BIOLOGY OF *Colletotrichum capsici* AND MANAGEMENT OF ANTHRACNOSE OF CHILLI

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CERTIFICATE

This is to certify that the thesis entitled, **"BIOLOGY OF *Colletotrichum capsici* AND MANAGEMENT OF ANTHRACNOSE OF CHILLI''** submitted to the Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka in the partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN PLANT PATHOLOGY,** embodies the result of a piece of bonafide research work carried out by bearing **Registration No. 11-04409,** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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

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ABSTRACT

Four isolates of *Colletotrichum capsici* infecting chilli in four major growing areas of Bangladesh were collected and purified to characterize in terms of cultural, morphological, physiological and pathogenicity. Another field experiment was conducted to search out a suitable management option against anthracnose disease of chilli. Five treatments viz. planting in raised bed, space planting (50x50cm), Micro tech-1 @ 10ml/L water, Metaril 72 WP @ 2g/L water and Control were applied. The isolates varied significantly in their cultural, morphological and pathogenic traits- viz. colony color, shape, margin, texture and production of conidia. The optimum temperature and pH for the radial mycelial growth of *Colletotrichum capsici* were recorded at 25-30°C and pH 6.0-6.5, respectively. No growth was observed at 10°C and 35°C temperature. Excellent (++++) sporulation was recorded at temperature 25 and 30°C and pH 6.0 and 6.5 for all the isolates. The pathogen *Colletotrichum capsici* grew well on PDA medium. The highest (87.00 mm) radial mycelial growth was obtained on PDA. Good (+++) sporulation was recorded at CDA media for all the isolates. No growth and sporulation was found in V-8 A and WA media. The length of conidia varied from 9.9-51.8µm. Mean length of conidia was maximum 28.40 µm in isolate BoCC-1 and minimum 2.88 µm in isolate PaCC-1. The breadth of conidia ranged from 0.33-12.40 µm. The highest mean breadth 4.56 µm was observed in isolate BoCC-1 and the lowest 2.88 µm in isolate PaCC-1. The highest disease score was found in Isolate BoCC-1 and RaCC-1 in case of red (7.00) and green (5.00) chillies, respectively. On the other hand, the lowest disease score was found in Isolate GaCC-1 and PaCC-1 in case of red (5.00) and green (3.00) chillies, respectively. The lowest plant infection (25.45%) and highest yield (456.3 g plant^{-1}) was obtained from planting in raised bed followed by application of Metaril 72 WP @ 2g/L water.

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LIST OF SOME ABBREVIATE FORM AND THEIR ELABORATIONS

ABBREVIATE FORM	ELABORATION
%	Percentage
% RH	Percent Relative Humidity
@	At the rate
µl	Microlitre
µg	Microgram
µm	Micromililitre
°C	Degree Celcius
AEZ	Agro-ecological Zone
C. capsici	*Colletotrichum capsici*
cm	Centimeter
CV	Coefficient of Variance
Conc.	Concentration
et al.	Co- workers
ml	Millilitre
mm	Millimeter
BARI	Bangladesh Agricultural Research Institute
CDA	Czapeks Dox Agar
CMA	Corn meal agar
g	Gram(s)
hrs	Hours
ha	Hectare(s)
PDA	Potato dextrose agar
V-8 A	V-8 Juice agar
WA	Water agar
NaOH	Sodium Hydroxide

WP	Wettable Powder
CRD	Completely Randomized Design
DAI	Days After Inoculation
DMRT	Duncan’s Multiple Range Test
LSD	Least Significance Difference
Viz.	Videlect

CHAPTER I

INTRODUCTION

Chilli (*Capsicum annuum*) is an important spice as well as vegetable crop cultivated worldwide. It is the most important spice crop in Bangladesh under the family Solanaceae. It is used in many cuisines as spice but also found to have many medicinal properties. Green chillies are rich source of vitamins especially vitamin A, C, B1, B2 (Saimbhi and Nandpuri, 1977). Pungency in chilli, which is due to the presence of capsaicin, is a digestive stimulant and a cure for rheumatic trouble. Chilli contains l.29mg protein, l1mg calcium, 870 l.U. vitamin A, l7.5mg ascorbic acid, 0.06 mg thiamin, 0.03 mg riboflavin and 0.55 mg niacin per 100 g edible fruit (Joshi and Sing, 1975).

The fruit of Capsicum has a variety of names, such as ‘chilli’, ‘chilli pepper’ or ‘pepper’ depending on place and type of fruits. The term “chilli” in most of the world refers exclusively to the smaller, hot types of Capsicum (Wikipedia, 2007). It forms an indispensable adjunct in every house in the tropical and sub-tropical world (Bose and Som, l990). The most important producers and exporters of chilli include China, India, Mexico, Pakistan, Thiland and Turkey. Chilli is also a cash crop, grown in all seasons and all areas of Bangladesh. So it has great demand in both green and ripen condition. In Bangladesh, chilli is grown in large scale commercial field as well as in small kitchen garden. In Bangladesh, the crop is cultivated in an area about 89268 ha with a total production of 109337 tons dried chilli in 2009 (FAOSTAT, 2011). But the production in terms of yield (12248 kg ha^{-1}) is not satisfactory as compared to the other chilli growing countries in the world (Anonymous, 2007).

The production per unit area is very low in Bangladesh. Among the various factors responsible for the low yield of the crop in the locality, seed borne fungal diseases play a vital role because most of the farmers in this locality use low quality seeds which are not certified and tested for seed health.

Colletotrichum is one of the vital phytopathogen worldwide causing the economically important disease anthracnose in a wide range of hosts including cereals, legumes, vegetables, perennial crops and tree fruits (Jeger and Bailey, 1992). Among these hosts, chilli is a core economic crop worldwide (Poulos, 1992), which is severely infected by *Colletotrichum capsici*, causal agent of anthracnose of chilli that may cause yield losses up to 50% during high severity (Pakdeevaraporn *et al*., 2005). Therefore, like other countries in Bangladesh, chilli anthracnose reduces the annual production, which ultimate affect the total requirements and as such large quantity is to be imported every year. 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 postharvest damage to chilli fruits causing anthracnose lesions. Even 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.

Traditionally, identification and characterization of *Colletotrichum spp*. is based on morphological characters such as size and shape of conidia and appressoria, existence of setae or presence of a teleomorph, and cultural characters such as colony colour, growth rate and texture (Smith and Black, 1990). Management strategies for this disease include use of disease free seed, resistant/tolerant cultivars and fungicidal sprays. Chemicals are the most common and practical method to control anthracnose disease. However, fungicide tolerance often arises quickly, if a single compound is relied upon too heavily (Staub, 1991).

The fungicide traditionally recommended for anthracnose management in chilli is Mancozeb group. It was reported that Mancozeb and Carboxin completely

controlled the seed borne *Colletotrichum capsici* (Mridha and Chowdhury, 1990).

Fungicide is being used to control this disease now a days. But the practice is highly risk for public health as the green fruit is eaten with or without cooking. These toxic chemicals directly enter into human tissue, get deposited and at critical concentration cause serious health hazards. This realization emphasizes the importance of non-chemical approach in controlling the disease, which are safe for human health as well as for the environment.

Therefore, on the view of above facts, the present study is proposed with the following objectives.

1. To collect, isolation and identification of *Colletotrichum sp.* Causing anthracnose disease of chilli.

2. To study the morphological characteristics of *Colletotrichum sp.*

3. To study the physiological requirements for growing of *Colletotrichum sp.*

4. To find out a suitable management option against anthracnose disease of chilli.

CHAPTER II

REVIEW OF LITERATURE

Anthracnose disease of chilli is caused by *Colletotrichum species*. The anamorphs are *Glomerella* species. The available information relevant to the present study has been reviewed in this chapter.

2.1. Geographical distribution

The widespread occurrence and destructive nature of the pathogen caught attention of several scientists all over the world. The disease has been studied and reports have been made from many countries.

Devi and Singh (1999) observed In November 1998, a severe anthracnose occurrence of chilli (*C. annuum*) at an experimental farm in Imphal, Manipur, India, causing 80% seedling mortality. The causal agent was identified as *C. gloeosporioides* [*Glomerella cingulata*] and its pathogenicity was confirmed.

Mathew *et al.* (1995) surveyed *C. annuum* in Vellanikkara, Trichur, Kerala, India, showed that Alternaria leaf blight, die back and fruit rot (caused by *C. capsici*), were serious problems during the rainy season.

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

Ekbote (2002) conducted a survey of the prevalent diseases of chilli (*C. annuum*) in 6 talukas (Byadagi, Hirekerur, Haveri, Ranebennur, Savanur and Shiggo) in the Haveri district of Karnataka, India was conducted from 1998-2000. Fruit rot caused by *C. capsici* was the most prevalent disease (36.4%) of chilli, followed by the Murda complex (34.56%), powdery mildew caused by *Levellula taurica* (17.54%) and leaf spot caused by *Cercospora capsici*

(12.11%). Among the taluks surveyed, Ranebennur recorded the highest mean incidence of Murda complex (56.25%) and powdery mildew (24.50%), whereas Savanur recorded the highest mean incidence of fruit rot (42.00%) and leaf spot (15.00%).

C. capsici (Sydo) Butler and Bisby causing anthracnose, die-back and fruit rot of chilli (*C. annum L.*). *C. capsici* was identified from various parts of Odisha according to their cultural, morphological characters and pathogenenicity test and squash mount study under microscope (Ranasingh *et al*. 2013).

Anthracnose caused by *C. capsici* is considered to be a dry fruit rot described by Pearson *et al*. (1984) in chilli. *C. capsici* and *C. gloeosporioides* are the two main casual agents 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.

Winch *et al.* (1984) tested pathogenicity of *C. gloeosporioides* using immature leaves and shoots of *Dioscoria alala* cut from the plants and put on moist blotter in glass petridish which they inoculated by drop of spore suspension.

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

Four *Colletotrichum species*, *C. capsici*, *C. gloeosporioides*, *C. acutatum* and *C. coccodes* have been reported as causal agents of pepper anthracnose in many countries although the major species are *C. capsici* and *C. gloeasporioides* (Hadden and Black I987).

Eastbum and Gubler (1990) tested the pathogenicity of anthracnose of strawberry caused by *C. aculatum* using the same method.

Seeds of *C. annum* infected by *C. capsici* were collected from Chittagong, Bangladesh. The fungus was recovered from the seed coat, endosperm and embryo (Basak 1998).

Anthracnose caused by *Colletotrichum spp*. is considered to be the major fungal disease of chilli as reported by Nalinee (2000).

Hegde and Ekbote (2002) reported that chilli fruits at the red stage having the highest percent disease index (PDI; 22.35-38.42%) due to anthracnose.

Azad *et al.* (2005) showed that the dimension of conidia of *Colletotrichum capsici* was 17-24X3.8-4.4 um.

2.2. Host range

Gautam (2014) reported various plant diseases caused by *Colletotrichum* in India with special reference to present century (i.e. 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 hosts.

2.3. Symptomatology

Colletotrichum, one of the major plant pathogenic genera responsible to anthracnose causes a plant disease on a variety of hosts from trees to grasses (Dean *et al*., 2012). The disease is characterized by sunken spots of various colours on leaves, stems, fruits or flowers. These spots often enlarge, and lead to wilting, withering, and dying of infected plant tissues (Hiremath *et al*., 1993).

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

Fungal isolates from chilli (*Capsicum spp*.) fruits in Thailand that showed typical anthracnose symptoms were identified as *C. acutatum, C. capsici and C. gloeosporioides* (Than *et al.* 2008).

