CONTROL OF COTTON BOLL ROT BY USING SOME SELECTED CHEMICALS

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ABSTRACT

An experiment was conducted at the Research Farm of Sher-e- Bangla Agricultural University, Dhaka, Bangladesh during the kharif season of 2013-2014 to study the effect of some selected chemicals to control cotton boll rot disease. The experiment was carried out under *in- vitro* and in field conditions. The field experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. Cotton variety CB 9 was used in the experiment. Three of fungi viz: *Fusarium* spp., *Alternaria* spp., *Aspergillus flavus* and *A. niger* were isolated from seeds of cotton and diseased bolls of cotton. *Sclerotium rolfsii* was also isolated from infected bolls of cotton. Three chemicals namely Mancozeb, Cupravit 50 WP and Streptomycin sulphate were used against the fungi. In *in- vitro* test combined effect of Mancozeb and Cupravit 50 WP (0.4%) showed the best result which inhibited the radial mycelial growth of all fungal species followed by Cupravit while Streptomycin sulphate showed no effect on mycelial growth. In field condition, seed treatment with Mancozeb + Cupravit 50 WP (0.4%) along with three foliar sprays proved to be most effective to control boll rot of cotton followed by seed treatment with Cupravit 50 WP (0.4%) along with foliar spray for three times. Seed health study of harvested cotton seeds revealed that seed treatment followed by foliar spray with Mancozeb + Cupravit reduced the incidence of seed borne fungi partially compared to control. In all the cases Streptomycin sulphate (0.1%) showed no significant effect.

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CERTIFICATE

This is to certify that the thesis entitled, "Control of Cotton Boll Rot by Using Some Selected Chemicals" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirement of the degree of Master of Science in Plant Pathology, embodies the result of a piece of bona fide research work carried out by Syed Moaz Mahmood, Registration no. 08-02670, under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma.

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

Dated: 20 November, 2014
Dhaka, Bangladesh

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Supervisor

CHAPTER 1
INTRODUCTION

Cotton, “The king of Fibers” is one of the most renowned, reliable fiber yielding crops as well as cash crops around the world including Bangladesh. Cotton is mostly grown in temperate and tropical regions more than seventy countries in the world. Hot and dry weather with adequate amount of moisture obtained through irrigation is required for its cultivation. It is harvested as seed cotton and ginned to separate seed and lint. (Tripathi *et al.*, 2011).

The word cotton refers to four species in genus of *Gossypium* (Family : Malvaceae) namely *G. hirsutum* L, *G. arborium* L, *G. harbacium* L, *G. barbadense* L. All of those were domesticated all over the world independently as the elementary source of textile fiber. Economically two of the varieties, those are – *Gosypium hirsutum* and *Gosypium barbadense* are most important (Percival and Kohel, 1990). Globally *Gosypium* genus has about fifty species. (Frixel and Rheeda, 1992).

Cotton is the most important cash crop next to jute in Bangladesh (Hussain, 2013). In Bangladesh cotton production was in forecast at 120000 bales in 2013/14 (11% higher than the previous years) and at the same period of time area under cotton cultivation was 45000 hectares where in 2012 it was 40000 hectares (Forecast, 2013). In May 2012/13 Bangladeshi yarn production was estimated at 688000 tons and an increase of about 12% from may 2012/ 13 production. Under normal condition domestic cotton in Bangladesh can only meet about 3% of countries current demand of raw cotton (Hussain, 2013). At this condition we are one of the biggest importers of cotton in the whole world (Cotton: trend in global production 2013).

Global production of cotton was expected to be 116.7 million bales in 2013/24 and in the same time area under cultivation was expected to be 33.1 million hectares and worlds` average yield is 766 kilograms/ha (Cotton: trend in global production 2013).

Each year, cotton production is being subdued due to the presence of grievous pathogens. The most common fungi associated with cotton diseases in field are *Fusarium* spp, *Colletotrichum* spp, *Rhizopus* spp, *Pythium* spp. (Roy and Bourland, 1982). Overall the most deteriorating pathogens associated with cotton boll rot are *Rhizoctonia* spp, *Fusarium* spp, *Alternaria* spp, *Aspergillus* spp, *Diplodia* spp, *Sclerotium* spp, *Rhizopus* spp and several other fungi and bacteria (Fulton and Bollenbacher, 1959, Alfred, 1963, Seneewong *et al.*, 1999, Mansoori and Hamdolahzadeb, 1995, Palmateer, 2004). Globally fungi associated with cotton are mostly *Fusarium, Helminthosporium, Curvularia, Alternaria, Mucor, Rhizopus, Penicillium, Aspergillus, Sclerotium, Cephalosporium, Myrithecium, Rhizoctonia , Tricoderma and Xanthomonas*. (Khan and Kausar, 1967). In this list some are more malign, rife and elementary in our country while others are extraneous.

Considering the prevalence of the pathogens and damage caused by them, an immediate redress seems to be exigent to palliate the present dilemma in cotton industry. Fungicides are known to be the supreme defensive component to control cotton boll rot disease and they have broad spectrum activities with protectant and systemic capabilities against most fungal pathogens. Generally, seed treatment fungicides are proved to be sufficient measure to control the seed born diseases of cotton and seedling disease (Minton *et al.*, 1982; Chambers, 1995). In search of the effective control measure different fungicides were used worldwide in order to minimize the damage of cotton bolls and among them fungicides originated from Copper and Mancozeb group were proved to be most promising.

Considering all the aforementioned facts stated above the present research was undertaken with following objectives.

1) To identify the causal agents of cotton boll rot and

2) To find out most effective Chemicals against cotton boll rotting pathogens.

CHAPTER 2
REVIEW OF LITERATURES

2.1 Seed Borne Diseases of Cotton

Hilocks (1992) conducted experiment on cotton diseases and reported that *Fusarium* spp is the causal agent on failure of infected xylem to meet the water requirement of the plants.

Adeoti *et al.* (1992) also reported that seed borne pathogen *Fusarium* spp is causing seedling rots in Nigeria.

Pizzinatto and Menton, (1991) stated that *Fusarium solani* and *F. equiseti* were approximated 60 % and 30 % of all fungi isolated from diseased seedling.

Colyer (1988) also conducted an experiment with a view to identifying the seed borne pathogens of cotton and isolated *Fusarium* spp. as root rot pathogen of cotton.

Sparnicht and Roncardori, (1972) conducted an experiment to identify the seed associated pathogens of cotton and their effect on cotton boll and noted that *Fusarium* spp. is the causal organism for delay boll formation of cotton.

King and Presly (1942) conducted an experiment and noted that most common fungi associated cotton diseases are *Fusarium* spp, *Colletotrichum gossippi, Rhizopus* spp, *Thialavispsis basicola and Pythium* spp .

Woodroof (1927) undertook an experiment to find out the seed borne pathogens of cotton and isolated *Fusarium* spp as the first root rot pathogen of cotton.

2.2 Cotton Seed Health Test in Laboratory

Tempe, (1953) conducted an experiment on Blotter method of seed health testing programme and noted that main objectives of seed health testing are related to actual policy towards seed improvement, seed trade and plant protection.

2.3 Pathogens Isolated From Cotton Seeds

Palmateer *et al.* (2004) conducted an survey to find out different seed borne fungi of cotton and isolated fifty eight species of fungi belonging to thirty seven genera, including nine species of *Fusarium* spp. where *F. oxysporum, F. solani and F. equiseti* were most frequent species .

Mansoori and Hamdolahzadeb, (1995) initiated an experiment to find out and isolate the seed related fungi of cotton seed and isolated *Alternaria* spp., *Fusarium* spp., *Pythium* spp . *Rhizopus* spp. from cotton seed.

Wang *et al.* (1992) conducted an experiment and reported about high frequency of *Fusarium moniliform* and *F. semitetcum* from cotton seedlings and bolls while *F. oxysporum, F. solani, F. compactum* were found with less frequency.

Seneewong *et al.* (1991) carried out an experiment and reported that *Fusarium* spp. was the most prevalent fungal species isolated from the cotton seed coat and from the embryo of hundred randomly selected seeds.

Kuch (1986) conducted an experiment to get acquainted with the seed associate fungi of cotton and isolated *Fusarium equiseti* and *Fusarium semitectum* for more than 10 percents of seed at any sampling in the northern USA.

Khan and Kausar (1967) conducted an experiment on seed borne pathogens in cotton seeds and stated that *Fusarium* spp, *Carvularia* spp, *Alternaria* spp, *Mucor* spp, *Aspergillus* spp, *Sclerotium* spp. were associated with cotton seeds.

Kamal and Khan (1964) reported that seed borne microorganisms cause a great deal of hazard to the yield contributing characters of cotton and cause a vast economic loss.

Alfred (1963) initiated an experiment to find out different patogens in cotton seeds and noted that fungi belonging to the *Alternaria , Fusarium, Diplodia , Aspergillus genera* were associated with the seed hairs and actual seeds during the boll development.

Khan *et al.* (1960) conducted an experiment with a view to isolating the pathogens associated with cotton seeds and reported that seed borne microorganisms causes rottening of seeds and seedlings, leaf spot, boll rots and bacterial blight which resulted in reduced seed germination, ultimately low yield.

Fulton and Bollenbacher, (1959) initiated an experiment on seed borne pathogens of cotton with a view to identifying and isolating different pathogens present in cotton seeds and reported that *Fusarium oxxysporum, F. moniliforme, F. semitechum, Pytheum* spp were found in the examined cotton seeds .

2.4 Field Diseases of Cotton

Burgess (1981) committed an experiment to find out the general ecology of *Fusarium* spp. and noted that *Fusarium oxysporum* is a common soil borne fungus that is present in every type of soil around the world.

Garret (1970) initiated an experiment with a view to finding out the root infecting fungi of plants and noted that *Fusarium* spp has a large diversity of strains and is a very successful saprophyte.

Kamal and Moghal (1968) conducted a survey to find out the cotton diseases in south west of Pakistan and reported that cotton gets effected by boll rot, root rot, wilting, and bacterial leaf blight, some nemic diseases, different types of leaf spot diseases, premature opening of bolls, leaf curl and stenosis of cotton.

Park *et al.*, (1965) committed an experiment on the survival capability of different microorganisms in soil and noted that many *Fusarium spp*. persist on soil by means of chlamydospores.

2.5 Seed Treating Chemicals for Cotton

Phillip *et al.* (2003) reported that seed treating with the combination of Mancozeb and Fludioxonil up to ten days prior to planting can provide effective control for *Fusarium* decay of cut seed pieces.

Bagga (1969) initiated an experiment to determine pathogenicity of different fungi species and reported that solution of Mercuric chloride and solution of Sodium hypochlorite is effective seed treating agent for cotton.

2.6 Copper Fungicides as Seed Treating Agent of Cotton

Rathod and Power, (2013) reported in his experiment while working on the *in vitro* seed treatment of fungicides for the control of seed borne fungi of soybean variety Durga that the seed treatment with Copper oxychloride increased seed germination and at the same time decreased seed mycoflora.

Muthomi *et al.* (2007) found in his experiment that treating the seeds with Copper oxychlorides increased seedling emergence and also reduced seedling mortality while he was working on the effectiveness of different fungicides on legume root rot.

Brennan (1990) committed an experiment to find out the effectiveness of some copper compounds applied as foliar sprays in alleviating copper deficiency of 744 wheat grown on copper deficient soils of Western Australia and noted that Copper oxy chloride is a classical non- systemic fungicide and widely used to treat seeds on copper deficient soil.

Lungren and Durrell, (1928) found that copper seed treatment done by copper carbonate showed reduced disease incidence of stinking smut of wheat.

2.7 Mancozeb as Seed Treating Chemical for Cotton

Neeraj and Shilpi, (2010) committed an experiment on different *Alternaria* diseases of vegetable crops and new approaches for its control and reported that Mancozeb was proved effective as seed dresser.

Maroni *et al.* (2000) initiated an experiment and stated that Mancozeb is one of the most used pesticides in the world and reported scare persistence in the environment.

2.8 Chemical Control

Effect of Copper Fungicides on *Fusariun* spp

Alam *et al.* (2003) conducted a research to study the effect of copper fungicides on *Fusarium* spp. and reported that copper fungicide retarded the mycelial growth of *Fusarium oxysporum* .

Hossain *et al.* (2001) worked on the Efficacy of different fungicides in controlling purple blotch of onion seed crop and noted that complete zone of inhibition for *Fusarium* spp. was found using copper fungicide.

Effect of Streptomycin on *Fusarium* spp

Hossain and Bashar, (2011) undertook a research work to find out the *In vitro* effect of plant extracts, fungicides and antibiotics on fungal isolates associated with damping off disease of crucifer and reported that the use of antibacterial antibiotics stimulates the growth of *Fusarium* spp.

Effect of Copper Fungicides on *Alternaria* spp

Neeraj and Shilpi, (2010) reported that Copper oxy chloride was found as one of the most active fungicides in inhibiting the spore germination and growth of *Alternaria* spp.

Alam and Mahal, (1999) initiated an experiment to find a cure the rot of the chilly caused by *Alteranria* spp. and reported that copper fungicide was proved to be effective against *Alternaria* spp @ 500 to 2500 ppm concentration for five to thirty minutes.

Timmer and Zitko, (1997) reported in his experiment committed on Evaluation of different fungicides for the control of *Alternaria* brown spot and citrus scab and noted that copper fungicide provided surprisingly good result to control of *Alternaria* spp.

Effect of Copper Fungicide on *Aspergillus* spp.

Minamor (2013) reported in his research on the Effect of two fungicides Coacobre and Ridomil on rhizosphere micro flora of cocoa (*Theobroma cacao L.*) seedling that Copper fungicide (Ridomil) effectively reduced both *Aspergillus flavus* and *A. niger* population in field condition .

Belli *et al.* (2006) reported in his research which was the Impact of fungicides on *Aspergillus carbonarius* growth and ochratoxin A production on synthetic grape medium and grapes that fungicides those contains copper in their composition are very effective to reduce growth of *Aspergillus* spp.

