Published with Open Access at Journal BiNET
Vol. 05, Issue 02: 37-49

Journal of Bioscience and Agriculture Research

Home page: www.journalbinet.com/jbar-journal.html

Control of cotton boll rot through selected chemicals

Syed Moaz Mahmood[a], Nazneen Sultana[a], Md. Mahfuzar Rahman[b], Md. Jannatul Adan[a] and Md. Shah Newaz Chowdhury[c]

[a]Dept. of Plant pathology, Sher-e-Bangla Agricultural University, Dhaka
[b]Dept. of Agronomy, Sher-e-Bangla Agricultural University, Dhaka
[c]Dept. of Agricultural Botany, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh

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 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.

Key words: *Cotton boll rot, seed treatment, foliar spray and chemicals*

Please cite this article as: Mahmood, S. M., Sultana, N., Rahman, M. M., Adan, M. J. & Chowdhury, M. S. N. (2015). Control of cotton boll rot through selected chemicals. *Journal of Bioscience and Agriculture Research,* 05 (02), 37-49.

I. 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. It is harvested as seed cotton and ginned to separate seed and lint (i.e., Lint is the common name for visible accumulations of textile fibers and other materials) (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). Cotton is the most important cash crop next to jute in Bangladesh

Corresponding author email: jannatsau419@gmail.com (Adan, M. J.)
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(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. In May 2012-13, Bangladeshi yarn (i.e., *Yarn* is a long continuous length of interlocked fibers) production was estimated at 688000 tons and an increase of about 12% from May 2012-13 production. At this condition we are one of the biggest importers of cotton in the whole world. Global production of cotton was expected to be 116.7 million bales in 2013-14 and in the same time area under cultivation was expected to be 33.1 million hectares and worlds` average yield is 766 kilograms/ha (Anon., 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). 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 (Seneewong *et al.*, 1999; 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). 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 (Chaudhry, 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 above facts, this research was undertaken with to identify the causal agents of cotton boll rot and to find out most effective Chemicals against cotton boll rotting pathogens.

II. Materials and Methods

Cotton variety CB-9 was used in this experiment as it is a widely cultivated variety. 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 (ISTA, 1996). Ten seeds were placed on 4 layers of moist blotter paper in 5 cm petridishes maintaining uniform distance between them. 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. 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.

Experimental site and duration: The field experiment was carried out during the period 30th May to November, 2013 in the experimental field in Sher-e- Bangla Agricultural University, 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. The selected field for this experiment was properly ploughed and proper doses of required fertilizers were applied to the field. 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 was covered by 511.2 m^2.

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 re-culturing fungi identified on the basis of their characteristics under compound microscope. These fungi were identified following the keys of Kamal and Khan (1964) and Kuch (1986).

Corresponding author email: jannatsau419@gmail.com (Adan, M. J.)
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Table 01. Detailed particular of chemicals used in the experiment

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

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 was 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.

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 Petri dish 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.

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). 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.

Isolation of causal organisms and tissue planting method: The pathogens associated with boll rot were isolated by following tissue planting method (Tuite, 1969). 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. 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.

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Treatments: Ten treatments were selected for this experiment, which were 1. T_1: Seed treatment with Cupravit 50 WP @ 0.4%, 2.T_2: Seed treatment with Cupravit 50 WP @ 0.4% + Mancozeb 80 WP @ 0.4%, 3.T_3 : Seed treatment with Streptomycin sulphet @ 0.1% , 4.T_4 : Seed treatment with Cupravit 50 WP @ 0.4 % + Foliar spray with Cupravit 50 WP @ 0.4 %, 5. 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 %,6. T_6: Seed treatment with Streptomycin sulphate @ 0.1% + foliar spray with Streptomycin sulphate @ 0.1 %, 7. T_7: Foliar spray with Cupravit 50 WP @ 0.4 %, 8. T_8: Foliar spray with Cupravit 50 WP @ 0.4 + foliar spray with Mancozeb 80 WP @ 0.4 %, 9. T_9: Foliar spray with Streptomycin sulphate @ 0.1 % and 10.T_{10}: Control.

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.

Data recording and statistical analysis: Data was recorded on leaf spot incidence, severity of leaf spot in PDI, boll rot incidence, and different yield contributing characters (number of branches/plant, number of leaves per plant, number of bolls per plant, plant height, weight of bolls, yield). Ten treatments with three replications were used following Randomized Block Design (RCBD). Data were analyzed for ANOVA using MSTAT-C program (MSTAT, 1991). Least significant difference (LSD) were performed to determine the level of significant differences and to separate the means within the parameters (Gomez and Gomez, 1984).