2.4. Morphology and cultural characters

Studies on morphology and cultural characters have been conducted by several workers on species of *Colletotrichum.*

Acervuli are erumpent, cushion like mass of hyphae, light brown to dark brown, sometimes lined with setae and conidiophores bearing conidia at their apices. Various diameters of acervuli have been reported 50 –250/µ by Shrinivasan (1952) and 55–120/µ by Mishra and Dutta (1963). The sizes of acervuli have been reported to differ according to the plant part affected. Malabanan (1926) recorded the size of acervuli of *C. nigrum* 56–100/µ on leaves and 50–250/µ on fruits of chilli.

Setae of *C. capsici* have been described as light brown to dark brown, septate thick and tapering towards the end with light coloured apices. Setae may be straight (Sundaram, 1926) or curved (Rao and Rao, 1956). The range of length and breadth of setae is 48-298 x 2.5-7.5/µ however; shorter range (73-95/µ) has been reported by Tandon and Agnihotri (1961).

Conidial shape has been considered as major criterion for classification (Kulshreshtha *et al.,* 1976) and erected two master species – one having curved conidia (*Colletotrichum dematium*) and second having straight conidia (*Colletotrichum gloeosporioides*). The size of falcate and cylindrical conidia has been reported by different workers to range 16.5-30 x 2.3-5.2/µ. Sharma *et al*. (2005) reported the existence of 15 pathotypes of *C. capsici* from the Himachal Pradesh area of northern India based on quantitative

Twenty isolates of *C. capsici* from conventional chilli growing areas of Tamil Nadu. In culture, most of the isolates produced cottony, fluffy or suppressed colonies. 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 virulences in chilli populations. Pathotype Cc-1 was most virulent and attacked all the differential cultivars. The genetic relationship between 20 morphological

groups recognized within *C. capsici* was investigated using random amplified polymorphic DNA (RAPD) analysis (Raj *et al*. 2012).

2.5. Epidemiology

The effect of relative humidity levels on conidial germination of *C. capsici* and *Leveillula taurica* and disease development in chilli (var. Parbhani Tejas). Results indicated that conidia of *C. capsici* and *L. taurica* could not germinate at 10% RH up to 48 hours of incubation. Maximum conidial germination of both these species took place at 100% RH followed by 75, 50 and 25% RH. Symptoms of *C. capsici* on leaves were not observed at 10% RH and on fruits at 10 and 25% RH up to a fortnight. Incubation period was minimum at 100% RH and steadily increased as humidity levels decreased. Powdery mildew symptoms on leaves up to a fortnight were not observed at 10, 25 and 100% RH. These developed within a week's period at 50 and 75% RH (Hingole *et al*., 2011).

The pathogen requires warm and humid conditions to infect different plant hosts, including gymnosperm, angiosperms, ornamental and fruit plants, vegetables, crops or even grasses. As the primary inoculum is disseminated by wind or rain, the pathogen is cosmopolitan in distribution (Farr et al., 2006).

Chung and Lee (1986) observed conidial germination of *Colletotrichum dematium f.sp* capsicum at 28°C, pH 5.5 and in more than 90 percent relative humidity and best on potato sucrose broth with exogenous supply of C and N.

Light intensity and day length may influence the disease development, survival of inoculum, pathogenesis and consequently help in the expression of symptoms and sporulation too. Under laboratory conditions, *Colletotrichum capsici* showed maximum growth and sporulation at 30°C and pH 5. Cultures placed under continuous normal light and alternative with ultra violet showed better growth (Mazilan and Sariah, 1980).

2.6. Pathogenicity

Winch *et al*. (1984) tested pathogenicity of *C. gloeosporioides* using immature leaves and shoots of *Dioscoria alala* cut from the plants and put on moist blotter in glass petridish which they inoculated by drop of spore suspension. Similar method was applied by Daykin and Milholland (1984) to test the pathogenicity of *C. gloeosporioides* caused by ripe rot of muscadine grape.

Rajamanickam and Sethuraman (2014) collected *Collectotrichum capsici* from different part of Tamil Nadu and assayed for their virulence, age of susceptibility of fruits and method of inoculation. The pathogen *C. capsici* isolate 1 caused the maximum fruit rot intensity of 72.27 per cent, while *C. capsici* isolate 9 showed least intensity of 2.93 per cent. The four methods of inoculation were tested under in vitro condition to assess the effectiveness of infection. Among the various methods of inoculation, Spray spore suspension after pinpricking the fruits method was enabled the maximum infection. Chilli fruits at six different ages viz., 5, 10, 15, 20, 25 and 30 days were used to found the stage of susceptibility by the inoculation with virulent isolate of *C. capsici* under in vitro. Twenty five days old fruits were the most susceptible to the disease and ideal for artificial inoculation, while 5, 10, 15 and 20 days old green fruits were not infected by the pathogens.

2.7. Management of the disease

Management strategies for this disease include use of disease free seed, resistant / tolerant cultivars and fungicidal or botanicals sprays. Singh (1987) and Agrios (1988) also reported that anthracnose disease of chilli is seed, soil and air borne in nature.

Mistry *et al* (2008), evaluated effective fungicides, phytoextracts and bio-pesticides against *C. capsici* under in vitro condition were evaluated for their efficacy to control die-back of chilli (Cv. S-49) under field condition. Three sprays of seven fungicides. Two bio-agents and two botanical extracts were carried out at 15 days interval after the initiation of die-back symptoms The

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%).

Chilli anthracnose damage chilli fruits extensively at pre- and post- harvest stages causing anthracnose lesions. Even very small lesion of anthracnose on fruits of chilli reduces the market value of chilli crop. Fungi toxic effect of eight fungicides and Homeo-fungicides were tested in vitro through poisoned food technique. 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 (1000ug/ml) tested. Revus had also inhibited the mycelia growth of *Colletotrichum capsici* completely at three concentrations of 300, 500 and 1000 ug/ml, respectively. From our result 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 (Subhani *et al*. 2015).

Ragozzino and Travaglini (1970) reported that two fungi, *Colletotrichum piperatum* and *Colletotrichum capsici* were responsible for the 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.

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

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

Mali and Joi (1987) tested 7 fungicides against seed mycoflora of chilli and reported that Vitavax (Carboxin) was the most effective against colony growth and sporulation of *Colletotrichum capsici.*

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.

Eswaramurthy *et al.* (1988) found that spray of 10 fungicides twice at 15 days interval, the best control of *Colletotrichum capsici* on *Capsicum annum* 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.

Perene and Joi (1989) found that the treatment of chilli seeds with Thiram or Bavistin and fungicidal spray of seedlings with Dithan 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 Dithan M~45. Fruit rot was less with combination effect of seed treatment with Thiram and spraying with Mancozeb.

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

concluded that when the number of fungicide sprays increased from 4 to 7 the fruit infections were reduced and fungicidal efficacy was significantly improved.

Raj *et al*. (l990) stated that seed treatment with thiram + carbendazim followed by 3 sprays of carbendazim gave min. disease incidence of anthracnose of urd bean (*Colletotrichum capsici)* and max. yield per plot. Seed treatment and 3 sprays of mancozeb also increased yield and reduced disease incidence.

Sinha (1990) observed on 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.

Datar *et al*. (1990) conducted an experiment on fungicidal control of anthracnose of chillies and found that 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.

Park *et al*. (l992) found that the combination of Metalaxyl + Dithianon (10 + 30%) was the most promising for control of *P. capsici* and *Colletotrichum gloeasporioides* (*Glomerella cingulala*) among 4 wettable powder mixtures tested. All the formulations had suitable mixing and storage properties.

Biswas (1992) conducted a field trials of 6 fungicides against *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 I 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 *C. annum* in field trials. Srivastava and Soni (1993)

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

Rahman *et al.* (1993, 1994) evaluated 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 50 WP both in in vitro and in vivo condition in controlling *Colletotrichum dematium*.

The efficacy of nine commonly used fungicides was evaluated in controlling fruit rot and dieback in chilli caused by *Colletotrichum capsici* under field conditions in Tamil Nadu, India (Ebenezar and Alice 1996). The best control was achieved with mancozeb (0.2%), followed by carbendazim (0.2%), and copper oxychloride (0.2%).

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

Miller *et al.* (1998) reported that Maneb has been used for many years to control the development of anthracnose. This product had worked well in reducing economic loss due to anthracnose, but its efficacy has been questionable as a result of problems in disease control. Maneb is usually rotated or combined with copper sulfate or a similar copper compound to control bacterial spot (*Xanthomonas campesrris pv. vesicatori).*

Hegde and Anahosur (2001) also conducted a field experiment to determine the effective fungicide treatment against fruit rot disease caused by (*Colletotrichum capsici*) of chilli under rainfed conditions. The efficacy of 4 non-systemic (Mancozeb, Chlorothaloril, Copper oxychloride and lprodione at 0.3% concentration) and 4 systemic (Bavistin [Carbendazim], Triadimefon,

Propiconazole,and Hexaconazole at 0.1% concentration) and the botanical fungicide Nunbicidin at 3% were tested. Among the fungicides tested, Carbendazim recorded the least percent disease index (35.63%) followed by Propiconazole (36.53%) and Hexaconazole (35.63%). lprodione recorded the highest percent disease index (7l.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.

Deshmukh *et al.* (2002) conducted an experiment for comparison between Mancozeb at 0.25% and the biological pesticide Zetron at 0.2, 0.25and 0.4% in controlling anthracnose of chilli (*Capsicum annum*) caused by *Colletotrichum capsici.* They observed 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%.

Kumaran *et al*. (2003) evaluated chemical fungicide Mancozeb at 320 ppm and ethanolic extracts 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 extracts of *Abrus precatorious* and *Rauvolfia tetraphylla* showed significant inhibitory effects on both the conidial germination and radial growth of *Colletotrichum capsici*.

Rahman *et al.* (2004) conducted a field experiment in Mymensingh, Bangladesh to evaluate the efficacy of Bion (0.005%), Azoxystrobin and carboxin in hiducing 18 systemic resistance 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 from Bion and Azoxystrobin treated seeds. Bion treatment resulted in moderate resistance of plants to anthracnose, where Azoxystrobin and Carboxin treatment resulted in susceptibility of the crop to the disease.

CHAPTER 3

MATERIALS AND METHODS

3.1. Lab Experiment

The experiment was conducted to record the cultural, morphological, physiological and pathogenic variability of four *Colletotrichum capsici* isolates collected from major chilli growing areas of Bangladesh.

3.1.1. Experimental site

The experiment was done during January to July'16 at the Plant Pathology Laboratory, Bangladesh Agricultural Research Institute. Joydebpur, Gazipur.

3.1.2. Collection of isolates

The *Colletotrichum* isolates used in this study were obtained from different major chilli growing areas of Bangladesh (Table l). These isolates were collected during the month of October, 2015 from the infected chilli field. Then the specimens were taken to the Plant Pathology Laboratory, BARI and were subjected to the process of isolation.

3.1.3. Isolation and identification of the pathogens

The pathogens were isolated using tip culture techniques. The surface of the working clean bench was sterilized with ethanol (70 %). Then the infected chilli samples were taken into the clean bench and cut into small pieces (0.5-l.0 cm). The cut pieces were sterilized in $HgCl_2$ solution (l: l000)) for 1 and half minutes and then taken out with the help of sterile forceps and put on sterile distilled water in order to wash the samples and repeated 3 times. After washing, these cut pieces were placed on sterilized blotter paper in petriplates and also placed onto the PDA plates, incubated at 25°C under near ultraviolet light following ISTA rules (ISTA, I996). Seven days after incubation the fungal culture were studied under stereoscopic (Model: Olympus, SZ 61, Japan) and compound microscope (Model: Olympus, CX 21 FSI, Tokyo, Japan) for identification of the desired pathogens.