Srininivasan and Shanmugam, (2006) found in his experiment on Post harvest management of black mold rot of onion that Copper oxychloride completely inhibited the mycelial growth of *Aspergillus niger* .

Effect of Mancozeb on *Fusarium* spp.

Mamza *et al.* (2012) evaluated six fungicides on the sporulation of *Fusarium pallidonoseum* isolated from castor using Benomyl, Thiram, Mancozeb, Metalaxyl-M, Difenconazol, Tricyclazole and reported that Mancozeb was partially able to control the growth of *Fusarium* spp .

Nisa *et el.* (2011) conducted an experiment on the *In- vitro* inhibition effect of fungicides and botanicals on mycelial growth and spore germination of *Fusasrium*

oxysporum and reported that Mancozeb was found most effective in reducing mycellial growth of *Fusarium* spp. among the non systemic fungicides.

Shah *et el.* (2010) screened three fungicides viz: Carbendazim, Mancozeb, conjoint Carbendazim, Mancozeb and Sulpher against *Fusarium oxysporum* to find out their efficacy against the growth of *Fusarium* spp and reported that after 120 hours of incubation Mancozeb @ 10000 ppm was found most effective in the controlling of *Fusarium* spp.

Fravel *et al.* (2005) reported that Mancozeb alone and combination of Mancozeb and copper solution reduced the final colony of fungus *Fusarium oxysporum* @ 100 ppm .

Effect of Mancozeb on *Alternaria* spp.

Kumar *et al.* (2013) undertook an experiment to Evaluate the efficacy of different fungicides for the management of *Alternaria* leaf spot disease of chili and concluded that Mancozeb was found most effective in reducing the *Alternaria* spp growth in laboratory as well as field trial.

Balai and Sing, (2013) conducted an experiment on Integrated management of *Alternaria* blight of pigeon pea with some selected fungicides and antagonists in pot condition and reported that Mancozb was found effective to reduce *Alternaria* spp attack on pigeon pea in field condition.

Gondal *et el.* (2012) conducted trials with different fungicides to find out the effect of different doses of fungicides against *Alternaria* leaf spot blight of tomato and reported that disease caused by *Alternaria* spp was reduced by applying Mancozeb fungicide fourteen days after applying.

Neeraj and Shilpi, (2010) carried out an experiment on *Alternaria* disease of vegetable crops and reported that Mancozeb was found very effective in *in -vivo* condition.

Mesta *et al.* (2009) initiated a research work and evaluated several fungicides to thwart the growth of *Alternaria* blight of sunflower and concluded that Mancozeb was significantly superior to control *Alternaria* spp. among all the treatment combination.

Kamal *et al.* (2007) conducted an experiment to find out Field efficacy of bioagents and different fungicides against tomato (*Lycopersicon esculentum Mill.*) disease and found that *Alternaria* blight and *Alternaria* rot of tomato was lower when foliar spray was done with Indofil M 45 with disease incidence of 1.7 and 4.0 %, respectively .

Thippeswamy *et al.* (2006) committed an experiment on leaf spot of brinjal caused by *Alternaria solani* and used different fungicides to reduce disease and reported that Mancozeb was found to be most effective against *Alternaria solni*.

Narain *et al.* (2006) let to occur an experiment on the efficacy of fungicides against *Alternaria* leaf spot of broccoli and noted that seed treatment by Apron followed by foliar spray of Indofil M– 45 (@ 0.2%) reduced leaf blight caused by *Alternaria* spp.

Kumar *et al.* (2006) had conducted an experiment on the efficacy of some fungi toxicants against *Alternaria brassicae* causing *Alternaria* blight of radish and tested fungicides *in-vitro* against *Alternaria brassicae,* causal agent of *Alternaria* blight of radish where Mancozeb was proved to be one of the most effective fungicides.

Tiwary *et al.* (2004) initiated an experiment to find out the effect of the spray schedule of Mancozeb on early blight caused by *Alternaria solani* and reported that Mancoaeb was found economical when sprayed twice rather than once and thrice.

Sing and Rai, (2003) reported that Indofil M-45 was found as most effective in reducing the mycelial growth of *Alternaria alternata*, that caused leaf spot of brinjal in *in -vitro.*

Katiyar *et al.* (2001) reported that the best control of *Alternaria* leaf spot of bottle gourd was obtained by spraying by recommended (@ 0.2%) Indofil (Mancozeb) in field condition.

Babu *et al.* (2001) reported that Mancozeb (0.2%) was found very effective against *Alternaria solani* while he used different fungicides to thwart the *Alternaria* blight of tomato.

Sing *et al.* (2001) evaluated different fungicides to subside the severity of *Alternaria* blight of tomato and reported that effectiveness of Mancozeb in controlling of early blight of tomato was quite satisfying.

Sing *et al.* (1997) conducted an experiment to determine the efficacy of different fungicides to control early blight of potato caused by *Alternaria solani* and reported combination of Indofil M- 45 (Mancozeb) with Emison -6 was found to be the most effective in controlling the *Alternaria* blight of potato.

Shtiensherg and Kremer, (1993) carried on an experiment in order to find out the Influence of physiological age of pima cotton on need for fungicidal treatment to suppress *Alternaria* leaf spot and recorded that Mancozeb showed effective action to reduce the severity of *Alternaria* spp leaf spot of cotton in field.

Ansari *et al.* (1990) undertook an experiment with a view to Evaluating some fungicides for seed treatment and foliar application to manage damping off of seedling blight of rapeseed caused by *Alternaria brassicae* and reported that seed treated with six fungicides checked and the pre emergence and post emergence of loss of seedlings to a varying extent against *Alternaria brassicae* infected rape seed mustard and found Mancozeb was most effective followed by Copper oxychloride.

Choulwar *et al.* (1989) experimented several fungicides to seek out the efficacy of fungi toxicants on the mycelial growth of *Alternaria solani* and reported that Mancozeb (0.2%) was found most effective for inhibiting the mycelial growth of *Alternaria solani.*

Effect of Mancozeb on *Aspergillus* spp.

Wani and Nisa, (2011) conducted an experiment on management of black mold rot of onion and reported that among the non systemic fungicides Mancozeb was found most effective against *Aspergillus niger*.

Mateo *et al.* (2011) screened several fungicides in his experiment where they used three fungicides Mancozeb, Copper oxychloride and Sulfur compound to reduce the growth of *Aspergillus spp.* and reported that Mancozeb was the best followed by Copper oxychloride and Sulfur compound to hinder *Aspergillus* spp. growth.

Prakash *et al.* (2007) conducted an experiment on the effectiveness of novel combination fungicide against Downey mildew incidence, fruit quality, shelf life and post harvest pathogens of grape vine in India and noted that Mancozeb and Secure 69 WDG (Mancozeb 50% + Fenamidone 10%) was the most effective against *Aspergillus niger* .

Syed *et al.* (2001) reported in his experiment that Mancozeb alone and combination of Mancozeb and garlic extract was proved to be most effective to reduce *Aseprgillus* spp, *Alternaria* spp, *Fusarium* spp, *Rhizopus* spp present in sorghum.

Effect of Mancozeb on *Sclerotium* spp.

Manu, (2012) used five fungicides in an experiment to find out the efficacy of fungicides against *Sclerotium rolfsii* causing foot rot disease of finger millet under *in-vitro* conditions using Hexaconazole, Propiconazole, Vitavax, Carbendazim and Mancozeb and reported that Mancozeb retarded the growth of *Sclerotium* spp successfully.

Madhavi and Bhattiprolu, (2011) screened some fungicides in an experiment on Integrated disease management of dry rot of chilly incited by *Sclerotium rolfsi* including different systemic and non systemic fungicides including Hexaconoxzole, Propiconazole, Tebuconazole, Difenoconazole, Copper oxy chloride, Mancozeb, Carbendazim and reported that among these fungicides Mancozeb showed satisfying effectiveness against the growth of *Sclerotium* spp than the others .

Yaqub and Shahzad, (2006) conducted an experiment using the following fungicides Benomyl, Sancozeb, Thiovit, Carbendazim, Topsin M and concluded that Mancozeb group fungicide, i.e. the Sancozeb showed better result to control *Sclerotium rolfsii* .

Paksha *et al.* (2003) carried out an experiment to determine the bio efficacy of fungicides against collar rot of cotton caused by *Sclerotium rolfsi* using different fungicides including Carbendazim, Tridemormg, Propiconazole, Captan, Thirum, Copper oxy chloride and Mancozeb and reported that Mancozeb (@ 0.4%) was effective against the growth of *Sclerotium* spp.

Patil *et al.* (1986) conducted an experiment on chemical control of wilt of Betel vine using some fungicides including Mancozeb and Copper based fungicides and reported that Mancozeb fungicide showed much more affective on *Sclerotium* spp than the other fungicides.

Kumar and Pandurangegauda, (1984) tested different fungicides including Mancozeb and Copper oxy chloride to allay the growth of *Sclerotium* spp. and reported that Mancozeb showed good effect against the growth of *Sclerotium* spp.

2.9 Comparison between Fungicides and Antibiotics as Seed Treating Agent for Cotton

Chaudhury *et al.* (2008) conducted an experiment to determine the efficacy of different fungicides and antibiotics against bacterial leaf blight of rice including Bordeaux mixture, Copper oxychloride, Streptomycin sulphate and reported that Bordeaux mixture alone was the most effective chemical to control bacterial leaf blight of rice, followed by the proper combination of copper fungicide (Copper oxychloride) and Streptomycin.

2.10 Average Cotton Production in the World

Chaudhury, (1995) depicted in an conference held in Poland with a heading “Worlds Cotton Yields Are Rising Slowly” that between 1983/84 and 1991/92, the world average yield rose from 450 kilograms per hectares to nearly 600 kilograms and an extrapolation

of the 44-year regression line through world yield indicates an average yield in 2000/01 of about 620 kilograms per hectares.

Johnson *et al*. (2014) in his conference with a heading "The World and United states Cotton Outlook" reported that worlds average cotton production is around 746 KG per hectare.

CHAPTER 3
MATERIALS AND METHODS

3.1. Variety Used

Cotton variety CB 9 was used in this experiment.

3.2. Collection of Seeds

The seeds of cotton variety CB-9 were collected from Khamar Bari, Farm gate, Dhaka .

3.3. Seed Health Study

Seeds of cotton variety CB-9 were collected from Khamar Bari, Farm gate, Dhaka. Then four hundred seeds were selected randomly for laboratory seed health study. Collected seeds were sterilized with 1% Clorox (NaOCI) for 5 minutes and rinsed with sterilized water for 3 minutes. Seed germination was determined by the blotter method according to the International Rules For Seed testing Agency (ISTA, 1996). Ten seeds were placed on 4 layers of moist blotter paper in 5 cm petridishes maintaining uniform distance between them. The petri dishes and blotter papers were sterilized properly before use. Each of the plates was incubated in 25 ± 4° C temperature for 7 days in incubation chamber with an alternation of twelve hours light and dark. After 7 days of incubation, plates were collected and examined under stereomicroscope for primary identification of the Pathogenic organism(s). Then the identified fungi were transferred to PDA plates for proper sporulation and purification. Hyphal tip culture method was used to make the pure culture of the fungi (Mian, 1995; Tuite, 1969).

Seeds obtained from the field experiment were also tested under same procedure described before following ISTA (1996) rules in order to find out seed borne boll rot pathogens present in them to determine the efficacy of different treatments to subdue the engender of cotton boll rot. The difference between of pathogenic presence in two different seeds of the same cotton variety was then calculated.

3.4. Isolation of Seed Borne Fungi from Incubated Seeds

Fungi grown over the incubated seeds were aseptically transferred on to PDA medium with the help of a sterile needle and the PDA plates were kept in incubation at 25±2° c and 12 hours alternating cycle of light and darkness for 7 days. Purification was done by reculturing fungi identified on the basis of their characteristics under compound microscope. These fungi were identified following the keys of Khan *et al.* (1960), Kamal and Khan, (1964) and Kuch, (1986).

3.5. Preservation of Culture Collection

Different fungal stock cultures were prepared in PDA slant and preserved in refrigerator at 4° c for further use.

3.6. Evaluation of the Efficacy of Some Selected Chemicals Against Seed Borne Fungi of Cotton

Trade name	Common name	Chemical name	Active ingredient (%)
Cupravit 50 WP	Cupravit	Copper oxychloride	50
Indofil M- 45	Mancozeb	Mancozeb	45
Streptomycin	Streptomycin	Streptomycin	01

		sulphate	

Table 01:

Detailed particular of chemicals used in the experiment

First, chemical suspensions were prepared as per following concentration, 0.4% for the fungicides viz: Cupravit 50 WP and Mancozeb 80 WP and 1 ppm for the antibiotic viz: Streptomycin sulphate. A fungal mycelial block was cut from a 7 days old fungal culture and transferred on a PDA.

An *in- vitro* evaluation was conducted to find out the effect of chemicals against the seed borne fungi of cotton on PDA following well method. Discs of mycelia (5 mm diameter) from each of the isolated fungi were cut from the edge of the actively growing fungal colony with a cork borer. One mycelial disc of each fungus was placed on the edge of each PDA plate and simultaneously on the other side a 5 mm well was prepared and on that well 80 µl of chemical suspension was poured and these plates were incubated at 25±2° c for 7 days. In case of the control plate, only the fungal mycelial block was placed without any chemical. after 7 days of incubation, radial mycelial growth of control plate and plates with fungicides were measured in diameter.

The following formula (Kantwa *et al.*, 2014) was used to determine the inhibition zone of fungal myecelia

$$\% \text{ inhibition} = \frac{C - T}{C} \times 100$$

C = Radial growth of control plates.

T = Radial growth of fungicide and antibiotic treated plates.

