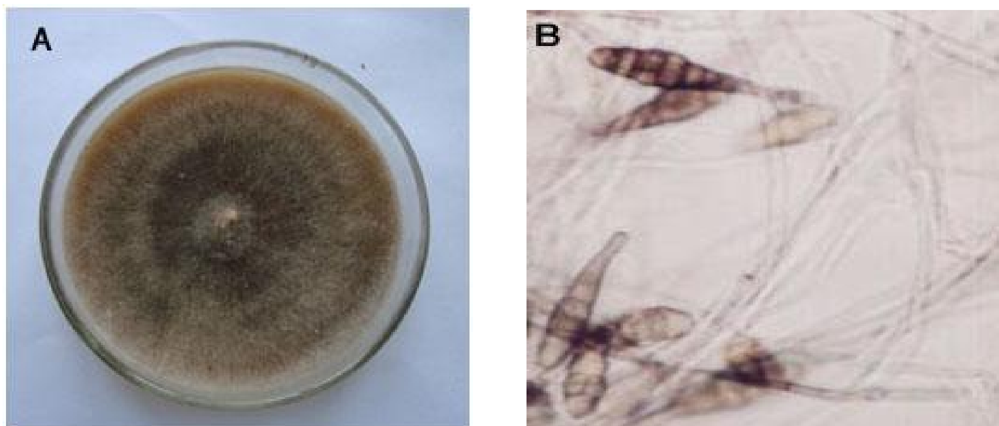


Plate 4. A. Pure culture of *Alternaria* sp isolated from diseased cotton boll. B. Conidia of *Alternaria* sp under compound microscope (40x).

4.4. Determination of seed germination(%) of selected three cotton varieties

Seed germination percentages differ insignificantly among the variety in blotter method (Plate 5). The highest seed germination (88.67%) was recorded in CB-9 cotton variety followed by CB-11 (87.0%) and lowest seed germination (83.33%) was observed in CB-12 coton variety (Table 4).

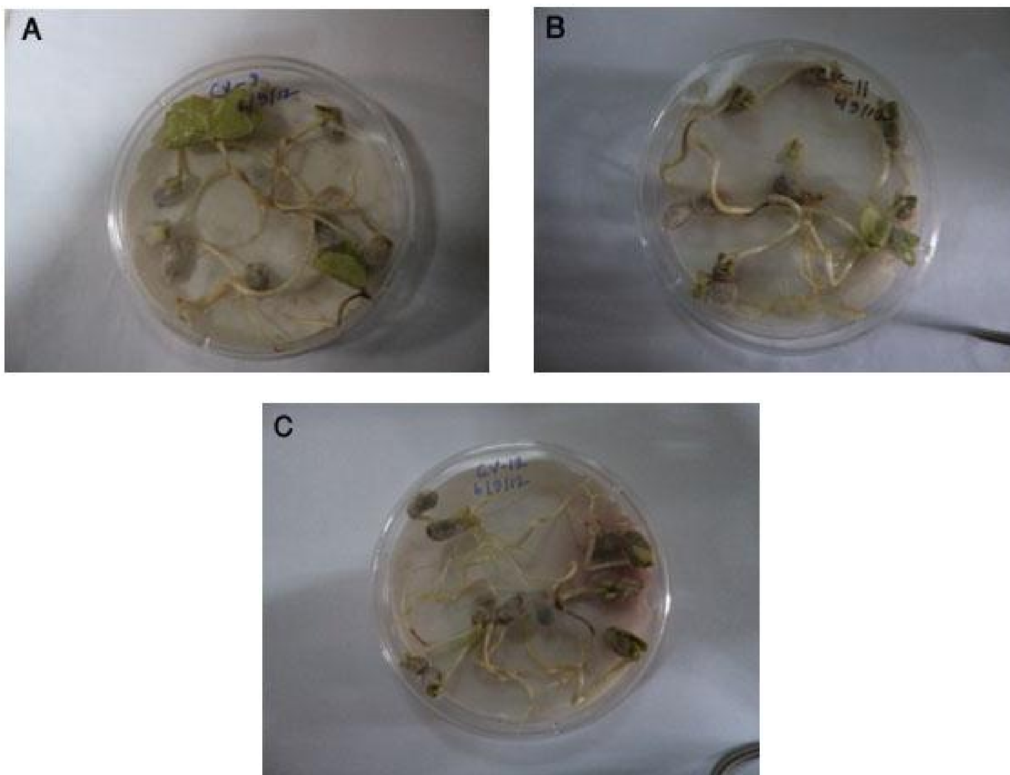


Plate 5. Seed germination of different cotton varieties A. CB-9, B. CB-11 and C. CB-12 (blotter test)

Table 3. Germination (%) of different cotton varieties on blotter plate

Variety	Germination (%)
CB-9	88.67 a
CB-11	87.00 ab
CB-12	83.33 b
LSD	4.004
%CV	3.69

Each data represents the mean value of three raplicats.

4.5. *In vitro* pathogenicity test of of *Fusarium oxysporum* on cotton seedlings

Seeds placed in Petri dish containing agar media inoculated with mycelial blocks of *Fusarium oxysporum* caused seed decay due to attack of the pathogen (Plate 5.B). Seedling mortality ranged from 52.33-69.0% was observed. The highest mortality was observed in CB-12 and the lowest mortality was observed in CB-9. Cottony myclial growth of the fungus was observed all over the infected seeds. On agar medium, the pathogen attacked whole seedling caused seedling mortality. The pathogen attacked leaves of young seedlings and produced characteristics brown leaf spot and leaf rot symptoms (Plate 6.D).When seeds were placed on agar media seedling mortality was only 12.67% in un-inoculated water agar (control).

Cotton seeds germinated on agar media without the pathogen produced healthy seedlings. Ungerminated seeds did not show any sign of fungal growth. The healthy seedlings had white roots and stem base, and brown color at crown zone (Plate 6.C).

Table. 4. ***In-vitro*** **pathogenicity of** ***F. oxysporum*** **on cotton varieties**

Variety	% seedling mortality
CB-9	52.33 c
CB-11	60.00 b
CB-12	69.00 a
Control	12.67 d
LSD	5.306
%CV	4.75

Each data represents the mean value of three replicats.

