MANAGEMENT OF ANTHRACNOSE DISEASE OF CHILLI CAUSED BY (*Colletotrichum capsici*) THROUGH SELECTED TREATMENTS FOR SEED PRODUCTION

REGISTRATION NO. 19-10105

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BY

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Dedicated to
my beloved parents
and
my respected teachers

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CERTIFICATE

This is to certify that the thesis entitled, **"MANAGEMENT OF ANTHRACNOSE DISEASE OF CHILLI CAUSED BY (*Colletotrichum capsici*) THROUGH SELECTED TREATMENTS FOR SEED PRODUCTION"** *submitted to the Department of Plant pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural university, Dhaka, in partial fulfillment of the requirement for the degree of* **MASTER OF SCIENCE IN PLANT PATHOLOGY** *embodies the results of a piece of bona fide research work carried out by* **KAWSAR MAHMUD**, **Registration No. 19-10105** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma elsewhere in the country or abroad.*

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

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The author

MANAGEMENT OF ANTHRACNOSE DISEASE OF CHILLI CAUSED BY (*Colletotrichum capsici*) THROUGH SELECTED TREATMENTS FOR SEED PRODUCTION

ABSTRACT

The *in vivo* experiment was conducted in the Central Farm of Sher-e-Bangla Agricultural University (SAU), Dhaka and *in vitro* experiment was conducted in the laboratory of the Department of Plant Pathology, SAU. The effect of eight treatments viz., Tilt 250 EC (Propiconazole), Ridomil gold 68 WG (Mancozeb+ Metalaxyl), Dithane M-45 (Dithiocarbarnate), Score 250 EC (Difenoconazole), *Trichoderma harzianum* (Bio-Control), Neem leaf extract (*Azadirachta indica*) Allamanda leaf extract (*Allamanda cathartica*) and a Control (T_0) were explored against Anthracnose of chilli (*Colletotrichum capsici*) during winter season 2019-2020. The efficacy of the treatments varied significantly in terms of disease incidence, disease severity and yield attributes of chilli. Among the treatments, Tilt 250 EC, Dithane M-45 showed promising performance against the disease. The lowest leaf incidence (4.31%) and leaf area diseased - LAD (4.16%) were recorded Tilt 250 EC. Similarly, the lowest fruit infection (3.93 %) and fruit area diseased - FAD (3.55%) were recorded in Tilt 250 EC (4.95%) and Dithane M-45 (4.41%), respectively. Reduction of disease incidence and severity of anthracnose of chilli by spraying Tilt 250 EC also reflected in case of fruit yield, seed yield and subsequent seed health of harvested chilli seed. The highest fruit yield (10.23 t/ha) and 1000 seed weight (4.21 gm) were also recorded in case of application of Tilt 250 EC. The maximum seed germination of harvested chilli seed (87.5%) was also observed in Tilt 250 EC applied plots. Among the plant extract, neem leaf extract found to give good yield (8.04 t/ha) against anthracnose disease which was even better than Ridomil Gold 68 WG (7.9 t/ha) and Score 250 EC (7.67 t/ha).

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	= Analysis of variance
AEZ	= Agro Ecological Zone
BARI	= Bangladesh Agricultural Research Institute
cm	= Centimeter
CV	= Coefficient Variance
DAS	= Days after sowing
DAT	= Days after transplant
DMRT	= Duncan's Multiple Range Test
EC	= Emulsifiable Concentrate
etc.	= Etcetera
et al.	= And others
Fig.	= Figure
FAD	= Fruit area diseased
FAO	= Food and Agriculture Organization
ha	= Hectare
kg	= Kilogram
LAD	= leaf area diseased
LSD	= Least Significant Difference
m	= Meter
m^2	= Square meter
ml	= Milliliter
MP	= Muriate of Potash
PDA	= Potato Dextrose Agar
pH	= Potential of hydrogen
psi	= Pounds per square inch
RCBD	= Randomized Complete Block Design
t	= Ton
TSP	= Triple Super Phosphate
WG	= Wettable Granular
W/V	= Weight per volume
^oC	= Celsius
%	= Percentage

INTRODUCTION

CHAPTER I

INTRODUCTION

Chilli is one of the most significant marketable crops of Bangladesh. Chillies (*Capsicum annum* L) are the berries or fruits of plant belonging to the solanaceae family. Chilli is a native plant of South America and Portuguese were the first to acquaint it to India from Brazil towards the end of 15th century. India is considered to be the secondary centre of diversity for chilli, especially of *C. annuum*, the most important cultivated species (Dhaliwal *et al*., 2014). There are more than 400 different varieties of Chilli's found all over the world. It is also called as hot pepper, sweet pepper, bell pepper, etc. Both chilli and capsicum belong to same family as well as same genus that is *Capsicum*.

Capsicum not only gives attractive color and flavor to the foods but also provides capsaicin, large amounts of vitamin C, small amounts of carotene (Provitamin A), vitamin B, vitamin B6, vitamin E and minerals. The capsaicin which is alkaloid present in the placenta and pericarp of the chilli fruits. Capsaicin has also anti-diabetic, anti-bacterial, analgesic and anti-carcinogenic properties (Geetha and Selvarani, 2017). There are thought to be 25-30 species of Capsicum of which 5 species i.e., *C. annum*, *C. frutescens*, *C. chinense*, and *C. pubescens* have been domesticated and cultivated (Kothari *et al.,* 2010).

In Bangladesh the crop is grown in an area of about 252 thousand acres and the production is 130 thousand ton and the average yield 1.32 ton/ha (BBS 2016). India is at the top in terms of international trade by sharing about 40% and Bangladesh about 5% of the total world chilli production. The largest importer of Indian chilli is Malaysia (30%) followed by other traditional importers like Bangladesh (20%), Sri Lanka (15%), the USA (9%) and the UAE (8%) (FAO, 2014). Chilli have been domesticated in our country and cultivated commercially mainly in Kharif and Rabi seasons in Bangladesh.

Most of the farmers faced many problems relating to the cultivation of chilli like adverse climate, pest and disease attack, lack of high yielding varieties,

unavailability of pesticides, lack of technical know hob and storage facilities etc. The production per unit area is very low in Bangladesh. Among the various factors responsible for the low yield of the crop, seed borne fungal diseases play a vital role because most of the farmers use low quality seeds which are not certified and disease free. The major diseases of chilli are anthracnose, wilt, damping off, grey mould, bacterial leafspot, chilli leaf curl virus, chilli mosaic virus etc. Among the diseases chilli anthracnose is a major constraint in chilli production leading to huge economic losses worldwide.

Colletotrichum is one of the most destructive phytopathogen worldwide causing the economically important disease anthracnose. Chilli anthracnose is a polycyclic devastating pathogen, where the initial disease is induced by the spores of *Colletotrichum capsici* which survive in and on seed in the form of acervuli and microsclerotia (Montri *et al.*, 2009). The spores disseminate, deposit and germinate on the surface of the chilli plants. From appressoria, the germinating spore penetrate into the cuticle layer of plant and produce the infectious hyphae results in development of lesions (Yu *et al.*, 2013). Typical anthracnose symptoms caused by *Colletotrichum capsici* appear as sunken necrotic tissues, with concentric rings of acervuli on chilli fruit. Anthracnose causes extensive pre and post-harvest damage to chilli fruits causing anthracnose lesions. Fruits showing small anthracnose lesions affect on chilli marketability (Manandhar *et al*., 1995). It is found that around 27°C temperature, about 80% relative humidity and soil pH 5-6 promote favorable infection and disease progress of chilli anthracnose. During rainy weather, severe losses occur because the spores are washed or splashed to other fresh fruits resulting in more infections (Roberts and Kuchrek, 2001). In Bangladesh, anthracnose of chilli is frequently observed in summer and winter.

Management strategies for this disease include use of healthy seeds, use of resistant cultivars and fungicidal sprays. Plant diseases are mainly managed by using chemical fungicides. However, indiscriminate use of chemicals is not only hazardous to living being but also to the ecological balance as they kill the

antagonist microorganisms unjudicially. Resistant variety of chilli against anthracnose disease is not available in the country. Among the control measures, seed treatment is probably the cheapest and safest method to control anthracnose pathogen. It was reported that Mancozeb and Carboxin completely controlled the seed borne *Colletotrichum capsici* (Mridha and Chowdhury, 1990). Control of seed-borne pathogens of many crops by applying botanicals treatment in many laboratories and field trials showed that crude extract from leaf, rhizome, stolon fruits and cloves of garlic (*Allium sativum*), neem (*Azadirachta indica*), and ginger (*Zingiber officinale*) significantly reduced the infestation of seed-borne fungi (Khan and Fakir, 1995). Neem extract minimize the ripe fruit rot of chilli. Garlic extract could effectively control chilli anthracnose (Harbant *et al.,* 1999). Considering the facts described above, the present research work was designed to test the efficacy of some treatments in controlling anthracnose and increase seed yield of chilli.

Objectives:

1) To isolate and identify the causal pathogen of anthracnose of chilli.
2) To evaluate the eco-friendly components for the management of anthracnose of chilli for seed production.
3) To determine the prevalence of pathogen in harvested chilli seeds.

CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Management strategies for the anthracnose disease of chilli relevant information including use of fungicidal, biological, botanical sprays and prevalence of seed borne pathogen have been reviewed in this chapter:

2.1. Geographical distribution

Gautam (2014) described various plant diseases caused by *Colletotrichum* in India with special reference to present century (2000-2012). About 25 plant diseases were caused by different species of *Colletotrichum* namely, *C. gloeosporioides, C. capsici, C. falcatum, C. truncatum, C. sansevieriae, C. acutatum and C. coccodes.* The study showed that even a single species of *Colletotrichum* can affect multiple host plant.

Anamika *et al*. (2012) assess the incidence of anthracnose of chilli in five locations in Rewa Province, India. The percent incidence of anthracnose affected fruits under field conditions was more on green fruits which ranged from 55.53% to 71.10% and it was revealed the predominance presence of the anthracnose disease is the major constraints to profitable cultivation of chilli in Rewa region.

Dean *et al*. (2012) stated *Colletotrichum*, was one of the major plant pathogenic genera responsible to anthracnose causes a plant disease on a variety of hosts from trees to grasses.

Raj *et al*. 92012) reported twenty isolates of *Colletotrichum capsici* from conventional chilli growing areas of Tamil Nadu, India. However, no significant differences were noticed in shape and size of conidia. The reaction of 20 isolates on an indigenously developed differential set of *Capsicum* cultivars indicated the existence of different virulence in chilli populations.

Hadden and Black (1987) reported four *Colletotrichum species, C. capsici, C. gloeosporioides, C. acutatum* and *C. coccodes* have been reported as causal

pathogens of pepper anthracnose in many countries although the major species were *C. capsici* and *C. gloeasporioides*.

Ahmed and Hossain (1985) reported at least 12 different *Colletotrichum spp.* which cause diseases of 65 different plants belonging to different taxonomic groups.

Pearson *et al*. (1984) reported anthracnose disease caused by *Colletotrichum capsici* was considered to be a dry fruit rot of chilli. *C. capsici* and *C. gloeosporioides* are the two main casual pathogens of pepper anthracnose in the hot humid tropics of Asia. *C. capsici* and *C. gloeosporioides* are the most important *Colletotrichum spp.* in reducing marketable fruit yields of pepper.

2.2 Symptomatology

Manandhar *et al*. (1995) reported the typical anthracnose symptoms on chilli fruits include necrotic tissues, with concentric rings of acervuli. Fruits showing blemishes have reduced yield losses.

Kulshreshtha *et al.* (1976) found the conidial shape has been considered as major criterion for classification and erected two master species–one having curved conidia (*Colletotrichum dematium*) and second having straight conidia (*Colletotrichum gloeosporioides*).

Tandon and Agnihotri 91961) reported that setae of *Colletotrichum capsici* have been described as light brown to dark brown, septate thick and tapering towards the end with light colored apices, Setae may be straight (Sundaram, 1926) or curved (Rao and Rao, 1956).

Shrinivasan (1952) found that acervuli are erumpent, cushion like mass of hyphae, light brown to dark brown, sometimes lined with setae and conidiophores bearing conidia at their apices.

2.3 Management through fungicides, biological and botanical sprays

Patel *et al.* (2020) reported that different isolates of *Trichoderma* spp. significantly inhibited the growth of *Colletotrichum capsici* causing die-back and fruit rot in chilli and maximum inhibition of 39.29% was recorded by *Trichoderma* isolate T2(REWA) after 96 hrs of incubation period. In field conditions, among treatments with different *Trichoderma* isolates, it was observed that minimum PDI of 19.40% was recorded in seed treatment and three foliar sprays with T2 isolate of *Trichoderma* with maximum yield of 69.55 qa/ha. Among different fungicides evaluated, minimum PDI of 21.47% was recorded in foliar spray with propiconazole @ 0.1%. This was followed by foliar spray with Thiram + Carboxin @ 0.2% where 23.73 % PDI was recorded.

Pooja and Simon (2019) conducted field experiment to test the effect of organic amendments with *Pseudomonas fluorescens* and carbendazim are used against *Colletotrichum capsici,* anthracnose disease of chilli and chilli growth parameters. Minimum disease intensity was recorded in T1-NPK + carbendazim (26.13%) with maximum yield (103.83 qa/ha) followed by T5-FYM + *P. fluorescens* (26.40%) with yield (95.79 qa/ha),T3-P. *fluorescens* seedling treatment + *P. fluorescens* (28.56%), with yield (92.52qa/ha), T2-Neem cake + *P. fluorescens* (32%), with yield (90.25 qa/ha), T6-Sheep manure + *P. fluorescens* (33.14%) with yield (85.44 qa/ha), T7-Poultry manure + *P. fluorescens* (35.55%) with yield (82.11 qa/ha), T4-Groundnut cake + *P. fluorescens*-(37.77%) with yield (77.86 qa/ha) maximum disease intensity was recorded in control (49.03) with lowest yield ((36.71 qa/ha).

Birari *et al.* (2018) conducted experiment during 2015-2017 to evaluate the effectiveness of extract of Bavchi seeds (*Psoralea corylifolia*), Datura leaves (*Datura* sp.) and Ghaneri leaves (*Lantana camera*), biocontrol agents against *Colletotrichum capsici.* Among the *Pseudomonas fluorescens* and *Trichoderma viride* tested by dual culture technique. *T. viride* suppressed the growth of *C. capsici* by 80.00%. The three botanicals Bavchi, Datura and Ghaneri at different concentrations 250µl, 500µl, 750µl and 1000µl were tested by poisoned food

technique. Methanolic extract of Bavchi @ 1000µl showed the highest inhibition against *C. capsici*. Treatment with *P. fluorescens* (culture filtrate @ 0.5%) + *T. viride* (culture filtrate @ 0.5%) + methanolic extract of @ 2% proved highly effective in reducing disease intensity (80%) in chilli under detached fruit bioassay.

Vivekanand *et al.* (2018) evaluated the efficacy of different management methods under in vivo condition two chemicals, Captan and Mancozeb (2.5g/kg), two bioagents *Trichoderma harzianum* and *Pseudomonas fluorescens* (5g/kg) against anthracnose of chilli. Mancozeb among chemicals was found effective to reduce per cent disease index (PDI) at 75 and 105 days after transplanting (DAT) whereas, Captan was found to reduced maximum PDI at 90 DAT. Among the combination treatments (Captan + Neem extract, Mancozeb + Garlic extract, *T. harzianum* + Captan and *P. fluorescens* + Carbendazim) in comparison to *T. harzanium* + Captan was found most effective with least per cent infected fruits (9.29%), disease incidence (6.67%), disease severity (5.36%) and disease index (4.70, 7.03 and 9.62%) at 75, 90 and 105 DAT respectively.