Table 1. List often *Colletotrichum capsici* isolates with their locations

Isolates	Host	Place of collection	*Colletotrichum Species*
RaCC-1	Chilli	Rangpur Sadar, Rangpur	*Colletotrichum capsici*
PaCC-1	Chilli	Ishurdi, Ishurdi	*Colletotrichum capsici*
BoCC-1	Chilli	Bogura Sador, Bogura	*Colletotrichum capsici*
GaCC-1	Chilli	Gazipur Sadar, Gazipur	*Colletotrichum capsici*

3.1.4. Purification and preservation of *Colletotrichum capsici*

After identification of *Colletotrichum capsici* organism was purified for further study. Purification of *Colletotrichum capsici* was done following single spore isolation technique. The stock culture of the isolates was maintained on potato dextrose agar in test tubes slant at 4±0.5°C in a refrigerator for further use.

3.1.5. Preparation of culture medium and culture plates

Extra pure dehydrated potato dextrose agar (PDA) manufactured by Hi-media,India was used for this experiments. The dehydrated PDA was hydrated in distilled water @ 39 g litre^{-1} and cooked for 3 minutes in a microwave oven (Model: 31 Power, Rangs). The pH of the medium was adjusted with 0.1N HCI or NaOH solution and utilizing a pH metre (Hariba pH metre, Model D-I2). After adjustment of the required levels of pH (5.0) the medium was poured into a series of conical flasks (250 ml) and autoclaved (Hl. 36-E. Tokyo. Hirayama manufacturing corporations) at 121°C under 15 psi for 30 minutes.

3.1.6. Cultural and morphological variations of *Colletotrichum capsici*

Cultural characteristics were noted on the potato dextrose agar (PDA) after three days of incubation at 25°C. Cultural features were observed both microscopically and naked eyes like colony color, Shape, margin and texture of four *Colletotrichum capsici* isolates.

Length and breadth of conidia was measured using ocular micrometer and ocular micrometer was calibrated by comparing the ocular micrometer scale with a pre-calibrated stage micrometer (Model: Erma). To determine the conidial size each isolates of *Colletotrichum capsici* was measured 50 times in length and breadth wise.

3.1.7. Effect of different temperature levels on mycclial radial growth of four *Colletotrichum capsici* isolates

Four days old cultures of *Colletotrichum capsici* were used in this study. Inoculated plates were incubated in an incubator (Incubator-SANYO MIR-553, SANYO Electric Co., Ltd. Japan.) with seven different levels of temperature viz., 10, 15, 20, 25, 30 and 35±0.5°C. The experiment was conducted at a Completely Randomized Design (CRD) comprising 3 replications. Sixteen (16) ml of melted PDA medium was poured in each petriplates using a media dispenser (Model: Rudolf, GMBH+Co.) and then autoclaved at 121°C for 30 minutes. After taking out the petriplates from the autoclave, then kept in a laminar air flow (Model: VS-1400 LVN, Vision Scientific Co., Ltd.). Five (5) mm mycelium discs was cut from the periphery of the seven days old culture of *Colletotrichum capsici* with the help of a flame sterilized cork borer and then transferred into the centre of the petriplates containing solidified PDA medium. Data were recorded till covering the entire petriplates of any isolates.

3.1.8. Effect of different pH levels on mycelial radial growth of four *C. capsici* isolates

The isolates were inoculated onto PDA medium having 5 pH levels viz., 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 in 90 mm diameter glass petriplates and incubated at 25±0.5°C with altenating I2 hrs of light and I2 hrs of dark period in an incubator (Incubator-SANYO MIR-553, SANYO Electric Co., Ltd. Japan.). The design of experiment was the same as mentioned under temperature studies. After l day of incubation, data on mycelial radial growth recording was started and continued till covering the whole glass petriplates of any isolates of *C. capsici.*

3.1.9. Effect of different nutrient media on mycelial radial growth, of *C. capsici* isolate

Seven different non-synthetic culture media such as Potato dextrose agar (PDA), V-8 Juice agar (V-8 A), Corn meal agar(CMA), Czapek's Dox agar (CDA) and Water agar (WA) media were used in this experiment and described in Table 2. The experimental design used for the study was Completely Randomized Design (CRD) having 3 replications. Data were recorded on mycelial radial growth of *C. capsici* after one day of incubation till covering the glass petriplates.

3.1.10. Spore counting in all the experiments

Five mm block was cut from a 10 day old culture and was taken in a test tube containing 1 ml of distilled water. Then the test tube was vortexed for 3 minute. One drop of spore suspension was taken on a glass slide and mounted with a glass cover slip. The slide was observed under a compound microscope (Model: Erma) with x 400 magnification. One microscopic field was considered for counting the spores. Finally no. of spore per microscopic field was graded following the scale described by Chavan Smita and Dhutraj D.N. (2017).

Table 2. The scale is described by following

Sl. No.	Score	Grade	Description (conidia/ microscopic filed [100 X])
1	++++	Excellent	>150
2	+++	Good	101 – 150
3	++	Fair	51 – 100
4	+	Poor	1 – 50
5	-	No sporulation	-

Table 3. Composition of nutrient media used in the experiment

Culture Media	Composition
PDA	Slice potato - 200 g, dextrose - 20 g, agar - I7 g and distilled water - I000 ml
V-8 A	V-8 juice - I00 g, CaCO3 - 3 g, agar - l7 g and distilled water - 1000 ml
WA	Agar- l7 g and distilled water - I000 ml
CDA	Sucrose-30g, Sodium nitrate-2g, Dipotassium phosphate-1g,Magnesiumsulphate-0.5g, Potassium chloride-0.5g, Ferrous sulphate-0.01g and Agar-15g.
CMA	Corn Meal-50g, Dextrose-2g and Agar-15g.

3.1.11. Pathogenicity testing

All the four isolates were used for pathogenicity testing. Isolates were cultured on PDA at 25°C. Conidia from 7 day-old cultures were harvested by adding 5-10 ml of sterilized distilled water onto the culture, which was then gently swirled to dislodge the conidia. The conidial suspension was filtered through two layers of muslin cloth. BARI Morich-1 variety of chilli was used in this study. Non-infected fruits (both red and green) were surface sterilized with 1% sodium hypochlorite for 5 min and washed twich with distilled water. The fruits were blotted dry and inoculated using the wound/drop method (Lin *et al*., 2002). The wound/drop method involved pin-pricking the chilli fruit wall and then placing ca. 5µl of conidial suspension (10^5 conidia ml^{-1}) over the wound.The inoculated fruits were incubated at 25°C, 98% RH.

Desease reactions of the host were evaluated by measuring the length, width and area of the typical anthracnose lesion which developed on the fruits. Symptoms were evaluated 9-15 days after inoculation (DAI). Desease reaction was scored on a 0-9 point scale described by Than *et al*. (2008): 0 (highly

resistant), no infection; 1 (resistant), 1-2% of the fruit with a necrotic lesion or a larger water soaked lesion surrounding the infection site; 3 (moderately resistant), > 2 to 5% of the fruit with a necrotic lesion, possibly acervuli may be present, or a watery lesion covering up to 5% of the fruit serface; 5 (susceptible), > 5 to 10% of the fruit showing a necrotic lesion, possibly acervuli may be present, or a watery lesion covering up to 25% of the fruit serface; 7 (very susceptible), > 10 to 25% of the fruit covered with a necrotic lesion with acervuli; and 9 (highly susceptible), > 25% of the fruit showing necrosis, lesion often encircling the fruit, abundant acervuli.

The data were analyzed statistically for calculating the mean values and for test significance. The means were compared the following Duncan's Multiple Range Test (DMRT).

3.2. Field Experiment

3.2.1. Experimental Site

The experiment was conducted at the research farm and laboratory of Plant Pathology Division, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur. The location of the site was about 40 Km North of Dhaka city between 24° 05' N latitude and 90° 25' E longitudes with an elevation of 8.4 meter from the sea level.

3.2.2. Soil and Climate of the area

The soil of the experimental field belongs to Salna series under the Agroecological Zone (AEZ)-28: Madhupur Tract. The texture of the soil was silty clay in surface layer and silty clay loam in subsurface region. The pH of the soil ranges from 6.0 to 6.5. The climate of the area is characterized by heavy precipitation during April to September and scanty or no rainfall during November.

3.2.3. Raising of Seedlings

At first germination test was conducted in the laboratory of Plant Pathology Division, BARI. At least 85% germination of seeds was confirmed. The nursery bed was prepared properly by using cow-dung and other fertilijers. Then the seeds were mixed with sand and sown in nursery bed in line on 16 October 2016. Irrigation, weeding and other operations were done as and when necessary.

3.2.4. Land Preparation

The experimental plots were prepared with tractor ploughing followed by harrowing and laddering to bring the desired tilth. Drains between beds and between replications were maintained. Final land and bed preparation was done about one week before transplanting of seedlings.

3.2.5. Application of Manure and Fertilizers

Fertilizer dose was used as Urea-210, TSP-300, MP-200, and Gypsum- 110 kg/ha as suggested by Spices Research Centre (Anon. 2005). Cow-during was used @ 10 ton/ha. All the fertilizers except urea were applied during final land preparation. Urea was applied in three splits, at 25, 50 and 75 days after transplanting.

3.2.6. Experimental Design and Layout

The experiment was conducted using Randomized Complete Block Design (RCBD) with three replications.

3.2.7. Transplanting of Seedlings and Intercultural Operations

About five weeks old seedlings were transplanted in 2.50 m x 2.00 m plot at BARI farm on November 20, 2016. There were 15 plots in the field. Irrigation, drainage, weeding, application of fungicides and other intercultural operations were done as and when required.

3.2.8. Selection of Treatments

Fungicides are gaining importance in crop protection in view of their selective properties, low cost and safety to ecosystem. Many fungicides as a chemical have been identified to be effective in the control of plant diseases. Use of bio-pesticide and other cultural management options were also found to be effective against plant diseases.

Four treatments was employed in this experiment viz. i) Planting in raised bed (6-8 inch raised), ii) Space planting (50x50cm) iii) Micro tech-1 @ 10ml/L water (bio-pesticide) and iv) Metaril 72 WP @ 2g/L water along with a untreated control.

3.2.9. Preparation of Fungicidal Solution

The spray solution of this fungicide was prepared by dissolving 2g of Metaril 72WP in one litre tap water and mixed thoroughly. In case of Micro tech-1, the suspension was prepared in the same way.This spray solution was sprayed in the each plots of specific treatment in all the replications.

3.2.10. Application of Fungicide

The spray solution of Metaril 72WP and Micro tech-1 was sprayed with compressed hand sprayer. Required amount of spray solution sprayed as test treatment on the chilli plants. The test fungicides was sprayed three times at 7 days intervals starting from the first appearance of disease symptoms. Special attention was given to complete coverage of the growing plants with the spray material from one plot to another plot.

3.2.11. Identification of the pathogen

The infected fruits were selected and seeds were placed onto blotting paper and incubated at room temperature for 7 days. The fungal pathogen was identified by observing the morphological features, acervuli formation, presence or absence of setae, cultural and conidial characters under compound microscope as described by Sutton (1980, l992).The length and width of the conidia were

measured separately by observing under compound microscope equipped with a calibrated ocular micrometer in an eyepiece.

The value of one small division of ocular micrometer was calculated by the following formula

$$1 \text{ small division of oculometer} = \frac{\text{No. of divisions of stage micrometer}}{\text{No. of divisions of oculometer}} \times 10\mu m$$

3.2.12. Data collection

3.3.12.1. Number of infected plants per plot: Total number of infected plants was recorded in each plot using the following formula described by Rashid *et al.* (2015).

$$\text{Plant infection (\%)} = \frac{\text{Number of infected plant}}{\text{Total number of plant}} \times 100$$

3.3.12.2. Fruit yield: Chilli fruits were harvested 2-3 times. Last harvest was done at 85 days after transplanting Sum of all harvest was considered as fruit yield.