Field Experiment

3.7. Experimental Site

The field experiment was carried out in the experimental field in Sher- e- Bangla Agricultural University, Sher- e- Bangla Nagar, Dhaka. *In- vitro experiment* was conducted in the Seed Pathology Laboratory and the M.S. Laboratory of the Department of Plant Pathology of Sher- e- Bangla Agricultural University, Dhaka.

3.8. Experimental Period

The following experiments were conducted during the period 30th May to November, 2013.

3.9. Experimental Plot

The selected field for this experiment was properly ploughed and proper doses of required fertilizers were applied to the field. The amount of the fertilizers applied to the field was as following, Urea – 13 kg, TSP- 9 kg, MOP- 9 kg, Gypsum- 6 kg, Borax- 1.5 kg and Zinc sulphate- 1.5 kg was applied in order to maintain desired growth of the cotton seedlings in the field. Clods, weeds in the rim of the field were removed and the whole field was properly leveled.

In the field experiment thirty plots were prepared for different treatments. Each plot was 3 meters in length and 2 meters in width where row to row distance was 2.8 m and plot to plot distance was 0.5 m. The total area covered by the field was 511.2 square meter.

3.10. Selection of Seed Treating Chemicals

As it has been mentioned before that fungicides show reliable effect to control boll rot disease of cotton, two most reliable fungicides groups originated from Copper and Mancozeb, being used worldwide were chosen as seed treating agent and what is more, an Antibiotic Streptomycin sulphate was also assigned to treat the experimental seeds.

3.11. Seed Treatment

Total required amount of seed for the field experiment was separated and divided in to three equal parts. Then one part was treated with Cupravit 50 WP @ 0.4%, another part was treated with a combination of Cupravit 50 WP @ 0.4% and Mancozeb 80 WP @ 0.4% and third part of the seeds were treated with antibiotic Streptomycin sulphate @ 0.1% . To treat the seeds with fungicides, first required amount of seed were kept in a Petridish and then the fungicide was added there. Then the Petridish was covered with the lid and it was shacked thoroughly for a few minutes so that the fungicide covers total surface of the seed coat. To treat seeds with antibiotic first, a regular bottle was filled with 100 ml sterile distilled water and 1 gm streptomycin sulphate was mixed to it. Then selected seeds were poured in the bottle and the bottle cap was attached. All three treated seed items were kept overnight till the next morning as it was the sowing day. In case of control plot, seeds were treated with sterile distilled water only.

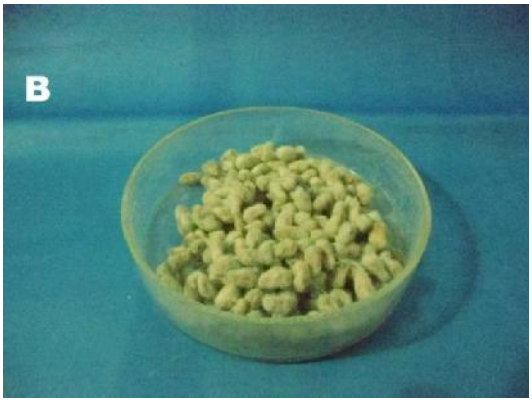

Figure 1: Cotton seed treatment with different chemicals A) Cupravit 50 WP, B) Mancozeb + Cupravit C) Streptomycin sulphate.

3.12. Seed Sowing

Prior to sowing cotton seeds in field, a series of operations were undertaken. These operations included tillage to loose the soil, application of different fertilizers to retain fertility of land. Considering all the apropos soil and climatic factors for cotton the treated and non treated experimental cotton seeds were sown in the selected experimental field at dawn according to its assigned plots those were randomly selected before sowing. Five cotton seeds were sown in one pit where there were nine pits per plot.

3.13. Intercultural Operations

Adequate amount of irrigation was given to non germinated seeds just after sowing in the field and after germination. Proper amount of fertilizers and pesticides was applied in

the field considering the fertility of the land and abundance of different insect pests. To ensure a commodious milieu in the experimental plots three major operations thinning, gap filling and weeding were done in due time, usually seven days interval in order to maintain proper growth of cotton seedlings.

3.14. Isolation of Causal Organism(s) of Cotton Boll Rot

3.14.1. Collection of Diseased Bolls

Infected cotton bolls those showed ostensible identical symptoms depicted by the previous onerous researches were collected from experimental field. The visible suspicious symptoms of the disease were recorded and disease was identified based on the symptoms (Hillocks, 1992; Watkinson, 1981). To prevent from being dried, collected bolls were kept in polythene bag immediately after collection. Then these samples were taken to the Plant Pathology Laboratory, Sher-e- Bangla Agricultural University. Collected bolls were wrapped with two layers of brown paper and kept in refrigerator at 4°C until isolation of the fungi was done.

3.14.2. Isolation of Causal Organisms

The pathogens associated with boll rot were isolated by following tissue planting method (Tuite, 1969).

3.15.1(a). Tissue Planting Method

The parts of bolls associated with disease were cut in to small pieces and surface sterilized with 0.1% Clorox (NaOCI) for 3 minutes and washed for three times in distilled and sterilized water. Then it was placed on moist filter papers (Whatman no.1). Two pieces of filter papers were dipped in sterile water to keep it moist. The covered petridishes containing the specimens were brought in the Seed Pathology Laboratory and kept under incubation for three days. After incubation those plates were observed

under stereomicroscope for the primary identification of the organisms (fungi). Then the fungi were transferred to PDA plate for proper sporulation and purification.

3.15.2. Preservation of Culture Collection

Different fungal stock cultures were prepared in PDA slant and preserved in refrigerator at 4° c for further use.

3.16. Treatments

Ten treatments were selected for this experiment.

T_1 : Seed treatment with Cupravit 50 WP @ 0.4%

T_2 : Seed treatment with Cupravit 50 WP @ 0.4% + Mancozeb 80 WP @ 0.4%

T_3 : Seed treatment with Streptomycin sulphet @ 0.1%

T_4 : Seed treatment with Cupravit 50 WP @ 0.4 % + Foliar spray with Cupravit 50 WP @ 0.4 %

T_5: Seed treatment with Cupravit 50 WP and Mancozeb 80 WP both @ 0.4 % + Foliar spray with Cupravit 50 WP and Mancozeb 80 WP both @ 0.4 %

T_6: Seed treatment with Streptomycin sulphate @ 0.1 @ + Foliar spray with Streptomycin sulphate @ 0.1 %

T_7: Foliar spray with Cupravit 50 WP @ 0.4 %

T_8: Foliar spray with Cupravit 50 WP @ 0.4 + foliar spray with Mancozeb 80 WP @ 0.4 %

T_9: Foliar spray with Streptomycin sulphate @ 0.1 %

T_{10}: Control

3.17. Foliar Application

After formation of bolls in the cotton plants, treatments were randomly assigned to different plots were applied to them for total four times with a certain interval. These

treatments were applied, both seed and foliar spray or only foliar spray. The treatments associated with seed treatment were done prior to the sowing of cotton seeds in the field.

% Boll/Leaf area diseased	Grade
0 %	0
0.1 %	1
5.1-12 %	2

3.18. Data Recording

Data was recorded on leaf spot incidence, severity of leaf spot in PDI, boll rot incidence, and different yield contributing characters as following methods

i) Leaf spot incidence: In field experiment, to determine the leaf spot incidence, four plants were randomly selected. Incidence was measured by following formula

$$\text{Leaf spot incidence} = \frac{\text{No. of infected leaves}}{\text{No. of total leaves}} \times 100$$

ii) Severity of leaf spot in PDI: Previously randomly selected plants those were used to measure the leaf spot incidence were taken and disease severity in % PDI was measured by following formula

$$\text{PDI of leaves} = \frac{\text{Sum of disease ratting}}{\text{Total no. of observations} \times \text{Highest grade in scale}} \times 100$$

Table 2. Disease ratting scale of Harsfall and Berette, (1945):

12.1-25 %	3
25.1-50 %	4
>50 %	5

iii) Incidence of boll rot:

Four plants were selected from each plot randomly as the previous step and disease incidence in boll rot was measured by following formula

$$\text{Boll rot incidence} = \frac{\text{No. of infected bolls}}{\text{No. of total bolls}} \times 100$$

iv) Number of branches/ plant: Number of branches per plant was counted from the same selected plants those were used to determine boll rot incidence.

v) Number of leaves per plant: Number of leaves per plant was counted from the same plants.

vi) Number of bolls per plant: Total number of bolls in the selected plants were counted and recorded.

vii) Plant height: The height of the selected plants were measured and recorded.

viii) Weight of bolls: Weight of bolls from different selected plants was measured and recorded.

ix) Yield: Total yield of different plots under different treatments were measured and recorded.

3.19. Seed Health Study (Harvested Seeds)

Seed health study of harvested seeds was done following the same procedure described in point 3.3 following the rule of ISTA (1996).

3.20. Statistical Analysis

In this experiment ten treatments with three replications were used following Randomized Block Design (RCBD). Data were analyzed for ANOVA using MSTAT-C program (Steel and Torie, 1980). Duncan`s Multiple Range Test (DMRT) and Least significant difference (LSD) were performed to determine the level of significant differences and to separate the means within the parameters.

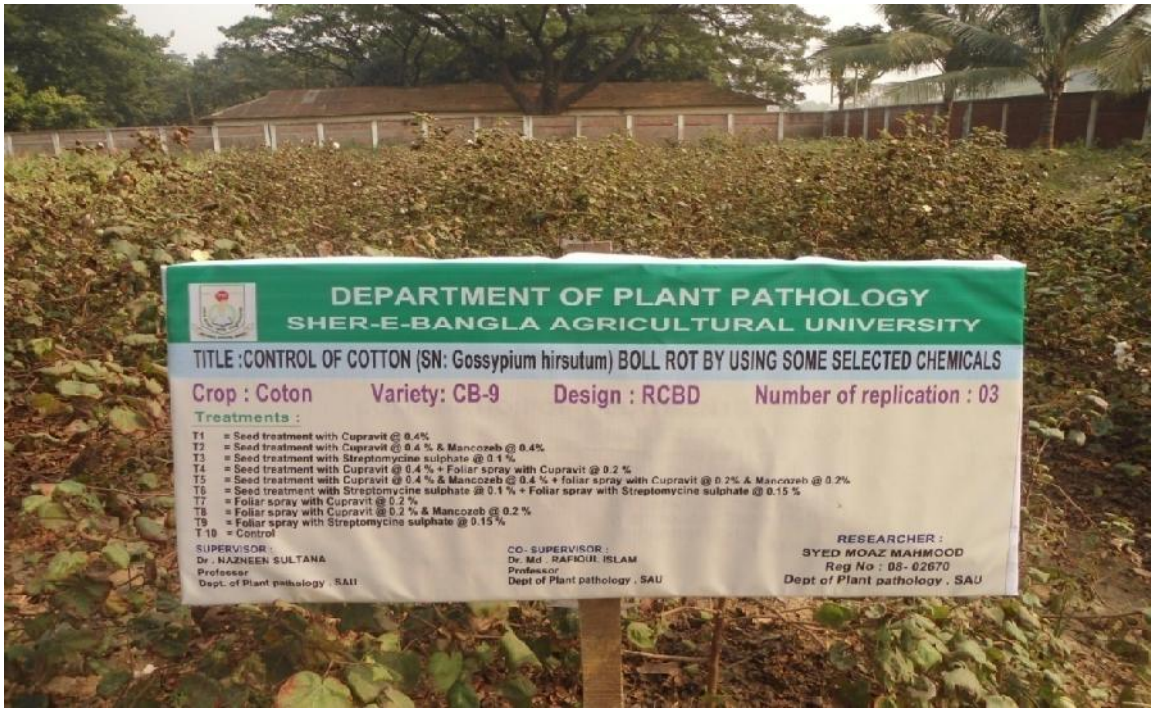

Figure 2: View of the experimental cotton field.

CHAPTER 4
RESULTS

4.1. Seed Health Study of Collected Cotton Seeds

In Blotter method, four species of fungi under three genus were observed after seven days of incubation. The observed fungi were *Fusarium* sp., *Alternaria* sp., *Aspergillus flavus* and *Aspergillus niger*.

Table 03: Incidence of different fungi in collected cotton seeds

Fungi	% present in cotton seeds
Fusarium sp.	4
Alternaria sp.	2
Aspergillus flavus	3
Aspergillus niger	2

4.2. Isolation and Identification of the Seed Borne Fungi of Cotton

In this experiment, four species of three fungal genera were isolated from seeds of cotton. The fungi were *Alternaria* sp., *Fusarium* sp., *Aspergillus flavus* and *A. niger.*

In case of *Alternaria* spp., conidiophores were dark, septate, determinate and conidia were dark, muriform (longitudinal and transverse septum present), beaked, obclavet and frequently borne acropetally in simple or branched conidiophores.

In case of *Fusarium* spp. conidiophores were slender, short, conidia were found two types, macroconidia those had 3-5 septations, slightly curved and microconidia those were one celled and oval shaped.

In case of *Aspergillus* spp. two different species were found where, *A. flavus* produced greenish colored colony and *A. niger* produced blackish colored colony. In both species, they had long, erect conidiophore standing on a thick walled foot cell and vesicle that had globose head like structure that was formed on the conidiophore.