Choudhury *et al.* (2017) tested different treatments like chloroform extract of Ginger, rhizome, Clerodendrum mature leaf and methanol extract of Polyalthia mature leaf against *Colletotrichum capsici* radial growth, biomass production and spore germination on following poisoned food technique at 20, 100, 200 and 400 µg/ml and carbendazim at 1, 5, 10, 20µg/ml was taken as standard fungicide control. However, the fungicide carbendazim showed highest activity than the botanical formulations at lower doses. Under in vivo condition, all the treatments showed reduced lesion diameter but Clerodendrum-chloroform showed less lesion diameter compared to other treatments. Based on the results, the plant extract of Clerodendrum could be developed and used as an effective alternative to synthetic chemicals for postharvest anthracnose of chilli both under field and post-harvest condition.

Mandal *et al.* (2017) set an experiment during rabi season of 2012-13 and 2013-14 for the management of anthracnose of chilli. The treatments included seed

treatment with Carboxin + Thiram @ 0.2%, seed treatment and spraying with biocontrol agent *Pseudomonas fleuroscens* @ 1.0%, spraying with Copper Oxychloride @ 0.4%, spraying with Difenoconazole @ 0.1%, spraying of Tebuconazole @ 0.1% and their various combinations. Among the treatments, a combination treatment combining seed treatment with Carboxin + Thiram and foliar spraying of Difenoconazole was found most effective during both the years of study. Two years pooled mean revealed that the maximum disease control (74.8%) and maximum increase in yield (79.3%) were also achieved with the same treatment.

Yadab *et al.* (2017) used three promising fungicides, tebuconazole (0.1%), carbendazim + mancozeb (0.1%) and propiconazole (0.1%) and two botanicals extract, NSKE (5%) and neem leaf extract (5%) for the control of pathogen (*Colletotrichum capsici*) in the field against fruit rot disease of chilli. Maximum reduction in disease (69.30%) with minimum percent disease (17.24) and maximum fruit yield (8.23q/ha) were obtained with the application of propiconazole (0.1%) as seed treatment and foliar spray of neem (NSKE5%) followed by application of propiconazole (0.1%) as seed treatment and foliar spray with neem leaf extract (5%) resulted in decreased PDI and increased fruit yield.

Naznin *et al.* (2016) conducted experiment in the laboratory and field of Spices Research Centre, BARI, Shibganj, Bogra, Bangladesh during Rabi season of 2014-2015 to test the efficacy of some new fungicides in controlling anthracnose/die-back disease and increase yield of chilli. *In vitro* tests, Orion 72 WP (0.2%) significantly inhibited the radial mycelial growth of the pathogen (*Colletotrichum capsici*) in the PDA culture media. Among the fungicides, Orion 72 WP showed the best performance.

Oo and Oh (2016) reported that use of appropriate integrated management practices, such as cultural, mechanical, chemical, and biological control, are important in chilli anthracnose disease prevention and control. Emphasis is laid

on the use of biological control because it is cost effective and eco-friendly, and is an appropriate approach for disease management.

Begum *et al.* (2015) evaluated different botanicals, fungicides and bioagents under *in vitro* and field condition against *Colletotrichum capsici*. Amongst fungicides, it was found that at 150 g/ml concentration 100% inhibition in mycelial growth was recorded in Tebuconazole, Mancozeb and Trifloxystrobin + Tebuconazole and minimum inhibition in mycelial growth was recorded in Carbendazim (6.71%). From three of all bioagents viz., *Trichoderma harzianum,* T. *viride* and *Pseudomonas fluorescens,* T. *harzianum* isolate Th-2 was found most effective giving 77.78% inhibition on the mycelial growth followed by T. *harzianum* isolate Th-1 inhibiting 74.00%. Among four botanical oils Garlic, Neem, Polyalthia and Citronella were tested for their *in vitro* efficacy at concentrations of 0.05%, 0.1% and 0.2%. Garlic oil at all concentrations and neem oil at 0.1% gave percent inhibition of mycelial growth of the fungus followed by Polyalthia at 0.20% (84.45%).

Owe *et al.* (2015) isolated fungus *Colletotrichum capsici* and found pathogenic & responsible for anthracnose in chilli. Different fungicides, bioagent and botanical were evaluated (*in vitro*). Maximum radial mycelial growth inhibition (85.18%) of *Colletotrichum capsici* was recorded in Hexaconazole followed by *Pseudomonas fluorescens* and Karanj leaf extract comparatively least effective in restricting the radial mycelial growth of *C. capsici.* Minimum percent disease incidence (PDI) and PDI was found in Hexaconazole @ (0.1%). However, sprays of *P. fluorescens* (0.4%) and Karanj leaf extract (10%) were least effective in reduction of disease incidence of *C. capsici* under natural field condition.

Rashid *et al.* (2015) carried out an investigation to determine the efficacy of some organic materials to manage anthracnose (*Colletotrichum capsici*) of chilli (*Capsicum annuum* L.) under natural field condition at Bangladesh Agricultural University, Mymensingh, Bangladesh. As chemical, the combined application of neem, mahogany and garlic extract also showed significant impact on disease reduction as well as on yield of chilli. Relatively, this organic cocktail gave a

higher benefit (BCR=3.97) with minimum production cost and this approach is proposed to the chilli-growing farmers to mitigate the chilli anthracnose. So, organic combination might be a better option to control anthracnose of chilli rather than chemical control as it is cost-effective and eco-friendly.

Subhani *et al.* (2015) examined anthracnose damage chilli fruits extensively at pre- and post- harvest stages causing anthracnose lesions. There was a significantly decrease in mycelial growth of fungus with an increase in fungicides and homeo-fungicides concentration in all the tested fungicides over the control. Benomyl proved to be the best which gave highest control efficiency (100%) at all the concentrations. While Protest proved to be the least effective (48.7%) at highest concentration (1000 ug/ml) tested. Revus had also inhibited the mycelia growth of *Colletotrichum capsici* completely at three concentrations of 300, 500 and 1000 ug/ml, respectively. It is clear that with increase in fungicides concentrations decreased the mycelial growth of fungus and homeo-fungicides can be used as an alternative to the synthetic fungicides to reduce the hazardous impact on the environment.

Chauhan *et al.* (2014) reported on chilli anthracnose disease caused by *Colletotrichum capsici* in India. Different fungicidal treatments were significantly superior over control. Minimum disease intensity of 19.67% was recorded in carbendazim 0.05% which was statistically at par with spraying of 0.2% mancozeb (20.71 %), 0.2% carbendazim + mancozeb (22.51%) and 0.2% copper oxychloride (23.16%). Among all the treatments revealed that carbendazim 0.05% or mancozeb 0.2% sprayed at 15 days interval starting from the appearance of the disease was most effective for the control of anthracnose disease of chilli and get higher healthy fruit yield and cost benefit ratio.

Islam *et al.* (2010) tested effect of six fungicides and five plant extract against anthracnose disease of chilli during winter season. The efficacy of the treatments varied significantly in terms of disease incidence, disease severity and yield of chilli. Application of Tilt 250 EC increased fruit yield of chilli by 195.9% followed by Proud 250 EC (177.5%) and Bavistin 50 WP (159.5%). Cost-benefit

analysis showed that the application of Tilt 250 EC (T6) gave the highest BCR (4.07) followed by Proud 250 EC (3.68), Bavistin 50 WP (3.47), Allamanda leaf extract (3.16) and Dithane M-45 (2.95).

Mistry *et al.* (2008) evaluated effectiveness fungicides, plant extract and biopesticides against C. *capsici* under *in vitro* condition for their efficacy to control die-back of chilli under field condition. Carbendazim (0.05%) was the most effective against C. *capsici.* The other fungicides in order of merit were Chlorothalonil (0.20%) followed by Foltaf (0.20%), Benomyl (0.025%), Shield (0.03%), biopesticides *Trichoderma viride*. (108 cfu), Cosko (0.03%) and botanical Ginger extract (5%).

Roy (2005) conducted an experiment of using eleven fungicides to control against *Colletotrichum capsici* on chilli. Fild 250 EC at 0.05%, give the effective result followed by Propicon 250 EC, Copper 50 WP and Indofil Z-78. These compounds also could control of the die back phase as well as anthracnose disease and increased yield of chilli.

Joi *el al.* (2004) found a field trial during kharif season of 2002/03 to evaluate the efficacy of RIL 006/CI at 0.05, 0.1, 0.15 and 0.2% concentration against anthracnose of chilli caused by *Colletotrichum capsici* at Maharastro, India. They also used Shan at 0.1%. Contaf (Hexaconazole) at 0.3% and 0.1%. They observed all treatments except for RIL 006/CI at 0.05% were significantly superior over the untreated control in reducing intensity of leaf anthracnose and fruit rot infection. RIL 006/CI at .02%, Tata Shan at 0.1 % + Contaf at 0.3% and RIL 006/CI at 0.1% reduced anthracnose and fruit infection 70.7, 67. l and 62.6%, respectively over control.

Rahman *et al.* (2004) evaluated the efficacy of Bion (0.005%), Azoxystrobin and carboxin in 18 systemic resistant to anthracnose (*Colletotrichum capsici*) in chilli (*Capsicum annum*). They observed die-back symptom did not appear in Bion treated seeds but was recorded in Azoxystrobin and carboxin treated seeds, lesion size, leaf infection and leaf area damage were less in plants grown. Bion treatment resulted in moderate resistance of plants to anthracnose, where

Azoxystrobin and Carboxin treatment resulted in susceptibility of the crop to the disease.

Ekbote (2003) conducted an experiment during the 2000/01 and 2001/02 kharif seasons in Karnataka, India to evaluate the efficacy of Prochoraz compared with Carbendazim and Mencozeb against fruit rot and die-back (*Colletotrichum capsici*) of chilli. The treatments comprised of 0.05, 0.75, 0. 1 and .0125% Prochoraz 45 EC; 0.25% Mencozeb; 0.25% Copper oxychloride 75 WP and .01% Carbendazirn. Among these fungicides 0.125% Prochoraz gave the lowest percent disease index of die back and fruit rot and highest yield performance.

Kumaran *et al.* (2003) evaluated that the chemical fungicide of Mancozeb at 320 ppm and ethanolic extract of the roots of 18 different plant species for their fungitoxic activity against anthracnose of chilli (*Capsicum annum*) caused by *Colletotrichum capsici*. They observed ethanolic root extract of *Abrus precatorious* and *Rauvolfia tetraphylla* showed significant inhibitory effects on both the conidial germination and radial growth of *Colletotrichum capsici*.

Mandal and Beura (2003) determine the efficacy of different application of 0.05% Mono crotophos + 0.25% Mancozeb against chilli thrips (*Scirtothrips dorsalis*); anthracnose and ripe fruit rot (*Colletotrichum capsici*). The pesticide combination was applied at 25, 45 and 65 days after transplant (DAT)~ at 25 and 45 at 25 and 65, and 45 and 65 DAT~ and at 25, 45 and 65 DAT. Leaf curling rate, percent disease index (PDI) of anthracnose and PDI of ripe fruit rot were reduced.

Deshmukh *et al.* (2002) compared between Mancozeb at 0.25% and the biological pesticide Zetron at 0.2, 0.25% and 0.4% in controlling anthracnose of chilli (*Capsicum annum*) caused by *Colletotrichum capsici*. They found Zetron significantly reduced the growth of the fungus relative to the control. The development of lesions on chilli fruits was considerably slower with the lowest lesion development resulting from the Mancozeb treatment followed by Zetron at 0.4, 0.25 and 0.2%.

Ekbote (2002) evaluate the efficacy of Copper hydroxide at 0.10, 0.15, 0.20 and 0.25%; Chlorothalonil at 0.20%; and Carbendazim at 0. 10% against fruit rot of chilli. The lowest disease index (30.47%) resulted from the application of 0.10% Carbendazim (30.47) followed by copper hydroxide (37.70%) sprayed at 0.25% concentration which indicated the potential of using Copper hydroxide in controlling *Colletotrichum capsici* to avoid the development of the resistance of the pathogen to carbendazim.

Hegde and Anahosur (2001) examined the effective fungicide treatment against fruit rot disease caused by *Colletotrichum capsici* of chilli under rainfed conditions. Among the fungicides tested, Carbendazim recorded the least percent disease index (35.63%) followed by Propiconazole (36.53%) and Hexaconazole (35.63%). Iprodione recorded the highest percent disease index (71.57%). Carbendazim the best fungicide in controlling fruit rot disease. The fungicides did not only reduce the disease incidence but also helped in obtaining maximum capsicin, ascorbic acid and total sugar contents in chilli fruits.

Saleem (2000) conducted a survey during 1996-1999 and more than 150 diseased Piper beetle specimens were collected in Thatta and Pakistan. *Colletotrichum capsici* and *Colletotrichum gloeosporides* were isolated and identified. Benoyl and Thiophanate-methyl gave good control of those pathogens.

Harbant *et al.* (1999) got some plant extract for the control of *Colletotrichum capsici*. The efficacy of neem (*Azadirachta indica*), garlic (*Allium sativum*) and Tagak-tagak (*Rhinocanthus indica*) at 5000 ppm on chilli was compared with the fungicide carbendazim (Bavistin) at 100 ppm. Garlic extract performed well under room humidity, while Tagak-tagak extract showed good control of chilli anthracnose under high moisture conditions. Neem extract minimized the chilli anthracnose.

Haque *et al.* (1998) tested 5 fungicides against seed borne fungi of chilli and reported that Vitavax-200 completely eliminated the seed borne pathogen of *Colletotrichum capsici*.

Khan *et al.* (1998) found 4 neem-based products namely Nemokil, Nernokil-S, SOS and SOC had antifungal activity against the guava wilt and the anthracnose pathogen. Those neem-based products proved less effective against *Colletotrichum gloeosporioides*.

Singh *et al.* (1997) repoted that plant extract of *Catharanthus roseus*, *Azadirachta indica*, *Pongamia pinnata*, *Tagetes erecta* and onion bulb extract and garlic extract at I, 2, 3 and 4% concentrations for radial growth and spore germination of *Colletotrichum capsici*, causing of dieback of Chilli. Extract of garlic bulb at 3% concentration completely inhibited the growth and spore germination of C. *capsici* whereas, 4% extract of onion bulbs, A. *indica* leaf. P. *glabra* leaf and T. *erecta* leaf gave complete inhibition of fungal mycelial growth and spore germination.

Ebenezar and Alice (1996) observed the efficacy of 9 commonly used fungicides in controlling fruit rot and dieback of chilli caused by *Collectotrichum capsici* in Tamil Nadu, India. The best control was achieved with Mancozeb (0.2%) followed by Carbendazim (0.2%) and Copper oxychloride (0.2%).

Acharya and Das (1995) conducted an experiment where foliar spray of Bitertanol (0.05 o/o), Mancozeb (0.2%) Ziram (0.1 o/o) and Bordeaux mixture (0.5%) were found to be effective against the *Collectotrichum capsici* causing anthracnose of Piper betel. A higher cost-benefit ratio was observed for Mancozeb and Bordeaux mixture.

Rahman *et al.* (1994) concluded experiment using Tilt 250 EC, Pencozeb 80 WP, Topsin M 70 WP, Knowin 50 WP and Fungi-kill 50 WP against *Colletotrichum lindemuthianum* causing anthracnose of country bean. Among the fungicide tested Knowin 50 WP was found to be the best followed by Tilt 250 EC and Topsin 70 WP both in *in vitro* and in vivo control of the *Colletotrichum lindemuthianum*. Tilt 250 EC was found to be the best followed by Knowin 50WP both in *in vitro* and in vivo condition in controlling *Colletotrichum dematium*.