CHAPTER 4

RESULTS

4.1. Lab Experiment

4.1.1. Colony characteristics of 4 *Colletotrichum capsici* isolates on PDA

Colony color, shape, margin and texture

All the isolates exhibited variation in colony characteristics such as color, shape, margin and texture. Colony colors were cottony white, ashy white, off white, and light ash.; colony shape irregular, regular with sector and regular without sector; colony margin was irregular, and entire; colony texture as fluffy, effuse and velvet (Table 4, Plate 1).

Ashy white colony color was found in RaCC-1, off white in Pacc-1, light ash in BoCC-1and cottony white in GaCC-1. (Table 4, Plate 1).

Marked variability was found in colony shape. Irregular colonies were observed in isolates RaCC-1, whereas regular with sector colonies were observed in isolates BoCC-1. Regular without sector colonies were found in isolates PaCC-1 and GaCC-1 (Table 4, Plate 1).

Colony margin was observed irregular in isolates RaCC-1, PaCC-1 and entire margin in isolates BoCC-1, GaCC-1 (Table 4, Plate 1). .

Distinct differences of the four isolates were obtained in terms of colony texture. Fluffy texture was observed in isolates and velvet texture was found in isolates PaCC-1, BoCC-1. Effuse type texture was noted in RaCC-1 (Table 4, Plate 1).

Table 4. Colony characteristics of 4 *Colletotrichum capsici* isolates on PDA

Isolates	Characteristics features
	Colony color
RaCC-1	Ashy white
PaCC-1	Off white
BoCC-1	Light ash
GaCC-1	Cottony white
	Colony shape
RaCC-1	Irregular
PaCC-1	Regular without sector
BoCC-1	Regular with sector
GaCC-1	Regular without sector
	Colony margin
RaCC-1	Irregular
PaCC-1	Irregular
BoCC-1	Entire
GaCC-1	Entire
	Colony texture
RaCC-1	Effuse
PaCC-1	Velvet
BoCC-1	Velvet
GaCC-1	Fluffy

RaCC-1

PaCC-1

BoCC-1

GaCC-1

Plate 1. A Colony characteristics of 4 *Colletotrichum capsici* isolates on PDA medium

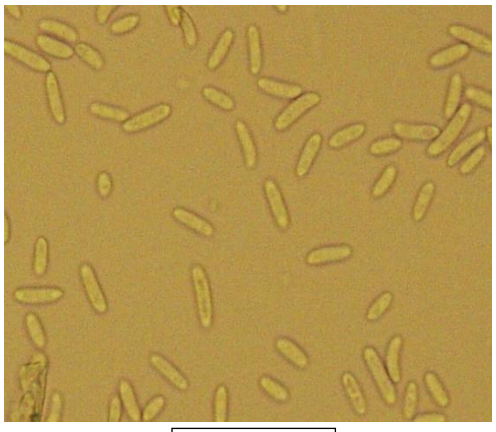
4.1.2. Variability in conidial shape and size of *Colletotrichum capsici* isolates

The conidial shape of different isolates of *C. capsici* on PDA were studied. The results revealed that all the isolates of *C. capsici* produced cylindrical conidia. Significant variations were observed with respect to conidial dimensions among the isolates. The length of conidia ranged from 9.9-51.8µm. Highest length of conidia was observed in RaCC-1 isolate (32.70 µm) followed by BoCC-1 isolate (28.40µm) and shortest conidia was recorded in PaCC-1 isolates (22.20 µm). Breadth of the conidia ranged from 0.33-12.40 µm. Isolate BoCC-1 recorded the highest breadth of conidium (4.56 µm) and was followed by RaCC-1(3.50) and GaCC-1 (3.07 µm). Lowest breadth was observed in PaCC-1 (2.88 µm) isolate (Table 5, Plate 2).

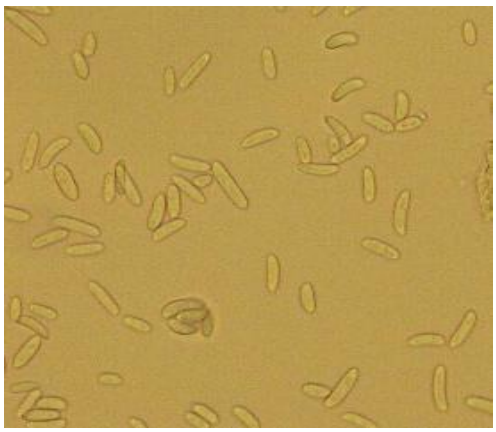
Table 5: Variability in conidial shape and size of *Colletotrichum capsici* isolates

	Length (µm)*		Breadth (µm)*		Shape
Isolate	Range	Mean	Range	Mean	
BoCC-1	16.5-44.4	28.40	1.65-12.40	4.56	Cylindrical
RaCC-1	13.2-51.8	32.70	2.65-4.880	3.50	Cylindrical
GaCC-1	16.5-26.4	18.09	1.65-3.300	3.07	Cylindrical
PaCC-1	9.9-33.00	22.20	0.33-3.300	2.88	Cylindrical

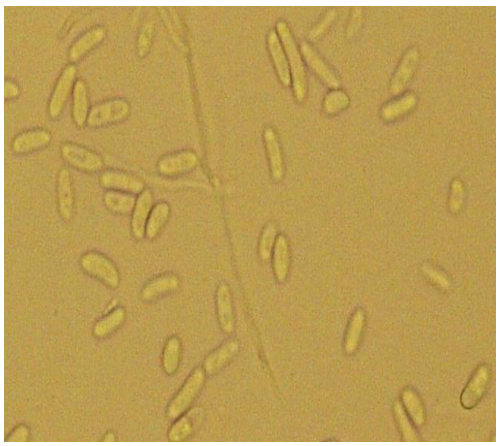
* Mean of 50 observations



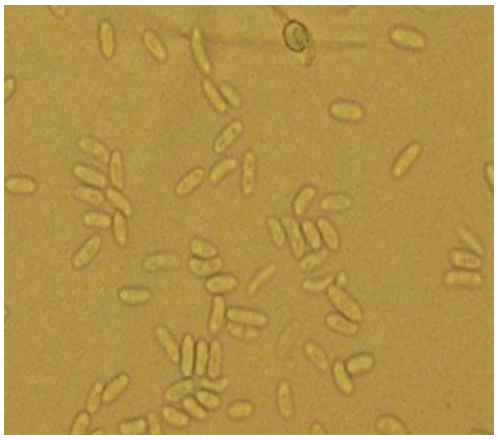
BoCC-1



RaCC-1



GaCC-1



PaCC-1

Plate 2. Conidia of four isolate

4.1.3. Radial growth of *Colletotrichum capsici* at different temperature (°C) levels

The effect of temperatures on the radial mycelial growth of *C. capsici* on PDA is presented in Table 6. The growth of *Colletotrichum capsici* was increased with the time for a certain period for each temperature. The radial mycelial growth gradually increased up to 30°C. No growth was observed at 35°C and 10°C temperature. The mycelial colony diameter was highest for all four isolates at 30°C. At 30°C maximum colony diameter (88.00 mm) was obtained in isolate RaCC-1 followed by GaCC-1 (87.67 mm), and those were statistically identical. The lowest colony growth (80.67 mm) was noted at 30°C incase of isolate PaCC-1 preceded by BoCC-1 (8l.l7 mm). (Plate 3, Fig 1)

From this investigation, it appeared that 25-30°C temperature was suitable for radial mycelial growth of *Colletotrichum capsici.*

Table 6: Radial mycelial growth of *Colletotrichum capsici* at different temperature (°C) levels

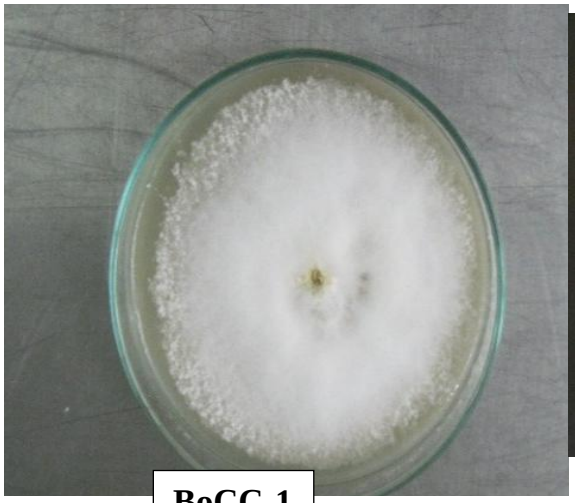
Temperature	Radial colony growth[1] (mm)			
	RaCC-1	**PaCC-1**	**BoCC-1**	**GaCC-1**
10°C	0.00	0.00	0.00	0.00
15°C	35.00 d	34.00 c	33.67 c	32.00 d
20°C	53.33 c	50.33 b	55.00 b	54.00 c
25°C	73.00 b	79.67 a	85.67 a	80.67 b
30°C	88.00 a	80.67 a	81.67 a	87.67 a
35°C	0.00	0.00	0.00	0.00
LSD (.05)	11.82	9.30	12.11	6.862
CV (%)	6.26	4.89	6.25	3.56

[1]Means of three replications for each isolate

Numbers with similar letter do not differ significantly at 5 % level according to Duncan's Multiple Range Test (DMRT

RaCC-1

PaCC-1



BoCC-1

GaCC-1

Plate 3. Maximum radial mycelial growth of *Colletotrichum capsici* at 30°C temperature.

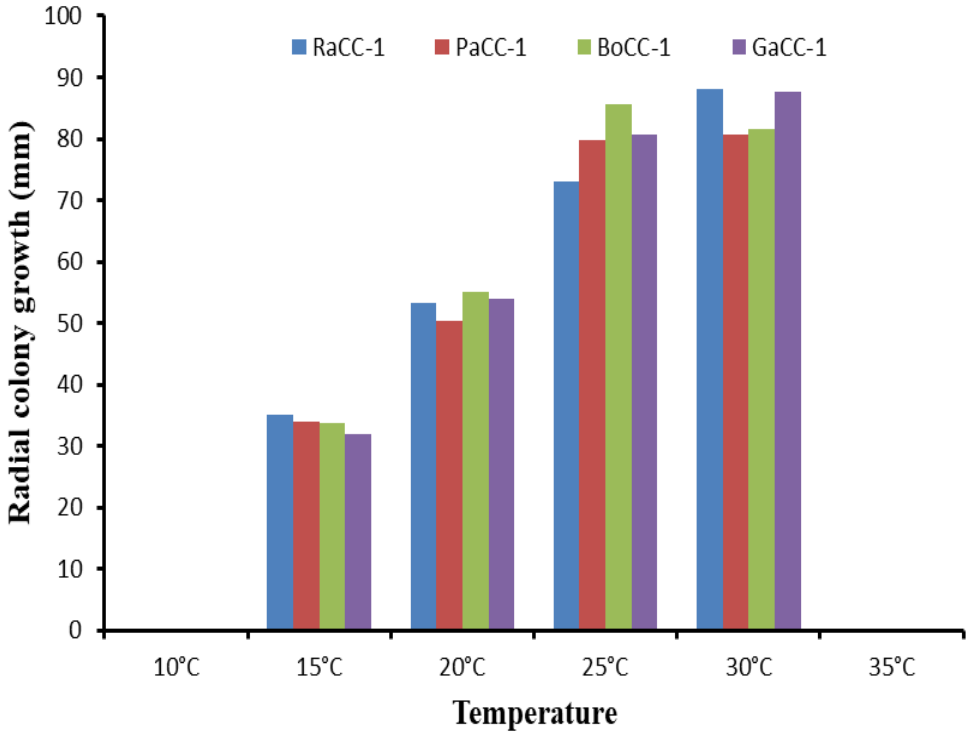


Fig 1. Radial mycelial growth of *Colletotrichum capsici* at different temperature (°C) levels