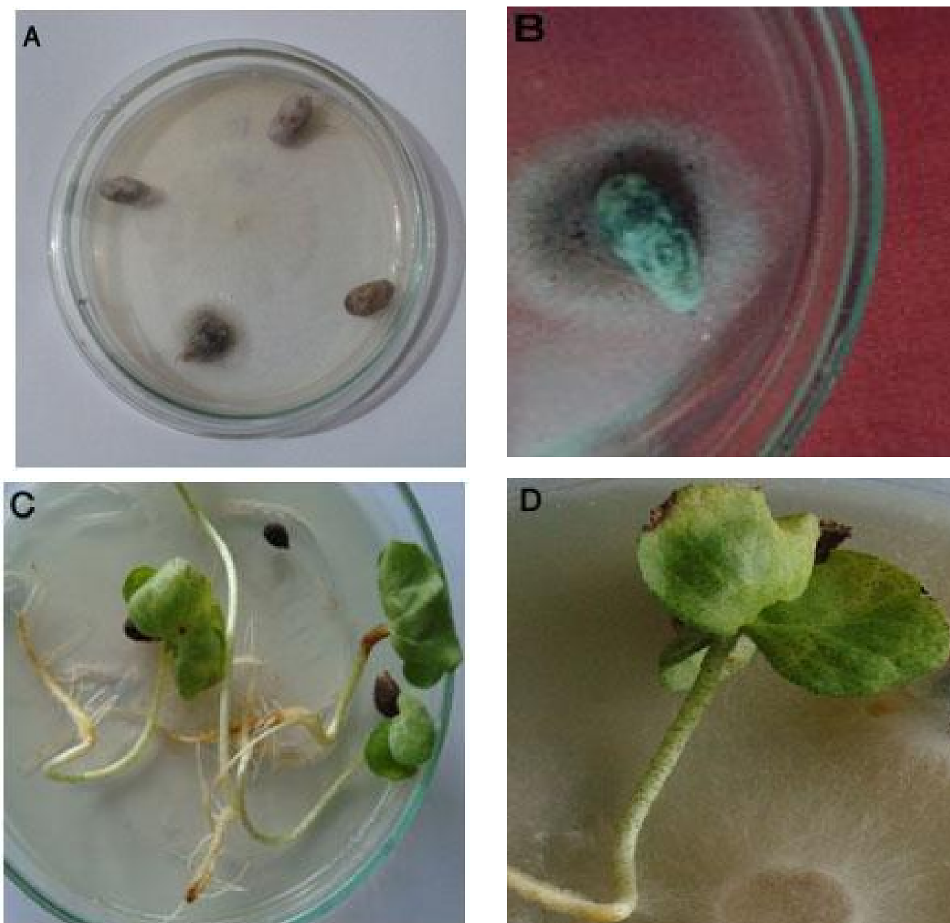


Plate 6. Seed germination, seedling growth and infection on agar media A. Healthy seeds, B. ungerminated and infected seeds,C. Healthy seedlings, D. Emerging seedling with mycelium growth.

4.6. Effect of inoculum level of *Fusarium oxysporum* on seedling

mortality of three cotton varieties

Pre-emergence as well as total mortality of cotton seedlings at different inoculum levels of *Fusarium oxysporum* were observed and it varied significantly among the tested varieties. In variety CB-9 it was found that the pre-emergence mortality was 13.20%, 33.41%, 52.03% and 73.33% and total mortality was 21.51%, 56.70%, 89.81% and 94.04% at 0, 10, 20 and 30 g inocula^{-1kg} of soil respectively. In variety CB-11, pre-emergence mortality ranged for 12.01% to 71.40% and total mortality was ranged from 17.77% to 94.32%. The Pre-emergence mortality was 12.88%, 34.39%, 52.25% and 73.58% and total mortality was 22.80%, 62.65%, 79.89% and 96.95% at 0, 10, 20 and 30 g inocula per kg of soil in variety of CB-12 (Table 5). The lowest pre-emergence mortality and total mortality of cotton seedling were recorded from pots under control (Plate 7A). The highest levels of inocula (30 g/kg) corresponded to highest severity of seedling mortality (Plate 7B). In three varieties of cotton pre-emergence mortality and total mortality positively correlated with inoculum level and their relationship was significant (Figure 1, 2 and 3).

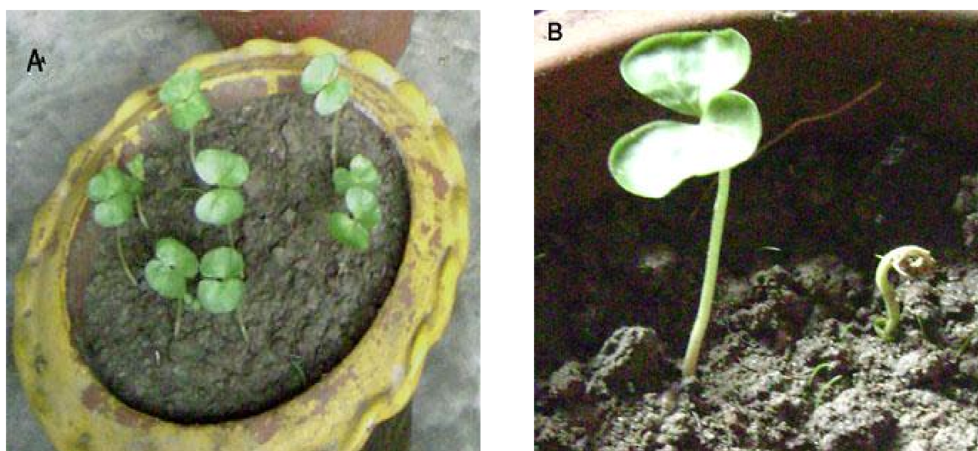
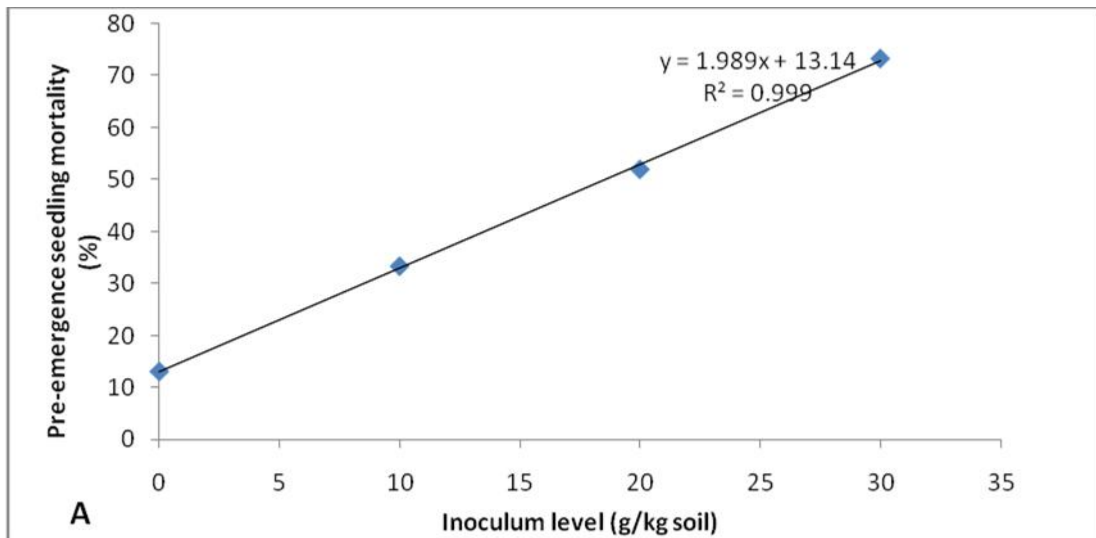