Srivastava and Soni (1993) reported that 0.1% Bavistin and 0.25% Dithan M-45 (mancozeb) were effective fungicides for the control of the anthracnose disease of chilli under laboratory and field conditions.

Biswas (1992) conducted that *Colletotrichum capsici* on *Capsicum annum*, where the best control was given by Bavistin (Carbendazim) at 0.1%, applied once in the nursery bed before transplanting and again 1 month and 2 months after transplanting. The best control was showed by Bavistin (carbendazim) at 0.1% when six fungicides were evaluated against C. *capsici* on chilli in field trials.

Datar *et al.* (1990) reported that fungicidal control of anthracnose of chilli in field trials over three seasons, 1984-87, three sprays of 0.25% mancozeb, fortnightly from one month after transplanting gave the best control of *Colletotrichum capsici* on the susceptible *Capsicum annum* cv. Jwala, with highest yields and lowest incidence of fruit rot.

Raj *et al.* (1990) reported that seed treatment with thiram + carbendazim followed by 3 sprays of carbendazim gave minimum disease incidence of anthracnose of bean (*Colletotrichum capsici*) and maximum yield per plot. Seed treatment and 3 sprays of mancozeb also increased yield and reduced disease incidence.

Sinha (1990) conducted field trials during 1984-87, with 7 commonly available fungicides where the best control of *Colletotrichum capsici* on *Capsicum annum* was given by Foltaf (Captafol). The best cost-benefit ratios were obtained with Dithane M-45 (Mancozeb) and Blitox 50 (Copper oxychloride) and these were recommended for the control of this disease in Bihar.

Hossain (1989) observed that, Topsin M, Rovral 50WP and Rovral-F low, completely inhibited the growth of *Colletotrichum gloeosporioides* of guava anthracnose. When applied on plants Topsin M significantly reduced fruit infection and disease severity followed by Rovral-Flow, Rovral 50 WP and Dithane M-45.

Perene and Joi (1989) reported that the treatment of chilli seeds with Thiram or Bavistin and fungicidal spray of seedlings with Dithane M-45, Blitox, carbendazim were more effective than either of the single treatments in controlling *Colletotrichum capsici*. The incidence of the disease was least after seed treatment with Bavistin and spraying with Dithane M-45. Fruit rot was less with combination effect of seed treatment with Thiram and spraying with Mancozeb.

Eswaramurthy *et al.* (1988) found that the best control of *Colletotrichum capsici* on chilli was given by Foltaf (Captafol) at 0.2%, followed by Fytolan (Copper oxychloride) at 0.25% and Bavistin (Carbendazim) at 0.l%. Those compounds also gave good control of the die-back phase of the disease and increased yields.

Setty *et al.* (l988) treated chilli seeds with Emisan gave effective control of *Aspergillus, Colletotrichum* and *Rhizopus* spp. and improved germination of *Capsicum annum* seeds which have to be stored during the wet and humid months.

Mali and Joi (1987) tested seven fungicides against seed mycoflora of chilli and found the most effective result against colony growth and sporulation of *Colletotrichum capsici* by using Vitavax (Carboxin).

Ahmed (1986) recommended that fungicides like Delsan (0.2%) and Cuman-L (0.5%) against anthracnose of chili caused by *Colletotrichum capsici*; Bordeaux mixture against anthracnose of betel vine caused by *Colletotrichum capsici*; Dithane M-45 (0.2o/o) against anthracnose of mango caused by C. *gloeosporioides* and Copper oxychloride (0.3%) or Dithane M-45 (0.2%) against anthracnose of guava for the management of the diseases respectively.

Akanda and Fakir (1985) evaluated Vitavax-200 (0.2%) was recommended for seed treatment to control three major seed borne pathogens of jute including *Colletotrichum chorcori* out of nine fungicides tested.

Anon. (1985-2005) evaluated the fungicides namely, Indofil, Dithane M-45, Topsin M and Tilt 250 EC for controlling the anthracnose of chilli. Data

indicated that incidence of anthracnose significantly lower in Topsin M treated plot which was significantly similar to Indofil sprayed plot. Tilt 250 EC and Dithane M-45 were also gave effectively control against anthracnose of chilli.

Raju and Rao (1985) applied Dithane M-45 (Mancozeb), to control *Colletotrichum capsici* on Capsicum, was compatible with 6 different insecticides that effectively control of fruit rot and 3 insect pests was given by 6 rounds of combined applications of Mancozeb + Monocrotophos at 15 days interval.

Bhuiyan and Fakir (1982) obserbed that about 90% control of *Colletotrichum dematium* and *Macrophomina phaseolina* when treated the soybean seeds with Captan (0.5%), Homai 80 WP (0.25%), Topsin M 70 WP (0.5%) and Vitavax-200 (0.35%) while all these fungicides controlled 70 to 95% seedling diseases caused by the two fungi in pot experiment. The found Homai 80 WP and Captan as the best fungicide to control two major seed borne pathogen of soybean.

Juangbhanich and China (1975) got that seed treatment with Ceresan (Ethoxyethyl mercury) at 0.16% or Desline (Methyl 2-benzimidazole carbamate) at 0.8% of seed wt gave complete control of *Colletotrichum piperatum* and *Colletotrichum capsici*.

Ragozzino and Travaglini (1970) reported that two fungi like *Colletotrichum piperatum* and *Colletotrichum capsici* were responsible for anthracnose disease. The first one was more common and virulent, especially in wet years and affected the ripe as well as unripe fruits. It occasionally caused leaf lesion. Control measures included seed disinfection and the use of Zineb and Maneb in the field. Immersion of seed for 10 minutes in water at 51°C also gave good results and did not affect germination.

2.4 Seed health prevalence

Welideniya *et al.* (2019) identified the different species of *Colletotrichum* that infect capsicum pepper, and the nature of this infection by employing standard tests which was isolated from the capsicum pepper seed samples collected from

three different agro ecological regions in Sri Lanka. It found that *Colletotrichum capsici* and *Colletotrichum gloeosporioides* can invade the important parts of the seeds internally and externally, causing higher germination losses during both the pre and post emergence stages of capsicum pepper; the *Aspergillus* sp. and *Fusarium* sp. were only found seed borne externally.

Chauhan *et al.* (2018) conducted experiment *in vitro* where occurrence of seed borne pathogens in chilli was carried out by two different methods viz., Standard blotter paper method and Potato Dextrose Agar (PDA) method. In standard blotter paper method *Aspergillus niger* was found dominant fungus in sterilized and unsterilized seeds & in PDA method percent disease incidence was not recorded in sterilized seeds by *A. niger*, *Aspergillus flavus, Fusarium* sp., *Rhizopus* sp., *Colletotrichum capsici* and *Penicillium* sp. while maximum percent disease incidence *A. niger* was found in unsterilized seeds.

Karim *et al.* (2017) found that prevalence of the total as well as the individual seed-borne fungal infections recorded in jhum morich significantly varied with respect to sources of seed collection. Nine different fungi in jhum morich seeds, in order of prevalence, were- *Penicillium sp., Fusarium sp., Alternaria alternata, Colletotrichum capsici, Doratomyces sp., Curvularia lunata, Alternaria solani, Aspergillus candidus, Cercospora capsici* varied significantly depending on the seed sources.

Alam *et al.* (2014) found five different seed borne fungi viz. *Colletotrichum capsici, Curvularia lunata, Rhizopus stolonifer, Aspergillus flavus* and *Fusarium moniliforme* were associated with the tested chili seed samples. Among these fungi, *Curvularia lunata* was the most prevalent one which was followed by *Rhizopus stolonifer, Colletotrichum capsici, Fusarium moniliforme* and *Aspergillus flavus.*

Hemannavar *et al.* (2009) investigated anthracnose of chilli collected from different chilli growing districts of northern Karnataka. They found from the chilli seed samples, fungi viz., *Colletotrichum capsici,* species of *Cercospora,*

Alternaria, *Penicillium* and *Aspergillus*. *C. capsici* was the most predominant fungi encountered (71.24%).

Telang, S. M. (2010) found eighteen fungi from the seeds of Chilli varieties, Pusa Jwala, Phule jyoti and Guntoor-4. More incidence of mycoflora was recorded on agar plates than on blotters. *Aspergillus flavus, Aspergillus niger*, *Rhizopus nigricans, Alternaria tenuis, Rhizopus stolonifer* and *Curvularia lunata* were the common and dominant seed borne fungi. Rest of other pathogens were *Alternaria alternata, A. solani, Aspergillus fumigatus, Fusarium moniliforme, F. solani, Rhizoctonia solani, Penicillium digitatum, Chaetomium sp., Pythium debaryanum, Phytopthora capsici, Cladosporium herbarum* and *Helminthosporium speciferum* were common seed borne fungi.

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

The experiment was carried out in the Central Farm of Sher-e-Bangla Agricultural University, Dhaka. During the period of November 2019 to May 2020 for the management of anthracnose of chilli through fungicides, biological agent and plant extract. The details of the materials and methods of this research work have been described in this chapter under the following headings and sub-headings:

3.1 Experimental sites

The *in vivo* experiment was conducted in the Central Farm of Sher-e-Bangla Agricultural University (SAU), Dhaka. The experimental field is located at the $23^0$74 N latitude and $90^0$35 E longitude with an elevation of 8.2 m. from sea level. The *in vitro* experiment was conducted in the laboratory of the department of Plant Pathology, SAU (Appendix 1).

3.2 Experimental period

The experiment was carried out during the Rabi season from November 2019 to May 2020. Seeds were sown on 17th November, 2019 and were harvested on 22-25th May 2020.

3.3 Soil type

The soil of the experimental site belongs to the Agro-ecological region of "Madhupur Tract" (AEZ No.: 28). It was Deep Red brown Terrace soil and belongs to "Nodda" cultivated series. The top soil is clay loam in texture. Organic matter content was very low (0.82%) and soil pH varied from 5.47-5.63. The information about AEZ 28 is given in (Appendix 2).

3.4 Weather

The monthly mean of daily maximum, minimum and average temperature, relative humidity, monthly total rainfall and sunshine hours received at the experimental site during the period of the study have been collected from

Bangladesh Meteorological Department, Agargaon, Dhaka and presented in (Appendix 4)

3.5 Experimental design and layout

The experimental plots were arranged in Randomized Complete Block Design (RCBD) with (3) replications (Appendix 3).

The experiment details were given bellow:

- Total plot area : 200 m^2
- Number of plot : 24
- Plot size : 4 m^2
- Block to block distance : 1.0 m
- Plot to boundary distance : 0.5 m
- Plot to plot distance (Lengthwise) : 0.7 m
- Plot to plot distance (breath wise) : 0.5 m
- Plant to plant spacing : 0.4 m

3.6 Multiple treatment experiments

Multiple treatments were applied in the experiments. Altogether treatments were applied comprising different number of sprays as follows.

T_0 = Control (No field spraying)

T_1 = Field spraying with Tilt 250 EC @ 0.05%

T_2 = Field spraying with Ridomil Gold 68 WG @ 0.5%

T3 = Field spraying with Dithene M-45 @ 0.44%

T_4 = Field spraying with Score 250 EC @ 0.05%

T_5 = Field spraying with *Trichoderma harzianum* @ 0.3%

T_6 = Field spraying with Neem leaf extract 1:2 (W/V)

T_7 = Field spraying with Allamanda leaf extract 1:2 (W/V)

3.7 Variety Selection:

Chilli (*Capsicum annuum* L.) variety (BARI Morich-3) was used in the experiment.

3.8 Collection of Chilli seed:

The seed were collected from Regional Spice Center, Bangladesh Agricultural Research Institute (BARI) at Joydebpur Gazipur.

3.9 Seed Treatment:

Seeds of chilli were treated before sowing. Seeds were soaked or dipped for 12 hours in fresh water. Seeds were dipped into the respective solution separately for seed treatment. Seeds were soaked for five minutes. After soaking, seeds were transferred to a newspaper sheet for drying. Then it was ready for sowing in the seedbed (Plate 1).

3.10 Raising of seedlings:

Seedlings were raised in Farm's seedbed of Sher-e-Bangla Agricultural University with proper care and management. The seedbed was prepared by mixing of Urea, TSP, MP and well decomposed cow dung. The seed was sown in seedbed and covered with soil. The seeds were sown on 17 November, 2019. Watering was done to maintain the soil moisture. Shade was provided to save the young and delicate seedling from heavy showering and scorching sunlight.

3.11 Land Preparation:

The experimental field was ploughed with power tiller drawn rotovator. After ploughing the field, it was left to nature for 10 days for sun and nature to work upon. Subsequent cross ploughing was done followed by laddering to make the land level. Then the soil clods were broken by a wooden hammer and all weeds, stubbles and residues were removed from the field. Later, Krishibid Organic Manure and chemical fertilizer like Urea, Triple Super Phosphate (TSP) and Muriate of Potash (MP) was mixed with soil during final land preparation. Finally, the land was properly leveled before seed sowing. and plots were prepared as per the Experimental design (Appendix 3 and Plate 1).

3.12 Application of Fertilizers:

The experimental field was fertilized with Nitrogen (in the form of Urea), Phosphorus (in the form of Triple Super Phosphate -TSP), Potassium (in the form

of Muriate of Potash -MP), Gypsum. As per the treatment, whole quantity of TSP, MP, Gypsum and one fourth of Urea were applied at final plot preparation. The rest third fourth Urea and Gypsum were applied later in three installments on (25, 50 and 70 days after transplanting). Fertilizer was applied as per recommended doses (BARI). Applied doses were as follows:

Table 1: Doses of chemical fertilizers

Name of the nutrient element	Name of the Fertilizer	Fertilizer dose (kg/ha)	Fertilizer applied during final land preparation (kg/200 m^2 land)	Rest installments (Urea) (kg/200 m^2 land)		
				1st	2nd	3rd
N	Urea	210	----	1.4	1.4	1.4
P	TSP	330	6.6	---	---	---
K	MP	200	1.3	0.9	0.9	0.9
S	Gypsum	110	2.2	---	---	---
Manure	Krisibid Organic Manure	1000	20	---	---	---

Ref. Bangladesh Agricultural Research Institute

3.13 Transplanting of seedlings convert:

The healthy seedlings were selected for transplanting in experimental plots. The seedlings were transplanted maintaining row to row distance 0.4m and plant to plant distance 0.4m. Healthy and uniform sized seedling of 35 days old were uprooted from the seedbed in the treatment wise by avoiding any injury to the roots and transplanted on 22th December 2019 in main field. Sixteen seedlings were transplanted in each plot in the afternoon. For easy lifting of the seedlings from the seedbed moistened enough before uprooting.

3.14 Intercultural operation

3.14.1 Irrigation

Watering was done immediately after transplanting the seedlings and continued up to keep the soil moisture in suitable condition as per requirement of the land

with regular intervals. Irrigation was continued up to harvesting of crop. Water cane with perforated mouth piece was used for soft discharged of water.

3.14.2 Weeding

Weeding was done when required to keep the crop free from weeds, for better soil aeration and conserve soil moisture. The common weeds were *Cynodon dactylon* (Durba grass), *Cyperus rotundus* (Mutha) etc. Weeding was done carefully keeping the delicate plants undisturbed.

3.14.3 Gap filling

Gap filling was done when any seedling failed to be established and died in the field. More than 60 seedlings were re-transplanted to fill the gaps.