4.1.4. Sporulation of *Colletotrichum capsici* at different culture temperature (°C) levels

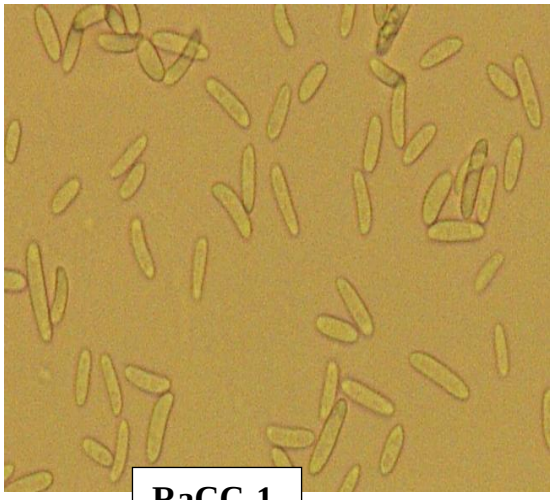
The fungus *Colletotrichum capsici* was grown on PDA medium at different temperatures viz., 10, 15, 20, 25, 30 and 35°C to know the suitable temperature requirement for their maximum radial growth and sporulation. Results (Table 7, Plate 4) revealed that, all the temperature regimes exhibited a wide range of sporulation from no (-) to excellent (++++) in all the four isolate. However excellent (++++) sporulation was recorded at temperature 25 and 30°C. Good (+++) sporulation was recorded at temperature 20°C. It was fair (++) at 15°C. At the temperatures of 10 and 35°C there was no sporulation of the test pathogen

In the present study, the excellent fungal growth and sporulation was observed at 25 and 30°C. Hence, the temperature range of 25 to 30°C can be suitable to obtain excellent fungal growth and sporulation of *C. capsici.*

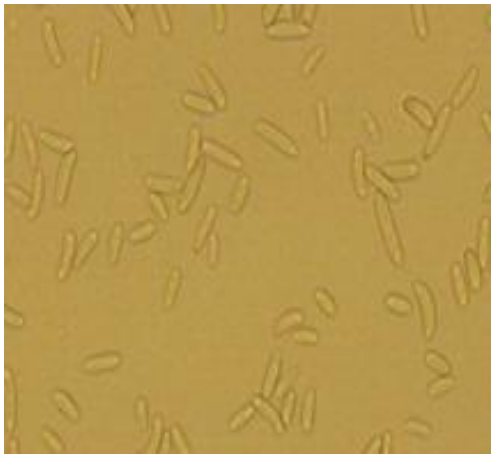
Table 7. Sporulation of *Colletotrichum capsici* at different culture temperature (°C) levels

Isolates	Sporulation					
	10°C	**15°C**	**20°C**	**25°C**	**30°C**	**35°C**
RaCC-1	_	++	+++	++++	++++	_
PaCC-1	_	++	+++	++++	++++	_
BoCC-1	_	++	+++	++++	++++	_
GaCC-1	_	++	+++	++++	++++	_

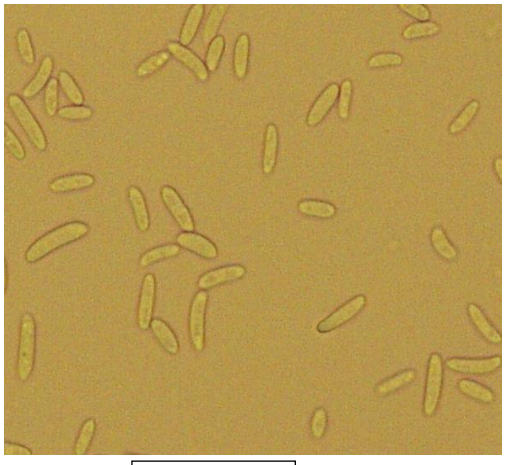
*Average of three replications ++++: Excellent; +++: Good; ++: Fair; + : Poor; - : No



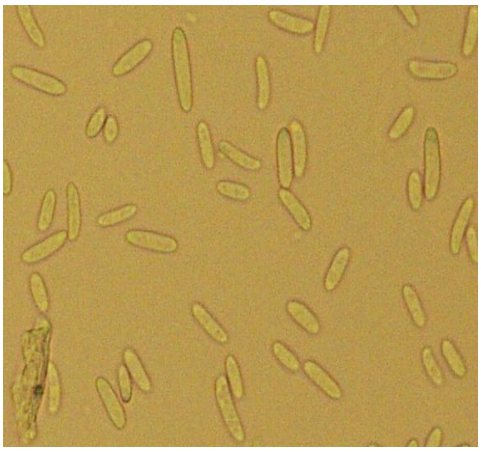
RaCC-1



PaCC-1



BoCC-1



GaCC-1

Plate 4. Maximum sporulation of *Colletotrichum capsici* at 30°C temperature.

4.1.5. Radial growth of *Colletotrichum capsici* at different pH levels on PDA

The pH of the culture medium had a significant role on colony growth of *Colletotrichum capsici*. The fungus grew well on PDA medium with a wide range of pH 5.5 to 8 in this study (Table 8, Plate 5, Figure 2). Deviating from this range caused gradual retardation of radial colony growth of different isolates. Profound growth was recorded at pH 6 and 6.5 pH than the other pH levels. Among the pH levels, luxuriant radial growth exhibited all of the isolates at pH 6.5 followed by 6.0 and radial growth decreased for the rest of other pH. The highest colony diameter was noted for the isolate BoCC-1 (87.00 mm) at pH 6.5 followed by RaCC-1 (86.33 mm) and GaCC-1 84.67 (84.67 mm) at pH 6. The lowest radial growth was recorded in isolate GaCC-1 (43.00 mm) preceded by BoCC-1 (45.33 mm) at pH 8.

The results of the present investigation showed that *Colletotrichum capsici* is an acid loving and showed variability in all isolates at different pH level in respect of mycelial radial diameter on PDA.

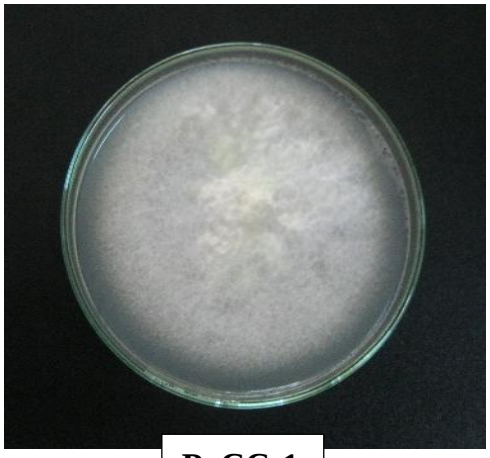
Table 8. Radial mycelial growth of *Colletotrichum capsici* at different pH levels

pH level	Radial colony growth[1] (mm)			
	RaCC-1	**PaCC-1**	**BoCC-1**	**GaCC-1**
pH 5.5	64.00 e	59.33 d	70.00 ab	69.67 c
pH 6	86.33 a	74.00 b	77.33 ab	84.67 a
pH 6.5	81.67 b	80.00 a	87.00 a	82.67 a
pH 7	77.33 c	74.00 b	75.67 ab	76.33 b
pH 7.5	77.33 c	68.00 c	57.00 ab	60.00 d
pH 8	67.00 d	56.33 e	45.33 b	43.00 e
LSD (.05)	1.614	2.82	35.79	2.659
CV (%)	0.82	1.59	20.12	1.48

[1]Means of three replications for each isolate

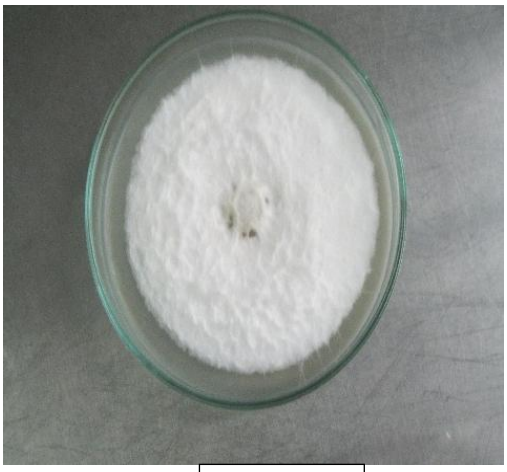
Numbers with similar letter do not differ significantly at 5 % level according to Duncan's Multiple Range Test (DMRT)

RaCC-1



PaCC-1

BoCC-1



GaCC-1

Plate 5. Maximum radial mycelial growth of *Colletotrichum capsici* at pH 6.5 of different isolate

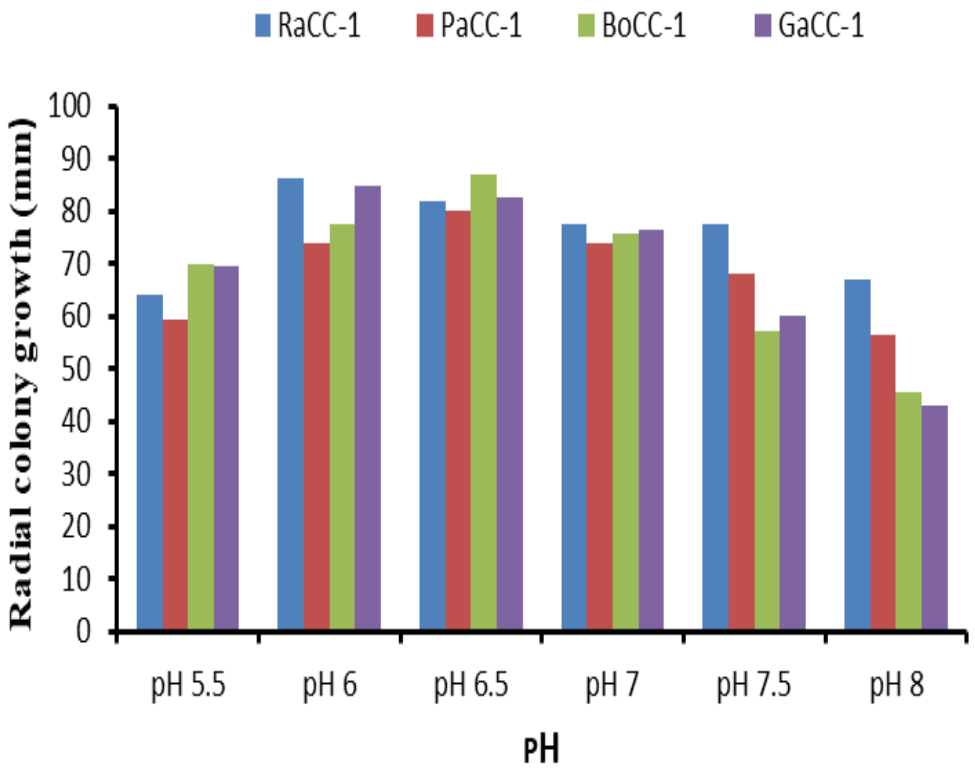


Figure 2. Radial Mycelial growth of *Colletotrichum capsici* at different pH levels