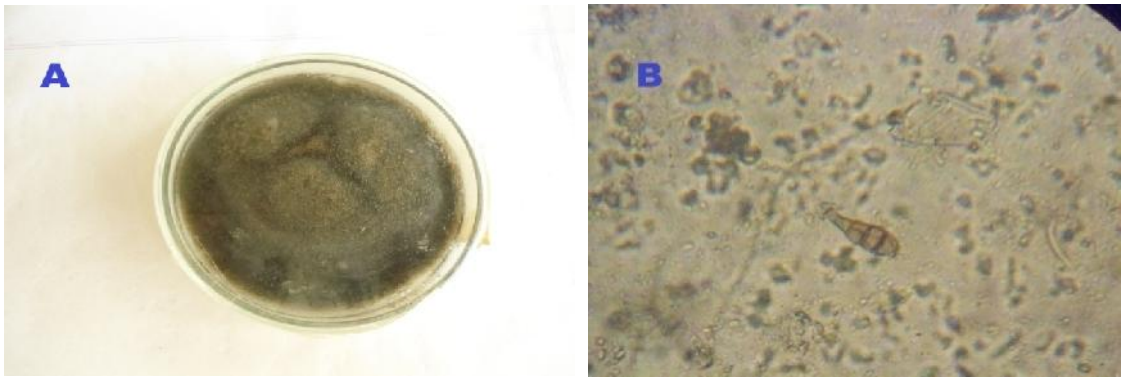

Figure 3: A) Pure culture of *Alternaria* sp. B) Conidium of *Alternaria* sp.

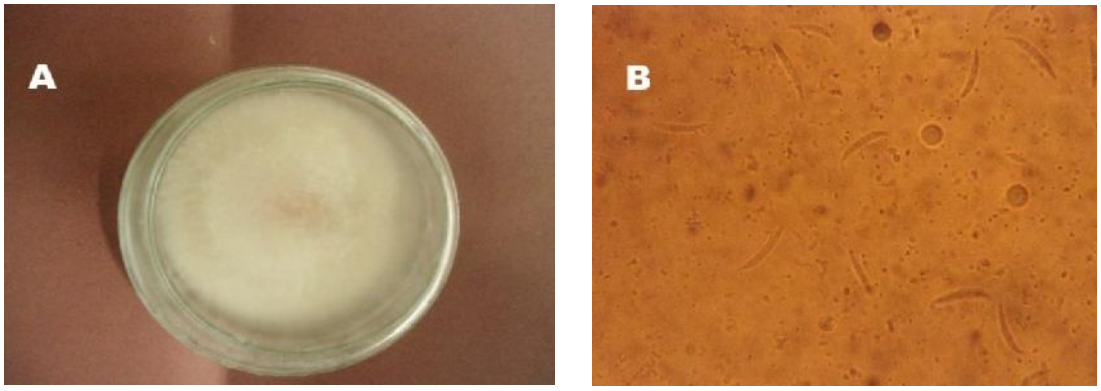

Figure 4: A) Pure culture of *Fusarium* sp. B) Conidia of *Fusarium* sp.

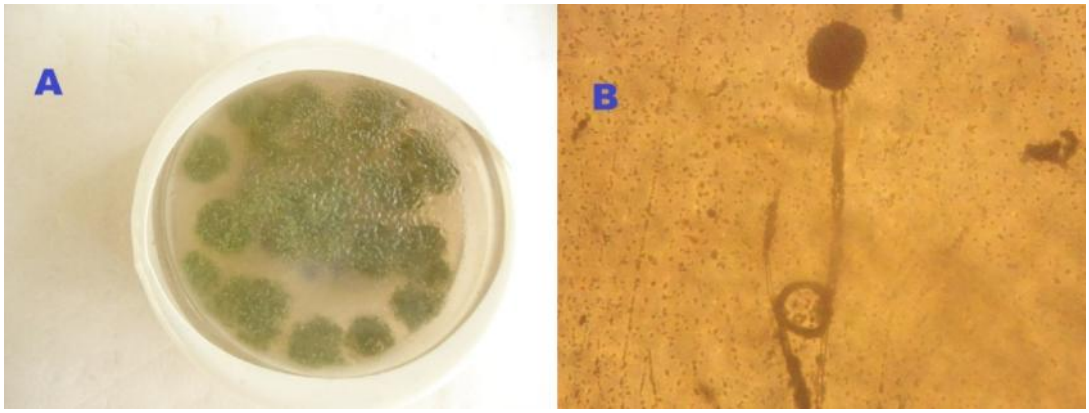

Figure 5: A) Pure culture of *A. flavus* B) Conidia and conidiophores of *A. flavus*

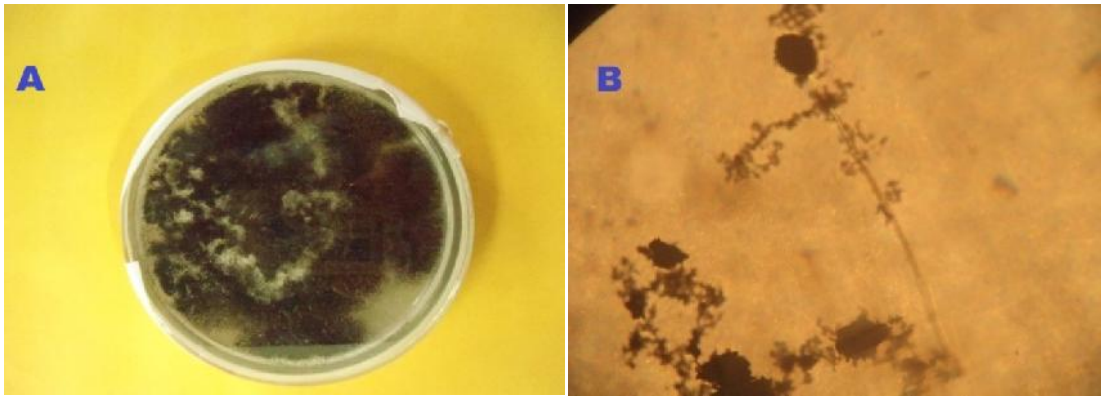

Figure 6: A) Pure culture of *A.* niger. B) Conidia and conidiophores of *A. niger.*

4.3 Efficacy of Selected Chemicals on Radial Mycelial Growth of Cotton Seed Borne Fungi

The effect of chemicals on the radial mycelial growth of *Fusarium* spp. is shown in Figure 1. In case of the *Fusarium* spp, the lowest mycelial growth (3.43 cm) was found in treatment 1 (T_1) (Mancozeb + Cupravit @ 0.4%), preceded by treatment 2 (T_2) (Cupravit @ 0.4%). The highest radial mycelial growth (9.0 cm) of *Fusarium* spp. was recorded in untreated control (T_4) followed by treatment 3 (T_3) (Streptomycin sulphate @ 0.1%).

The efficacy of chemicals on the radial mycelial growth of *Alternaria* spp is shown in Figure 1. In case of *Alternaria* spp. the lowest mycelial growth (4.5 cm) was recorded in treatment 1 (T_1) (Mancozeb + Cupravit @ 0.4%), preceded by treatment 2 (T_2) (Cupravit @ 0.4%). The highest mycelial growth (9.0 cm) of *Alternaria* spp was recorded in untreated control (T_4) followed by treatment 3 (T_3).

The efficacy of chemicals on reducing the mycelial growth of *Sclerotium* spp. is shown in Figure 1. In case of *Sclerotium* spp. lowest mycelial growth (5.03 cm) was recorded in treatment 1 preceded by treatment 2 (T_2). The highest mycelial growth (9.0 cm) of *Sclerotium* spp. was recorded in untreated control (T_4), followed by treatment 3 (T_3).

The efficacy of chemicals on radial mycelial growth of *Aspergillus flavus* is shown in Figure 1. Here, the lowest mycelial growth (5.93 cm) was observed in treatment 1 (T_1), preceded by treatment 2 (T_2). The highest mycelail growth (9.0 cm) was recorded in the untreated control (T_4), followed by treatment 3 (T_3).

The effect of chemicals on mycelial growth of *Aspergillus niger* is shown in Figure 1. Here, the lowest mycelial growth (6.01 cm) was observed in T_1 (Mancozeb + Cupravit

@ 0.4%), preceded by T_2 (Cupravit @ 0.4%). The highest mycelial growth (9.0 cm) was found in untreated control (T_4), followed by treatment T_3 (Streptomycin sulphate 0.1%).

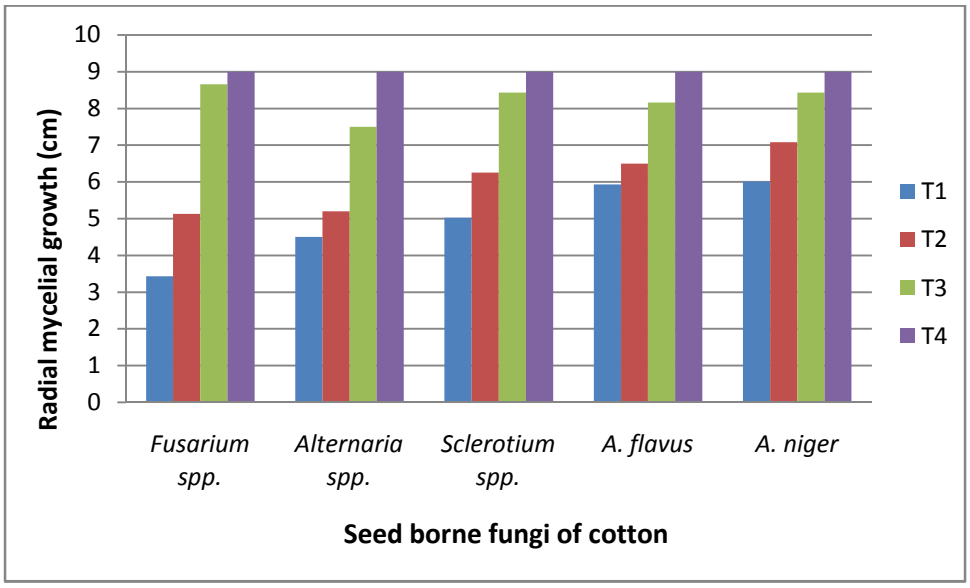

Figure 7: Effect of the selected chemicals on Mycelial growth of fungi in *in - vitro* condition

T_1: Mancozeb + Cupravit (Both @ 0.4%)

T_2: Cupravit 50 WP (@ 0.4%)

T_3: Streptomycin sulphate (@ 0.1 %)

T_4: Distilled water

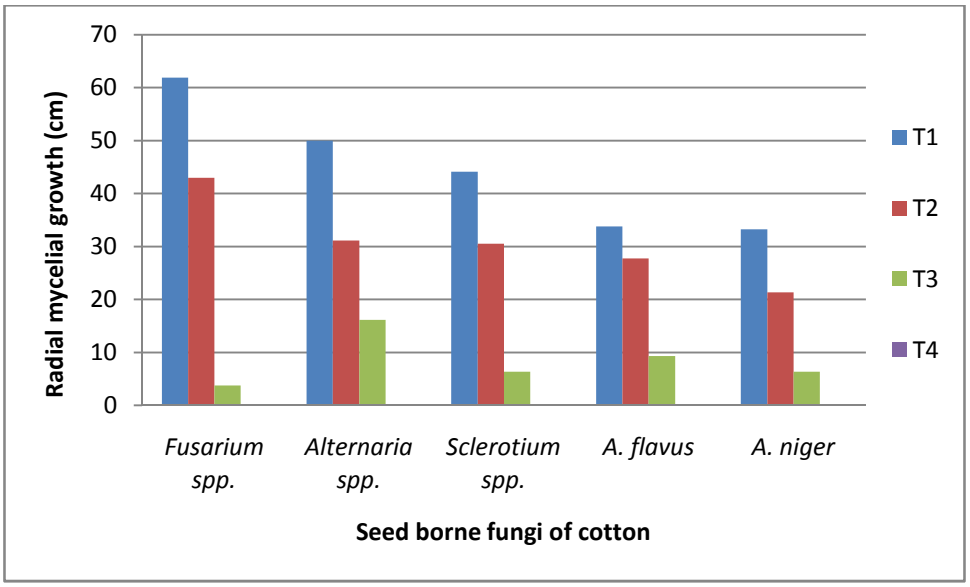

Figure 8: Percent inhibition of the fungi caused by the selected chemicals in *in - vitro* condition

T_1: Mancozeb + Cupravit (Both @ 0.4%)

T_2: Cupravit (@ 0.4%)

T_3: Streptomycin sulphate (@ 0.1%)

T_4: Distilled water

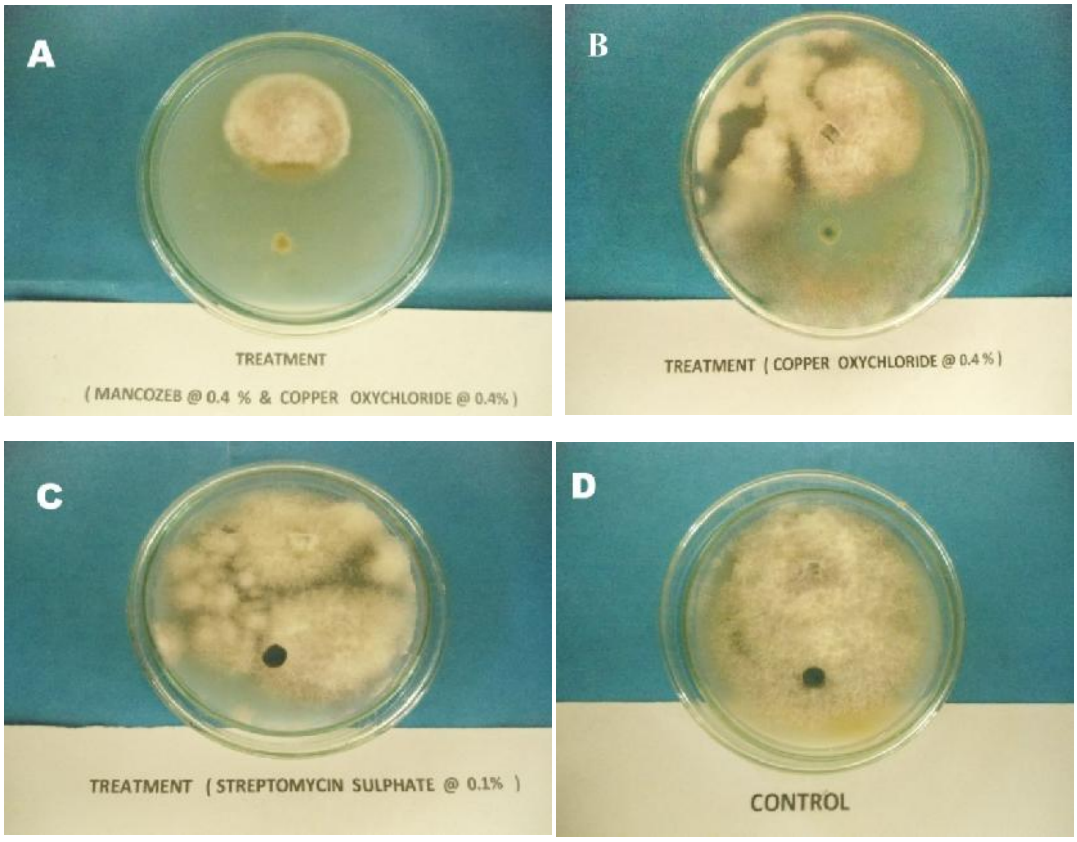

Figure 9: Effect of selected chemicals on radial mycelial growth of *Fusarium* sp. (Well method)

A) Mancozeb +Cupravit against *Fusarium* sp.