3.15 Application of fungicides solution

The spray solution was prepared by mixing with required amount of doses with tap water. The experiment was monitored regularly to observe the on-set of anthracnose disease. Sprays were applied at 60 days after transplanting. Every time the treatments were freshly prepared prior to application and the spray tank was thoroughly cleaned before filling with another treatments. All together 4 sprays were done with 15 days intervals. Adequate precaution was taken to avoid drifting of spray materials from one plot to neighboring ones. Fungicides was presented in (Plate 2).

Table 2: Fungicide used in the experiment

Treatments with Common name	**Chemical name**	**Active ingredients**	**Doses used**
Tilt 250 EC	Propiconazole	Propiconazole 25%	0.5 ml/litre
Ridomil Gold 68 WG	Mencozeb+Metalixil	4% Mencozeb +68% Metalixil	5 g/litre
Dithane M-45	Manganous ethylene bisdilhio - carbamate	Dithiocarbarnate 80%	4.4 g/litre
Score 250 EC	Difenoconazole	Difenoconazole 25%	0.5 ml/litre

3.16 Collection and preparation of plant extract solution

Two plants extract namely Allamanda leaf, Neem leaf were used in this study (Plate 2). For extraction of juice, required amount of respective parts of each pant parts were taken, washed in tap water and crushed in a mortar and pestle. The crushed materials were blended in an electric blender and the blended materials were filtered. The supernatant was diluted in equal amount of sterile water for 1:2 solutions. The extract was shaken thoroughly before use.

Table 3: Plant extract used in the experiment

Common Name	Scientific name	Plant parts used	Ratio
Neem	*Azadirachta indica*	Leaf	1:2
Allamanda	*Allamanda cathartica*	Leaf	1:2

3.17 Tagging and data collection:

Randomly five plants were selected from each plot for determining of leaf infection, leaf area diseased, fruit infection and fruit area diseased of each treatment in the experimental plot.

3.18 Isolation and identification of pathogen

Infected leaves, twigs and fruits were collected for Isolation of the fungi from experimental plot. Cut the pieces of infected sample and surface sterilized in Chlorox (10%) solution for few seconds and washed three times in sterile water. After washing, the cut pieces were placed on sterilized blotter paper (Whatman No.1) in petridish and also placed onto the PDA plates, incubated at $25^0C \pm 2^0C$ under 12 hours light alternating with 12 hours dark. Five to seven days after incubation the fungal culture were studied under stereoscope (Model: Motic, SMZ-168) and compound microscope (Model: Omano, OMTM-85) for identification of the desired pathogens. The fungus was identified following the appropriate keys (Kulsherestha *et al.,* 1976 and Sulton, 1980).

3.19 Preparation of culture medium, purification and identification of the organism

Potato Dextrose Agar (PDA) was used in this experiment. At first potato extract was taken and boiled 100 g potato slice in 500 ml water. Then 10g agar and 10g dextrose was dissolved in potato extract. The PDA medium was autoclaved at121^{o}C under 15 psi for 30 minutes. After autoclaving the medium was kept few minutes for cooling and added 50-60 drops of lactic acid. About 15-20 ml medium was poured into each sterile petridish for growth of the fungal colony. Pure culture of the organism was prepared by means of hyphal tip culture method aseptically and repetition of sub-culture into PDA to at least 3-5 times. Colony characters, mycelial growth, color and sporulation of the fungi were studied and identified the organism following the methods of Singh (1982).

3.20 Collection of data

The following parameters were considered for data collection.

- **Disease incidence and severity**
  - a. Percent leaf infection
  - b. Percent leaf area diseased (% LAD)
  - c. Percent fruit infection
  - d. Percent fruit area diseased (% FAD)
- **Yield and yield contributing characters**
  - a. Plant height (cm)
  - b. Yield (t/ha)
  - c. Dry weight of 50 chilli fruit (gm)
  - d. 1000-seed weight (gm)
- **Seed health test**
  - a. Percent seed germination and seed infection.
  - b. Prevalence of seed borne pathogen.

3.21.1 Percent leaf infection

Five plants per plot were selected and tagged for collection of data. Data on percent leaf infection were recorded at 60 days after transplanting by visual observation of symptoms. Percent leaf infection was calculated by the following formula.

$$\% \text{ Leaf infection} = \frac{\text{Number of infected leaf}}{\text{Number of total inspected leaf}} \times 100$$

3.21.2 Percent leaf area diseased

Data on percent leaf area diseased (LAD) were recorded at 60 days after transplanting by visual observation of symptoms. Percent leaf area diseased was calculated by the following formula.

$$\% \text{ Leaf area diseased} = \frac{\text{Leaf area infected}}{\text{Total leaf area inspected}} \times 100$$

3.21.3 Percent fruit infection

Data on percent fruit infection were recorded at 90 days after transplanting by visual observation of symptoms. Percent fruit infection was calculated by the following formula.

$$\% \text{ Fruit infection} = \frac{\text{Number of infected fruit}}{\text{Number of total inspected fruit}} \times 100$$

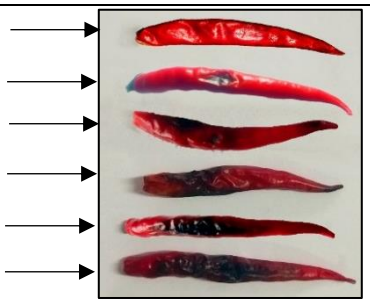
3.21.4 Percent fruit area diseased

Data on percent fruit area diseased were recorded at 90 days after transplanting by visual observation of symptoms. Percent fruit area diseased was calculated by the following formula.

$$\% \text{ Fruit infection diseased} = \frac{\text{Fruit area infected}}{\text{Total fruit area inspected}} \times 100$$

3.21.5 Disease severity scale:

Using "0-5" scale (Horsfall and Barratt, 1945) we calculated the disease severity. "0-5" scale is given bellow-

Grade/Rating	% Area Infected	Area Infection Photo
0	0	
1	0.1 - 5.0 % Area Infected	
2	5.1 - 12.0 % Area Infected	
3	12.1 - 25.0 % Area Infected	
4	25.1 - 50.0 % Area Infected	
5	>50.0 % Area Infected	

3.22 Yield of chilli fruit

After vegetative growth chilli plant continues to produce flower and fruit for 3 to 4 months. But all the fruits are not matured and ripen at a time. Weight of fresh and ripe fruits per treatment was recorded at the time of every harvest. Harvesting was done with four times. The weight of fruits per plot was recorded and converted into ton per hectare yield.

3.23 Testing of Seed health and transmission of anthracnose pathogens

Seed health study was done by blotter method following the procedure of ISTA to examine *Colletotrichum capsici* and others seed borne & air borne pathogen in the harvested seed. Seeds were placed on three layers of moist blotting paper (Whatman no.1) contained in petridishes. In each petridish, 25 seeds were placed in petridish for 400 seeds of each treatment. All the plates with seeds were incubated at room temperature (25+/-2^{o}C) under 12 hours cycle of alternate Near Ultra Violet light and darkness for 7 days. (ISTA., 1976). Watering was done as and when required. After 7-10 days of incubation the plates were examined under stereo-microscope for detection of fungi. Each individual incubated seed was observed under stereo-microscope at 10X and 25X magnifications in order to record the incidence of *Colletotrichum capsici* and other pathogen. Most of the associated fungi were detected by observing their growth characters on the incubated seeds on blotting paper. For proper identification of fungi temporary

slides were prepared from fungal colony and observed under compound microscope (Ram Nath *et al.*, 1970).

The fungi were identified to species level wherever possible. The germination and seed infection occurred by incidence of *Colletotrichum capsici* and others air borne and seed borne pathogen were recorded.

3.24 Experimental Design and Statistical analysis:

The collected data for different parameters were compiled and tabulated in proper form. Appropriate statistical analysis was made by MSTAT computer package program. The treatment means were compared by Duncan's Multiple Range Test (DMRT). Statistical program ‘Stat 10’ was used for analysis of the experimental data.

Plate 1: Different events in field experiment

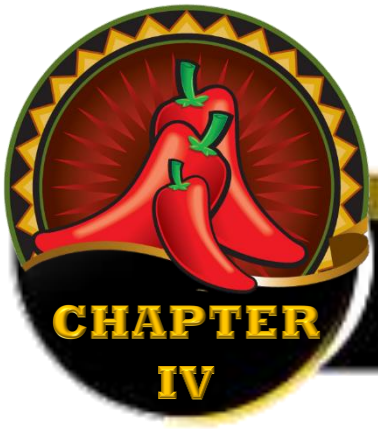
A: Fertilizer mixing at *in vivo* experimental plot
B: Final land preparation
C: Seed sowing in seedbed
D: Raising of seedlings

Plate 2: Treatment used in the experiment

A: Different chemical fungicides

B: Biological agent *Trichoderma harzianum*

C: Neem and Allamanda leaf extract



RESULTS

CHAPTER IV
RESULTS

4.1 Identified symptoms of anthracnose disease of chilli in the experimental plot

The symptoms of anthracnose disease appeared on leaves as small and irregular yellow, brown, dark brown or black spots (Plate 3). However, symptoms on leaves are often rare, even in the field. When the fruit came out and getting mature, small, circular and masses of spores covered the sunken lesions with black margins appeared on the ripen fruits surface (Plate 4). The disease appeared on ripen fruits and therefore sometimes the disease is called "Ripe fruit rot". Sometimes dark acervuli appeared on the affected parts when it gets favorable condition and dropped down prematurely resulting high yield losses.

4.2 Isolation and identification of the organism of anthracnose diseases of chilli

Firstly, infected fruits were collected for isolation of the causal fungus from diseased field. Infected sample cut down into pieces and surface sterilized in Chlorox (10%) solution for few seconds and washed three times in sterile water. After washing, the cut pieces were placed on sterilized blotter paper (Whatman No.1) in petridish incubated at 25^{o}C ± 2^{o}C under 12 hours light alternating with 12 hours dark (Plate 3). Five to seven days after incubation the fungal culture were studied under stereoscope and compound microscope and identified the pathogen. The pathogen, *Colletotrichum capsici* was identified based on the asexual reproductive structure under compound microscope. Acervuli were disc shaped. waxy, sub epidermal, typically with dark needle like septate setae, conidiophore short, simple, conidia hyaline, one celled, ovoid or oblong. In the PDA media, pure culture of the organism was observed and identified as *Colletotrichum capsici* based on colony characters, mycelial growth, color and sporulation of the fungi (Plate 5).

4.3 Effect of different fungicides and plant extract on disease incidence and severity of anthracnose disease of chilli

4.3.1 Effect of different treatments on % Leaf infection and % Leaf Area Diseased (LAD) of anthracnose disease of chilli

Effect of different fungicides on percent leaf infection and percent leaf area diseased (%LAD) are presented in Table 4. The lowest leaf infection (4.31%) of anthracnose of chilli was recorded in case of foliar application of Tilt 250 EC (0.05%) preceded by Dithane M-45 (5.33%), Ridomil Gold 68 WG (5.56%) and Score 250 EC (5.92%). The highest percent leaf infection was recorded in control (16.10%) followed by *Trichoderma harzianum* (6.52%), Allamanda leaf extract (6.25%) and Neem leaf extract (6.13%).

In respect of leaf area diseased, the treatment effect varied significantly compared to control. Among the treatments, Tilt 250 EC also showed the best performance in reducing the leaf area diseased (LAD). The lowest percent of leaf area diseased (4.16%) was noted in case of foliar application of Tilt 250 EC preceded by Allamanda leaf extract (4.88%), Score 250 EC (%.10%), Dithane M-45 (5.21%), Ridomil Gold 68 WG (5.44%), Neem leaf extract (5.62%) and *Trichoderma harzianum* (6.23%) respectively. The highest percent leaf area diseased was recorded in control (15.37%).

Table 4: Effect of spraying different treatments on % Leaf infection and % Leaf Area Diseased (LAD) due to anthracnose disease of chilli

Treatment	% Leaf infection	% LAD
T_0=Control	16.10 a	15.37 a
T_1=Tilt 250 EC	4.31 c	4.16 d
T_2=Ridomil Gold 68 WG	5.56 bc	5.44 bc
T_3=Dithane M-45	5.33 bc	5.21 bcd
T_4=Score 250 EC	5.92 b	5.10 bcd
T_5=*Trichoderma harzianum*	6.52 b	6.23 b
T_6=Neem leaf extract	6.13 b	5.62 bc
T_7=Allamanda leaf extract	6.25 b	4.88 cd
LSD (0.05)	1.48	1.15
CV %	12.07	10.10

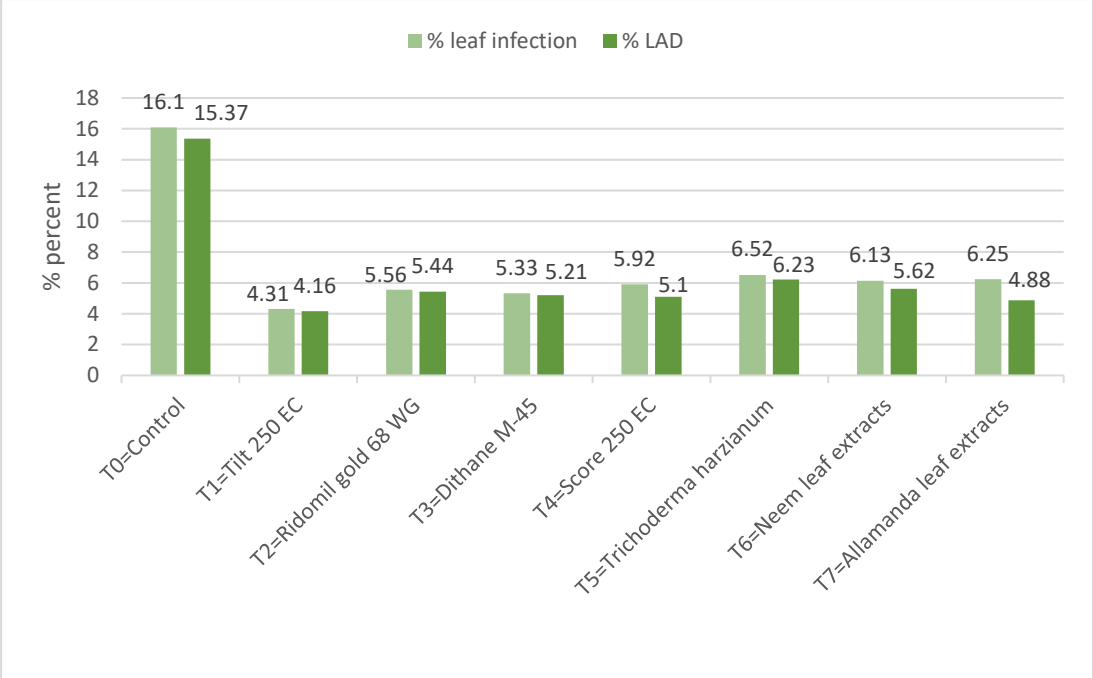


Fig 1: Effect of using different treatments on % Leaf infection and % Leaf Area Diseased (LAD) due to anthracnose disease of chilli

4.3.2 Effect of different treatments on % Fruit infection and % Fruit Area Diseased (FAD) of anthracnose disease of chilli

Effect of different fungicides and plant extract on percent fruit infection and percent fruit area diseased were found statistically significant (Table 5). The lowest percent of fruit infection was observed in Tilt 250 EC (3.93%) and the highest percent of fruit infection was observed in control (32.4%). The second highest percent fruit infection was recorded in Allamanda leaf extract (7.18%) which was statistically similar to that of Neem leaf extract (6.91%) followed by *Trichoderma harzianum* (6.27%), Ridomil Gold 68 WG (5.92%) and Score 250 EC (5.23%), respectively. Dithane M-45 (4.95%) also gave good result in percent fruit infection next to Tilt 250 EC (3.93%).