III. Results

Seed health study of collected cotton seeds

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

Table 02. 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

Isolation and identification of the seed borne fungi of cotton

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 conidiophores standing on a thick walled foot cell and vesicle that had globose head like structure that was formed on the conidiophores.

Efficacy of selected chemicals on radial mycelial growth of cotton seed borne fungi

In case of the *Fusarium* spp, the lowest mycelial growth (3.43 cm) was found in (T_1) (Mancozeb + Cupravit @ 0.4%), preceded by (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 (T_3) (Streptomycin sulphate @

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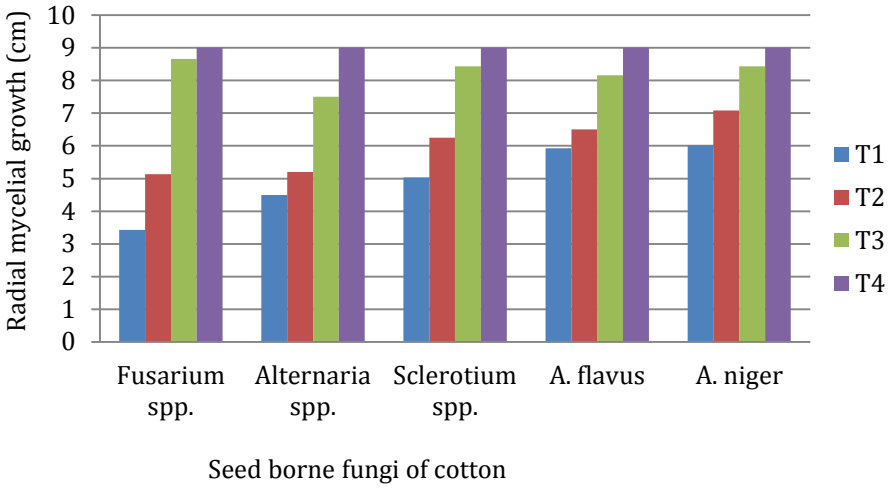
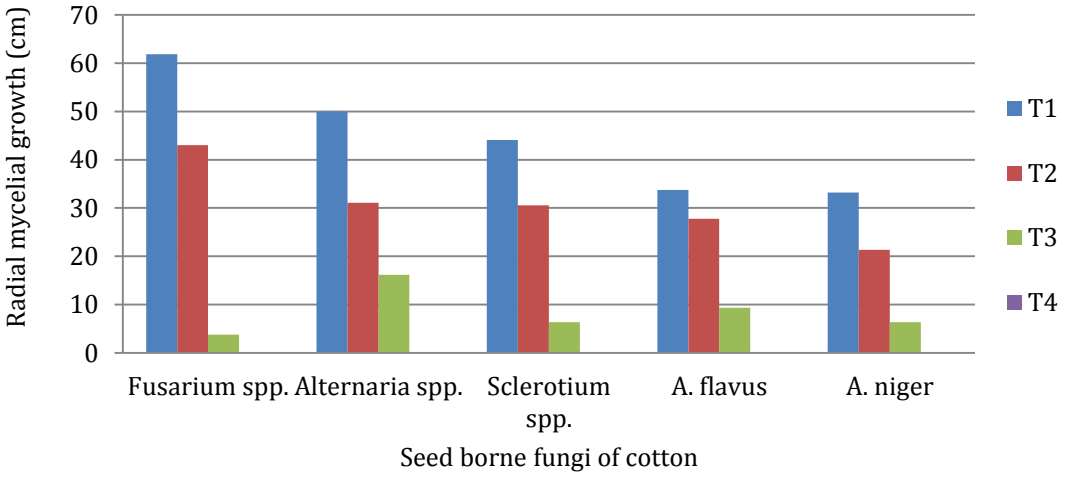
0.1%).The efficacy of chemicals on the radial mycelial growth of *Alternaria* spp is shown in Figure 01. In case of *Alternaria* spp. the lowest mycelial growth (4.5 cm) was recorded in (T_1) (Mancozeb + Cupravit @ 0.4%), preceded by (T_2) (Cupravit @ 0.4%). The highest mycelial growth (9.0 cm) of *Alternaria* spp was recorded in untreated control (T_4) followed by (T_3). In case of *Sclerotium* spp. lowest mycelial growth (5.03 cm) was recorded in treatment 1 preceded by (T_2). The highest mycelial growth (9.0 cm) of *Sclerotium* spp. was recorded in untreated control (T_4), followed by (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 (T_2). The highest mycelail growth (9.0 cm) was recorded in the untreated control (T_4), followed by (T_3). 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 T_3 (Streptomycin sulphate 0.1%).