4.1.6. Sporulation of *Colletotrichum capsici* at different pH levels

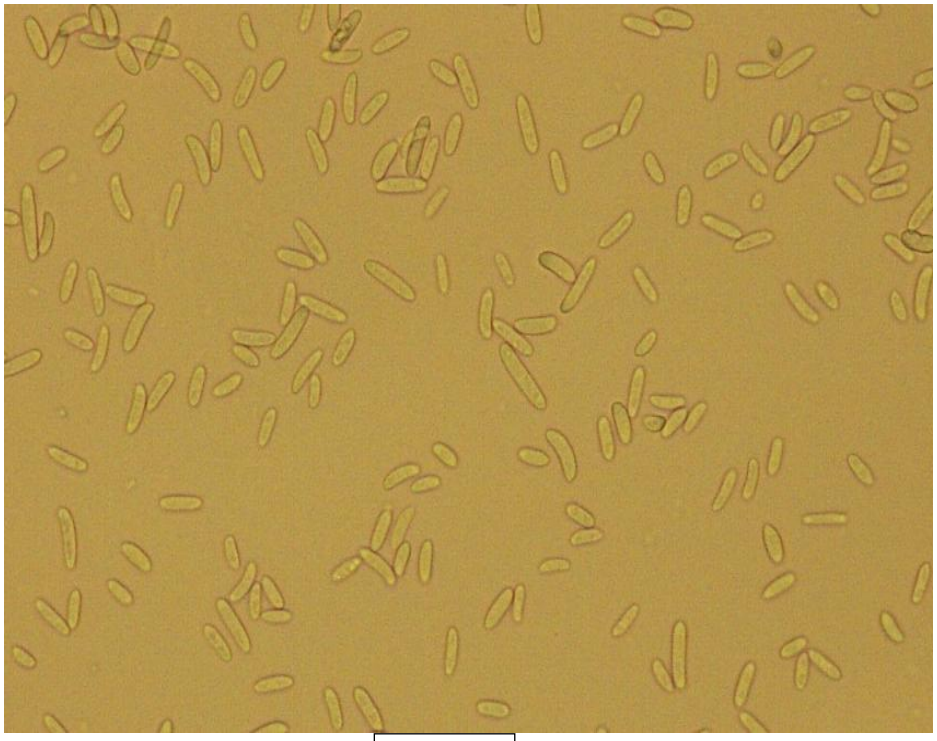
The fungus *Colletotrichum capsici* was grown on PDA media at different pH viz., 5.5, 6.0, 6.5, 7.0, 7.5, and 8 to know the suitable pH requirement for their maximum radial growth and sporulation. Results (Table 9) revealed that, all the pH regimes exhibited a wide range of sporulation from poor (+) to excellent (++++). However excellent (++++) sporulation was recorded at pH 6 and 6.5 for the all isolate (Plate 6). Good (+++) sporulation was recorded at pH 7 and 5.5 in case of isolate PaCC-1 and BoCC-1. It was fair (++) in all isolate at pH 7 except PaCC-1 and pH 7.5. Only GaCC-1 isolate showed fair at pH 5.5, 7.0, and 7.5. Poor (+) sporulation was recorded at pH 5.5 in case of isolate RaCC-1 and PaCC-1. All isolate showed poor (+) spuralation at pH 8.

In the present study, the excellent fungal growth and sporulation was observed at pH 6 and 6.5. Hence, the pH range of 6 to 6.5 can be suitable to obtain excellent fungal growth and sporulation of *C. capsici.*

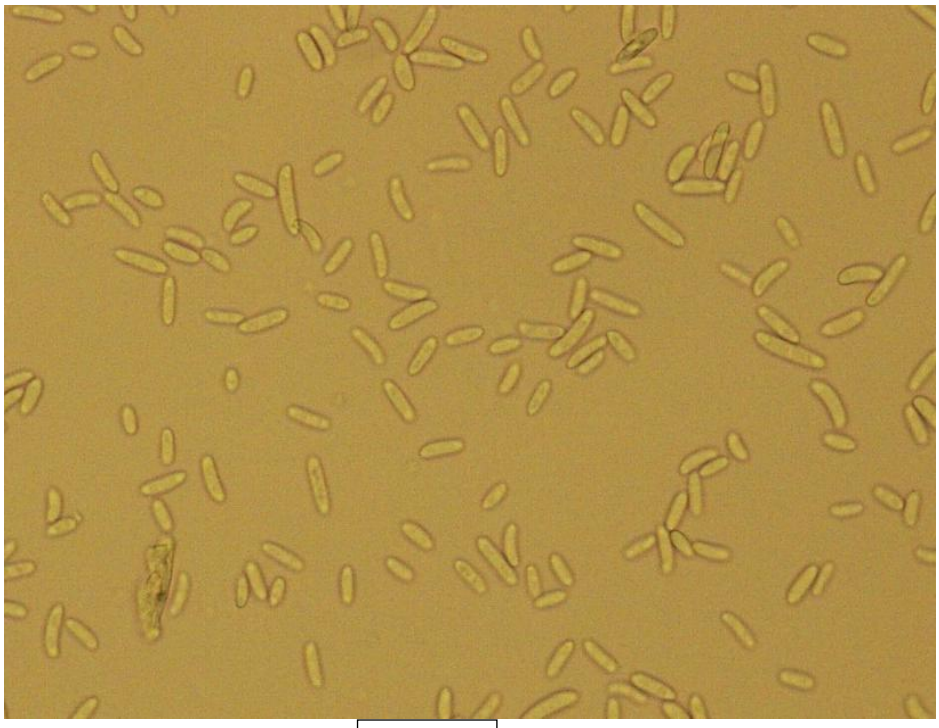
Table 9. Sporulation of *Colletotrichum capsici* at different pH levels

Isolates	Sporulation					
	pH 5.5	**pH 6**	**pH 6.5**	**pH 7**	**pH 7.5**	**pH 8**
RaCC-1	+	++++	++++	++	++	+
PaCC-1	+	++++	++++	+++	++	+
BoCC-1	+++	++++	++++	++	++	+
GaCC-1	++	++++	++++	++	++	+

*Average of three replications ++++: Excellent; +++: Good; ++: Fair; +: Poor; - : No



pH 6.0



pH 6.5

Plate 6. Excellent (++++) sporulation of different isolates.

4.1.7. Radial growth of *Colletotrichum capsici* on different culture media.

The effect of different culture media on mycelial radial growth of *Colletotrichum capsici* varied significantly and presented in Table 10 and Plate 7.

The results of the studies on culture media indicated that, the radial growth of *C. capsici* was maximum (87 mm) on PDA in RaCC-1. It was significantly superior over all other media. Statistically similar growth was observed on PDA and CDA in case of all the four isolates. No mycelial radial growth was fouud in V-8 A and WA media. Maximum colony diameter (87.00 mm) was obtained in isolate RaCC-1 on PDA media followed by rest of isolate. The lowest colony growth (35 mm) was obtained in PaCC-1 preceded by RaCC-1 (49.33 mm) on CMA media.

Table 10. Radial mycelial growth of *Colletotrichum capsici* on different culture media.

culture media	Radial colony growth[1] (mm)			
	RaCC-1	**PaCC-1**	**BoCC-1**	**GaCC-1**
PDA	87.00 a	83.67 a	86.67 a	86.67 a
CDA	79.67 a	60.00 ab	83.00 a	75.67 a
CMA	49.33 b	35.00 b	85.00 a	69.33 a
V-8 A	0.00	0.00	0.00	0.00
WA	0.00	0.00	0.00	0.00
LSD (.01)	23.97	42.66	20.40	6.69
CV (%)	8.86	19.05	6.39	19.53

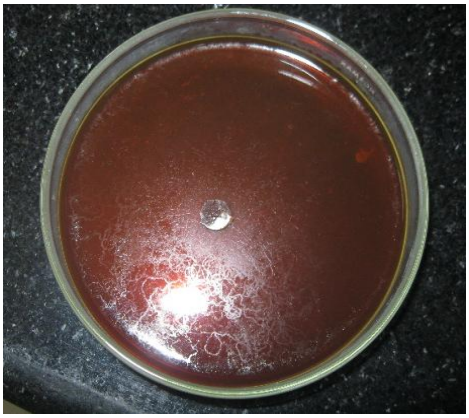
°Means of three replications for each isolate

Numbers with similar letter do not differ significantly at 1 % level according to Duncan's Multiple Range Test (DMRT)

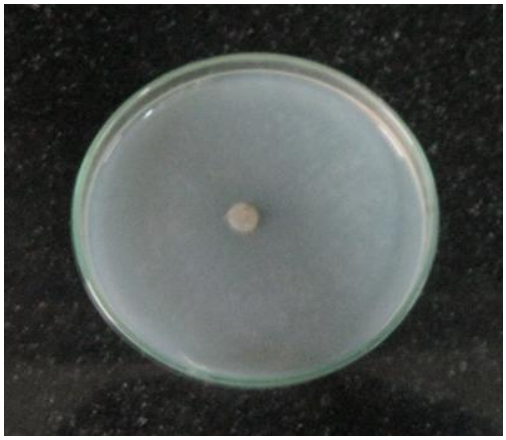
CDA

PDA

CMA



V-8 A



WA

Plate 7. Radial Mycelial growth of *Colletotrichum capsici* on different culture media.

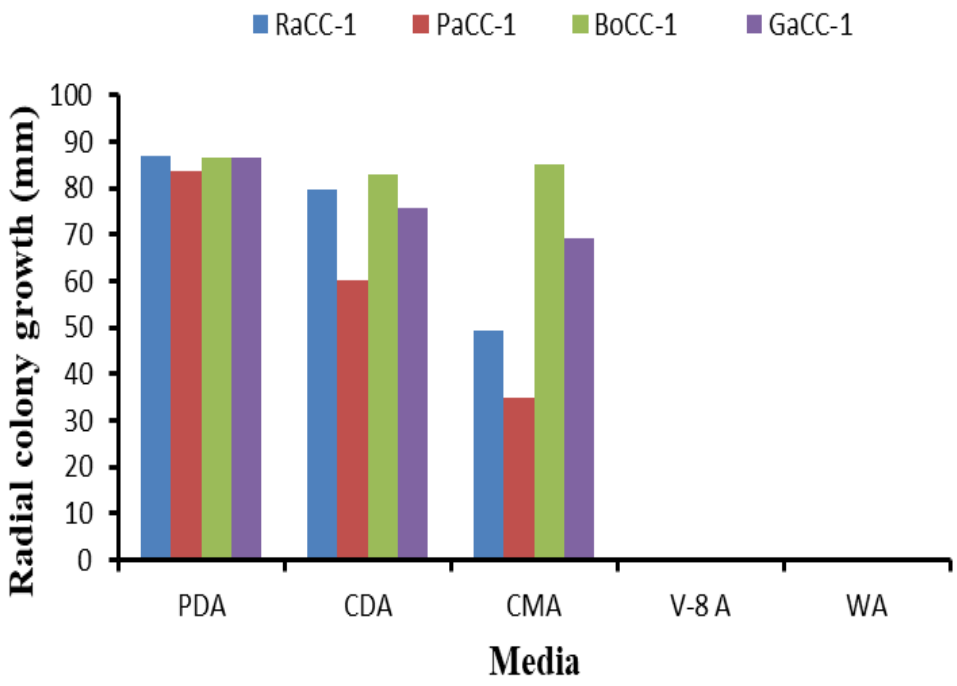


Figure 3. Radial mycelial growth of *Colletotrichum capsici* on different culture media

4.1.8. Sporulation of *Colletotrichum capsici* on different culture media.

The fungus *Colletotrichum capsici* was grown at different culture media viz., PDA, V-8 A, WA, CDA and CMA to know the suitable media for sporulation. Results (Table 11) revealed that, all the media regimes exhibited a wide range of sporulation from no (-) to good (+++). However, Good (+++) sporulation was recorded at CDA media for all the four isolates. Fair (++) sporulation was recorded at PDA and CMA media for all the isolate. All the four isolates failed to spuralation on V-8 A and WA media.