Plate 7. Cotton seedling growth on pot A. Healthy cotton seedlings in sterilized soil without inocula of *F. oxysporum* B. Cotton seedlings grown in *F. oxysporum* inoculated soil

Table 5. Effect of inoculum level of *Fusarium oxysporum* on seedling mortality of three cotton varieties

Inoculum level (g/kg soil)	Seedling mortality (%)					
	CB-9		CB-11		CB-12	
	Pre emergence	Total	Pre emergence	Total	Pre emergence	Total
0	13.2 d	21.51 d	12.01 d	17.77 d	12.88 d	22.8 d
10	33.41 c	56.7 c	36.2 c	57.17 c	34.39 c	62.65 c
20	52.03 b	89.81 b	52.9 b	88.11 b	52.25 b	79.89 b
30	73.3 a	94.04 a	71.4 a	94.32 a	73.58 a	96.95 a
LSD $_{(0.05)}$	3.572	3.398	5.614	3.492	5.245	7.067
CV (%)	4.16	2.6	6.52	2.72	6.07	5.39

Values in a column with same letter(s) do not differ significantly (p=0.01). Each data represents the mean value of three replicats.



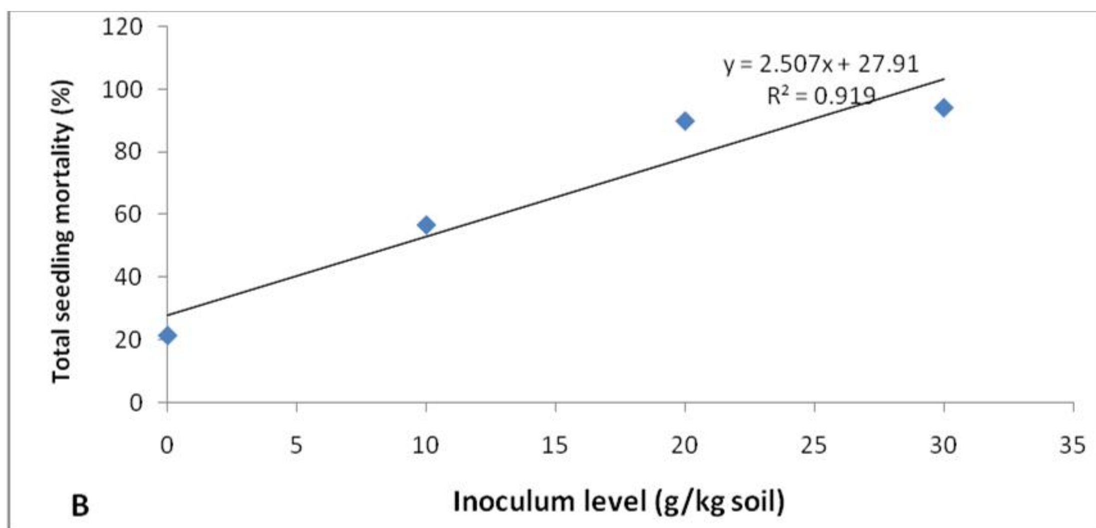
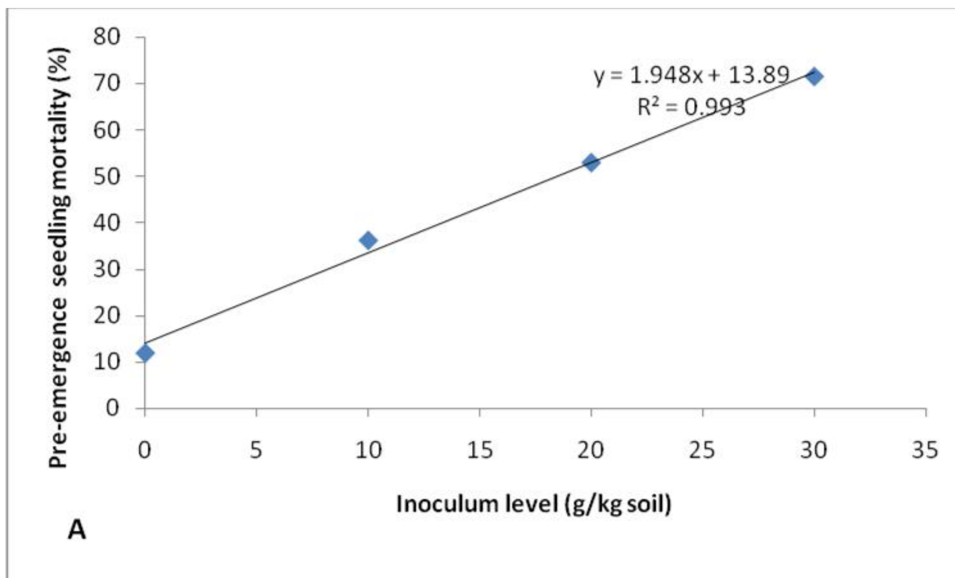


Figure 1. Relationship of A. pre-emergence seedling mortality and B. total seedling mortality of cotton variety CB-9 with inoculum level of *Fusarium oxysporum*



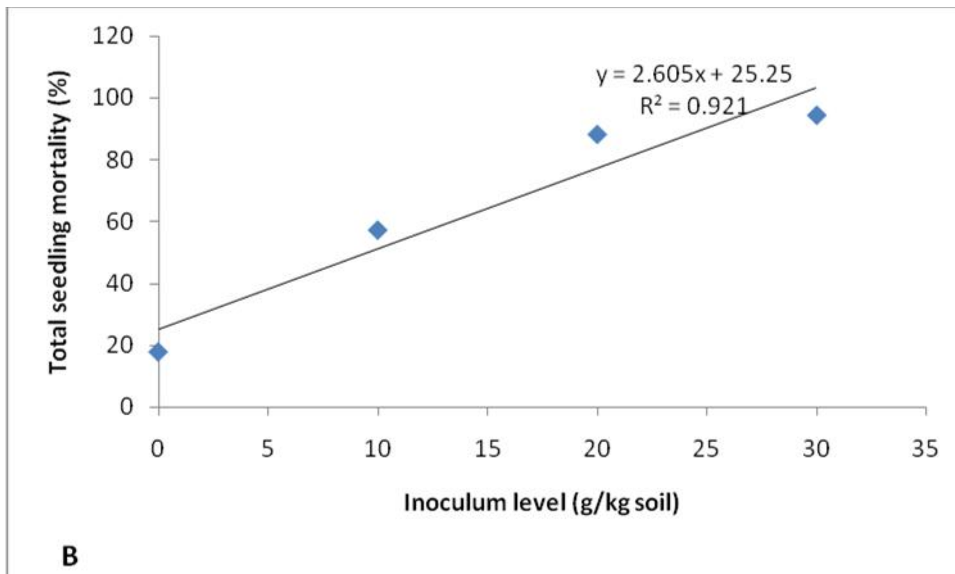


Figure 2. Relationship of A. pre-emergence seedling mortality and B. total seedling mortality of cotton variety CB-11 with inoculum level of *Fusarium oxysporum*