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 01. Effect of 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 02. Percent inhibition of fungi caused by selected chemicals in *in - vitro* condition

Symptoms of cotton boll rot

The initial stage symptoms appeared on bolls as small brown or dark brown to black spots with depressed center. Then the superficial growth of fungal mycelia appeared on bolls. Later spots turn in

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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.

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.

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, i.e, foliar spray with Streptomycin sulphate 0.1%. The lowest leaf spot incidence (10.03%) was recorded in T_5 (seed treatment with foliar spray with Cupravit + Mancozeb @ 0.4%) and this was statistically similar to T_4 and T_2. 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 and lowest value was recorded from T_4 (18.09%) 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 03. Effect of selected chemicals on leaf spot incidence and leaf spot severity (% PDI) of cotton

Treatments	Leaf spot incidence in leaves (%)			% reduction over control at 6th month	PDI (%)			% reduction over control at 6th month
	Days				Days			
	120	150	180		120	150	180	
T_1	15.75 d	21.43 e	27.93 c-e	68.02	34.63 e	46.18 e	42.13 f	53.26
T_2	11.17 e	17.57 f	21.27 de	75.62	43.03 d	54.09 d	43.50 ef	51.74
T_3	26.50 b	32.47 c	17.36 e	80.12	8.80 f	13.17 g	62.13 c	31.08
T_4	11.50 e	12.23 g	18.09 e	79.29	41.50 d	51.29 d	54.22 d	39.85
T_5	10.03 e	11.40 g	19.08 e	78.15	6.40 g	13.17 g	13.17 g	85.39
T_6	49.57 a	60.73 b	75.21 b	13.90	34.63 e	46.18 e	45.13 f	49.93
T_7	23.17 bc	25.43 d	32.95 c	62.28	43.03 d	54.09 d	43.50 ef	51.74
T_8	21.77 c	25.43 d	30.80 cd	64.74	8.80 f	13.17 g	62.13 c	31.08
T_9	49.83 a	60.10 b	83.52 ab	4.39	41.50 d	51.29 d	54.22 d	39.85
T_{10}	48.23 a	67.23 a	87.36 a	0	60.17 a	70.14 a	90.15 a	0
Lsd (0.05%)	*3.89*	*3.41*	*10.17*		*3.89*	*2.91*	*3.49*	
CV (%)	*8.49*	*5.95*	*11.34*		*6.64*	*3.90*	*3.86*	

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 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 sulphate @ 0.1%) , T_{10}= Control

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%

Corresponding author email: jannatsau419@gmail.com (Adan, M. J.)
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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 04. Effect of selected chemicals on cotton boll rot incidence

Treatment	Incidence of boll rot (%)			% reduction over control at 180 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*	

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 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 sulphate @ 0.1%) , T_{10}= Control

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. 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%).

Number of branches per plant

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 T_8 (15.33 branches) and T_7 (15 branches).

Number of leaves per plant

The highest number of leaves per plant (889) was counted in T_5 (seed treatment + foliar spray with Mancozeb + Cupravit @ 0.4%) followed by T_4 (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 (509 leaves) and T_3 (527.30 leaves).

