VARIABILITY, PATHOGENICITY AND *IN VITRO* MANAGEMENT OF *BOTRYTIS CINEREA* CAUSING BOTRYTIS GRAY MOLD IN CHICKPEA (*CICER ARIETINUM* L.)

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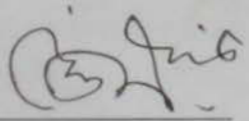

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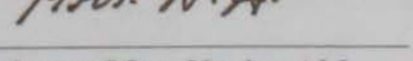

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This is to certify that the thesis entitled, "*VARIABILITY, PATHOGENICITY AND IN VITRO MANAGEMENT OF BOTRYTIS CINEREA CAUSING BOTRYTIS GRAY MOLD IN CHICKPEA (CICER ARIETINUM L.)*" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of *MASTER OF SCIENCE IN PLANT PATHOLOGY*, embodies the result of a piece of bona fide research work carried out by *MD. IQBAL HOSEN*, *Registration No. 00894*, under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma.

I further certify that any help or sources of information, as has been availed of during the course of this inquire have been duly acknowledged and the contents & style of the thesis have been approved and recommended for submission.

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ABSTRACT

A total of ten isolates of *Botrytis cinerea* infecting chickpea in most chickpea growing areas of Bangladesh were characterized in terms of cultural, morphological, physiological and pathogenicity. The isolates varied significantly in cultural, morphological and pathogenic traits- colony color, shape, margin and texture; production and arrangement of sclerotia on PDA medium. The optimum temperature and pH for the mycelial radial growth of *Botrytis cinerea* were recorded at 20°C and pH 4.5, respectively. The mycelial radial growth of all ten isolates increases with the time for a certain period. No growth was observed at 35°C temperature. The pathogen *Botrytis cinerea* grew well on CDA medium. The highest (79.17 mm) mycelial radial growth was obtained on CDA. The quickest (5 days) sclerotia initiation was observed on CDA and LDA culture media but the highest number (2.5×10^4 ml^{-1}) of spores were counted on LDA medium. The length of conidia varied from 5.00 to 15.00 µm. Mean length of conidia was maximum 12.00 µm in isolate AHI-9 and minimum 7.50 µm in isolate AHI-1. The breadth of conidia ranged from 5.00 to 10.00 µm. The highest mean breadth 8.25 µm was observed in isolate AHI-9 and the lowest 6.00 µm in isolate AHI-4. The isolates exhibited different reaction of highly susceptible to resistant to a set of chickpea cultivars and AHI-9 and AHI-10 were found the most virulent isolates among the others. The antagonist *Trichoderma harzianum* appeared to be a good bio-control agent against *Botrytis cinerea*. Among the seven tested fungicides namely- Bavistin® 50WP (Carbendazim), CP-Zim 50WP (Carbendazim), Sunphanate 70WP (Thiophanate methyl) and Rovral 50WP (Iprodione) were the most effective fungicides to inhibit the mycelial radial growth of *Botrytis cinerea* at a lower concentration (500 ppm).


Dedicated To My
Beloved Parents
&
Departed Grandfather

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TABLE OF CONTENTS

Cont'd.....

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF SOME ABBREVIATE FORM AND THEIR ELABORATIONS

ABBREVIATE FORM	ELABORATION
%	Percentage
%RH	Per cent Relative Humidity
@	At the rate
µg	Microgram
µl	Microlitre
µm	Micromililitre
^{0}C	Degree Celsius
B. cinerea	*Botrytis cinerea*
BARI	Bangladesh Agricultural Research Institute
BC	*Botrytis cinerea*
BGM	Botrytis Gray Mold
$CaCO_3$	Calcium carbonate
CBDA	Chickpea Barley Dextrose Agar
CDA	Chickpea Dextrose Agar
cm	Centimeter
Conc.	Concentration
CRD	Completely Randomized Design
CV	Coefficient of Variance
DMRT	Duncan's Multiple Range Test
et al.	And Others
FAO	Food and Agricultural Organization
g	Gram(s)
ha	Hectare(s)
HCl	Hydrochloric Acid
$HgCl_2$	Mercury Chloride
HR	Highly Resistant
hrs	Hours
I	Immune
ISTA	International Seed Testing Association

Cont'd.....

ABBREVIATE FORM	ELABORATION
Kv	Kilovolt
LDA	Lentil Dextrose Agar
LSD	Least Significance Difference
mA	Miliampere
ml	Millilitre
mm	Millimeter
N	Normal
NaOH	Sodium Hydroxide
Pa	Pascal
PDA	Potato Dextrose Agar
PP	Polypropylene
psi	Per Square Inch
R	Resistant
S	Susceptible
SEI	Secondary Electron Image
SEM	Scanning Electron Microscope
T. harzianum	*Trichoderma hazianum*
T. viridae	*Trichoderma viridae*
V8 Juice	V8- Juice Agar
Viz.	Videlicet
WA	Water Agar
WP	Wettable Powder


CHAPTER 1
INTRODUCTION

CHAPTER 1

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the third most important legume crop in the world and is grown in at least 44 countries of the world (Bakr *et al.* 2002; Pande *et al.* 2006). Chickpea is valued for its nutritive seeds with high protein content ranged from 25.3 to 28.9 % (Hulse, 1991). Chickpea cultivated on about 11.12 million ha, adding 8.62 million tones of grain to the global food basket (FAO, 2005). In Bangladesh it was occupying the third position next to lathyrus and lentil but due to some major constraints for the last several years, its area and production has been declined to only 3 % of the total pulse area in Bangladesh, occupying the fifth position among pulses (Bakr *et al.* 2002).