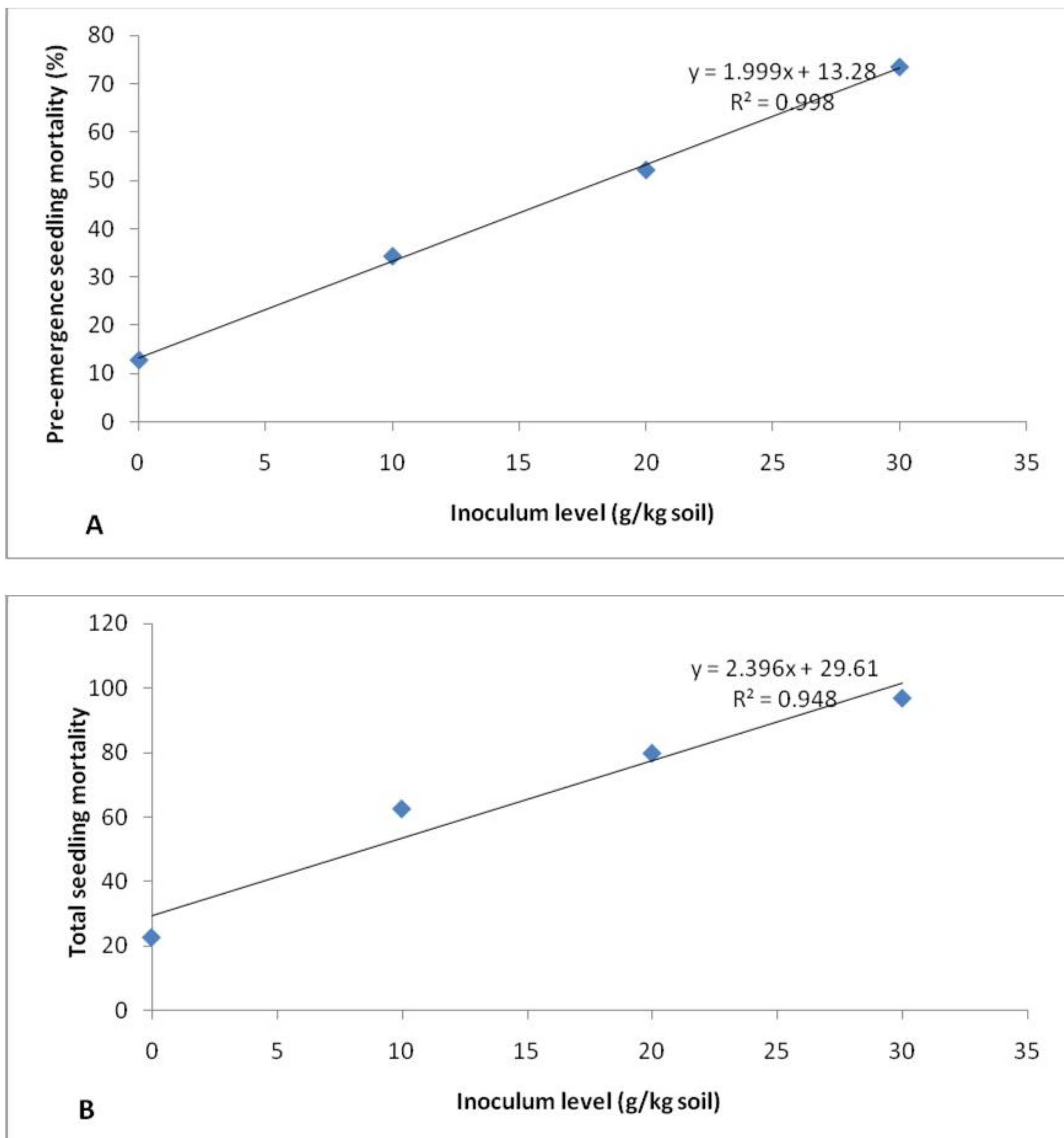


Figure 3. Relationship of A. pre-emergence seedling mortality and B. total seedling mortality of cotton variety CB-12 with inoculum level of *Fusarium oxysporum*

4.7. Efficacy of fungicide against *Fusarium oxysporum*

It has been observed that Proud 250 EC showed significant effect in inhibition of radial mycelial growth over untreated control (Table 6 and

Plate 8. B) even at 100 ppm. Proud 250 EC completely inhibited the radial growth of *Fusarium oxysporum* up to120 hours of incubation period.

Bavistin 50 WP showed significant effect in inhibition of radial mycelial growth over untreated control (Table 7 and Plate 8. C) even at 100 ppm. Bavistin 50 WP completely inhibited the radial growth of *Fusarium oxysporum* up to120 hours incubation period.

It has been observed that Provax 200 WP showed significant effect in inhibition of radial mycelial growth over untreated control by 43%, 53% and 55% at 100, 200 and 300 ppm concentration (Table 8 and Plate 8. D). The results of the experiment showed that all the selected concentrations of Provax 200 partially inhibited the radial mycelia growth of *Fusarium oxysporum* up to120 hours incubation period.

It has been observed that Rovral 50 WP showed significant effect in inhibition of radial mycelia growth over untreated control by 38%, 61% and 67% at 100, 200 and 300 ppm concentration (Table 9 and Plate 8.E). The results of the experiment showed that all the selected concentrations of Rovral 50 WP partially inhibited the radial mycelial growth of *Fusarium oxysporum* up to120 hours incubation period.

It has been observed that Ridomil gold MZ 68 showed less significant effect in inhibition of radial mycelial growth over untreated control by 0.4%, 9% and 2% at 100, 200 and 300 ppm concentration (Table 10 and Plate 8.F). The results of the experiment showed that all the selected concentrations of Ridomil gold MZ partially inhibited the radial mycelial growth of *Fusarium oxysporum* up to120 hours incubation period.