B) Cupravit against *Fusarium* sp.

C) Streptomycin sulphate against *Fusarium* sp.

D) Control

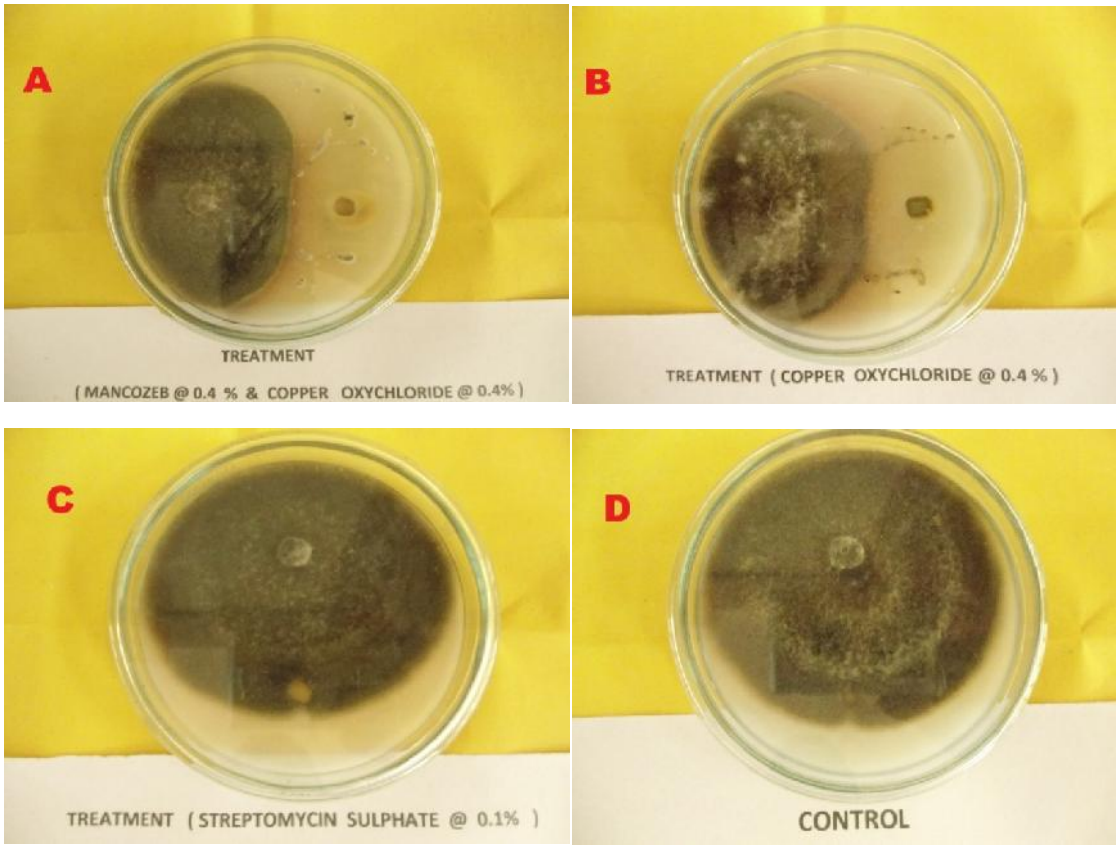

Figure 10: Effect of selected chemicals on radial mycelial growth of *Alternaria* sp. (Well method)

A) Mancozeb +Cupravit against *Alternaria* sp.

B) Cupravit against *Alternaria* sp.

C) Streptomycin sulphate against *Alternaria* sp.

D) Control

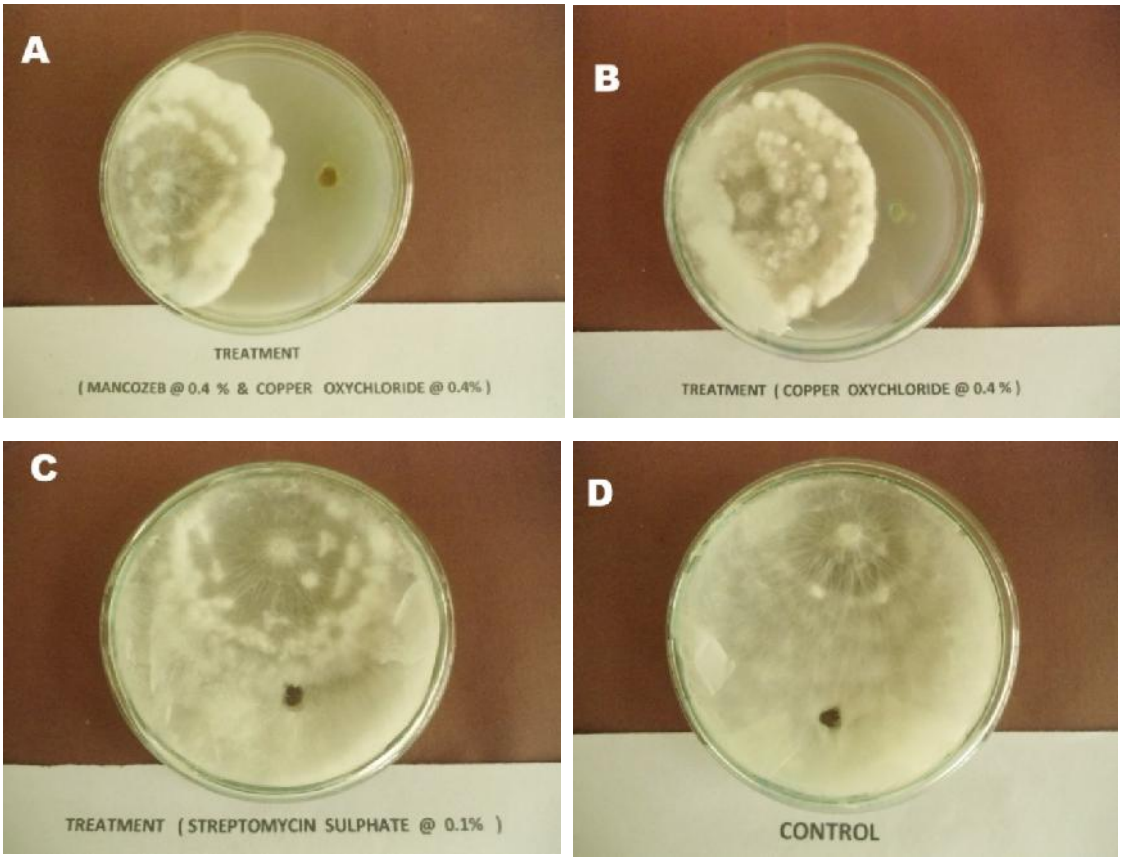

Figure 11: Effect of selected chemicals on radial mycelial growth of *Sclerotium* sp. (Well method)

A) Mancozeb +Cupravit against *Sclerotium* sp.

B) Cupravit against *Scleroium* sp.

C) Streptomycin sulphate against *Sclerotium* sp.

D) Control

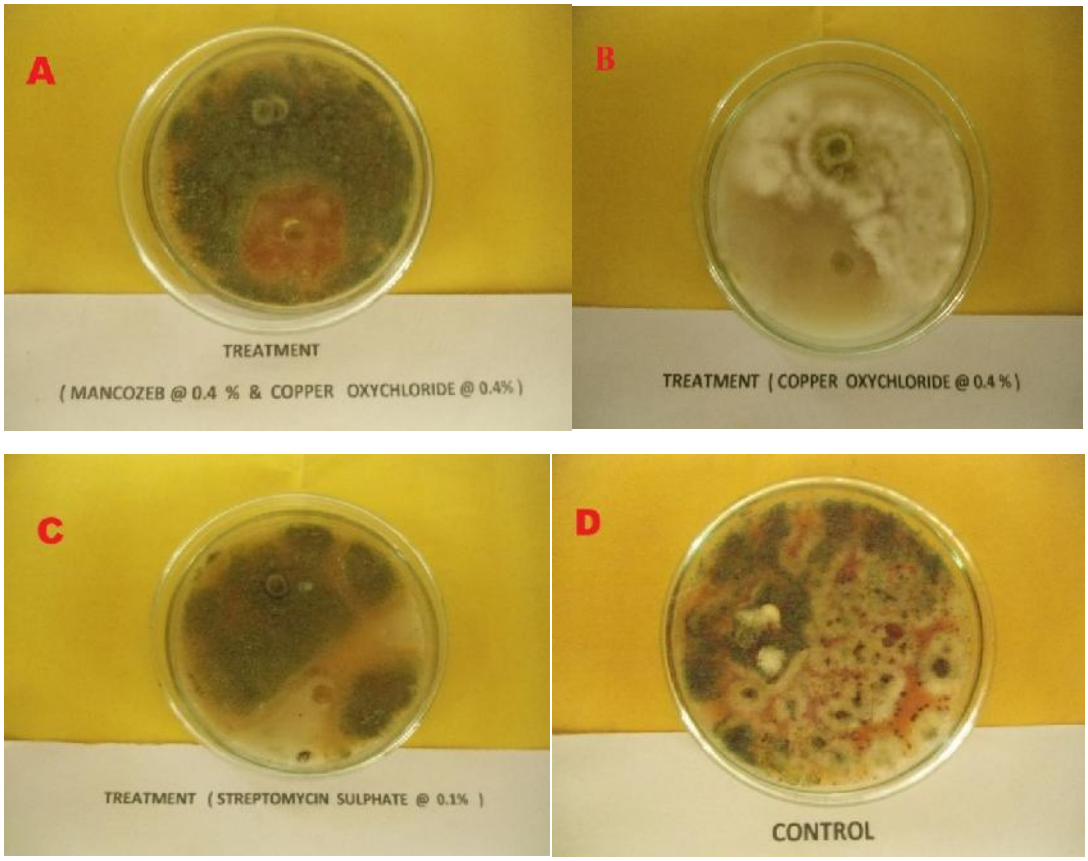

Figure 12: Effect of selected chemicals on radial mycelial growth of *Aspergillus flavus*. (Well method)

A) Mancozeb +Cupravit against *A. flavus*.

B) Cupravit against *A. flavus.*

C) Streptomycin sulphate against *A. flavus.*

D) Control

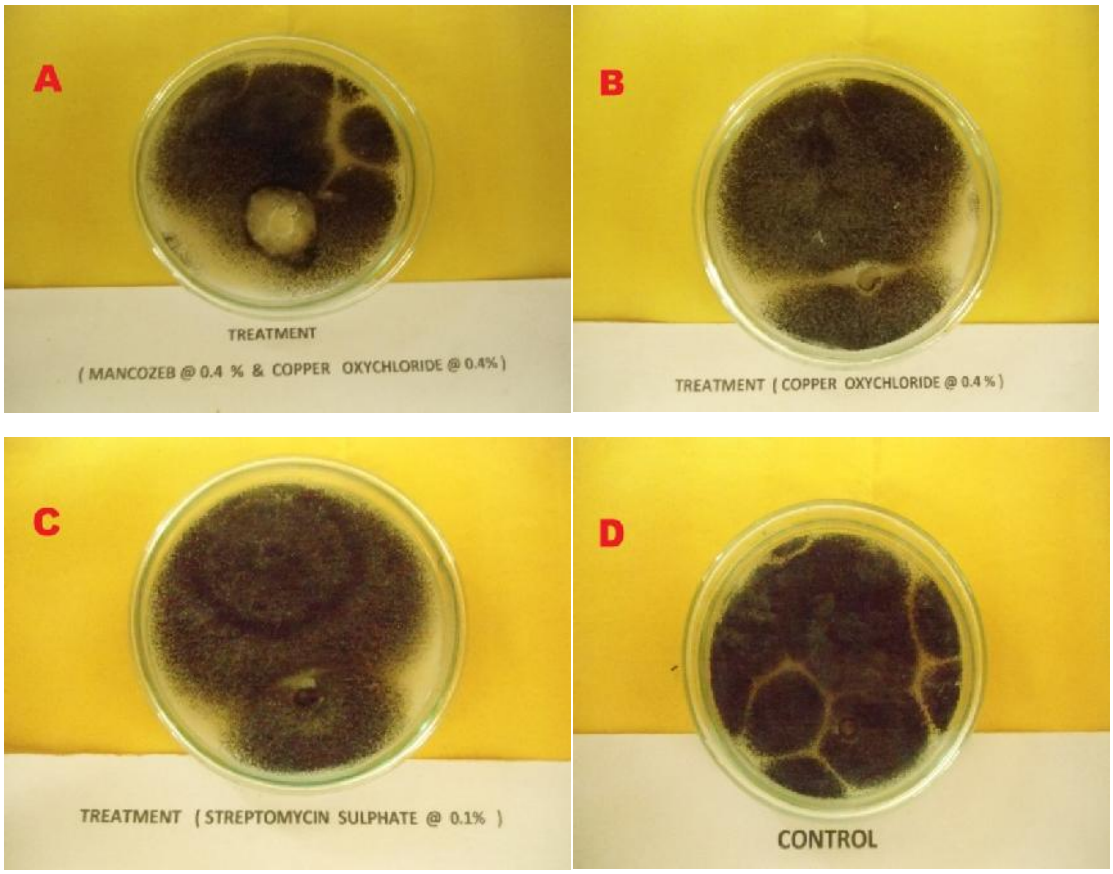

Figure 13: Effect of selected chemicals on radial mycelial growth of *Aspergillus niger*. (Well method)

A) Mancozeb +Cupravit against *A. niger*

B) Cupravit against *A. niger.*

C) Streptomycin sulphate against *A.niger*.