In case of percent Fruit Area Diseased (FAD), the lowest % FAD was recorded in case of Tilt 250 EC (3.55%) and the highest % FAD (7.07%) was recorded in Allamanda leaf extract. The second highest % FAD (6.63%) was noted in Neem leaf extract that was statistically quite similar to *Trichoderma harzianum* (5.95%), Ridomil Gold 68 WG (5.65%), Score 250 EC (4.77%) and Dithane M-

45 (4.41%). The Allamanda leaf extract also showed better performance in reducing FAD in comparison to control.

Table 5: Effect of different treatments on % Fruit infection and % Fruit Area Diseased (FAD) due to anthracnose disease of chilli

Treatment	% Fruit infection	% FAD
T_0=Control	32.4 a	31.32 a
T_1=Tilt 250 EC	3.93 c	3.55 d
T_2=Ridomil Gold 68 WG	5.92 bc	5.65 bcd
T_3=Dithane M-45	4.95 bc	4.41 cd
T_4=Score 250 EC	5.23 bc	4.77 bcd
T_5=*Trichoderma harzianum*	6.27 bc	5.95 bcd
T_6=Neem leaf extract	6.91 b	6.63 bc
T_7=Allamanda leaf extract	7.18 b	7.07 b
LSD (0.05)	2.97	2.58
CV %	18.67	17.04

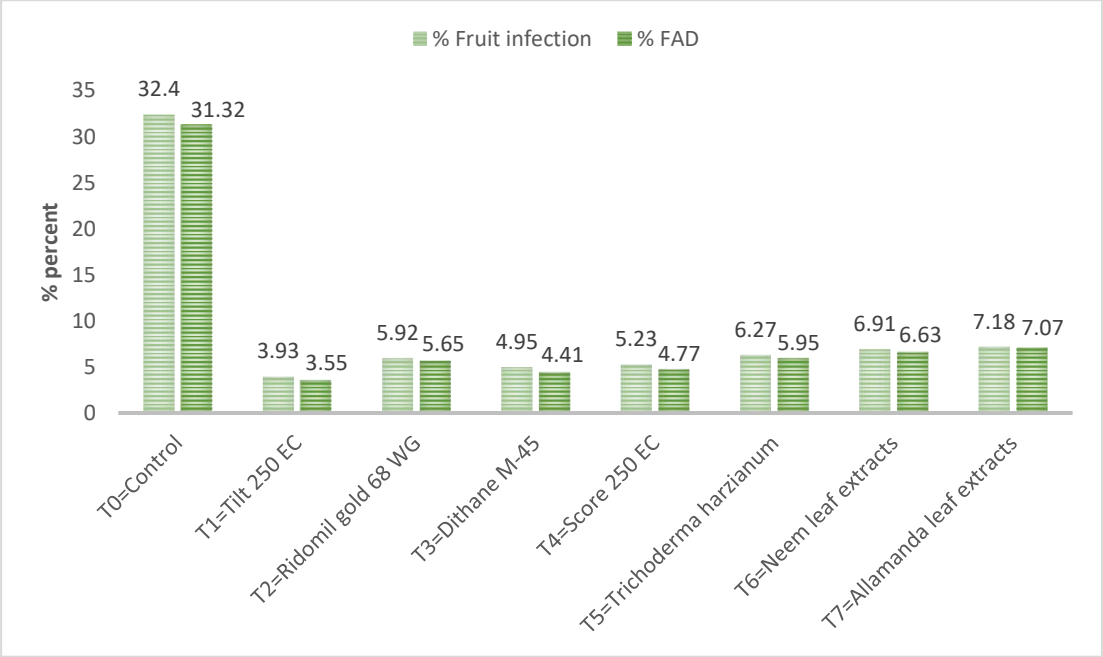


Fig 2: Effect of using different treatments on % Fruit infection and % Fruit Area Diseased (FAD) due to anthracnose disease of chilli

4.4 Effect of different fungicides and plant extract on fruit yield and yield contributing characters against anthracnose of chilli

4.4.1 Plant height

Effect of different treatments on plant height of chilli after 90 days of transplant was presented in table 6. The treatment effects were found significant with some extents. The highest performance was recorded in case of Tilt 250 EC (42.6 cm) followed by Neem leaf extract (41.23 cm), Allamanda leaf extract (41.6 cm), *Trichoderma harzianum* (41.2 cm), Dithane M-45 (41.17 cm), Score 250 EC (40.6 cm) and Ridomil Gold 68 WG (40.5 cm). The lowest plant height was recorded in control (35.7 cm).

4.4.2 Yield

Effect of different treatments on yield of chilli was measured and presented in table 6. The effect of different fungicides and plant extract on fruit yield of chilli against anthracnose was varied significantly. The highest yield (10.23 t/ha) was recorded in the plot treated with Tilt 250 EC. The lowest yield was recorded in control plot (5.42 t/ha) preceded by *Trichoderma harzianum* (7.21 t/ha). Treatments Dithane M-45 (8.84 t/ha), Ridomil Gold 68 WG (7.9 t/ha) and Score 250 EC (7.67 t/ha) also showed better performance in terms of fruit yield against anthracnose disease of chilli. Among plant extract, Neem leaf extract gave good yield (8.04 t/ha) against anthracnose disease which was even better than Ridomil Gold 68 WG (7.9 t/ha) and Score 250 EC (7.67 t/ha). Allamanda leaf extract (7.87 t/ha) also showed better performance in fruit yield of chilli against anthracnose disease compared to control.

4.4.3 Dry weight of 50 ripen chilli fruit

The highest dry weight of 50 chilli fruit was recorded in case of the treatment Tilt 250 EC (13.14 gm) and the lowest dry weight was recorded in control (9.53 gm). The other treatments Allamanda leaf extract (11.36 gm), Dithane M-45 (11.27), Neem leaf extract (11.15 gm), Ridomil Gold 68 WG (11.06 gm), Score

250 EC (10.76), *Trichoderma harzianum* (10.22 gm) showed statistically similar effect.

4.4.4 1000 seed weight

Effect of different fungicides and plant extract on 1000 seed weight of chilli against anthracnose was determined and the highest 1000 seed weight was recoded in case of treatment Tilt 250 EC (4.21 gm) and the lowest was recorded in control (3.64 gm). The second highest 1000 seed weight was recorded in treatment Ridomil Gold 68 WG (4.06 gm) followed by Dithane M-45 (3.98 gm). The effect of Allamanda leaf extract (3.96 gm), Neem leaf extract (3.91 gm) were found statistically similar. Score 250 EC (3.81 gm) and *Trichoderma harzianum* (3.72 gm) yielded the lowest 1000 seed weight in the experiment.

Table 6: Effect of different treatments on fruit yield and yield contributing characters due to anthracnose disease of chilli

Treatment	Plant Height (cm)	Yield (t/ha)	Dry weight of 50 ripen chilli fruit (gm)	1000 seed weight (gm)
T_0=Control	35.70 b	5.42 d	9.53 c	3.64 c
T_1=Tilt 250 EC	42.36 a	10.23 a	13.14 a	4.21 a
T_2=Ridomil Gold 68 WG	40.50 a	7.9 bc	11.06 b	4.06 ab
T_3=Dithane M-45	41.17 a	8.84 b	11.27 b	3.98 abc
T_4=Score 250 EC	40.60 a	7.67 bc	10.76 b	3.81 bc
T_5=*Trichoderma harzianum*	41.20 a	7.21 c	10.22 bc	3.72 bc
T_6=Neem leaf extract	41.23 a	8.04 bc	11.15 b	3.91 abc
T_7=Allamanda leaf extract	41.6 a	7.87 bc	11.36 b	3.96 abc
LSD (0.05)	3.77	1.78	1.17	0.39
CV %	5.32	8.51	6.08	5.8

4.5 Seed health status of harvested seeds

4.5.1 Seed germination and Seed infection of harvested chilli seed

Effect of using different treatments on percent seed germination and seed infection on harvested chilli seed was determined and presented in Table 7. The treatment effect on seed germination and seed infection of harvested chilli seeds

were found differ sharply in comparison to control. The highest seed germination was counted in case of Tilt 250 EC (87.5%) followed by Dithane M-45 (83.2%), Allamanda leaf extract (81.5%), Ridomil Gold 68 WG (80.4%) and Neem leaf extract (80.2%). The lowest seed germination (60.5%) was counted in control preceded by *Trichoderma harzianum* (76.7%) and Score 250 EC (79.5%). The trend of seed infection of harvested chilli seed from the experimental plot was found to differ remarkably compared to control. The highest seed infection was counted in control (60.5%) while the lowest seed infection was counted in case of Tilt 250 EC (24.5%) preceded by Dithane M-45 (33.75%), Neem leaf extract (35.5%), Ridomil Gold 68 WG (36.75%) and so on.

Table 7: Effect of different treatments on percent seed germination and seed infection of harvested chilli seed.

Treatment	% Seed germination	No. of seeds infected out of 400 seeds	% seed* infection
T_0 = Control	62.6	242	60.5
T_1 = Tilt 250 EC	87.5	98	24.5
T_2 = Ridomil Gold 68 WG	80.4	147	36.75
T_3 = Dithane M-45	83.2	135	33.75
T_4 = Score 250 EC	79.5	164	41
T_5 = *Trichoderma harzianum*	76.7	178	44.5
T_6 = Neem leaf extract	80.2	142	35.5
T_7 = Allamanda leaf extract	81.5	150	37.5

* No. of seed infections was calculated on the basis of 400 seeds tested as per ISTA Rules.

4.5.2 Frequency and occurrence of *Colletotrichum capsici* and others seed borne pathogen

Occurrence of *Colletotrichum capsici* causing anthracnose disease of chilli and others seed borne pathogen were counted and presented in Table 8. Overall, 1256 seeds out of 3200 seeds have the pathogenic infection by numerous seed borne pathogen like *Colletotrichum capsici, Aspergillus flavus, Aspergillus niger, Rhizopus* sp, Bacterial sp., and unknown pathogen. Out of 1256 infected seeds, *Colletotrichum capsici* was found on 292 seeds while *Aspergillus flavus* was

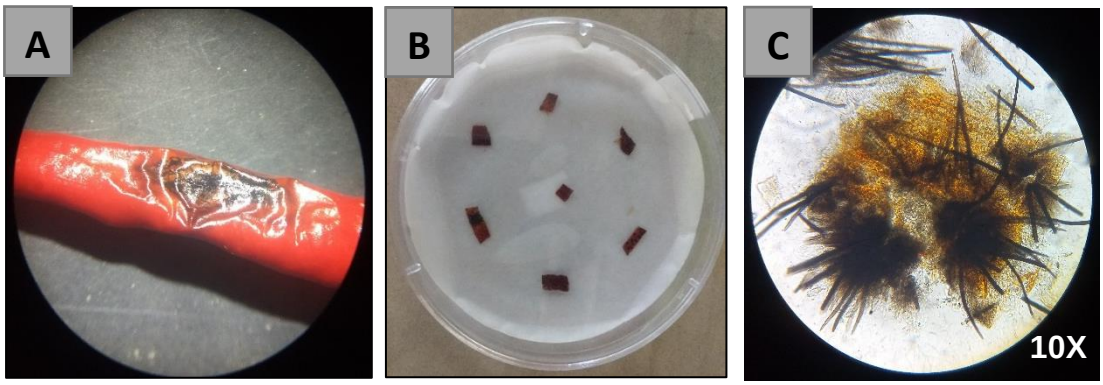
found on 358 seeds, *Aspergillus niger* was found on 345 seeds, *Rhizopus* sp. was found on 160 seeds, Bacterial sp. was found on 47 seeds and unknown pathogen was found on 54 seeds. The highest infection was recorded on *Aspergillus flavus* (28.50%) followed by *Aspergillus niger* (27.46%) *Colletotrichum capsici* (23.25%), *Rhizopus* sp. (12.74%), Unknown pathogen (4.31%) and Bacterial sp. (3.74%) respectively. The seed borne microflora observed on chilli seed under stereo-microscope and compound microscope were presented in Plate 7.

Table 8: Frequency and occurrence of various pathogenic microflora recorded on harvested chilli seeds

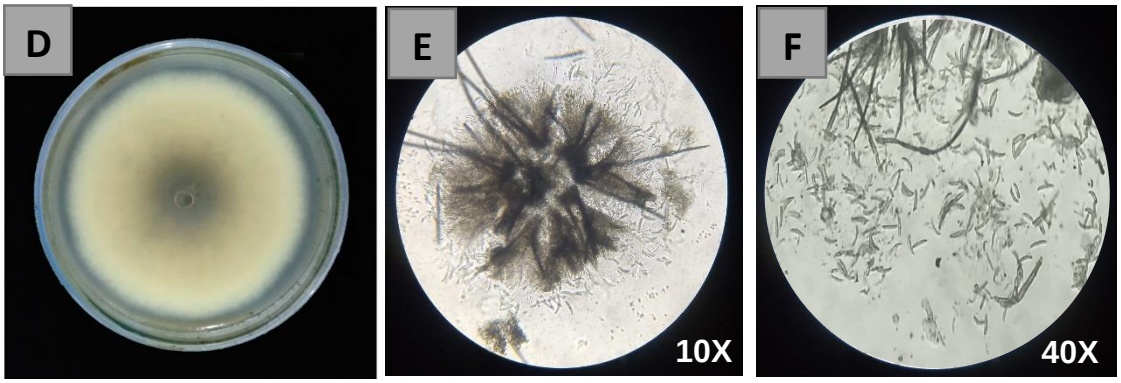
Fungi	No. of pathogenic infection	% of total infection
Colletotrichum capsici	292	23.25
Aspergillus flavus	358	28.50
Aspergillus niger	345	27.46
Rhizopus sp.	160	12.74
Unknown	54	4.31
Bacterial ooze	47	3.74
Total	1256	100

Plate 3: Symptom of anthracnose on chilli leaf showing irregular yellow, brown, dark brown spots

Plate 4: Typical symptom of anthracnose on chilli fruit showing masses of acervuli



Colletotrichum capsici observed from infected fruit part on blotter under compound microscope



Colletotrichum capsici from PDA medium seen under compound microscope

Plate 5: Identification of *Colletotrichum capsici* from infected fruit and PDA culture medium.