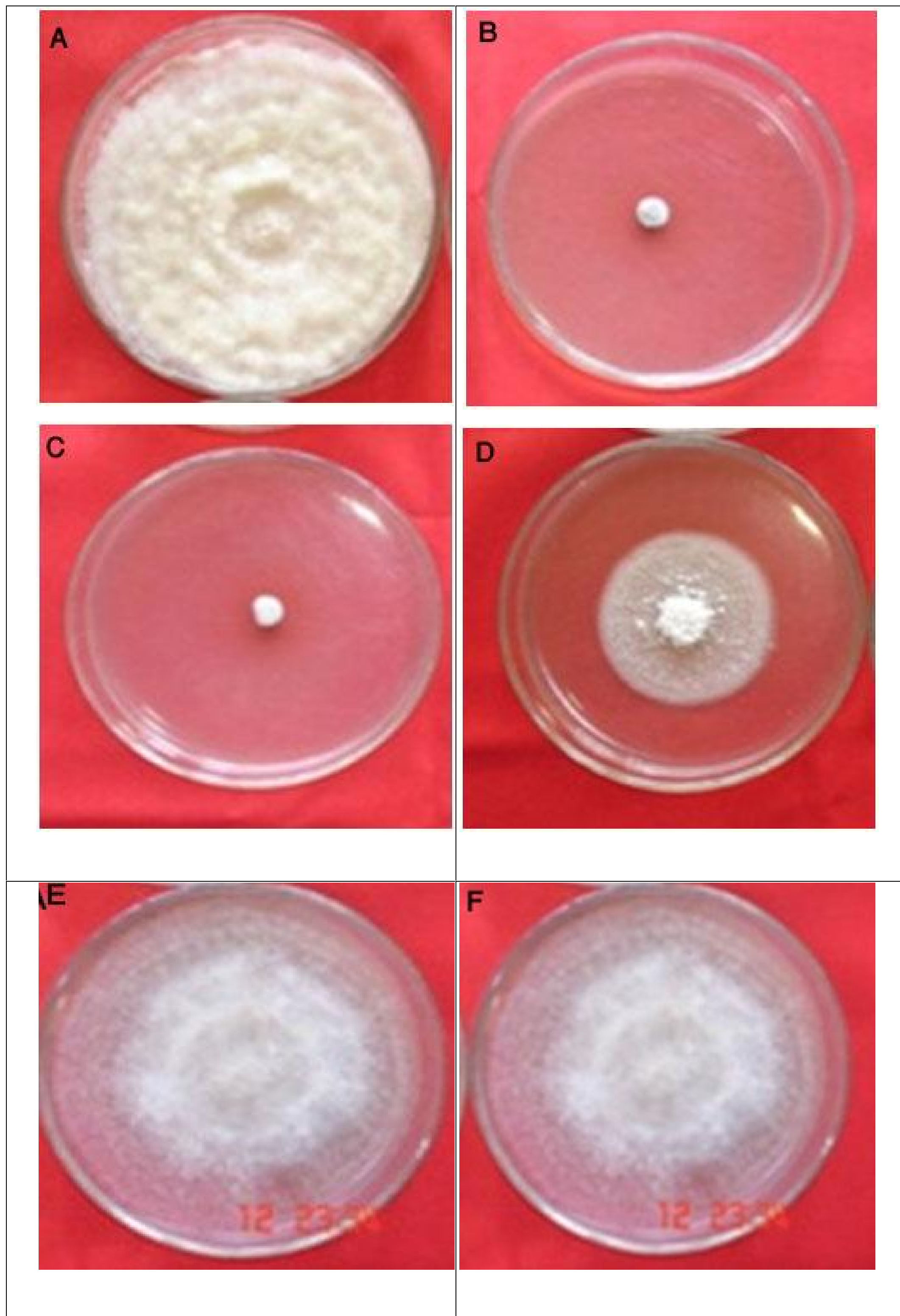


Plate 8. Mycelial growth of *Fusarium oxysporum* against different Fungicides A. Control, B. Proud 250 EC, C. Bavistin 50 WP, D. Provax-200 WP, E.Rovral-50WP and F. Ridomil gold MZ 68 WP

Table 6. Effect of Proud 250 EC on radial mycelial growth of *F. oxysporum*

Concentration (ppm)	Radial mycelia growth (cm)					
	24hr	48hr	72hr	96hr	120hr	% growth inhibition
100	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	100
200	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	100
300	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	100
Control	2.40 a	3.23 a	4.40 a	5.82 a	9.00 a	
$LSD_{(0.05)}$	0.49	0.32	0.35	0.39	0.02	
CV (%)	5.00	13.58	11.74	10.62	5.22	

Each data represents the mean value of three replications

Table 7. Effect of Bavistin 50 WP on radial mycelial growth of *F. oxysporum*

Concentration (ppm)	Radial mycelia growth (cm)					
	24hr	48hr	72hr	96hr	120hr	% growth inhibition
100	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	100
200	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	100
300	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	100
Control	2.40 a	3.23 a	4.40 a	5.82 a	9.00 a	
$LSD_{(0.05)}$	0.49	0.32	0.35	0.39	0.02	
CV (%)	5.00	13.58	11.74	10.62	10.15	

Each data represents the mean value of three replications

Table 8. Effect of Provax-200 WP on radial mycelial growth of *F. oxysporum*

Concentration (ppm)	Radial mycelia growth (cm)					
	27hr	48hr	72hr	96hr	120hr	% growth inhibition
100	0.0 b	1.82 b	2.70 b	3.64 b	5.15 b	43
200	0.0 b	1.59 b	2.12 c	2.88 c	4.22 c	53
300	0.0 b	1.00 c	2.00 c	2.70 c	4.07 c	55
Control	2.40 a	3.23 a	4.40 a	5.82 a	9.00 a	
$LSD_{(0.05)}$	0.49	0.30	0.45	0.42	0.23	
CV (%)	5.00	7.74	8.06	5.64	2.00	

Each data represents the mean value of three replications

Table 9. Effect of Rovral-50WP on radial mycelial growth of *F. oxysporum*

Concentration (ppm)	Radial mycelia growth (cm)					
	27hr	48hr	72hr	96hr	120hr	% growth inhibition
100	0.0 b	1.93 b	2.72 b	3.83 b	5.55 b	38
200	0.0 b	1.34 c	2.23 c	2.65 c	3.53 c	61
300	0.0 b	1.27 c	1.71 d	2.33 c	2.95 d	67
Control	2.40 a	3.23 a	4.40 a	5.82 a	9.00 a	
LSD(0.05)	0.48	0.34	0.35	0.45	0.11	
CV (%)	4.73	8.82	6.37	6.12	2.01	

Each data represents the mean value of three replications

Table 10. Effect of Ridomil gold MZ 68 WP on radial mycelial growth of *F. oxysporum*

Concentration (ppm)	Radial mycelia growth (cm)					
	27hr	48hr	72hr	96hr	120hr	% growth inhibition
100	0.0 b	2.06 b	4.99 a	6.54 a	8.96a	0.4
200	0.0 b	1.84 b	4.19 b	5.69 b	8.17b	9.2
300	0.0 b	1.21 c	3.22 c	4.92 c	7.21c	20
Control	2.40 a	3.23 a	4.40 b	5.82 b	9.00a	
$LSD_{(0.05)}$	0.49	0.33	0.36	0.42	0.13	
CV (%)	5.00	8.08	4.27	3.65	2.79	

Each data represents the mean value of three replications

4.8. Effect of *Trichoderma harzianum* against *Fusarium oxysporum* in dual culture technique

Trichoderma harzianum was tested against the of *Fusarium oxysporum*on PDA by dual culture technique and the results of the effect are presented in Fig 13. *Trichoderma* showed more than 77.77% inhibition of the radial mycelia growth of the test pathogen *Fusarium oxysporum* over control.