D) Control

4.4. Symptoms of Cotton Boll Rot

The initial stage symptoms appeared on bolls as small brown or dark brown to black spots with depressed centre. Then the superficial growth of fungal mycelia appeared on bolls. Later spots turn in black and the bolls became dried up. Some infected bolls showed hard lock symptoms where bolls remained closed and seed coat turned into a very flinty covering. At the end of disease progression, secondary infection of saprophytic fungi was also observed.

Figure 14: Visual symptoms of cotton boll rot A) Initial stage B) Next stage C) Fungal mycelia on boll D) Crack in boll E) Hard lock symptom F) Saprophytic growth on boll.

4.5. Isolation of Causal Fungi of Cotton Boll Rot from Infected Bolls

Two genera of fungi namely *Alternaria* sp and *Sclerotium rolfsii* were isolated from diseased cotton bolls. The fungi were identified by observing their colony morphology and characteristics under the compound microscope.

Figure 15: Pure culture of *Sclerotium rolfsii*

Figure 16: Effect of selected chemicals on cotton plant A) Treated plant

B) Untreated plant.

4.6. Effect of Selected Chemicals on Leaf Spot Incidence of Cotton

At 120 days after sowing the highest leaf spot incidence (49.83) was observed in control treatment which was statistically insignificant with T_9 treatment ie; foliar spray with Streptomycin sulphate 0.1%. The lowest leaf spot incidence (10.03%) was recorded in T_5 treatment (seed treatment with foliar spray with Cupravit + Mancozeb @ 0.4%) and this was statistically similar to T_4 (seed treatment with foliar spray with Cupravit @ 0.4%) and T_2 (Seed treatment with Mancozeb + Cupravit @ 0.4%).

At 150 DAS leaf spot incidence was recorded maximum in control (67.23%) and minimum (11.40%) in T_5 when seed treatment and foliar spray with Mancozeb +Cupravit @ 0.4% were used. This was statistically insignificant with T_4 where only Cupravit (0.4%) was used as seed treatment agent and foliar spray.

At 180 DAS leaf spot incidence varied from 17.36% to 87.36% where the highest value was found in control treatment and lowest value was recorded from T_4 (18.09%) treatment which was statistically similar with T_1 (Seed treatment with Cupravit @ 0.4%), T_2 (Mancozeb + Cupravit @ 0.4%), T_4 and T_5 (seed + foliar with Mancozeb + Cupravit @ 0.4%).

Table 4: Effect of selected chemicals on leaf spot incidence of cotton

Treatment	Leaf spot incidence in leaves (%)			% reduction over control at 6th month
	Days	Days	Days	
	120	150	180	
T_1	15.75 d	21.43 e	27.93 cde	68.02
T_2	11.17 e	17.57 f	21.27 de	75.62
T_3	26.50 b	32.47 c	17.36 e	80.12
T_4	11.50 e	12.23 g	18.09 e	79.29
T_5	10.03 e	11.40 g	19.08 e	78.15
T_6	49.57 a	60.73 b	75.21 b	13.90
T_7	23.17 bc	25.43 d	32.95 c	62.28
T_8	21.77 c	25.43 d	30.80 cd	64.74
T_9	49.83 a	60.10 b	83.52 ab	4.39
T_{10}	48.23 a	67.23 a	87.36 a	0
lsd $_{(0.05\%)}$	3.89	3.41	10.17	
CV (%)	8.49	5.95	11.34	

Figure in column, having same letter(s), do not differ significantly at 5% level of significance.

Here,

T_1= Seed treatment (Cupravit @ .4%)

T_2= Seed treatment (Mancozeb + Cupravit @ .4 %)

T_3 = Seed treatment (Streptomycin @ 0.1%)

T_4 = Seed+ Foliar (Cupravit @ 0.4 %)

T_5= Seed + Foliar (Mancozeb + Cupravit both @ 0.4 %)

T_6= Seed +Foliar spray (Streptomycin 0.1%)

T_7= Foliar (Cupravit @ 0.4%)

T_8= Foliar (Mancozeb + Cupravit @ 0.4 %)

T_9= Foliar (Streptomycin @ 0.1%)

T_{10}= Control

4.7. Effect of Selected Chemicals on Severity of Leaf Spot (% PDI) in Cotton

At 120 days after sowing the highest leaf spot severity was observed in control treatment which was statistically insignificant with T_9 treatment ie; foliar spray with Streptomycin 0.1%. The lowest leaf spot severity (6.40%) was recorded in T_5 treatment (seed treatment with foliar spray with Cupravit + Mancozeb @ 0.4%).

At 150 DAS leaf spot severity was recorded maximum in control (70.14%) and minimum (13.17%) in T_5 when seed treatment and foliar spray with Mancozeb + Cupravit @ 0.4% were used. This was statistically insignificant with T_8 where only foliar spray with Cupravit (0.4%) was used.

At 180 DAS leaf spot severity varied from 13.17% to 90.15% where the highest value was found in control treatment and lowest value was recorded from T_5 (13.17%) treatment.

Table 5: Effect of selected chemicals on leaf spot severity (% PDI)

Treatment	PDI (%)			% reduction over control at 6th month
	Days	Days	Days	
	120	150	180	
T_1	34.63 e	46.18 e	42.13 f	53.26
T_2	43.03 d	54.09 d	43.50 ef	51.74
T_3	8.80 f	13.17 g	62.13 c	31.08
T_4	41.50 d	51.29 d	54.22 d	39.85
T_5	6.40 g	13.17 g	13.17 g	85.39
T_6	34.63 e	46.18 e	45.13 f	49.93
T_7	43.03 d	54.09 d	43.50 ef	51.74
T_8	8.80 f	13.17 g	62.13 c	31.08
T_9	41.50 d	51.29 d	54.22 d	39.85
T_{10}	60.17 a	70.14 a	90.15 a	0
$lsd_{(0.05\%)}$	3.89	2.91	3.49	
CV (%)	6.64	3.90	3.86	

Figure in column, having same letter (s), do not differ significantly at 5% level of significance.

Here,

T_1= Seed treatment (Cupravit @ 0.4%)

T_2= Seed treatment (Mancozeb + Cupravit @0.4 %)

T_3 = Seed treatment (Streptomycin @ 0.1%)

T_4 = Seed+ Foliar (Cupravit @ 0.4 %)

T_5= Seed + Foliar (Mancozeb + Cupravit

T_6= Seed +Foliar spray (Streptomycin 0.1%)

T_7= Foliar (Cupravit @ 0.4%)

T_8= Foliar (Mancozeb + Cupravit @ 0.4 %)

T_9= Foliar (Streptomycin

both @ 0.4 %)

sulphate @ 0.1%)

T_{10}= Control

4.8. Effect of Selected Chemicals on Incidence of Boll Rot of Cotton

At 120 days after sowing the highest boll rot incidence (50.43) was observed in control treatment. The lowest leaf spot incidence (3.83%) was recorded in T_5 treatment (seed treatment with foliar spray with Cupravit + Mancozeb @ 0.4%) which was statistically similar with T_1 (seed treatment with Cupravit @ 0.2%) and T_2 (Seed treatment with Mancozeb + Cupravit @ 0.2%).

At 150 DAS, boll rot incidence was recorded maximum in control (55.37%) and minimum (4.47%) in T_5 when seed treatment and foliar spray with Mancozeb +Cupravit @ 0.4% were used. This was statistically similar with T_2 where Mancozeb+ Cupravit (0.4%) was used as seed treatment agent.

At 180 DAS boll rot incidence varied from 6.16% to 74.06% where the highest value was found in control treatment and lowest value was recorded from T_5 (6.16%) which was statistically similar to T_1 (Seed treatment with Cupravit @ 0.4%) and T_2 (Seed treatment with Mancozeb + Cupravit @ 0.4%).

Table 6: Effect of selected chemicals on cotton boll rot incidence

Treatment	Incidence of boll rot (%)			% reduction over control at 180 days
	Days	Days	Days	
	120	150	180	
T_1	6.03 fg	11.30 f	20.07 def	72.90
T_2	6.23 fg	7.70 fg	11.69 ef	84.21
T_3	22.43 d	31.50 c	35.57 c	51.97
T_4	8.63 f	11.53 f	20.64 de	72.13
T_5	3.83 g	4.47 g	6.16 f	91.68
T_6	32.74 c	44.43 b	58.45 b	21.07
T_7	20.02 d	25.67 d	51.56 b	30.38
T_8	13.68 e	17.90 e	33.66 cd	54.55
T_9	39.87 b	45.45 b	63.68 ab	14.01
T_{10}	50.43 a	55.37 a	74.06 a	0
lsd $_{(0.05\%)}$	3.96	3.71	13.34	
CV (%)	11.33	8.48	10.71	

Figure in column, having same letter (s), do not differ significantly at 5% level of significance.

Here,

T_1= Seed treatment (Cupravit @ 0.4%) T_6= Seed +Foliar spray

T_2= Seed treatment (Mancozeb + Cupravit @ 0.4 %)

T_3 = Seed treatment (Streptomycin @ 0.1%)

T_4 = Seed+ Foliar (Cupravit @ 0.4 %)

T_5= Seed + Foliar (Mancozeb + Cupravit both @ 0.4 %)

(Streptomycin 0.1%)

T_7= Foliar (Cupravit @ 0.4%)

T_8= Foliar (Mancozeb + Cupravit @ 0.4 %)

T_9= Foliar (Streptomycin sulphate @ 0.1%)

T_{10}= Control

4.9. Number of Branches Per Plant

The effect of selected chemicals on the number of branches per plant is shown in Figure 3. The highest number of branches (35) was found in plot under T_5 (seed + Foliar spray with Mancozeb + Cupravit @ 0.4%) treatment followed by T_4 (seed + foliar with Cupravit @ 0.4%) treatment having 23 branches which was statistically similar with T_1 (seed treatment with Cupravit) and T_2 (seed treatment with Mancozeb + Cupravit @ 0.4%). The lowest number of branches (10.33) was observed in plot under untreated control followed by treatment 8 (15.33 branches) and treatment 7 (15 branches).

4.10. Number of Leaves per Plant

A very profound effect of the selected chemicals on the number of leaves per plant is observed in Figure 3. The highest number of leaves per plant (889) was counted in T_5 (seed treatment + foliar spray with Mancozeb + Cupravit @ 0.4%) treatment followed by the next best T_4 treatment (746 leaves) and T_1 treatment (749 leaves) having no significant statistical difference between them. The lowest number of laves per plant

(339) was counted from untreated control plants followed by T_9 (Streptomycin sulphate @ 0.1%) treatment (509 leaves) and T_3 (527.30 leaves).

4.11. Number of Bolls per Plant

The effects of selected chemicals on number of bolls per plant varied significantly among different treatments (Figure 3). After 180 Days after sowing (DAS), the highest number of bolls (388) was counted in the plot where seed treatment with foliar sprays were applied with Mancozeb + Cupravit @ 0.4% treatment followed by T_4 (Cupravit @ 0.4%) treatment, having 244 bolls per plant, and T_2 treatment having 189 bolls per plant. The lowest number of bolls per plant (78.67) was found in untreated control (T_{10}) proceeded by treatment 9 (T_9) (101 bolls). However, treatment T_3 showed a bit increased bolls than the treatment 9 but there was no statistically significant difference between them.

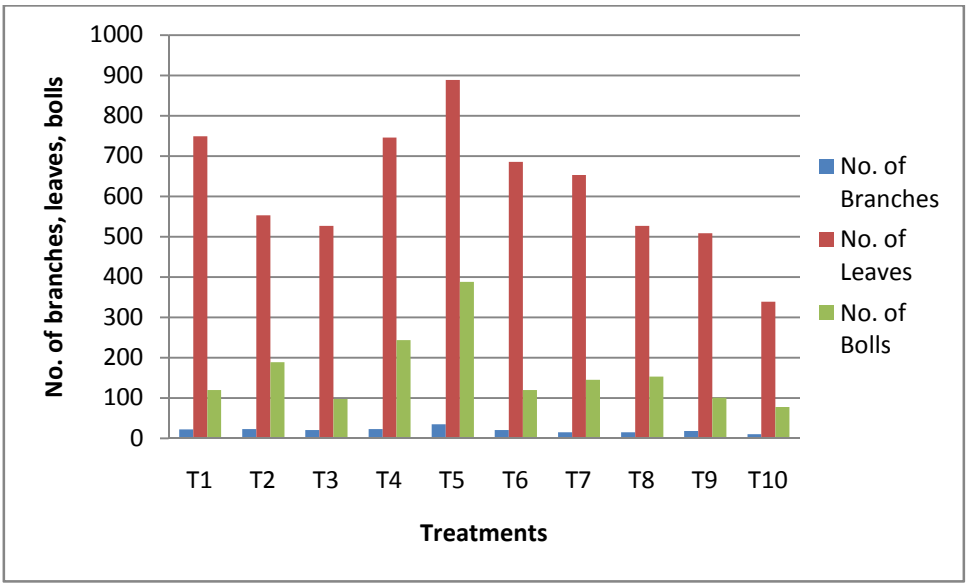

Figure 17: Effect of selected chemicals on number of branches, leaves and bolls of cotton

Here,

T_1= Seed treatment (Cupravit @ 0.4%)

T_2= Seed treatment (Mancozeb + Cupravit

T_6= Seed +Foliar sprqay (Streptomycin 0.1%)

@ 0.4 %)

T_3 = Seed treatment (Streptomycin @ 0.1%)

T_4 = Seed+ Foliar (Cupravit @ 0.4 %)

T_5= Seed + Foliar (Mancozeb + Cupravit both @ 0.4 %)

T_7= Foliar (Cupravit @ 0.4%)

T_8= Foliar (Mancozeb + Cupravit @ 0.4 %)

T_9= Foliar (Streptomycin sulphate @ 0.1%)

T_{10}= Control

The effect of selected chemicals on percent increase of branches, leaves and bolls over control is very vividly observed in figure 4. It is clearly observed that, number of branches per plant was increased up to 240% by using Mancozeb and Copper fungicide together (T_5) as seed treatment agent as well as foliar sprayer over the control plot. Number of leaves and number of bolls per plant, both increased as well up to 152% and 400% respectively in the plots under treatment 5 over the control plot.