Despite the large acreage under chickpea cultivation in the world, the total production and productivity are quite low in most of the chickpea growing areas (Pande *et al.* 2006). The climate and agro-ecological conditions of Bangladesh favors the rapid development and growth of various plant pathogens (Ahmed, 1996). So, susceptibility of chickpea to a number of fungal pathogens from seedling stage to maturity is the primary cause of low yield.

So far, 17 diseases of chickpea are recorded in Bangladesh (Bakr and Rashid, 2007). Among the diseases, Botrytis gray mold (BGM) caused by *Botrytis cinerea* Pers. Ex. Fr., is the most damaging disease of chickpea (Pande *et al.* 2006). The disease has threatened the chickpea crop so much that the area of the crop has come down to 16,000 ha from more than 10, 00,000 ha within a span of 10 years (BBS, 1999). Cool wet weather favors the disease development (Anonymous, 2006). BGM can devastate chickpea, resulting in complete yield loss in years of extensive winter rain and high humidity (Reddy *et al.* 1988 and Pande *et al.* 2002).

The occurrence of BGM in chickpea was first reported by Shaw and Ajrekar in 1915, but Carranza (1965) reported its first field incidence in Argentina, resulted in a crop loss of 95 %. The disease is of serious concern in Bangladesh, India, Nepal, Pakistan, Australia and Argentina (Bakr *et al.* 1993; Dhar *et al.* 1993; Pande *et al.* 2002; Davidson *et al.* 2004) where 100 % yield losses were reported under conduciveness conditions. Recently, more than 80 % crop losses due to BGM have been observed in the Indo-Gangetic plains of Bangladesh, Nepal and north-western India (Pande, 1998).

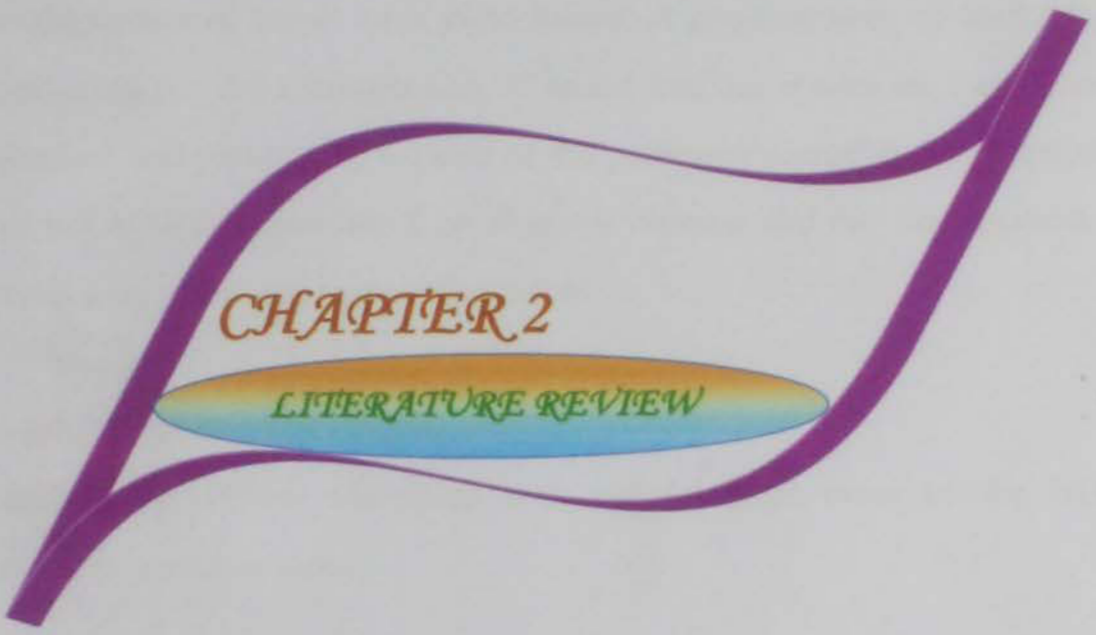
The disease was first documented in Bangladesh during 1981 and reached to a major production constrains in 1988, destroying almost all part of chickpea crop (Bakr and Ahmed, 1992). Currently, it is considered the most damaging foliar disease of chickpea in Bangladesh (Bakr *et al.* 2002). Mostly the disease is spread over the field through spores. This fungus can survive on infected chickpea debris and infect the next season crop (Singh and Tripati, 1992).