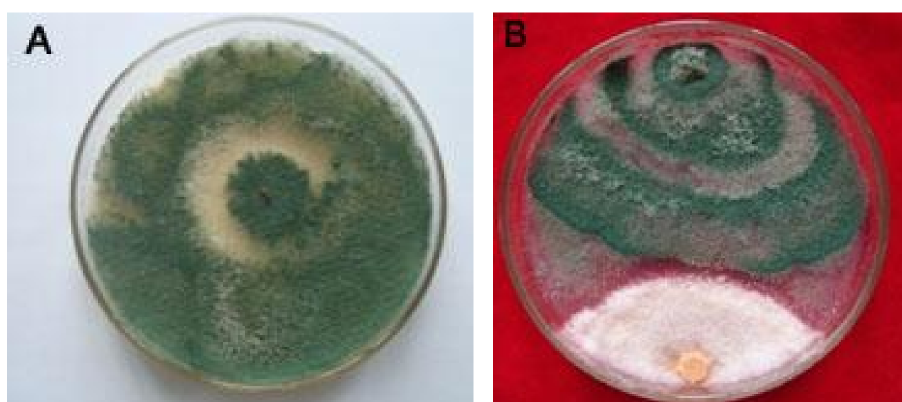


Plate 9. A. Growth of *Trichoderma harzianum* and B. Dual culture of *T. harzianum* and *F. oxysporum*

4. 9. Effect of seed treatment with fungicides against *Fusarium oxysporum* the causal agent of cotton boll rot

In cotton variety of CB-9, seed germination increased significantly over control when seeds were treated with fungicides. The highest seed germination (91.33%) was found in seed treated with Bavistin. The lowest seed germination (79.67%) was found in control treatment (Table 11). In case of pre-emergence seedling mortality varied from 8.66% to 30% when the maximum value recorded in control (30%) and the minimum value recorded in Bavistin 50 WP treated seeds. In case of post-emergence seedling mortality varied from 5.21% to 27.67% when the maximum value recorded in control (27.67%) and the minimum value recorded in Proud 25 EC treated seeds.

In cotton variety of CB-11, seed germination increased significantly over control when seeds were treated with fungicides. The highest seed germination (90.67%) was found in seed treated with Bavistin. The lowest seed germination (65%) was found in control treatment (Table 12). In case of pre-emergence seedling mortality varied from 9.33% to 35% when the maximum value recorded in control (35%) and the minimum value recorded in Proud 25 EC treated seeds. In case of post-emergence seedling mortality varied from 6.64% to 29.38% when the maximum value recorded in control (29.38%) and the minimum value recorded in Bavistin treated seeds.

In cotton variety of CB-12, seed germination increased significantly over control when seeds were treated with fungicides. The highest seed germination (90.0%) was found in seed treated with Bavistin. The lowest seed germination (61%) was found in control treatment (Table 13). In case of pre-emergence seedling mortality varied from 10.0% to 38.0%

when the maximum value recorded in control (38%) and the minimum value recorded in Bavistin treated seeds. In case of post-emergence seedling mortality varied from 6.26% to 29.38% when the maximum value recorded in control (33.0%) and the minimum value recorded in Bavistin treated seeds.

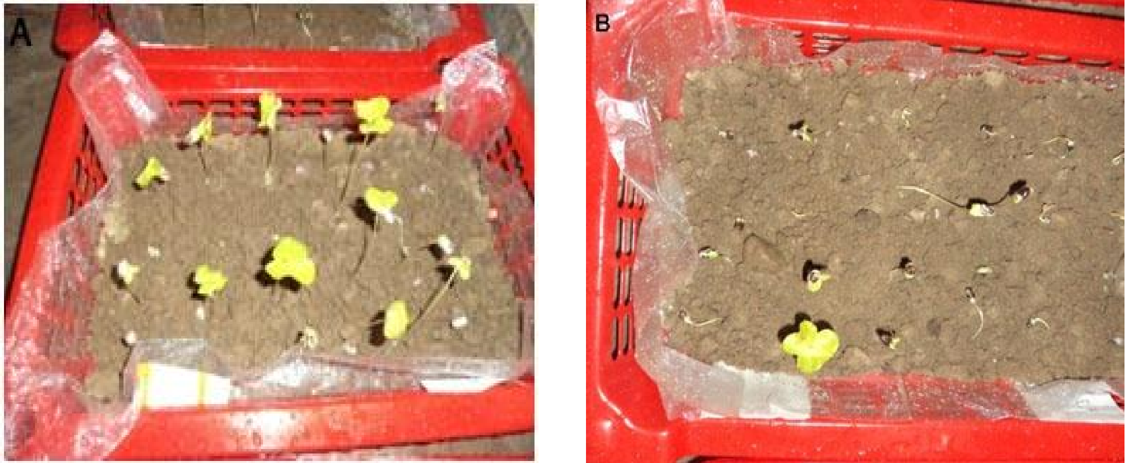


Plate 10. Raising of cotton seedling in plastic tray A. Healthy cotton seedlings in sterilized soil without inocula of *F. oxysporum*, B. Cotton seedlings grown in *F. oxysporum* inoculated soil and C. Effect of seed treatment with fungicides against *F. oxysporum* of different cotton varieties

Table 11. Effect of seed treatment of cotton variety CB-9 with fungicides on germination and seedling mortality in plastic tray

Fungicides	Germination%		Pre emergence seedling mortality%		Post emergence seedling mortality%	
Provax 200 WP	85.67	abc	14.33	c	11.64	b
Proud 25 EC	89.67	ab	10.33	d	5.21	d
Bavistin 50 WP	91.33	a	8.667	d	7.40	cd
Ridomil gold MZ 68 WP	83.00	bc	17.00	bc	10.27	bc
Rovral 50 WP	81.67	c	18.33	b	9.047	bc
Control	79.67	c	30	a	27.67	a
LSD $_{(0.05)}$	6.975		2.772		3.071	
CV	6.92		9.27		14.22	

Values in a column with same letter(s) do not differ significantly (P=0.01). Each data represents the mean value of three replications.

Table 12. Effect of seed treatment of cotton variety CB-11 with fungicides on germination and seedling mortality in plastic tray

Fungicide	Germination%		Pre emergence seedling mortality%		Post emergence seedling mortality%	
Provax 200 WP	83.67	b	17	c	14.49	bc
Proud 25 EC	88.67	a	11.33	d	11.2	cd
Bavistin 50 WP	90.67	a	9.333	d	6.643	e
Ridomil gold MZ 68 WP	84.00	b	16.00	c	10.51	d
Rovral 50WP	73.67	c	26.33	b	16.59	b
Control	65	d	35.00	a	29.38	a
LSD $_{(0.05)}$	3.249		3.404		3.764	

Values in a column with same letter(s) do not differ significantly (P=0.01). Each data represents the mean value of three replications.