Table 11. Sporulation of *Colletotrichum capsici* on different culture media

Isolates	**Sporulation**				
	PDA	V-8 A	WA	CDA	CMA
RaCC-1	++	_	_	+++	++
PaCC-1	++	_	_	+++	++
BoCC-1	++	_	_	+++	++
GaCC-1	++	_	_	+++	++

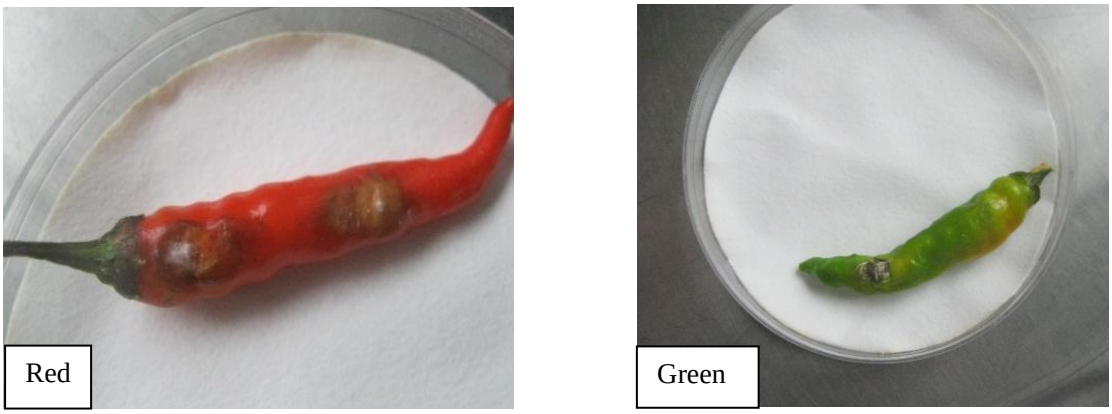
*Average of three replications ++++: Excellent; +++: Good; ++: Fair; +: Poor; - : No

4.1.9. Disease reaction of four *C. capsici* isolates inoculated on detached chilli fruits

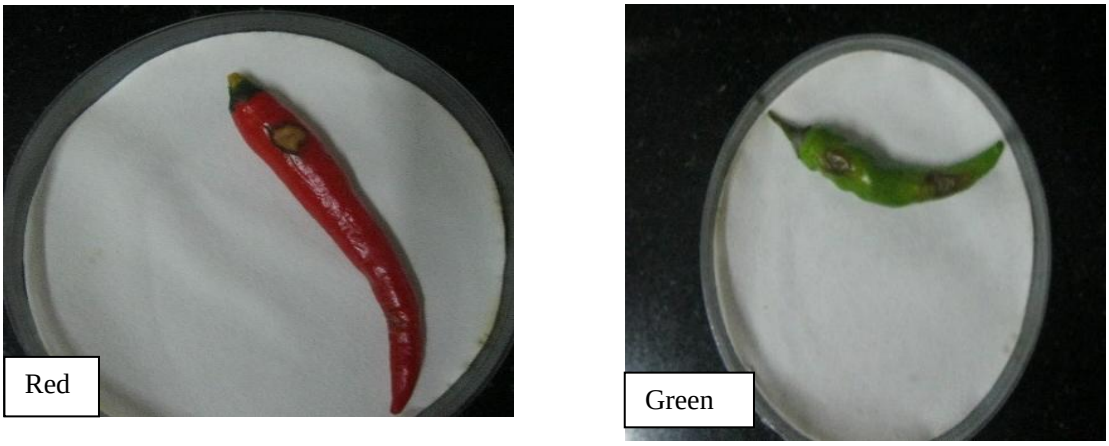
The results indicated that there is a positive correlation between disease reaction on red fruits and Green fruits observed under laboratory conditions on detached chilli fruits (Table 12). Ripened red fruits were found to be more susceptible with large lesion areas while green fruits were showing initial symptoms only at the colour changing stage. The highest disease score was found in Isolate BoCC-1 and RaCC-1 in case of red (7.00) and green (5.00) chillies. On the other hand, the lowest disease score was found in Isolate GaCC-1 and PaCC-1 in case of red (5.00) and green (3.00) chillies.

Table 12: Disease reaction of four *C. capsici* isolates inoculated on detached chilli fruits

	Disease score (0-9 scale)	
Isolate	**Red**	**Green**
BoCC-1	7.00	5.00
RaCC-1	7.00	5.00
GaCC-1	5.00	3.00
PaCC-1	5.00	3.00



BoCC-1 and RaCC-1



GaCC-1 and PaCC-1

Plate 8. Disease reaction of four *C. capsici* isolates inoculated on detached chilli fruits.

4.2. Field Experiment

4.2.1. Percent plant infection per plot due to anthracnose of chilli:

All the treatments reduced the incidence of anthracnose disease of chilli significantly in regarding to plant infection compared to the untreated control at 85 Days After Transplanting (DAT). Among the treatments, the highest (73.14%) infection of chilli plant by anthracnose was observed in control plots at 85 DAT, while the lowest (25.45%) plant infection was obtained from the treatment planting in raised bed followed by application of Metaril 72 WP @ 2g/L water (29.36%), Micro tech-1 @ 10ml/L water (41.21%) and Space planting (50x50cm) (56.27%) treated plots (table-10, Plate 9). Highest (65%) reduction of percent plant infections at 85 DAT were obtained from planting in raised bed plots followed by Metaril 72 WP @ 2g/L water (60%), Micro tech-1 @ 10ml/L water (44%) spraying and Space planting (50x50cm) (23%) plots (Table 13). This result indicates that all the treatments reduce the plant infection. Albeit the planting in raised bed showed highest reduction of in percent plant infection, the other treatments also worked as good as raised bed.

Table 13: Percent plant infection per plot due to anthracnose of chilli at 85 DAT as influenced by some management practices

Treatments	Plant infection at 85 DAT (%)	Reduction of plant infection over control treatment at 85 DAT (%)
Planting in raised bed	25.45 d	65
Space planting (50x50cm)	56.27 b	23
Micro tech-1 @ 10ml/L water	41.21 c	44
Metaril 72 WP @ 2g/L water	29.36 d	60
Control	73.14 a	-
LSD (0.05)	5.822	
CV (%)	7.68	

DAT: Days After Transplanting

Means followed by the same letter(s) in a column did not differ significantly at 5% level by DMRT.

Lowest infection in planting in raised bed

Highest infection in control

Plate 9. Highest and Lowest plant infection per plot due to at anthracnose of chilli at 85 DAT

4.2.2. Effect of different treatments on the yield performance of chilli

Effect of different treatments on yield of chilli in terms of dry weight (grams/plant) was determined. Fruit yield of chilli was significantly influenced by different treatments applied. Yield of chilli profoundly varied from one treatment to another treatment ranging from 211.1- 456.3 g plant^{-1}. It was found that the highest fruit yield 456.3 g plant^{-1} was recorded in planting in raised bed plots which was statistically similar with the treatment Metaril 72 WP @ 2g/L water 413.7 g plant^{-1}, followed by 360.3 g plant^{-1} in Micro tech-1 @ 10ml/L water and 302.7 g plant^{-1} in Space planting (50x50cm). The highest (54%) yield increase at 85 DAT was found in planting in raised bed followed by Metaril 72 WP @ 2g/L water (49%) treated plots (Table 14, Plate 10). This result suggests that all the treatments could increase the fruit of chilli. This result also suggests that planting in raised bed are more effective approach to restrict pathogen ingress efficiently, which increase yield of chilli fruit.

Table 14: Effect of different treatments on the yield performance of chilli

Treatments	Fruit yield plant^{-1} (g)	Yield increase over control treatment (%)
Planting in raised bed	456.3 a	54
Space planting (50x50cm)	302.7 c	30
Micro tech-1 @ 10ml/L water	360.3 b	41
Metaril 72 WP @ 2g/L water	413.7 a	49
Control	211.1 d	-
LSD (0.05)	45.33	
CV (%)	9.38	

Means followed by the same letter(s) in a column did not differ significantly at 5% level by DMRT,

Highest fruit yield in planting in raised bed

Lowest fruit yield in control

Plate-10. Highest and Lowest fruit yield of planting in raised bed and control respectively

CHAPTER 5

DISCUSSION

Chilli is a profitable cash crop in Bangladesh. In Indian sub-continent countries, chilli is also a popular member of spices and condiments for its special taste, flavour and colour. Anthracnose disease of chilli caused by *Colletotrichum spp.* is one of the most devastating diseases of chilli in Bangladesh as well as other chilli growing countries.

All four isolates showed variations in respect of their cultural, morphological, physiological and pathogenic characteristics. The isolates of *Colletotrichum capsici* showed variation in respect of colony color, shape, margin and textures. Remarkable variations found in colony color and shape. Colony colors were ashy white, off white and Cottony white in RaCC-1, PaCC-1 and BoCC-1, respectively. Colony color was light ash in BoCC-1. Colony margin were irregular and entire. Colony textures were velvet in PaCC-1and BoCC-1. On the other hand, effuses and fluffy type of texture were found in RaCC-1 and GaCC-1, respectively. The current findings were well supported by Akther and Singh (2007) who reported different colony color and margin of *Colletotrichum capsici* isolates.

The conidial shape of different isolates of *C. capsici* on PDA were studied. All the isolates of *C. capsici* were observed with cylindrical shape of conidia. The highest length of conidia was observed in RaCC-1 isolate (32.70 µm) followed by BoCC-1 isolate (28.40µm) and shortest conidia was recorded in PaCC-1 isolates (22.20 µm). The highest breadth of conidia (4.56 µm) was found in BoCC-1 and was followed by RaCC-1 (3.50) and GaCC-1 (3.07 µm) and the lowest breadth was observed in PaCC-1 isolate (2.88 µm). These finding are very similar to Jinyoung Lim *et al*. (2002) who reported that the conidia of *C. musae* isolates were aseptate, hyaline, mostly ellipsoid, ranging from 10-18 µm and 5-9 µm (average of 14.5-6.9 µm).

Influences of temperature have been observed on radial colony growth of *Colletotrichum capsici*. Radial mycelial radial growth was gradually increased with time in case of all the four isolates where there was different growth at different temperatures. All the collected isolates (RaCC-1, PaCC-1, BoCC-1, GaCC-1) grew well at varied range of temperatures (15 to 30°C) and gradually increased within the range of >15°C to <30°C temperatures but gradually decreased after >30°C. At temperature 10°C and 35°C growth was completely stopped. The highest (88.00 mm) colony diameter was noted in isolate GaCC-1 and the lowest (80.67) was obtained in isolate PaCC-1 both at temperature 30°C. From this investigation, it appears that 30°C temperature is suitable for mycelial radial growth of *Colletotrichum capsici*. The present findings are in agreement with other researchers (Jalal Sultani *et. al.* 2014) who found that 28°C was the optimum temperature for maximum colony diameter of *Colletotrichum gloeosporioides*. They also observed that the fungal growth was completely inhibited at 5°C and 35°C.

The highest sporulation was recorded at temperature 25 and 30°C and the lowest sporulation was recorded at temperature 15°C. Excellent (++++), Good (+++) and fair (++) sporulation was recorded at temperature 25-30°C, 20°C and 15°C, respectively. At the temperatures of 10 and 35°C there was no sporulation of the test isolate. The present results are in agreement with the results obtained by Mishra and Gupta (1994), Shirshikar (1995), Murthy (1997), Varaprasad (2000), Singh and Singh (2001) and Laxman (2006), who reported that optimum temperature for growth and sporulation of *C. truncatum* was 25 to 30°C. Hence, the temperature range of 25 to 30°C can be suitable to obtain excellent fungal growth and sporulation of *C. capsici*.