A: Infected fruit with masses of ascervuli

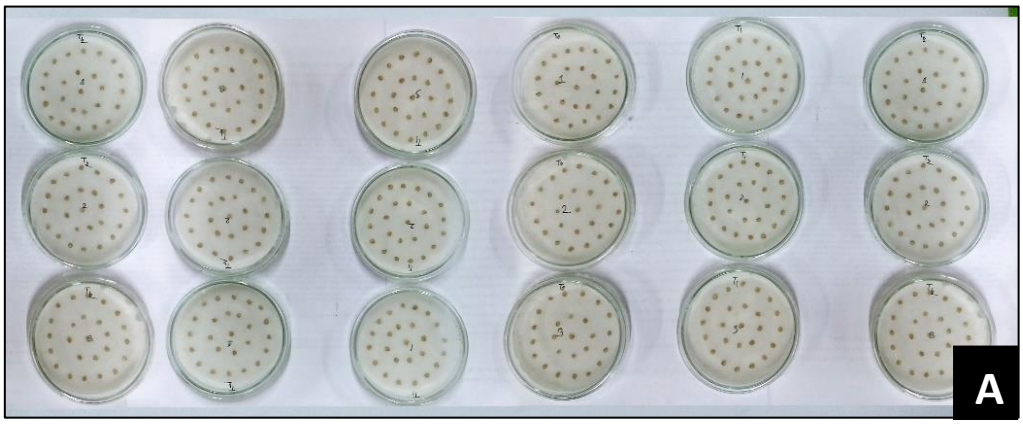
B: Tissue planted from infected chilli fruit on blotter paper

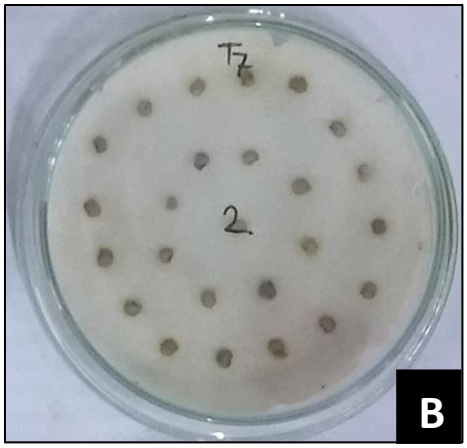
C: Acervuli of *Colletotrichum capsici* observed under compound microscope (10X) grown on blotter paper

D: Pure culture of *Colletotrichum capsici* on PDA medium

E: Ascervulus of *Colletotrichum capsici* seen under compound microscope (10X)

F: Crescent shape conidial masses of *Colletotrichum capsici* seen under compound microscope (40X)





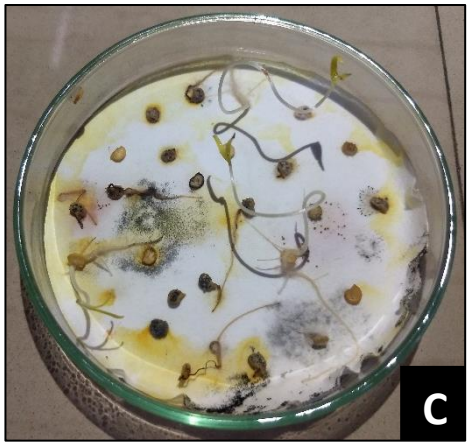


Plate 6: Seed health testing in blotter method

A: 25 seeds per plate and 400 seeds per treatment were tested in blotter method

B: 25 Seeds placed for seed health testing

C: Germinated and infected seeds on blotter paper

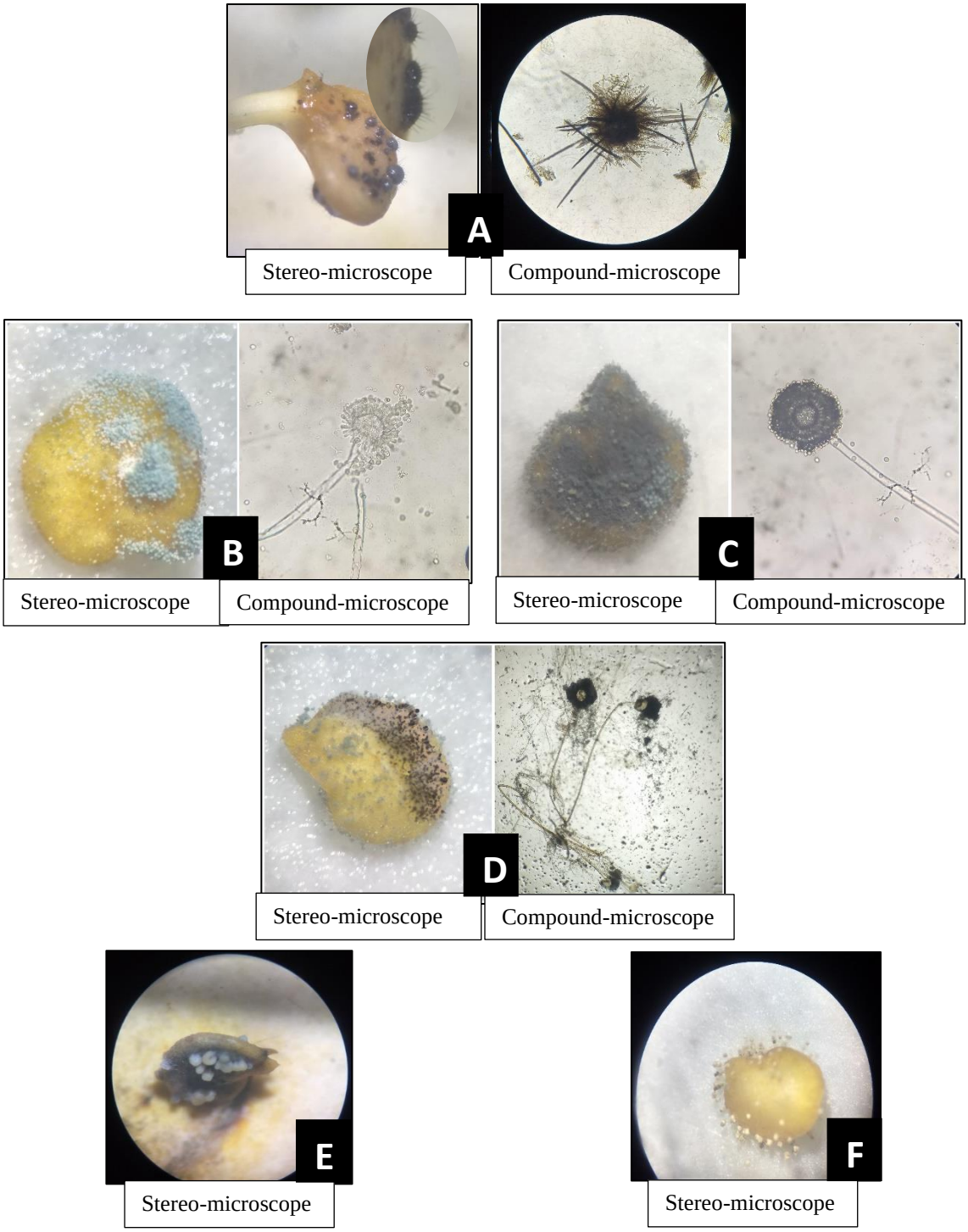


Plate 7: Identified seed borne pathogen of infected chilli seed under stereo-microscope and compound microscope

A: *Colletotrichum capsici*
B: *Aspergillus flavus*
C: *Aspergillus nigar*
D: *Rhizopus* sp
E: Bacterial sp.
F: Unknown pathogen

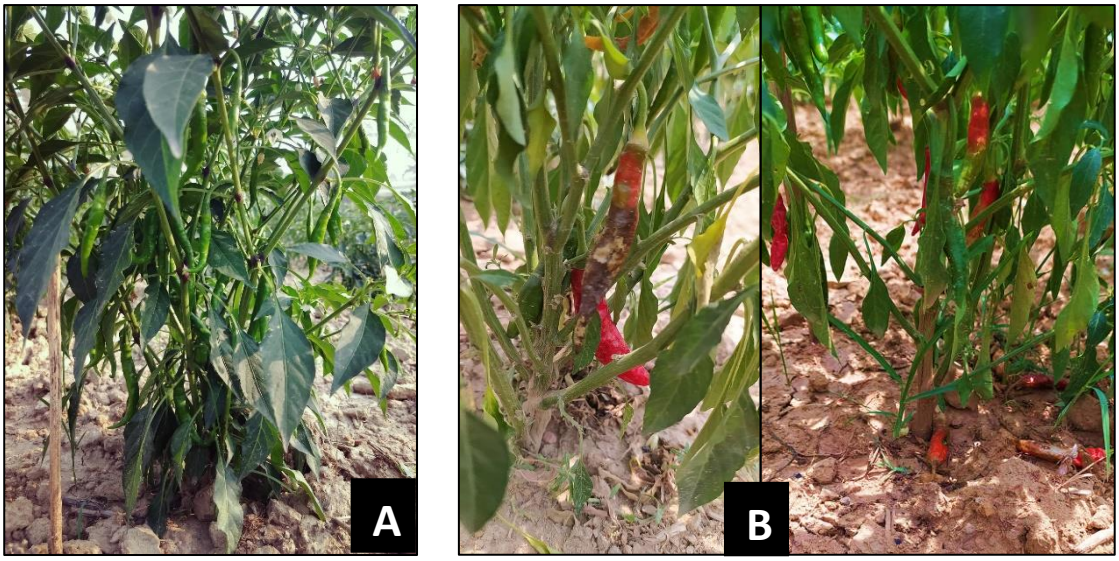


Plate 8: Healthy and infected plants in experimental plot

A: Healthy Plant
B: Infected Plant
C: Field view of the experimental plots


CHAPTER
V
DISCUSSION

CHAPTER V
DISCUSSION

Colletotrichum spp. has been rated as the most notorious pathogens in the world, causing severe crop losses worldwide (Dean *et al.*, 2012). The species under this genus are reported to cause anthracnose disease in more than 121 plant genera from 45 different plant families (Farr *et al.*, 2016). *Colletotrichum capsici* was identified based on the asexual reproductive structures acervuli seen under compound microscope. Acervuli were disc shaped, waxy, sub epidermal, typically with dark needle like septate setae; short conidiophore, hyaline, single celled, ovoid or oblong conidia (Kulsherestha *et al*., 1976 and Sulton, 1980). There are various methods of controlling plant disease. As no single strategy is found to be very effective in controlling chilli anthracnose disease, Agrios (2005) recommended an integrated disease management approach.

In the present i*n vivo* experiment, different fungicides and plant extract showed significant variation in performance against *Colletotrichum capsici* causing anthracnose disease of chilli. On the basis of effectiveness of the fungicides against the disease, Tilt 250 EC and Dithane M-45 found to be the effective fungicides. The lowest percent of leaf infection was recorded in Tilt 250 EC (4.31%) followed by Dithane M-45 (5.33%). On the contrary, the lowest percent leaf area diseased (LAD) was noted in Tilt 250 EC (4.16%) and Score 250 EC (5.10%) followed by Dithane M-45 (5.21%). The findings of the present investigation corroborated with the findings of Anon. (1985-2005), Rahman *et al.* (1994), Islam *et al.* (2010) Yadab *et al.* (2017).

Almost similar performance of the fungicides was observed in percent fruit infection and percent fruit area diseased (FAD). The lowest percent fruit infection was noted in Tilt 250 EC (3.93%) followed by Dithane M-45 (4.95) and Score 250 EC (5.23%). The lowest percent fruit area diseased (FAD) was noticed in Tilt 250 EC (3.55%) preceded by Dithane M-45 (4.41%) and Score 250 EC (4.77%). According to Anon. (2005) it was reported that Tilt 250 EC was found effective in controlling anthracnose disease of chilli. Rahman *et al.*

(1994) reported that Tilt 250 EC had promising effect in *in vivo* condition and *in vitro* condition against *Colletotrichum lindemuthianum* and C. *dematium.* Srivastava and Soni (1993) found that the most effective and economical control against *Colletotrichum capsici* performed by spraying with Dithane M-45 at (0.2%).

In case of effect of plant extract against anthracnose disease of chilli, better performance was recorded on percent leaf infection by applying Neem leaf extract (6.13%) but the lowest percent leaf area diseased (LAD) was noted in Allamanda leaf extract (4.88%). Similarly, the lowest percent fruit infection and percent fruit area diseased (FAD) were counted in Neem leaf extract (6.91%) and (6.63%) respectively. According to Yadab *et al.* (2017), foliar spray with neem leaf extract at (5%) resulted in decreased PDI and increased fruit yield.

Considering the yield, the fungicides and plant extract acted against anthracnose disease of chilli. The highest yield was recorded in Tilt 250 EC (10.23 t/ha) followed by Dithane M-45 (8.84 t/ha) and Neem leaf extract (8.04 t/ha). In case of 1000 seeds weight, the highest weight was counted in Tilt 250 EC (4.21 gm) followed by Ridomil Gold 68 WG (4.06 gm), Dithane M-45 (3.98 gm), Allamanda leaf extract (3.96 gm) and Neem leaf extract (3.91 gm). The present findings corroborated with the findings of Islam *et al.* (2010), Yadab *et al.* (2017).

Among 400 seeds tested from each treatment, the lowest seed infection was found in T_1-Tilt 250 EC (24.5%). The highest percent seed germination was also recorded in T_1 (87.5%). Out of 3200 harvested seeds, 1256 seeds have the pathogenic infection where found numerous seed borne pathogen like *Colletotrichum capsici* (292)**,** *Aspergillus flavus* (358), *Aspergillus niger* (345), *Rhizopus* sp (160), Bacterial sp. (47) and unknown pathogen (54). Alam *et al.* (2014) reported different most prevalent seed borne fungi viz. *Colletotrichum capsici, Curvularia lunata, Rhizopus stolonifer, Aspergillus flavus* and *Fusarium moniliforme* were associated with the tested chili seed samples.

Occurrence of *Colletotrichum capsici* causing anthracnose disease of chilli and other seed borne pathogen, 1256 seeds out of 3200 seeds have the pathogenic infection by numerous seed borne pathogen like *Colletotrichum capsici***,** *Aspergillus flavus, Aspergillus niger, Rhizopus* sp, Bacterial sp., and unknown pathogen. Out of 1256 infected seeds, *Colletotrichum capsici* was found on 292 seeds while *Aspergillus flavus* was found on 358 seeds, *Aspergillus niger* was found on 345 seeds, *Rhizopus* sp. was found on 160 seeds, Bacterial sp. was found on 47 seeds and unknown pathogen was found on 54 seeds. The highest prevalence of infection was recorded of *Aspergillus flavus* (28.50%) followed by *Aspergillus niger* (27.46%) *Colletotrichum capsici* (23.25%), *Rhizopus* sp. (12.74%), Unknown pathogen (4.31%) and Bacterial sp. (3.74%) respectively. The present findings are kept in with the findings of Alam *et al.* (2014) who reported that the most prevalent fungi associated with the chilli seeds were *Colletotrichum capsici*, *Curvularia lunata, Rhizopus stolonifer, Aspergillus flavus* and *Fusarium moniliforme*.

SUMMARY AND CONCLUSION

CHAPTER VI

SUMMARY AND CONCLUSION

The experiment was conducted at the central farm of Sher-e-Bangla Agricultural University during the period of November/2019-May/2020 to find out the effective fungicides and plant extract in controlling anthracnose of chilli. Four fungicides viz. Tilt 250 EC, Ridomil Gold 68 WG, Dithane M-45, and Score 250 EC one biological agent *Trichoderma harzianum* & two plants extract viz. Neem leaf extract, Allamanda leaf extract were applied in the *in vivo* experimental plot. The effect of fungicides and plant extract against the anthracnose disease of chilli were determined by recording data after different days of spraying in terms of disease incidence, disease severity, yield and seed health testing (*in vitro*). The effects of treatments were compared by Duncan's Multiple Range Test (DMRT).

Effect of different fungicides on percent leaf infection and percent leaf area diseased (LAD) were recorded after each of the spraying done. The lowest percent leaf infection (4.31%) and LAD (4.16%) were recorded in case of treatment Tilt 250 EC. The highest leaf infection was recorded in case of spraying treatment, *Trichoderma harzianum* (6.52%) which was statistically similar to that of Allamanda Leaf extract (6.25%).

In case of percent fruit infection and percent fruit area diseased, the lowest fruit infection was observed in Tilt 250 EC (3.93%) followed by Dithane M-45 (4.95%). Simultaneously the highest fruit infection (32.4%) and fruit area diseased (32.2%) were recorded in control.