Table 13. Effect of seed treatment of cotton variety CB-12 with fungicides on germination and seedling mortality in plastic tray

Fungicide	Germination%	Pre emergence seedling mortality%	Post emergence seedling mortality%
Provax 200 WP	82.33 b	17.67 b	13.49 b
Proud 25 EC	89.33 a	10.67 c	7.05 c
Bavistin 50 WP	90.00 a	10.00 c	6.263 c
Ridomil gold MZ 68 WP	79.33 c	20.67 b	13.42 b
Rovral 50 WP	79.00 c	21.00 b	7.653 c
control	61.00 d	38.00 a	33.00 a
$LSD_{(0.05)}$	2.552	3.764	3.914

Values in a column with same letter(s) do not differ significantly (P=0.01). Each data represents the mean value of three replications.

CHAPTER V

DISCUSSION

The present study was undertaken to studyefficacy of fungicides and*Trichodermaharzianum*against seed born pathogens of cotton. Experiment was conducted on the incidence of boll rot of cottonon three cotton varieties viz. CB-9, CB-11 and CB-12 in the experimental field during the period of April 2012 to March 2013. In the present study, the disease was identified by observing the symptoms on cotton boll following the description of Roy and Bourland (1982), Silva *et al*. (1995), Ribeiro*et al*. (2000), Belot and Zambiasi (2007) during survey. Three genera of fungi viz. *Colletotrichum*sp, *Alternaria*spand*Fusariumoxysporum*were isolated and identified. The pathogens has also been reported by many researchers throughout the world [Roy and Bourland (1982), Batson and Borazjani (1984), Colyer (1988), Silva *et al*. (1995), Ribeiro*et al*. (2000), Belot and Zambiasi (2007)]. According to Belot and Zambiasi (2007) there are many pathogens that can cause boll rot, such as *Alternaria* spp., *Ascochytagossypii*, *Aspergillusflavus*, *Bacillus pumilus*, *Colletotrichum* spp., *Diplodiagossypina*, *Erwiniaaroideae*, *Fusarium* spp., *Lasiodiplodiatheobromae*, *Myrotheciumroridum*, *Pantoeaagglomerans*, *Phomaexigua*, *Phomopsis* sp., *Phytophthora* spp., *Rhizoctoniasolani* and *Xanthomonasaxonopodis*pv. *malvacearum*. Various symptoms were observed may be due to the presence of complex of pathogens. The bolls were soft and blackened, and in some cases, arise from lesions in both the apex and at its base. Fructifications in various colors, from white to black were alsoobserved. The results obtained by Zancan*et al.* (2011) confirm reports from Belot and Zambiasi (2007) and Silva *et al*. (1995), who

found fungi associated with cotton bolls rot to be *Alternaria* spp., *Colletotrichum* spp., *Fusarium* spp., *Botryodiplodiatheobromae*, *Myrotheciumroridum* and *Aspergillus* spp.Significant variations in incidence of cotton boll rot disease were observed and it varies withinvarieties and growing periods. Among the three cotton varieties under study, the maximum incidence of boll rot disease was found in variety of CB-12 (6.32%) at March 2013 and the minimum incidence of boll rot was found in variety CB-9 (5.70%) at November 2012.According to Hillocks (1992) long periods with high atmospheric humidity is the main factor favoring the development of an epidemic of cotton bolls rot. Krishnamurthy and Verma (1974) and Restrepo*et al.* (1978) reported that *Fusariumsemitectum* is known to cause considerable damage by inciting cotton boll rot in India and Columbia under hot and humid conditions which indicates that seed colonization and thus seed inoculum potential may be related to the boll rot severity. The study period of the present experiment was mainly winter and early summer in Bangladesh condition where temperature and humidity were low and dry. This might be the reason of low incidence of the boll rot disease.

In vitro pathogenicity test of *Fusariumoxysporum*on cotton seedling showed that the highest seedling mortality was 69.00% in CB-12 and the lowest seedling mortality was 52.33% in CB-9. *Fusariumoxysporum*caused seed decay and seedling mortalitydue to attack of the pathogen. The pathogen attacked leaves of young seedlings and produced characteristics brown leaf spot and leaf rot symptoms. Pathogenicity on cotton seedlings has also been reported by many researchers throughout the world (Ray and Mclaughlin, 1942 and Dhingra, 1978).Dhingra (1978) reported that the emergence rate of seeds exposed to the fungal colonization for 24 h was similar to that of control,

but 36% of the seedlings showed a small (1 to 2 mm) reddish dark brown necrotic lesion on the collar regions, without apparent effect on the seedling growth. The lesion expansion was slow and did not girdle the collar. The isolation from these lesions always yielded *F. semitectum*. On the other hand, when the seeds were exposed to fungal colonization for 48 h the seedling emergence was reduced by 25% and all the emerged seedlings had some kind of lesion on the roots, collar or the primary leaves and the isolation from all such lesions yielded *F. semitectum.* Ray and Mclaughlin (1942) reported that role of seedborne inoculum in causing an array of diseases on cotton that involves, reduced seedling emergence, negative geotropism, leaf tearing, and collar rot leading to root rot. The appearance and the severity of symptoms, however depends upon the inoculum potential in the seed coat. All three levels of inocula caused significantly increasee in seedling mortality in three varieties of cotton. The highest levels of inocula corresponded to highest severity of seedling mortality showing maximum seedling mortality at 30 g/kg and the lowest at 10g/kg soil. In three varieties of cotton pre-emergence mortality and total mortality positively correlated with inoculum level and their relationship was significant.Klich (1986) and Davis (1977) stated that the lack of correlation between the proportion of *F. semitectum* infected seeds in cottonseed lots and appearance of symptom in seedling test as seen in seed pathology tests, can be explained on the basis of inoculum potential in the seed coat, which on the other hand is reported to be related to the climatic conditions during the reproductive phase. Dhingra (1978) reported that seedborne inoculum of *F. semitectum* in cotton can cause seed and root rot of seedlings and growing plants, only if the pathogen is internally seedborne, since no effect was observed when the seeds were surface inoculated. Even when internally seedborne, the degree of damage appears to be related to the quantity of inoculum

present in the seed coat, since neither emergence rate nor appreciable seedling damage occurred if the seeds were exposed to the fungal colonization for only 24 h. On the other hand, the exposure for 48 h resulted in appreciable seedling emergence loss and seedling damage, even though the fungus had not reached the embryonic tissues.