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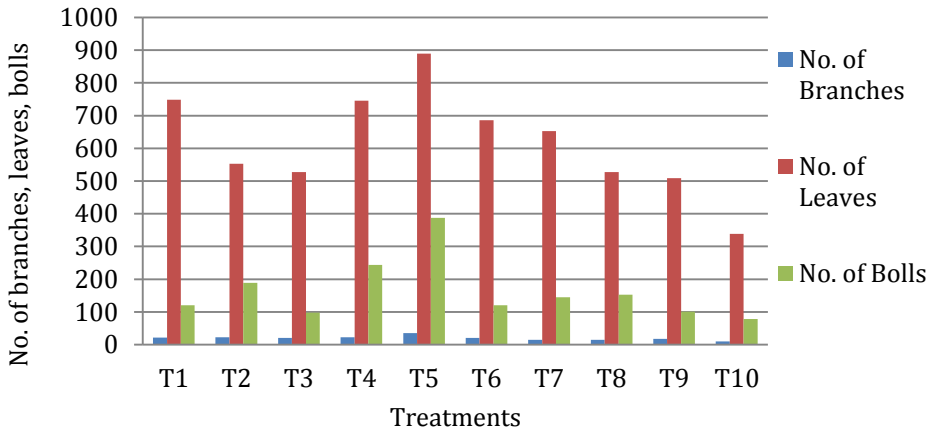
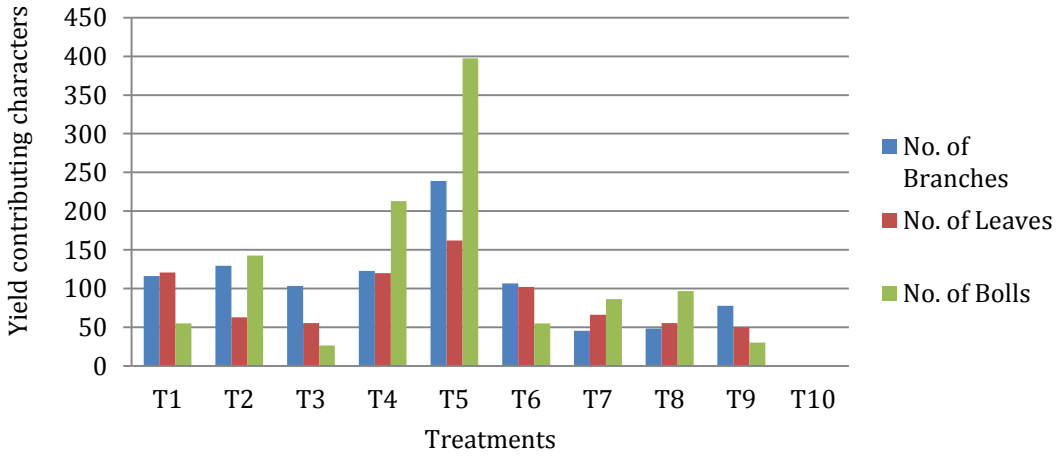
Number of bolls per plant

After 180 days after sowing, 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 control (T_{10}) proceeded by (T_9) (101 bolls). However, T_3 showed a bit increased bolls than the T_9 but there was no statistically significant difference between them.



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 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 sulphate @ 0.1%) , T_{10}= Control

Figure 03. Effect of selected chemicals on number of branches, leaves and bolls of cotton



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 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 sulphate @ 0.1%) , T_{10}= Control

Figure 04. Effect of selected chemicals on percent increase of branches, leaves and bolls over control in cotton

The effect of selected chemicals on percent increase of branches, leaves and bolls over control is very vividly observed (Figure 04). 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.

Corresponding author email: jannatsau419@gmail.com (Adan, M. J.)
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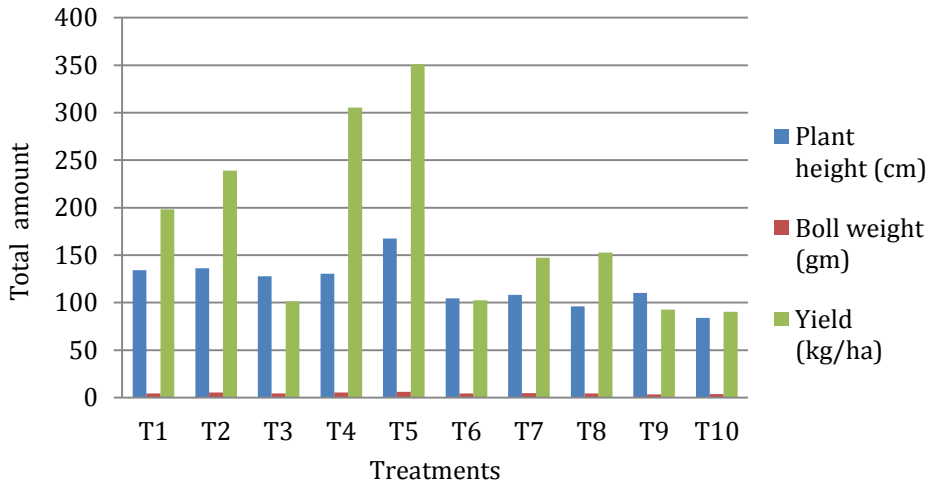
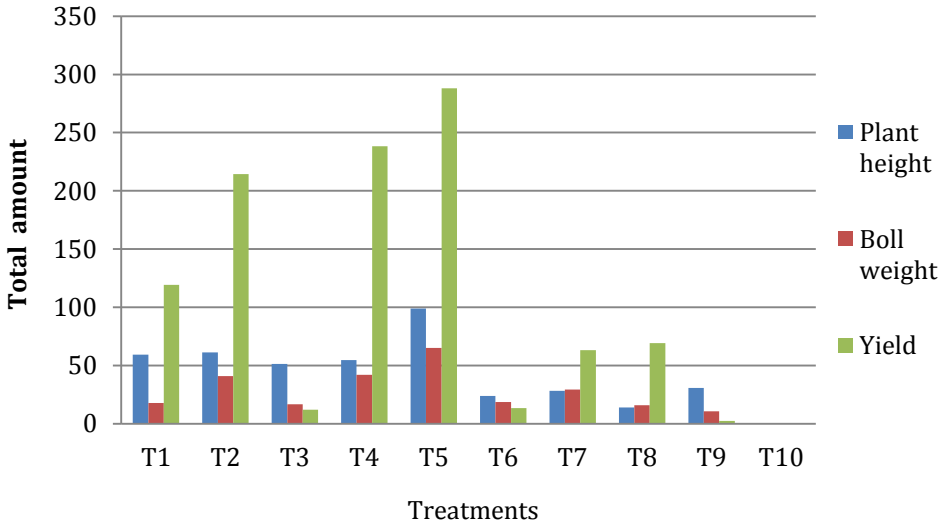
Plant height, boll weight and cotton yield