Effect of different treatments on yield and yield contributing attributes of chilli varied significantly and the highest yield (10.23 t/ha) was recorded in the treatment Tilt 250 EC followed by Dithane M-45 (8.84 t/ha), Ridomil Gold 68 WG (7.9 t/ha) and Score 250 EC (7.67 t/ha). Among the plant extract, Neem leaf extract gave good yield (8.04 t/ha) against anthracnose disease which was even better than Ridomil Gold 68 WG (7.9 t/ha) and Score 250 EC (7.67 t/ha).

Effect of different fungicides and plant extract on 1000 seed weight of chilli against anthracnose were found to differ. The highest 1000 seed weight was recorded in treatment of Tilt 250 EC (4.21 gm) followed by Ridomil Gold 68 WG (4.06 gm), Dithane M-45 (3.98 gm), Allamanda leaf extract (3.96 gm), and Neem leaf extract (3.91 gm).

The highest percent seed germination on harvested chilli was recorded in Tilt 250 EC (87.5%). Occurrence of *Colletotrichum capsici* (292)**,** *Aspergillus flavus* (358), *Aspergillus niger* (345), *Rhizopus* sp (160), Bacterial ooze (47) and unknown pathogen (54) were recorded on harvested chilli seeds out of 3200 seeds.

Considering the overall performances of the treatments applied in *in vivo* experiment, the chilli growers may be suggested to apply Tilt 250 EC and Dithane M-45 as foliar spraying.

REFERENCES

CHAPTER VII

REFERENCES

Acharya, A. and Das, J. N. (1995). Control of anthracnose of betel vine by fungicidal chemicals. *Current Agril. Res.* **8**: 58-60.

Agrios, G. N. (2005). Plant Pathology, 5th Ed. p.922. Academic Press, San Diego.

Ahmed, H. U. (1986). Recommendation on the method of disease management of crops in Bangladesh. Plant Pathology Division. Bangladesh Agricultural Research Institute. Joydebpur, Gazipur. Pp 47-107.

Ahmed, H. U. and Hossain, M. M. (1985). Crop disease survey and establishment of a herbarium at BARI. Plant Pathology Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur. P.107.

Akanda, A. M. and Fakir, G. A. (1985). Effect of seed dressing chemical for the control of major seed-borne pathogens of jute. *Bangladesh J. of Plant Pathol.* **1**(1): 13-19.

Alam, M. Z., Hamim, I., Ali, M. A. and Ashrafuzzaman, M. (2014). Effect of seed treatment on seedling health of chili. *J. Environ. Sci. & Natural Resources.* **7**(1): 177-181.

Anamika., Rhoda, S. and Nath, P. (2012). Survey of anthracnose disease in chilli crop in Rewa region. *Internat. J. Sci. Res*. **3**(8): 1851-1854.

Anonymous (1985-2005). Efficacy of different fungicides in controlling anthracnose of chilli. Plant Pathological Research. Plant Pathology Division, Bangladesh Agricultural Research Institute. P. 106.

Bangladesh Bureau of Statistics (BBS) (2016). Statistical Year Book of Bangladesh, Ministry of Planning, Government of the People's Republic of Bangladesh, Dhaka.

Begum, S., Yumlembam, R. A., Marak, T. R. and Nath, P. S. (2015). Integrated management of anthracnose of chilli caused by *Colletotrichum capsici* in

west Bengal condition. Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia-741 252 (West Bengal), India. *An International Quarterly Journal of Life Sciences.* **10**(1): 1901-1904.

Bhuyia, K. and Fakir, G. A. (1982). Control of two major seed borne pathogens of soybean with seed dressing fungicides. Proc. 6th and 7th Bangladesh Ann. Sci. Conf. Abs. Section-I: 69.

Birari, B. P., Gade, R. M. and Chuodhari, R. K. (2018). Antifungal efficacy of plant extract, biocontrol agents against *Colletotrichum capsici* causing anthracnose of chilli. *Journal of Pharmacognosy and Phytochemistry*. **7**(5): 1368-1373.

Biswas, A. (1992). Efficacy of fungicides in control of anthracnose disease of chilli in sondarban region of West Bengal. *J. myco Pathological Res*. India. **30**(1): 31-35.

Chauhan, R. T., Patel, P. R. and Thumar, V. M. (2018). Occurrence of seed borne pathogens in Chilli (*Capsicum frutescence* L.) cv. GVC 111 in vitro. *Int. J. Chem. Stud*. **6**(2):1374-1376.

Chauhan, Y. B., Patel, R. L., Chaudhary, R. F. and Rathod, N. K. (2014). Efficacy of different fungicides for the management of chilli anthracnose caused by *Colletotrichum capsici. An International Quarterly Journal of Life Sciences.* **9**(1): 399-402.

Choudhury, D., Saha, S., Nath, R., Kole, R. K. and Saha, J. (2017). Management of chilli anthracnose by botanicals fungicides caused by *Colletotrichum capsici. Journal of Pharmacognosy and Phytochemistry.* **6**(4): 997-1002.

Datar, V. V., Sontakke, M. B., Purandare, N. D. and Shinde, N. N. (1990). fungicidal control of anthracnose of chillies. *Indian J. Mycology and P. Pathol*. **20**(2): 156- 158.

Dean, R., Van Kan, J. A. L., Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P. D. (2012). The Top 10 fungal pathogens in molecular plant pathology. *Mol. Plant Pathol.* **13**: 414–430.

Deeksha, J., Tripathi, H. S. and Josi, D. (2002). Cultural. Biological and Chemical of anthracnose of Urdbean. *J. Mycology and Plant Pathology*. **32**(1): 86-87.

Dhaliwal M. S., Yadav A., Jindal S. K. (2014). Molecular characterization and diversity analysis in chili pepper using simple sequence repeats (SSR) markers. *Afr. J. Biotechnol.* **13**:3137-3143

Ebenezer, E. G. and Alice, D. (1996). Field evaluation of fungicide against fruit rot and die back of chilli. *Indian J. of Plant Protec*. **24**: 50-52.

Ekbote, S. D. (2002). Bio-efficacy and Copper hydroxide (coxid) against anthracnose of chilli. *Kamataka J. Agril. Sci.* **15**(4): 729-730.

Ekbote, S. D. (2003). Efficacy of Prochioraz 45 EC against fruit rot and die back of chilli. *Indian J. Plant Protection.* India. **31**(1): 139-140.

Eswaramurthy, S., Pappiah. C. M., Muthusamy, M., Muthusamy, S., Mariappan, V., Jeyasekar, R., Natarajan, S., David, P. M. M. and Gomathirayagam, P. (1988). Chemical control of fruit rot and dieback of chillies, *Pesticides.* **22**(3): 38-40.

FAO. (2014). FAO Yearbook, Production. Food and Agriculture Organization of United Nations, Rome, Italy.

Farr, D. F., Rossman, A. Y., Palm, M. E., and McCray, E. B. (2016). Fungal Databases, Systematic Botany and Mycology Laboratory, ARS, USDA.

Gautam, A. K. (2014). The genera *Colletotrichum*: an incitant of numerous new plant diseases in India. *J. New Bio. Rep*. **3**(1): 09-21

Geetha, R. and Selvarani, K. (2017). A study of chilli production and export from India. *International Journal of Advance Research and Innovative Ideas in Education*. **3**(2): 205-10.

Hadden, J. F. and Black, L. L. (1987). Comparison of virulence of tomato and pepper isolates of *Collelotrichum spp*. *Phytopathol.* **77**: 641.

Haque, A. H. M. M., Momin, A., Rahman, G. M. M. and Hossain, M. D. (1998). Fungi associated with chilli seeds and their *in vitro* control. *Bangladesh J. Seeds Sci. & Tech.* **2**(1 &2): 85-90.

Harbant, S., Korpraditskul, V., Singh, H., Vichai, K., Singh, P. P. and Saxena, R. C. (1999). Evaluation of some plant extract for the control of *Colletotrichum capsici* (SYD.) Butlar and Bisby, the causal agent of chilli anthracnose. In: "*Azadriachta indica* A. Juss" edited by R. P. P. Singh and R. C. Saxena. Science Publishers, India, 131-138 pp.

Harbant, S., Korpraditskul, Y., Singh, H., Vichai, K., Singh. P. P. and Saxena, R. C. (1999). Evaluation of some plant extract for the control of *Colletotrichum capsici* (SYD). Butlar and Bisby, the causal agent of chilli anthracnose. *A. Juss. Mara Inst of Tech.* **12**:131-138.

Hegde G. M. and Anahosur K. H. (2001). Evaluation of fungi toxicants against fruit rot of chilli and their effect on biochemical constituents. *Kamataka J. Agril. Sci*. **l4**(3): 836-838.

Hemannavar, V., Rao, M. S. L., Hegde, Y. and Mohankumar, H. D. (2008). Status of seed borne incidence of anthracnose of chilli in northern Karnataka and evaluation of seed health testing methods for the detection of *Colletotrichum capsici. Karnataka J. Agric. Sci.* **22**(4): 807-809.

Horsfall, J. G.; Barratt, R. W. (1945). An Improved Grading System for Measuring Plant Disease. *Phytopathology*. **35**: 655

Hossain, S. (1989). Survey and chemical control of guava anthracnose. Abstract of thesis (1966-1990), Dept. of Plant Pathol. Bangladesh Agricultural University, Mymensingh. p. 13.

Islam, M. M., Islam, M. R., Aminuzzaman, F. M., Faruq, A. N., and Siddiquee, T. A., (2010). Efficacy of Fungicides and Plant Extract on Management of Anthracnose (*Colletotrichum capsici*) of Chilli (*Capsicum annuum* L.). *The Agriculturists*, **8**(1):1-9.

Islam, M. M., Islam, M. R., Aminuzzaman, F. M., Faruq, A. N., and Siddiquee, T. A. (2010). Efficacy of Fungicides and Plant Extract on Management of Anthracnose (*Colletotrichum capsici*) of Chilli (*Capsicum annuum* L.). *The Agriculturist*. **8**(1):1-9.

ISTA. (1976). International Rules for Seed Testing, International Seed Testing Association. *Seed Sci. & Technol.* **4** :3-177.

Joi, M. B., Deshukh, D. P. and Khadatare, R. M. (2004). Efficacy of RIL 006/CI, a new fungicide for the control of anthracnose and fruit rot of chilli. *Annals of plant Protection Sciences.* **12**(2):463-464.

Juangbnich, P. and China, C. (1975). Efficacy of some fungicides against seed borne infection of *Colletotrichum sp.* and ripe rot of pepper *(Capsicum frutescens). Kasetsart. J.* **9**(2): 115-118.

Karim, M. M., Momotaz, R., Arifunnahar, M., Islam, M. N. and Rahman, M. M. E. (2017). Prevalence of seed borne fungi associated with jhum morich (jhum chilli) seeds. *Int. J. Sustain. Crop Prod.* **12**(3): 7-12.

Khan, A. A. and., Fakir, G. A. (1995). Seed treatment with garlic extract to control seed borne pathogens Jute. *Bangladesh Journal of Plant Pathology*, **11**:1-2.

Khan, M. S., Nasir, M. A. and Bokharis, S.A. (1998). *In vitro* evaluation of certain neem based product and systemic fungicide against different plant pathogen responsible for wilt and anthracnose of guava. Department

of Plant Pathology. University of Agriculture. Faisalabad, Pakistan. *Pakistan J. Phytopatho*. **10**(2): 72-74.

Kothari S. L., Joshi A., Kachhawaha S., Ochoa-Alejo N. (2010). Chili Pepper-a review on tissue culture and transgenics. *Biotechnol. Adv.* **28**: 35-48.

Kulsherestha, D. D.; Mathur. S. B. and Neergard, P. (1976). Identification of seed-borne species of *Colletotrichum*. *Friesia.* **11**: 116-126.

Kumaran, R. S., Gomathi, V. and Kannabiran, B. (2003). Fungitoxic effects of root extract of certain plant species *Colletotrichum capsici* causing anthracnose in *Capsicum annum*. *Indian Phytopathology*. **56**: 114-116.

Mali, J. B. and Joi, M. B. (1987). Control of seed mycoflora of chilli (*Capsicum annum*) with fungicides. *Curr. Rep*. **1**(1): 8-10.

Manandhar, J. B., Hartman, G. L. and Wang, T.C. (1995). Anthracnose development on pepper fruits inoculated with *Colletotrichum gloeosporioides*. *Plant Dis.* 79:380-383.

Mandal, D., Pal, R. and Mohanty, A. K. (2017). Management of anthracnose disease of chilli. Regional Research and Technology Transfer Station, O.U.A.T, Chiplima, Sambalpur, Odisha, Pin-768025, India. *Progressive Research – An International Journal*. **12**(4): 475-477.

Mandal, S. M. A. and Beura, S. K. (2003). Chemical control of leaf curl, anthracnose and ripe fruit rot in chilli. *Indian J. Plant Protection*. **31**(1): 137-138.

Mistry, D. S., Sharma, I. P. and Patel, S. T. (2008). Evaluation of fungicides, phytoextract and bio-pesticides against dieback of chilli. *J. Pl. Dis..Sci*. **3**(1):53-55.

Montri, P., Taylor, P. W. and Mongkolporn O. (2009). Pathotypes of *Colletotrichum capsici*, the causal agent of chili anthracnose in Thailand. *Plant Disease*. **93**(1): 17-20.

Mridha, M. A. U. and Chowdhury, M. A. H. (1990). Efficacy of some selected fungicides against seed borne infections of chilli fruit rot fungi. *Seed Research*. **18**(1):98-99.

Naznin, S., Khalequzzaman, K. M., Khair, A. (2016). Effect of New Fungicides in Controlling Anthracnose/Die Back Disease of Chilli. *Asain J. Appl. Sci. Eng.* **5**: 117-124.

Oo, M. M. and Oh, S. K. (2016). Chilli anthracnose (*Colletotrichum* spp.) disease and its management approach. *Korean Journal of Agricultural Science*. **43**:153-162.

Owe, S. G., Ingole, M. N., Ingle, S. T. and Gaikwad, M. S. (2015). Management of anthracnose of chilli caused by *Colletotrichum capsici*. *Trends in Biosciences.* **8**(22): 6313-6318.

Patel, A., Kumar, A., Tiwari, R. K. and Ramakrishnan, R. S. (2020). Management of Die back and Fruit Rot Disease of Chilli (*Capsicum annum* L.). *Int. J. Curr. Microbiol. App. Sci*. **9**(3): 1235-1242.

Pearson, M. N., Bull, P. B. and Speke, H. (1984). Anthracnose of Capsicum in Papua, New Guinea; varietal reaction and associated fungi. *Tropical Pest Management*. **30**(3): 230-233.

Perene, R. R. and Joi, M. B. (1989). Control of fruit rot and dieback of chilli by seed treatment and spray. *J. Maharashtra Agril. Univ*. **14**(3): 368.

Pooja, G. and Simon, S. (2019). Integrated approaches for management of anthracnose of chilli (*Capsicum annuum* L). *Journal of Pharmacognosy and Phytochemistry.* **8**(1): 422-427.

Ragozzino, A. and Travaglini, V. (1970). L‟ anthracnose del peperone (*Capsicum annum*) ed I soui agent in Campania. (Anthracnose of Capsicums and its causal agent in campanile) *Ann. Fae. Sci. Agarar*. **3**: 337-359.

Rahman, M. K., Islam, M. R. and Hossain, I. (2004). Effect of Bion, Amistar and Vitavax on anthracnose of chilli. *J. Food, Agriculture and Environment*. **2**(2): 210-217.

Rahman, M. L., Akanda, A. M., Malek, M. A. and Khan, A. L. (1994). Some aspect of *Colletotrichum dematium* a pathogen of country bean anthracnose. *Bangladesh J. of Plant Pathol*. **10**: 31-33.