Among the different fungicides evaluated in the present *in vitro* investigation, Proud 250EC and Bavistin 50WP exhibited significantly superior efficacy by completely inhibiting the radial mycelial growth of *Fusariumoxysporum*at even 100 ppm while Provax-200 WP and Rovral 50WP were found moderately effective and Ridomil gold MZ 68WP was least effective.Mayur*et al.*(2001) testedsystemic fungicidescarbendazim, difenoconazole, thiophanate-methyl, propiconazole, hexaconazole and ridomil (metalaxyl)and non-systemic thiram, ziram, mancozeb, captan and chlorothalonil) at 50, 100 and 200 ppm against *Fusariumoxysporium*and found carbendazim was the most effective, inhibiting the radial mycelial growth by 100% even at the lowest concentration, and with the maximum toxicity index of 300.This result is in accordance with the findings of the present study. Dhrub and Singh (1988) reported that an isolate of *F. solani* from wilted seedlings of Kagazi lime was completely inhibited in vitro by carbendazim. Similar result was also reported by Kapoorand and Usman (1996) who studied the relative efficacy of systemic (benomyl, carbendazim and thiophanate-methyl) fungicides against 2 isolates each of *F. oxysporum* and *F. solani* from infected tomatoesand foundcarbendazim and benomyl were most toxic. Pant and Mukhopadhayay (2001) utilized three fungicides viz. Carboxin (Vitavax) 75 WP, Thiram 75 WP and carbendazim (Bavistin) 50 WP for the soybean seed treatment against the seed and seedling rot complex of soybean caused by *Rhizoctoniasolani*, *Sclerotiumrolfsii*.

Macrophominaphaseolina and *Fusarium* spp. Carboxin gave 100% inhibition of *S. rolfsii* at all the three concentrations, while 82% inhibition of *R. solani*, 66% of *M. Phaseolina* and 52% inhibition of *Fusarium* spp. was obsrved at 50 µg concentration of carboxin. The results of these findings corroborates with the findings of the present study. The biocontrol agent *Trichodermaharzianum* was tested against the boll rot pathogen*F. oxysporum*and it was observed that *T.harzianum*grew over the *F. oxysporum*and inhibition zone was formed at the point of meeting.This findings is in accordance with the findings of Elias *et al*. (1993) who reported antagonistic efficacy of *T. harzianum* against *F. oxysporum*f.sp.*cucumerinum*. *Trichodermaharzianum* reduced mycelial growth of *F. oxysporum*f. sp. *cucumerinum* and *R. solani*. Katragadda and Murugesan (1996) studied the mechanism of antagonism of*T. harzianum*against the cotton wilt pathogen *F. oxysporum f. sp. vasinfectum*. *T. harzianum* potentially reduced the radial growth *F. oxysporum*f. sp. *vasinfectum.* Bernal *et al*. (2001) observed that the antagonistic effect between *Trichoderma* spp. and *Fusariumoxysporum* f. sp. *cubense* was studied using dual culture on potato-dextrose-agar medium. The competitive capacity and inhibition of radial growth of *F. oxysporum* due to *Trichoderma* spp. were evaluated. An inhibition zone of 70% was observed, followed by total invasion of the fungal surface.

Treatment of seeds with five fungicides viz. Proud 250EC (Propiconazole), Bavistin 50WP (Carbendazim), Provax-200 WP (Carboxin), Rovral 50WP (Iprodione), Ridomil gold MZ 68WP (MetalaxylM+Mancozeb) effect significantly on the germination of the seeds of cotton varieties.Bavistin 50WP (Carbendazim) and Proud 250 EC (Propiconazole) treated seeds showed highest germination and minimum pre and post emergence seedling mortality. Champawat (1990)

studied ten fungicides against *Fusariumoxysporum*and found Bavistin(carbendazim) was the most effective and also enhanced seed germination and seedling vigour.In another experiment with Kagazi lime.Dhrub and Singh (1988) found thiophanate-methylwas the most effective fungicide in reducing mortality of infected seedlings followed by carbendazim, at 0.1% concentration. The present study showed that,*Fusariumoxysporum*can be bettter controlled and germination(%) of different cotton varieties can be increased by using Bavistin 50WP and Proud 250EC as fungicides and *Trichodermaharzianum*as bio control agent.

CHAPTER VI

SUMMARY AND CONCLUSION

Boll rot is considered as the major disease problem of the crop. The important pathogens involved in cotton boll rot are *Alternaria* spp., *Colletotrichum* spp., *Fusarium* spp., *Botryodiplodia theobromae*, *Myrothecium roridum*, *Aspergillus* spp., *Ascochyta gossypii*, *Lasiodiplodia theobromae* and *Rhizoctonia solani* in different cotton growing countries. The present study was undertaken to study the cotton boll rot disease and its management through bioassay of causal agent (*Fusarium oxysporum*) and seed treatment against five fungicides and one bio controlling agent.

In the field experiment during the period of April 2012 to March 2013 in the three cotton variety viz. CB-9, CB-11 and CB-12 and observing the maximum incidence of cotton boll rot disease at March 2013 in CB-12 (6.32%) among three varieties.

In-vitro experiments were conducted in the Seed Pathology laboratory (SPL) and Laboratory of the department of Plant Pathology of Sher-e Bangla Agricultural University, Dhaka. Three genera of fungi viz. *Colletotrichum* sp., *Alternaria* sp. and *Fusarium oxysporum* were identified and isolated from infeted boll and seed.

In an *in vitro* pathogenicity test it was found that seed decay, cottony mycelial growth of the fungus all over the dead seeds, hypocotyl rot, roots rot of emerged seedlings, seedling mortality, brown leaf spot and leaf rot were common symptoms caused by *F. oxysporum*. Effect of inoculums level of *Fusarium oxysporum* on seedling mortality of three cotton varieties showed that all of the *F. oxysporum was* pathogenic on cotton seedling causing pre- and post-emergence seedling mortality.

Bioassay was done on the basis of radial mycelia growth of *Fusarium oxysporum* to five fungicides namely Proud 250EC (Propiconazole), Bavistin 50WP (Carbendazim), Provax-200 (Carboxin), Rovral 50WP (Iprodione), Ridomil gold MZ 68WP (Metalaxyl M+Mancozeb) at the rate of 100, 200 and 300ppm for 120 hours and bio control agent *Trichoderma harzianum.* Among the different fungicides Proud 250EC and Bavistin 50WP exhibited significantly superior efficacy in inhibiting the radial mycelial growth of *F. oxysporum* over untreated control. *Trichoderma* inhibited the growth of *F. oxysporum.* Incase of control (*F. oxysporum*) the fungus grew well and produce luxuriant mycelia. It was observed that *Trichoderma harzianum* grew over the *F. oxysporum* and inhibition zone was formed at the point of meeting.

Among the pathogens *F. oxysporum* occurs throughout the world and can damage any part or all of a plant. Seed treatment with fungicides is a primary component in a program to manage cotton boll rot diseases. The fungicides, such as Proud 250EC (Propiconazole), Bavistin 50WP (Carbendazim), Provax-200 (Carboxin), Rovral 50WP (Iprodione), Ridomil gold MZ 68WP (Metalaxyl M+Mancozeb) were used for seed treatment. Germination of the seeds of cotton variety has been found to differ significantly among the various treatments of fungicides. Among the fungicides Bavistin 50WP and Proud 250EC were more effective for highest germination and minimum Pre and post emergence seedling mortality in case of three cotton variety (CB-9, CB-11 and CB-12).

CHAPTER VII
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