The maximum and minimum radial mycelial growth was observed at pH 6.0 and pH 6.5, respectively for all four isolates. At pH 6.5 highest (87.00 mm) radial growth was obtained in isolates BoCC-1 followed by RaCC-1 (86.33 mm) and the lowest mycelial radial growth was obtained in isolates GaCC-1 (43.00 mm) preceded by BoCC-1 (45.33 mm)..

The excellent (++++) sporulation was recorded at pH 6.0 and 6.5 for all the isolates. Good (+++) sporulation was recorded at pH 7.0 and 5.5 in case of isolate PaCC-1 and BoCC-1. Poor (+) sporulation was recorded at pH 5.5 in case of isolate RaCC-1 and PaCC-1. All isolates showed poor (+) sporulation at pH 8. In the present study, the excellent fungal growth and sporulation was observed at pH 6.0 and 6.5. This finding is very similar to Naik *et al.* (1988) who found that pH 5.0 and 6.0 were the optimum pH for maximum fungal growth and sporulation of *Colletotrichum gloeosporioides.*

The highest (87.00) mm radial growth of *C. capsici* was observed on PDA in RaCC-1. PDA media was significantly superior over all other media. This was followed by CMA (85.00 mm) and CDA (83.00 mm) incase of isolate BoCC-1. No mycelial radial growth was fouud in V-8 A and WA media. The lowest colony growth (35 mm) was obtained in PaCC-1 preceded by RaCC-1 (49.33 mm) on CMA media except BoCC-1 isolate. Kenchaiah (1975) observed maximum radial growth and sporulation of *Colletotrichum capsici* on potato dextrose agar. Similarly, Kadu (1977) obtained good growth of *C. capsici* (90 mm) on potato dextrose agar.

Good (+++) sporulation was recorded at CDA media for the all isolate. Fair (++) sporulation was recorded at PDA and CMA media for all the isolates. No sporulation was found in V-8 A and WA media. Mamatham *et al.* (2006) observed that no sporulation of *Colletotrichum sp.* was found in V-8 A.

The highest disease score was found in isolate BoCC-1 and RaCC-1 in case of red (7.00) and green (5.00) chillies. On the other hand, the lowest disease score was found in Isolate GaCC-1 and PaCC-1 in case of red (5.00) and green (3.00) chillies. These results are in agreement with Than *et al.* (2008). They observed that red chillies were more prone to anthracnose than green chillies.

Different application practices like planting in raised bed, space planting (50x50cm), spraying Micro tech-1 @ 10ml/L water and Metaril 72 wp @ 2g/L water along with an untreated control was selected in this study to evaluate their management ability against anthracnose disease of chilli caused by

Colletotrichum capsici with cost effective and eco-friendly approaching. When the chilli plants were planted in raised bed had shown the lowest disease intensity as well as other severity parameters. The space planting (50x50cm) had shown the least disease control potential but significantly superior to the control. The bio-fungicide Micro tech-1 @ 10ml/L water have shown significantly better performance over control. The chemical treatments Metaril 72 WP @ 2g/L water had shown very strong response as single treatment, though less than the planting in raised bed. In this study, all the treatments had significant effect in reducing the disease incidence and increased the yield of chilli.

The highest reduction of fruit infection was measured from the planting in raised bed and chemical treatment of Metaril 72 WP @ 2g/L water and the lowest from the control treatment. As a result, the lowest yield was found in control (211.1 g plant^{-1}) plots. The highest yields was found in the plot planting in raised bed (456.3 g plant^{-1}) followed by Metaril 72 WP @ 2g/L water (413.7 g plant^{-1}). The results support the findings of Hegde and Ekbote (2002). They observed that planting in raised bed yielded lower infection than control.

CHAPTER 6

SUMMERY AND CONCLUSION

The present investigation on studies on the biology and the management of anthracnose disease of chilli *(Capsicum annuum L.*) caused by *Colletotrichum capsici* was carried at the Research farm and laboratory of Plant Pathology Division, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur. The findings of investigation were summerised as follows:

Colletotrichum capsici causing anthracnose in chilli is considered the most damaging disease in Bangladesh. Four isolates of *Colletotrichum capsici* were collected from four different chilli growing districts of Bangladesh. Colony of *C. capsici* was cottony white, ashy white, off white and light ash colored colony with irregular, regular without sector or with sector and irregular, entire margin; effuse, velvet and fluffy texture was observed. The conidia size varied within a range of 9.9 to 51.8 µm in length and breadth from 0.33 to 12.40 µm. The maximum mean conidial length was found in isolate RaCC-1 (32.70 µm) and minimum in isolate GaCC-1 (18.09 µm). The maximum mean breadth (4.56 µm) of conidia was found in isolate BoCC-1 and the lowest (2.88 µm) was found in isolate PaCC-1. Conidia of all the four isolates were cylindrical shaped. The fungus grew well in a varied range of temperature, pH and nutrient media. The maximum radial colony diameter was found at 25-30°C, pH 6.0-6.5 and PDA media for all the isolates. No growth was observed at 10°C and 35°C temperature. The highest sporulation were found at 25-30°C, pH 6.0-6.5 and CDA media for all the isolates. No sporulation were observed at temperature 10°C and 35°C and media WA and V-8 A media. The highest disease score was found in Isolate BoCC-1 and RaCC-1 in case of red (7.00) and green (5.00) chillies. On the other hand, the lowest disease score was found in Isolate GaCC-1 and PaCC-1 in case of red (5.00) and green (3.00) chillies. The highest chilli plant infection 73.14% by anthracnose was observed in control at the observation dates 85 DAT, while the lowest plant infection was obtained from planting in raised bed (25.45%). Highest reduction of percent plant infections at 85 DAT were obtained from planting in raised bed plots followed by Metaril

72 WP @ 2g/L water (65%), Micro tech-1 @ 10ml/L water (44%). The highest yield was recorded 456.3 g plant^{-1} in planting in raised bed followed by 413.7 g plant^{-1} in Metaril 72 wp @ 2g/L water, 360.3 g plant$^{-`1}$ in Micro tech-1 @ 10ml/L water and 302.7 g plant^{-1} in space planting (50x50cm) treatments but statistically significant by different from control. The highest yield increase (54%) of chilli was found in planting in raised bed followed by other treatments.

At the end of the above results it can be concluded that-

- Variability exist in chilli anthracnose pathogen (*Colletotrichum capsici*) prevailing in the chilli growing areas of Bangladesh.
- 25-30°C temperature is favourable for the growth and development of *Colletotrichum capsici.*
- Suitable pH for the growth and development of *Colletotrichum capsici* is 6.0-6.5.
- *Colletotrichum capsici* can grow well in PDA medium.
- *Colletotrichum capsici* cannot grow below 10°C and above 35°C temperature.
- All the isolates were not virulent equally to the tested chilli cultivar.
- Planting in raised bed was found effective against anthracnose disease of chilli.
- Fungicides from Mancozeb+Metalaxyl group like Metaril 72 WP @ 2g/L water was also found effective against anthracnose disease of chilli.
- Bio-pesticide Micro tech-1 @ 10ml/L water can also be used as an alternative of chemical fungicide against anthracnose disease of chilli.
- Molecular characterization should be done to confirm the variability exist in the present anthracnose isolates.

CHAPTER 7

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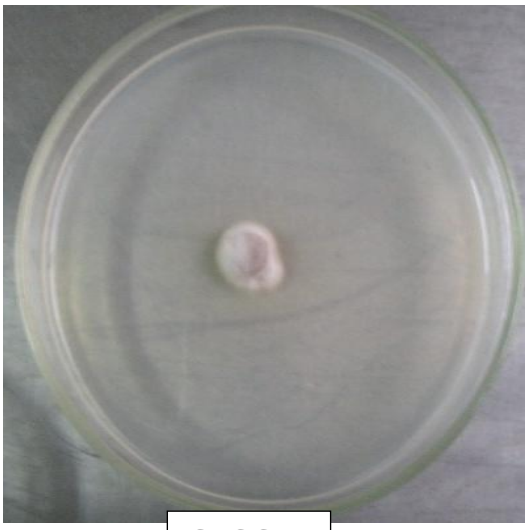
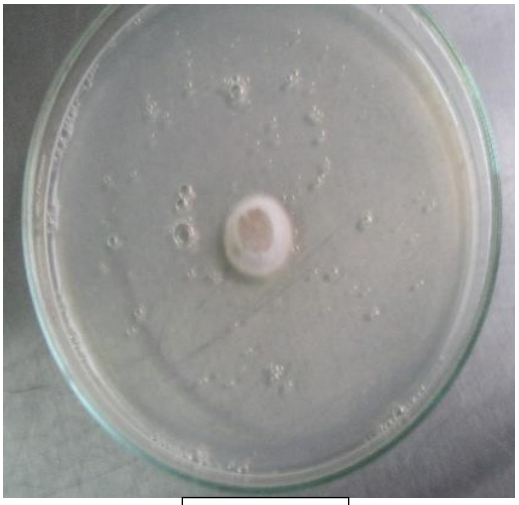
APPENDICES



GaCC-1

PaCC-1

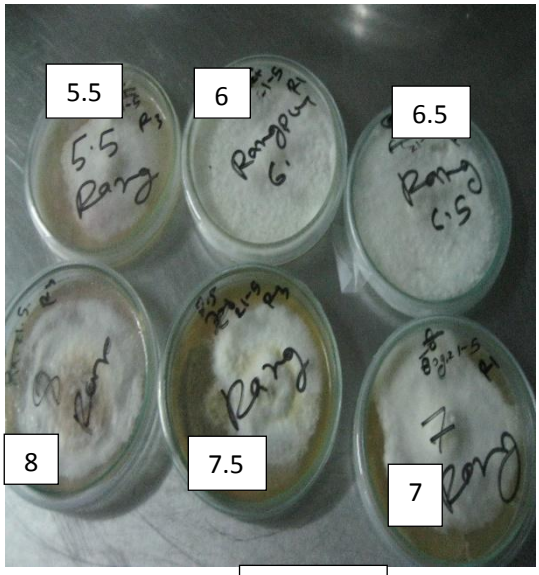


RaCC-1

BoCC-1

Appendix I. Completely retarded mycelial growth of different isolates at 10 and 35°

RaCC-1



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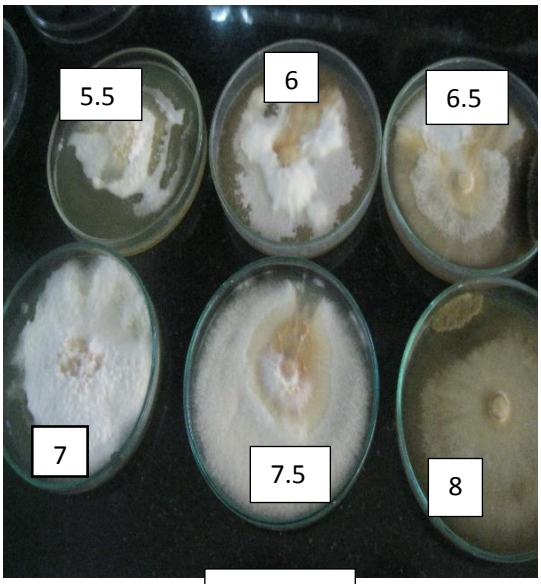
BoCC-1



GaCC-1

Appendix II. Radial mycelial growth of *Colletotrichum capsici* at different pH levels of different isolate

Appendix III. Field of experiment at BARI, GAZIPUR.

Planting in raised bed

Space planting (50x50cm)

Micro tech-1 @ 10ml/L water

Metaril 72 WP @ 2g/L water

Control

Appendix IV. The plots were shown by different treatment