Raj, K., Mukhopadyay, A. N., Kumar, R. (1990). Chemical control of anthracnose of bean in field conditions. *Indian Phytopath*. **43**: 102-105.

Raj, T. S., Christopher, D. J. and Suji, H. A. (2012). Morphological, cultural and genetic variabilities in fruit rot of chilli caused by *Colletotrichum capsici* in tropical region of South India. *Ann. Pl. Protec. Sci*. **20**(2): 421-425.

Raju, K. S. and Rao, G. S. (1985). Effect of combined application of Dithane M-45 with different insecticides to control fruit rot and pest complex on chilli. *Indian J. Mycology and P. Path*. **15**(3): 239-246.

Ramnath., S. B., Mathur and Neergaard, P. (1970). Seed borne fungi of mungbean (*Phaseolus aureus* Roxb.) from India and their significance. *Proc. Int. Seed. Test. Assoc.* **35**: 225-241.

Rao, G. P. and Rao, K. D. (1956). Anthracnose disease on *Cyamopsis tetragonoloba* Taub. *Sci. & Cult*, **21**: 457-458.

Rashid, M. M., Kabir, M. H., Hossain, M. M., Bhuiyan, M. R. and Khan, M. A. I. (2015). Eco-friendly management of chilli anthracnose (*Colletotrichum capsici*). *International Journal of Plant Pathology*. **6**(1): 1-11.

Roberts, D., Pernezny, K. L. and Kucharek, T. A. (2001). Anthracnose caused by *Colletotrichum sp*. On pepper. University of Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, Florida, USA. Pp. 178.

Roy, M. C. (2005). Efficacy fungicides in controlling die back/ anthracnose of chilli. MS. Thesis, Department of Plant Pathology, Bangladesh Agricultural University, Mymenshingh. 1-5.

Saleem, S. (2000). Anthracnose of betel vines in Pakistan. *Pakistan J. Bot.* **32**(1): 41-44.

Setty, T. A. S., Uthaiah, B. C., Rao, K.B. and Indiresh, K. M. (1988). Chemical control of seed micro flora of chilli. *P. Path. Newsletter*. **6**(l-2): 22.

Shrinivasan, K. V. (1952). Seedling blight of Sesbenia grandi flora Pers. *Curr. Sci*. **21**: 318.

Singh, R. S. (1982). Principles of Plant Pathology. (3rd Edn.) Oxfored and JBH Publishing Co. 66. Jonapath. New Delhi.

Singh, S. N., Yadav, B. P., Sinha, S. K. and Ojha, K. L. (1997). Efficacy of plant extract in inhibitory of radial growth and spore germination of *Colletotrichum capsici. J. of Applied Biology*. **7**: 82-84.

Sinha, P. P. (1990). Cost effective control of die-back and fruit rot of chillies. Vegetable *Sci.* **17**(1): 110-112.

Srivastava, K. K. and K. K. Soni. 1993. Seedling blight of Albizia falcaturia and its control. Annals of Forestry. **1**: 82-84.

Subhani, M. N. (2015). (Institute of Agri. Science) Asian Network for scientific information 2015, *Pakistan J. Nutrition.* **14**(6): 325-329.

Sulton, B. C. (1980). The Coelomycetcs. Commonwealth Mycological lnstitue, Kew, survey, England 696.

Sundaram, S. (1926). Some Vermicularia of economic importance in South India. Madras Agric. Dept. Year Book. Pp. 10-12.

Tandon, R. N. and Agnihotri, V. P. (1961). Pathological studies of *Colletotrichum capsici* (Syd.) B&B causing leaf spot disease of Pathos scandens (Wall.). *Proc. Nat. Acad. Sci*. **31**(1): 16-21.

Telang, S. M. (2010). Effect of extract of various plant parts on seed mycoflora and seed germination of chill. *An Asian Journal of Soil Science*. **5**(1): 42-45.

Thippeswamy, B., Krishnappa, M., Chakravarthy, C. N., Sathisha, A. M., Jyothi, S. U., Vasantha, Kumar, K. (2007). Pathogenicity and management of anthracnose and alternaria leaf spot of chilli. Internat. *J. Plant Sci.* **2**(1): 39-43.

Vivekanand, Ravi, S., Mishra, R. C. and Bahuguna, P. (2018). Evaluation Various Management Techniques against Chilli Anthracnose caused by *Colletotrichum capsici. Int. J. Pure App. Biosci.* **6**(2): 861-867.

Welideniya, W. A., Rienzie, K. D. R. C., Wickramaarachchi, W. A. R. T. and Aruggoda, A. G. B. (2019). Characterization of fungal pathogens causing anthracnose in capsicum pepper (*Capsicum annuum* L.) and their seed borne nature. *Ceylon Journal of Science.* **48**(3): 261-269.

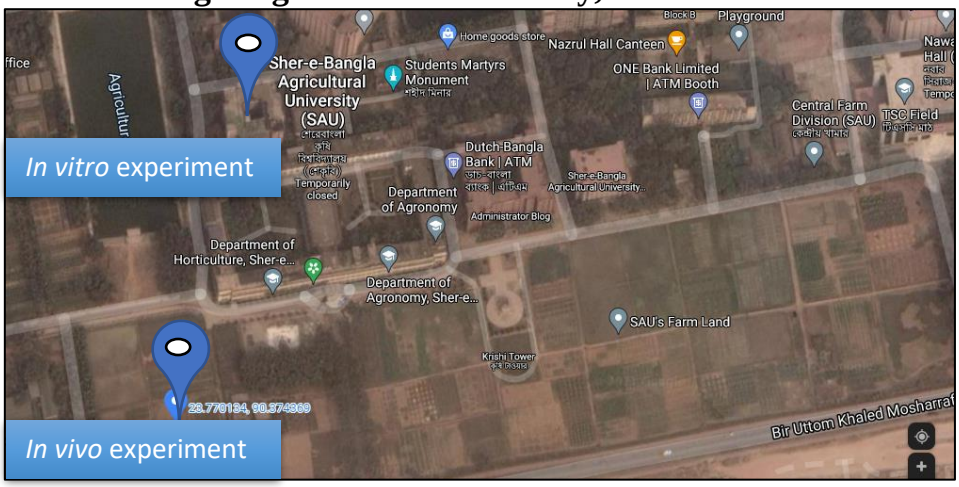
Yadav, A. L., Ghasolia, R. P., Choudhary, S. and Yadav, V. K. (2017). Exploitation of fungicides and plant extract for ecofriendly management of chilli fruit rot disease. *International Journal of Chemical Studies*. **5**(4): 1632-1634.

Yu, S. M., Ramkumar, G. and Lee, Y. H. (2013) Light quality influences the virulence and physiological responses of *Colletotrichum acutatum* causing anthracnose in pepper plants. *Journal of Applied Microbiology*.**115**(2): 509-16.

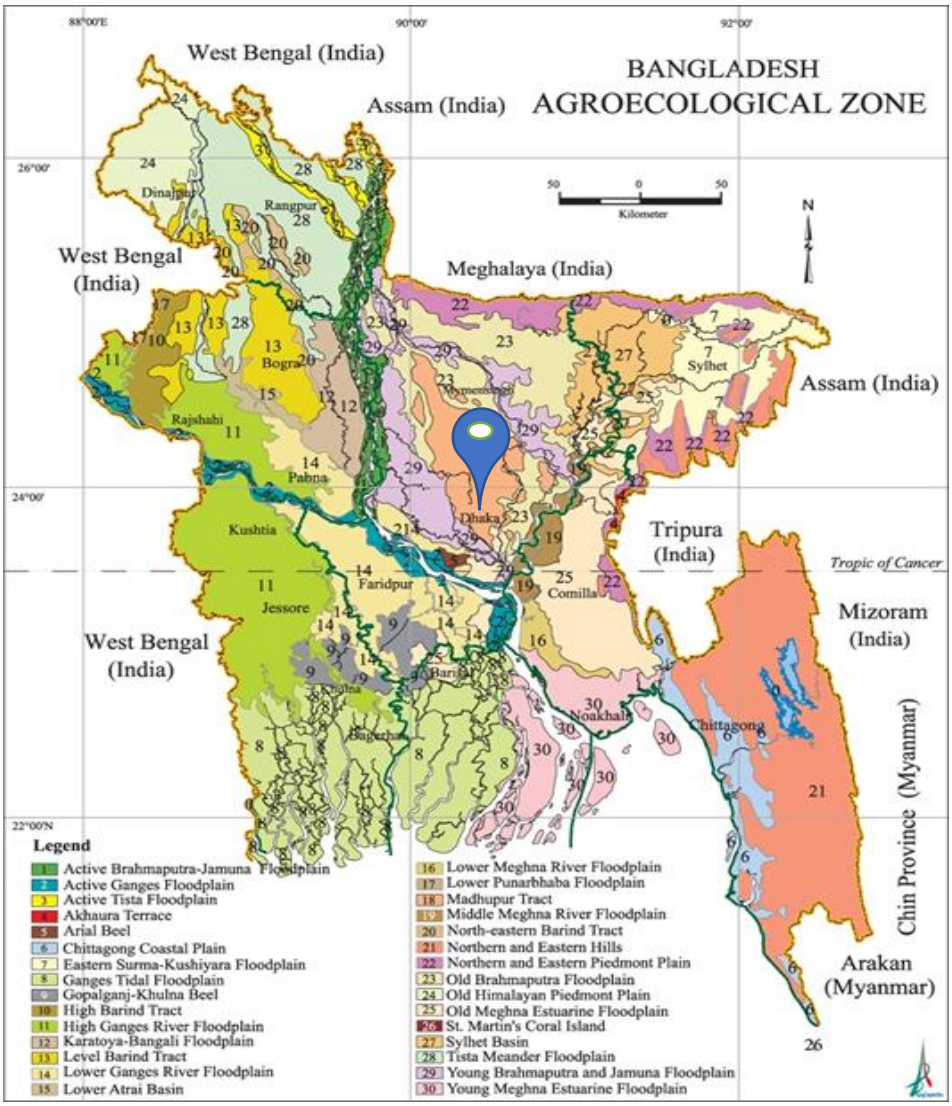

APPENDICES

APPENDICES

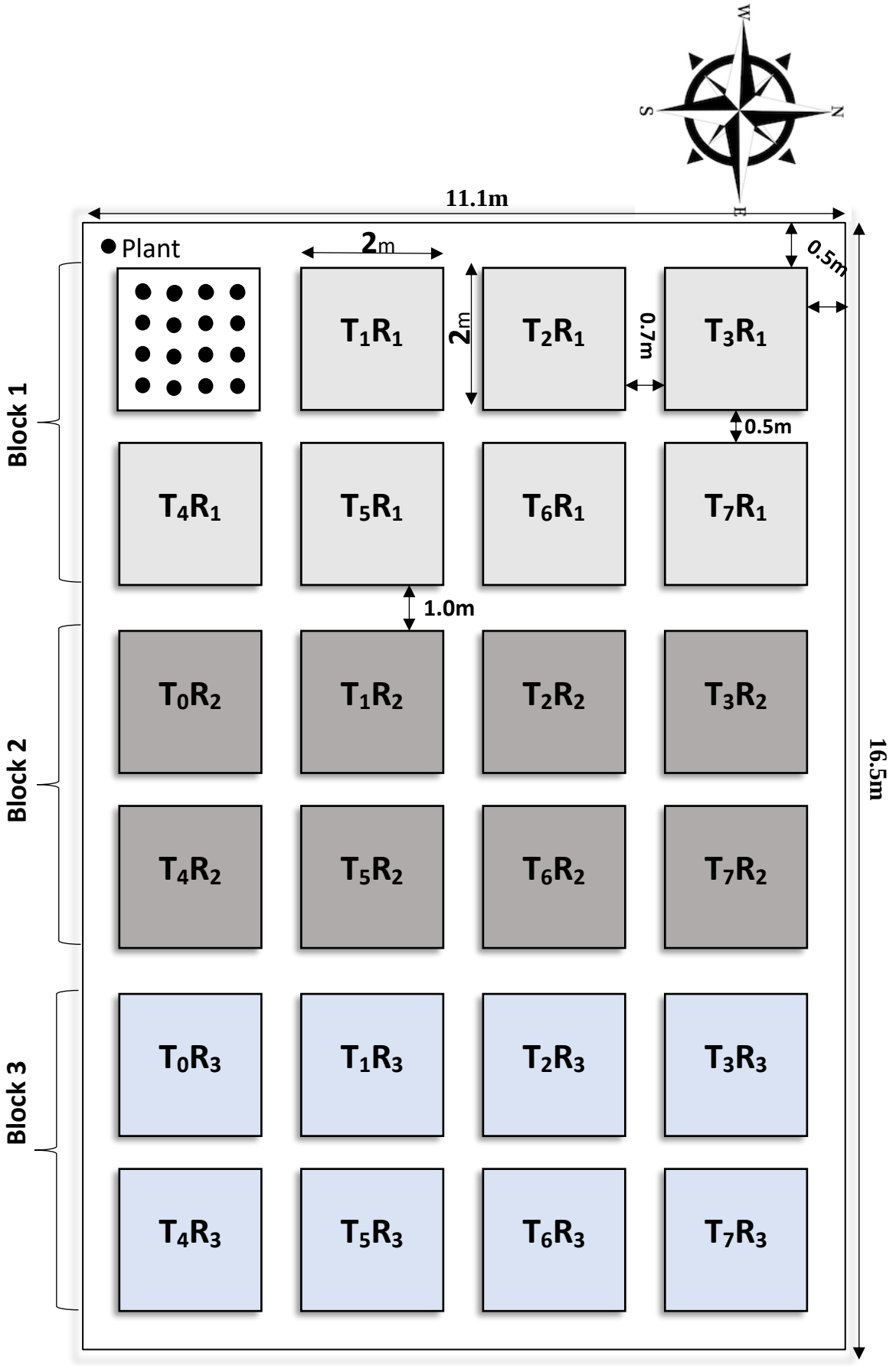
Appendix 1: The experimental field location at central farm of Sher-e-Bangla Agricultural University, Dhaka.



Appendix 2: Agro-ecological region of "Madhupur Tract" (AEZ No.: 28).



Appendix 3: Layout of *In vivo* experimental plot



Appendix 4: Mechanical and chemical properties of soil of experiment plot

Mechanical Properties	
Constituents	**Percent**
Sand	33.45 %
Silt	60.25 %
Clay	6.20 %
Texture class	Silty loam
Chemical Properties	
Soil properties	**Amount**
Organic matter	6.12 gm
Nitrogen (%)	1.32 %
Exchangeable K (%)	0.2 %
Available P (ppm)	20

Appendix 5: Monthly average temperature, relative humidity and total rainfall of the experimental site during the period from November 2019 to May 2020 are given below:

Month	**Temperature**		**Relative humidity (%)**	**Rainfall (mm)**	**Wind Speed (km/hr)**
	Max.	**Min.**			
November'2019	25.5	6.70	54.80	0.0	0.6
December'2019	23.80	11.70	46.20	0.0	1.3
January'2020	22.75	14.26	37.90	0.0	1.2
February'2020	35.20	21.00	52.44	20.4	1.4
March'2020	34.70	24.60	65.40	165.0	1.3
April'2020	35.10	24.86	67.80	170.2	1.2
May'2020	35.35	25.06	67.94	170.6	1.3

Source: Bangladesh Meteorological Department (Climate division), Agargaon, dhaka-1207.

Appendix 6. Composition of Potato Dextrose Agar (PDA) preparation

Components	**Composition**
Water	1000 ml
Potato	200 gm
Dextrose	20 gm
Agar	20 gm