The effect of the selected chemicals on height of cotton plants is projected in Figure 05. Here, the highest plant height (167.60 cm) was found in the plot treated with T_5 (Mancozeb + Cupravit @ 0.4%). 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 (96 cm). 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. The lowest weight (3.73 g) in bolls was found in the plot under untreated control followed by T_9 (3.33 g). The highest yield (351 Kg) was obtained in T_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).



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 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 sulphate @ 0.1%) , T_{10}= Control.

Figure 05. Effect of selected chemicals on plant height, boll weight and yield of cotton



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 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 sulphate @ 0.1%) , T_{10}= Control

Figure 06. Effect of selected chemicals on percent increase of plant height, yield and boll weight over control in cotton

Corresponding author email: jannatsau419@gmail.com (Adan, M. J.)
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Effect of selected chemicals on percent increase of plant height, yield and boll weight over control is very vividly observed in (Figure 06). 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.

Comparison between treated and untreated seeds

Presence of different seed borne fungi in untreated seeds is shown in figure 07. *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.

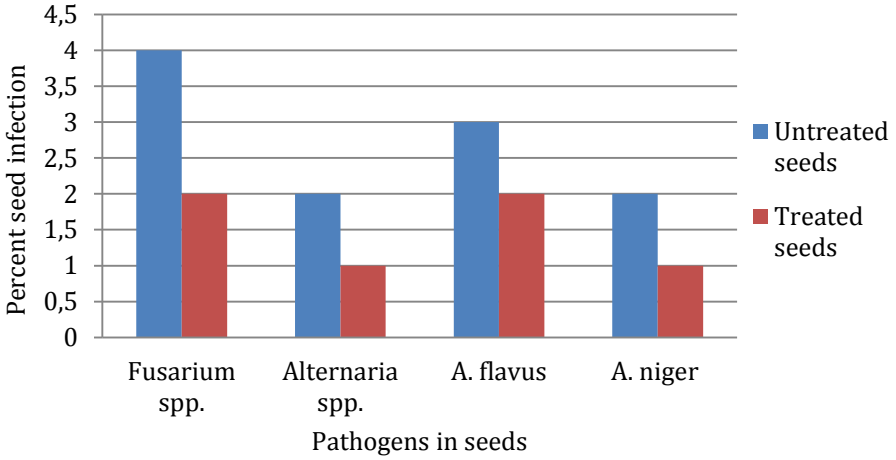

Figure 07. Comparative seed health study of untreated cotton seeds and harvested cotton seeds

IV. Discussion

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) and Coyler (1988). 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. 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), and 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 Minamor, (2013), Belly *et al.* (2006) and Wani and Nisa, (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%

Corresponding author email: jannatsau419@gmail.com (Adan, M. J.)
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disease reduction over control and combined effect of Mancozeb and Cupravit was on next showing 78.15% reduction over control. 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. 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; Mamza *et al*., 2012; Nisa *et al*., 2011; Minamor, 2013; Gondal *et al.* 2012; Kumar *et al*. 2013; 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.* (2001) reported the same result during their experiments.

Madhavi and Bhattiprolu (2011) used different fungicides including Hexaconazole, Propiconazole, Difanoconazole, 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). 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.

V. Conclusion

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. Thus, cotton growers can use Cupravit alone or Mancozeb + Cupravit as seed treating and foliar spray during cultivation.

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Corresponding author email: jannatsau419@gmail.com (Adan, M. J.)
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