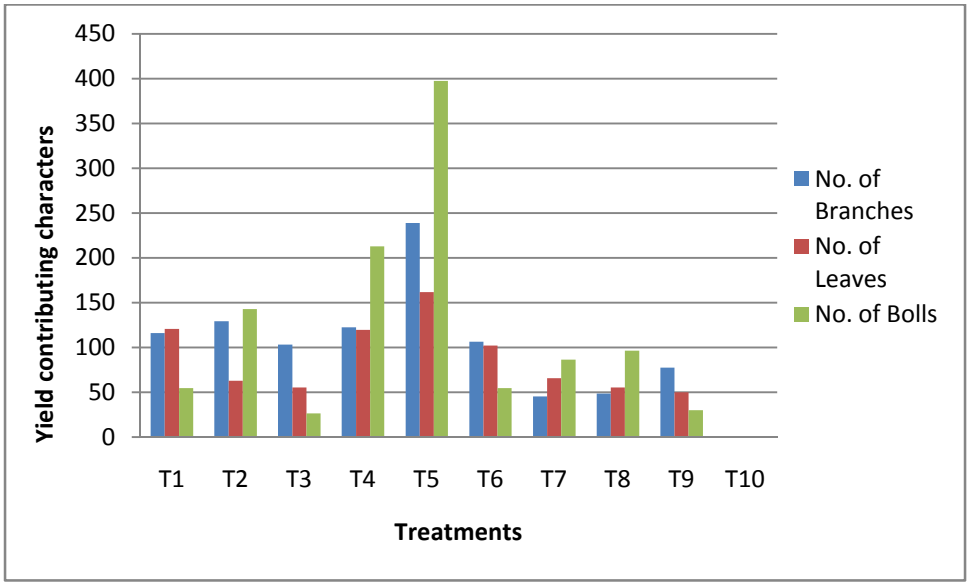

Figure 18: Effect of selected chemicals on percent increase of branches, leaves and bolls over control in cotton

Here,

T_1= Seed treatment (Cupravit @ 0.4%)

T_2= Seed treatment (Mancozeb + Cupravit @ 0.4 %)

T_3 = Seed treatment (Streptomycin @ 0.1%)

T_6 = Seed +Foliar (Streptomycin 0.1%)

T_7= Foliar (Cupravit @ 0.4%)

T_8 = Foliar (Mancozeb

T_4 = Seed+ Foliar (Cupravit @ 0.4 %) + Cupravit @ 0.4 %)

T_5= Seed + Foliar (Mancozeb + Cupravit both @ 0.4 %)

T_9 = Foliar (Streptomycin @ 0.1%)

T_{10} = Control

4.12. Plant Height

The effect of the selected chemicals on height of cotton plants is projected in Figure 5. Here, the highest plant height (167.60 cm) was found in the plot treated with T_5 (Mancozeb + Cupravit @ 0.4%) treatment followed by both T_1 (Seed treatment with Cupravit @ 0.4%) treatment (134.30 cm) and T_2 (Seed treatment with Mancozeb + Cupravit @ 0.4%) treatment (136 cm). The lowest plant height (84.25 cm) was found in plots under untreated T_{10} control treatment, the same result was found in plots treated with T_8 treatment (96 cm).

4.13. Boll Weight

The effect of selected chemicals on the weight of cotton bolls is in Figure 5. Bolls from the plants in the plots under T_5 (Mancozeb + Cupravit @ 0.4%) treatment obtained the highest weight (6.16 g) followed by bolls obtained from plants under T_4 (seed treatment with foliar spray with Cupravit @ 0.4%) treatment (5.30 g). The lowest weight (3.73 g) in bolls was found in the plot under untreated control followed by treatment 9 (3.33 g).

4.14. Cotton Yield

The effect of selected chemicals on yield of cotton is depicted in Figure 5. The highest yield (351 Kg) was obtained from the plot under treatment 5 (Seed treatment + foliar spray with Mancozeb + Cupravit @ 0.4%) followed by treatment 4 (305.40 kg). On the other hand, lowest yield (90.30 kg) was obtained from the plot associated with untreated control followed by T_9 (92.67 kg).

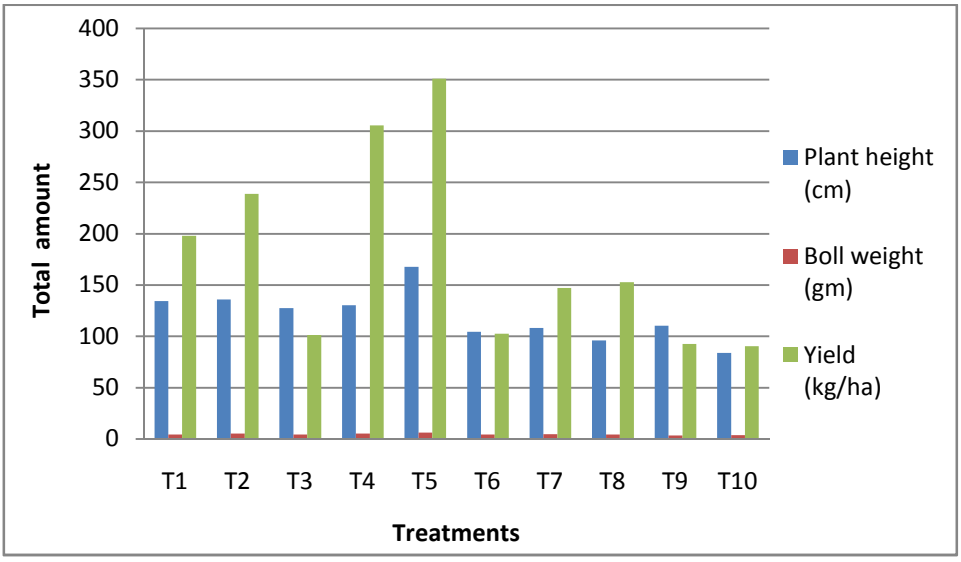

Figure 19: Effect of selected chemicals on plant height, boll weight and yield of cotton

Here,

T_1= Seed treatment (Cupravit @ .4%)

T_2= Seed treatment (Mancozeb + Cupravit @ .4 %)

T_3 = Seed treatment (Streptomycin @ .1%)

T_4 = Seed+ Foliar (Cupravit @ .4 %)

T_6= Seed +Foliar (Streptomycin .1%)

T_7= Foliar (Cupravit @ .4%)

T_8= Foliar (Mancozeb + Cupravit @ .4 %)

T_5= Seed + Foliar (Mancozeb + Cupravit both @ .4 %)

T_9= Foliar (Streptomycin @ 0.1%)

T_{10}= Control

The effect of selected chemicals on percent increase of plant height, yield and boll weight over control is very vividly observed in Figure 6. It is clearly observed that, plant height in the plots under treatment 5 increased up to 52% over the control plot. It is also observed that, cotton yield and boll weight also up to 270% and 53% respectively by applying treatment 5 over the control plot.

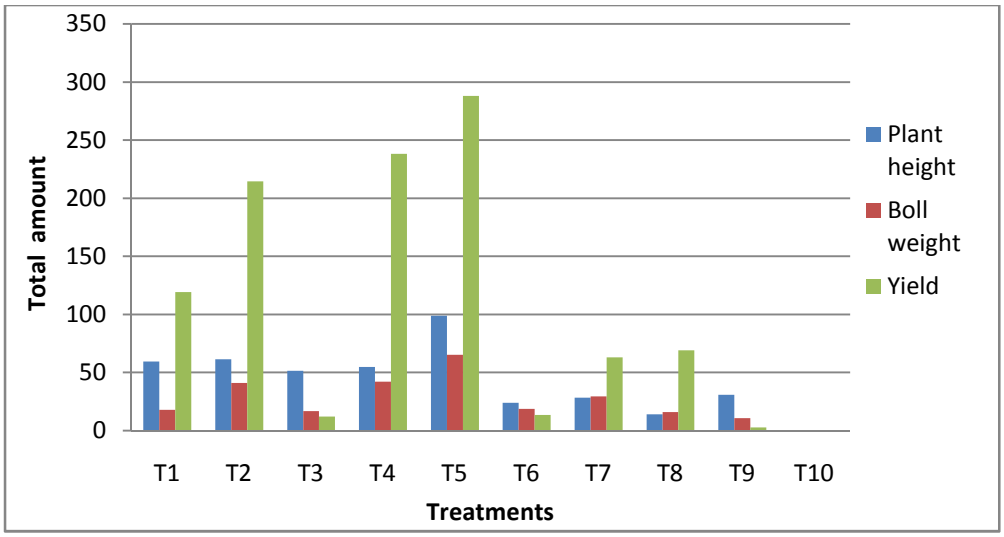

Figure 20: Effect of selected chemicals on percent increase of plant height, yield and boll weight over control in cotton

Here,

T_1= Seed treatment (Cupravit @ .4%)

T_2= Seed treatment (Mancozeb + Cupravit @ .4 %)

T_3 = Seed treatment (Streptomycin @ .1%)

T_4 = Seed+ Foliar (Cupravit @ 0.4 %)

T_5= Seed + Foliar (Mancozeb + Cupravit both @ 0.4 %)

T_6= Seed +Foliar (Streptomycin .1%)

T_7= Foliar (Cupravit @ .4%)

T_8= Foliar (Mancozeb + Cupravit @ 0.4 %)

T_9= Foliar (Streptomycin @ 0.1%)

T_{10}= Control

4.15. Comparison between Treated and Untreated Seeds

The presence of different seed borne fungi in untreated seeds is shown in figure 7. *Fusarium* spp and *A. flavus* recorded from untreated cotton seeds were 4% and 3% respectively and prevalence of *Alternaria* spp and *A. niger* were 2% of each. While in the harvested seeds, *Fusarium* spp and *A. flavus* were recorded 2% for both fungal genera and prevalence of *Alternaria* spp and *A. niger* were found 1% of each.

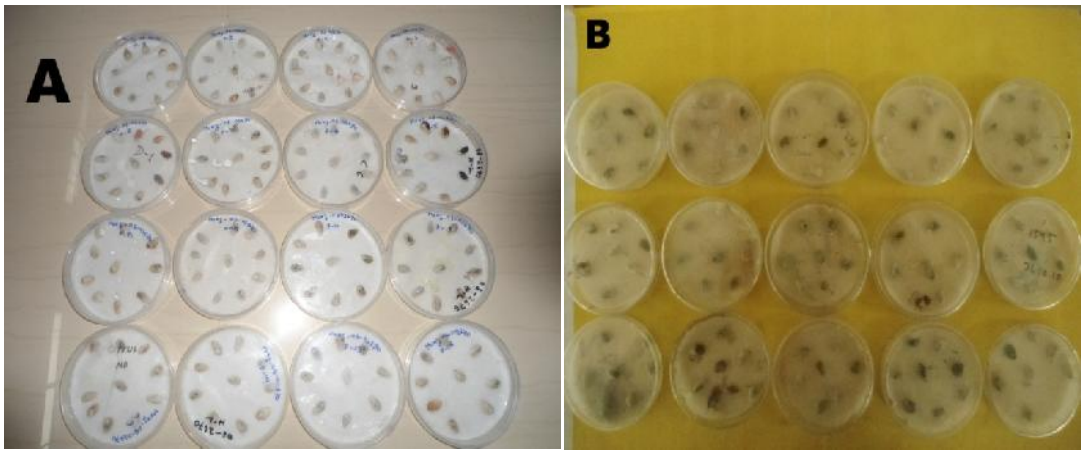

Figure 21: Seed health study A) Seed health study of treated seeds. B) Untreated seeds

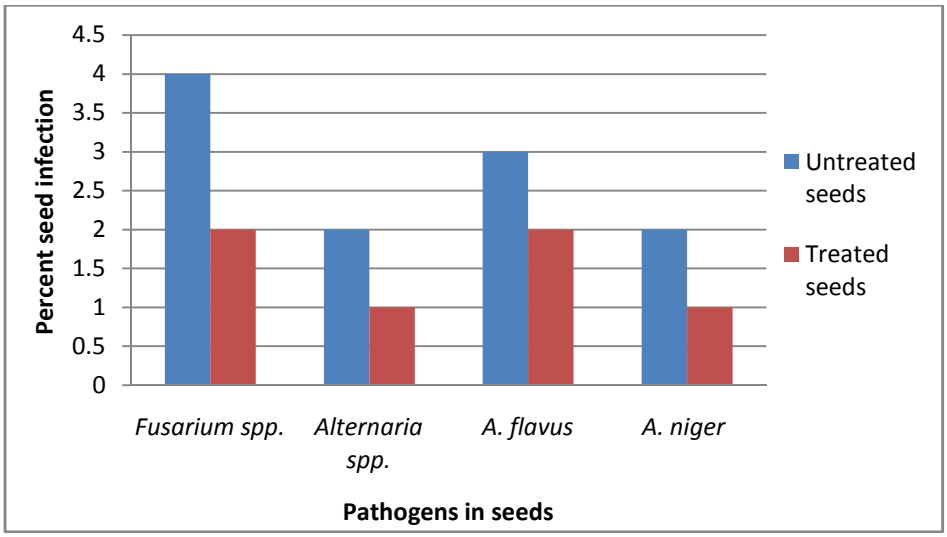

Figure 22: Comparative seed health study of untreated cotton seeds and harvested cotton seeds

CHAPTER 5
DISCUSSIONS

In blotter test, three genera of fungal pathogens appeared after seven days of incubation. The most frequent fungi were *Fusarium* spp*, Alternaria* spp*, Aspergillus flavus, A. niger*. This result is in accordance with the findings of Hillocks, (1992), Kings and Persley, (1942), Woodroof (1927), Coyler (1988). Khan and Kausar (1697), Fulton and Bollenbacher, (1959). They conducted different experiments to find out the pathogens associated with cotton seeds and all of them reported that the most abundant seed borne pathogens of cotton were *Fusarium* spp*, Carvularia* spp*, Alternaria* spp*, Aspergillus* spp*, Mucor* spp. and *Diplodia* spp.

Phillip *et al.* (2003) conducted an experiment on the seed treatment application timing options for *Fusarium* decay in cut seed pieces and reported that combination of Mancozeb and Fludioxonil up to ten days prior to planting can control *Fusarium* decay of seeds. Rathod and Pawar, (2013) conducted an experiment on *in vitro* seed treatment chemicals for soy bean and reported that Copper oxychloride not only increased the germination percentage of seeds but also decreased seed borne micro flora.

The chemicals assayed in the laboratory showed significant effect in reducing radial mycelial growth of five different funguses. It was observed that combination of Mancozeb and Cupravit 50 WP both @ 0.4% significantly reduced the mycelial growth of *Fusarium* spp*, Alternaria* spp*, Sclerotium* spp*, Aspergillus flavus* and *A. niger* after seven days of observation. This result is in accordance with a vast amount of research

findings of many researchers named Muthomi *et al*. (2007), Hussain *et al*. (2001), Nisa *et al*. (2011), Shah *et al*. (2010). Fravel *et al.* (2005) conducted an experiment to find out the efficacy of Mancozeb and Cupravit against the mycelial growth of *Fusarium oxysporum* and observed that Mancozeb and Cupravit both reduced the colony growth of *Fusarium* spp. This finding was supported by Timmer and Zitko, (1997), Alam and Mahal, (1999), Minamor, (2013), Belly *et al.* (2006), Srininivasan and Shanmugam, (2006), Mesta *et al.* (2009), Wani and Nisa, (2011), Mateo *et al.* (2011).

Muthomi *et al*. (2007) reported that Copper oxychloride completely obliterated the growth of *Fusarium graminearum* in *in- vitro* condition where Hossain *et al*. (2001) asseverated this finding in their report. Timmer and Zitko, (1997) evaluated some fungicides to control *Alternaria* brown spot and citrus scab and noted that copper fungicides provided surprisingly good result to thwart the growth of *Alternaria* spp. Copper fungicide was very handy to control *Aspergillus* spp in *in vitro* condition (Belly *et al* 2006). Shah *et al.* (2010) reported that Mancozeb was found most effective against *Fusarium* spp. growth. Wani and Nisa, (2011) reported that Mancozeb was best fungicide to wane the growth of *Alternaria* spp. Paksha, (2003) used different fungicides to control *Sclerotium rolsfii* in *in vitro* experiment named Carbendazim, Tridemormg, Propiconazol, Captan, Thirum, Copper oxychloride and Mancozeb and reported that Mancozeb @ 0.4% showed promising efficacy against growth of *Sclerotium* spp.

In case of disease incidence in leaves, effect of Cupravit showed promising effect in reducing disease incidence in leaves of cotton where it showed 79.29% disease reduction over control and combined effect of Mancozeb and Cupravit was on next showing 78.15% reduction over control. Antibiotic Streptomycin hardly left any negative impact on incidence of disease in leaves.

In this experiment it was revealed that, Combination of Mancozeb and Cupravit controlled the disease severity in leaves most successfully showing 85.39% reduction over control but no other treatments were proved to be very effective against disease

severity in leaves. Streptomycin was proved to be the most innocuous treatment against the fungal pathogens.

In case of disease incidence in bolls it was found that combined effect of Mancozeb and Cupravit both as seed treating agent and foliar application reduced disease incidence in bolls up to 91.68% over control at 180 DAS. Cupravit 50 WP was also found to be effective in reducing disease next to Combination of Mancozeb and Cuprvait and it showed 72.13% disease reduction over control. Antibiotic Streptomycin was proved ineffective to the incidence of disease.

The present result on effect of different fungicides on disease incidence and severity of cotton bolls and leaves is asseverated by previous researchers (Hussain *et al*. 2001; Alam *et al*. 2003; Mamza *et al*., 2012; Nisa *et al*., 2011; Neeraj and Shilpi, 2010; Alam and Mahal, 1999; Minamor, 2013; Srininivasa and Shanmugam, 2006; Gondal *et al.* 2012; Kumar *et al*. 2013; Ansari *et al*. 1990; Choular *et al.,* 1989; Sing *et al.*, 2001; Babu *et al*. 2001; Prasad and Naik, 2003; Neeraj and Shilpi, 2010; Taiwary *et al.*, 2004; Katiyar *et al.* 2001; Narain *et al*. 2006).

Narain *et al.* (2006) reported that Indofil M 45 (Mancozeb @ 0.2%) effectively countermanded leaf blight caused by *Alternaria* spp. Syed *et al.* (2011) and Prakash *et al.* (2007) reported the same result du;ring their experiments.

Madhavi and Bhattiprolu, (2011) used different fungicides including Hexaconazole, Propiconazole, Tebuconazole, Difanoconazole, Copper oxy chloride, Mancozeb and Carbendazim and reported that Mancozeb showed the best effect among the fungicides to reduce mycelial growth of *Sclerotium* spp. This finding was in accordance with findings of Manu *et al*. (2012), Patil *et al.* (1986). All of these depicted results accord with the findings of this experiment.

In case of yield and yield contributing characters, Mancozeb with Cupravit gave the best performance. Cupravit alone also showed good result in case of parameters recorded in this experiment. Seed health study also revealed that seed treatment with Mancozeb and

Cupravit along with foliar spray with these two chemicals reduced the incidence of seed borne fungi of cotton.

Therefore Mancozeb + Cupravit (0.4%) could be used as seed treating agent as well as foliar spray to control boll rot disease of cotton effectively.

CHAPTER 6
SUMMERY AND CONCLUSION

An experiment was conducted at the research farm of Sher- e- Bangla Agricultural University, Dhaka, during 30th May to November, 2013 to study the control of Cotton boll rot caused by different fungi species. The experiment was done in both *In vitro* and in field condition and the field experiment was laid out in a Randomized Complete Block Design (RCBD) with ten treatments viz: T_1 (Seed treatment with Cupravit @ 0.4%), T_2 (Seed treatment with Mancozeb + Cupravit @ 0.4%), T_3 (Streptomycin sulphate @ 0.1%), T_4 (Seed treatment +foliar spray with Cupravit @ 0.4%), T_5 (Seed treatment + Foliar spray with Mancozeb + Cupravit @ 0.4%), T_6 (Seed treatment + foliar spray with Streptomycin sulphate @ 0.1%), T_7 (Folair spray with Cupravit @ 0.4%), T_8 (Foliar spray with Mancozeb+ Cupravit @ 0.4%), T_9 (Foliar spray with Streptomycin sulphate 0.1%), T_{10} (Control).

Seed health study of blotter method revealed that seeds of cotton variety CB 9 yielded *Fusarium* spp. (4%), *Alternaria* spp. (2%), *Aspergillus flavus* (3%) and *A. niger* (2%). Efficacy of some selected chemicals against seed borne fungi of cotton variety CB 9 was studied and observed that Mancozeb + Cupravit @ 0.4% showed best result against all fungi. Cupravit also gave better result in reducing radial mycelial growth of seed borne fungi of cotton. Among the chemicals tested, all have significant effect except Streptomycin sulphate.

In field experiment, seed treatment + foliar spray with Mancozeb + Cupravit (@ 0.4%) exerted best performance in reducing percent leaf infection, Percent disease index in leaves, Percent boll rot incidence. This treatment increased number of branches, number of leaves, number of bolls, plant height, boll weight and yield up to 238, 161, 397, 98, 65 and 288% over control, respectively.

Seed treatment + spraying with Mancozeb + Cupravit (@ 0.4%) reduced seed borne fungal infection. Health study of harvested seeds revealed that the lowest abundance of seed borne fungi was found where seeds were treated with Mancozeb + Cupravit @ 0.4% along with foliar spray with the same chemicals.

The findings of the present study clearly pointed out that, among the chemicals used Mancozeb + Cupravit @ 0.4% appeared to be the best for its performance in controlling seed borne fungi of cotton as well as in decreasing boll rot incidence and increasing yield of cotton. So, cotton growers can use Cupravit alone or Mancozeb + Cupravit as seed treating and foliar spray.

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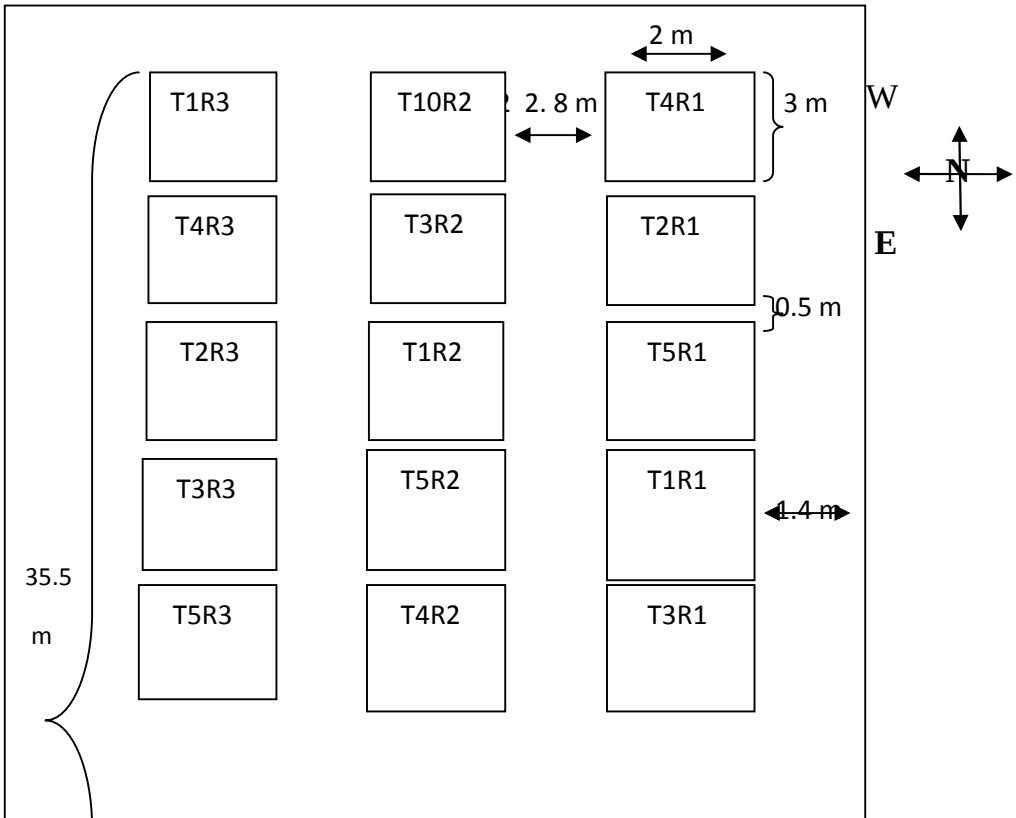
APPENDIX

Appendix 1: Layout of the experimental field (RCBD)



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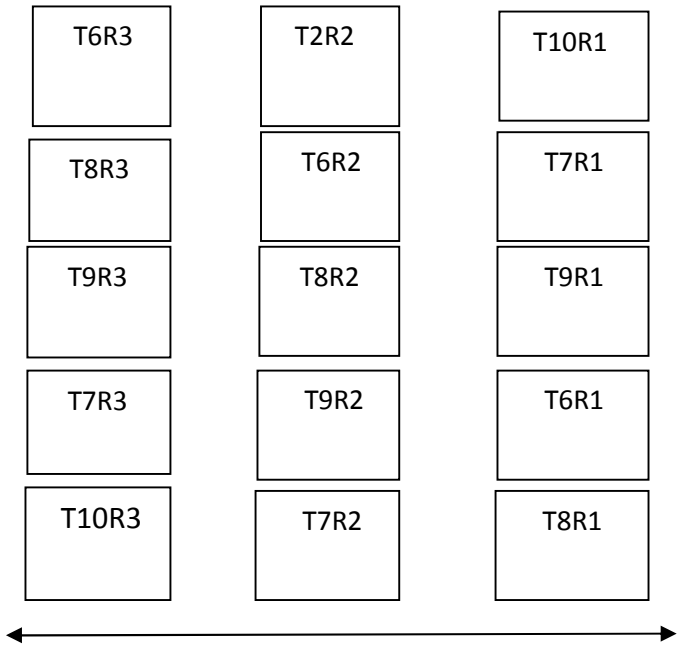

ABBREVIATIONS USED

@ = At the rate of

Anon. = Anonymous

cm = Centimeter

DAS = Days after sowing

DMRT = Duncan's Multiple Range Test

e. g. = Example

g = Gram

FAO = Food and Agriculture Organization

ha = Hectare

Kg = Kilogram

LSD = Least significant difference

PDA = Potato Dextrose Agar

RCBD = Randomized Complete Block Design

T = Treatment

t/ ha = Ton per hectare

°C = Degree centigrade

% = Percent