Although a number of various investigations were conducted on variability of others fungal pathogen but a little work was done on the variability of *Botrytis cinerea* pathogen. Thus, importance and urgent attention need to concentrate to know the information regarding the variability and pathogenicity of *Botrytis cinerea* for its management.

Hence, present research work was aimed to carry out detailed investigation on the variability of the isolates of *Botrytis cinerea*, collected from major chickpea growing regions of Bangladesh in respect to cultural, morphological, physiological and pathogenic variation and correlate them with the aggressiveness of *Botrytis cinerea* causing Botrytis gray mold disease (BGM) in chickpea. This is also crucial to establish the fundamental basis for more comprehensive studies on *Botrytis cinerea* for its management strategies.

Thus this piece of work was designed to achieve the following objectives-

1. To determine the cultural, morphological, physiological and pathogenic variability of *Botrytis cinerea* isolates
2. To know the virulence level of *Botrytis cinerea*
3. To find out the management option of the disease


CHAPTER 2
LITERATURE REVIEW

CHAPTER 2

LITERATURE REVIEW

Botrytis Cinerea (Perfect stage: *Botryotinia fuckeliana*), the causal fungus of the Botrytis gray mold (BGM) of chickpea is known to be a complicated and variable plant pathogens. But their level of the degree of variability and complexity is not yet identified perfectly. Variability in respect of cultural, morphological and pathogenic characteristics of the fungus has been found to be the main causes of broad term yield losses. A good number of works have been conducted for the management of BGM but the works on the cultural, morphological and pathogenic aspects of the pathogen scarce in the literatures. However the available literatures on *Botrytis cinerea* and the management of the Botrytis gray mold were presented below-

2.1. Variability of *Botrytis cinerea*

Singh and Bhan (1986a) identified four physiological races of the BGM pathogen from northern India.

Rewal and Grewal (1989b) reported five pathotypes of *Botrytis cinerea* on the basis of their reaction to five chickpea lines.

Variation exists in cultural and morphological characters of different isolates of *Botrytis cinerea* and the knowledge of pathogenic variability of the fungus is an important in order to develop resistant cultivars (Bakr *et al.* 2002).

However, no works have been done to know the cultural, morphological and pathogenic variability among chickpea isolates of Bangladesh origin. But the pathogen produces different pathotypes or races to cope with host diversity and fluctuating environmental conditions.

2.1.1. Cultural and morphological variations of *Botrytis cinerea*

Coley-Smith (1980) stated that the cultural characteristics, sporulation of the fungal pathogen of *Botrytis cinerea* varied with synthetic media, temperature and other ecological factors. The pathogen of *Botrytis cinerea* is known to produce sclerotia on crop stubbles of many host species that are thought to be the main means of survival for the long time.

Ahmed *et al.* (2007) observed that *Botrytis cinerea* isolates varied appreciably in their colony color. They observed that isolates BGMN and PNR produced grayish colored colony while light gray colony was found in BGMP isolates and white color in LDH. They also reported that marked variation were found in colony characters. Regular colonies observed in three isolate BGMN, BGMP and LDH whereas colony of PNR was wavy. Mat textured colony was found in two isolates (BGMN and BGMP), velvet colony in LDH and fluffy textured colony in PNR. *Botrytis cinerea* conidia varied in length (2 to 8.5 µm) and width (1 to 4.5 µm). They also observed the highest (2.45 µm) mean width of conidia in LDH and the lowest (1.65 µm) in BGMP.

Joshi and Singh (1969) measured the conidia developed by *Botrytis cinerea* on PDA were measured as 4-16×4-10 µm.

2.1.2. Effect of temperature on *Botrytis cinerea*

Varied in temperatures across the providence accommodates most plant diseases and the rapid development of the disease will occur when the temperature is optimal (Mwakutuya, 2006). Temperature remains as an important variable as it has an effect on almost all biological components of a plant pathosystem (Campbell and Madden, 1990). Temperature affects the extent of conidial germination and the time required for germination and germ tube elongation (Agrios, 2005).

Mahmood and Sinha (1990) reported that optimum growth and sporulation of the fungus laid at 25°C.

Bakr and Ahmed (1992) found that the BGM disease increased at of 17-28°C temperatures and 70-90 % relative humidity.

Tripathi and Rathi (1992) studied the epidemiology of Botrytis gray mold of chickpea in India and observed that the maximum disease severity was recorded at a temperature range from 25-30°C.

Bakr *et al.* (1997) reported that in Bangladesh, maximum disease severity was recorded at a temperature range of 20-28°C.

Singh (1997) observed that on chickpeas, the optimum temperature for sporulation and conidial germination was 25°C and 20°C respectively, while 5°C and 30°C being the minimum and maximum were extremes for conidial germination.

Haware (1998) found that the epidemics of BGM can spread rapidly at 95 % or above relative humidity and at temperature of approximately 25°C in a dense crop canopy. Under such conditions the disease cycle can be completed within in 7 days.

Ahmed *et al.* (2007) conducted an experiment of *Botrytis cinerea* with four isolates; the colony diameter increased gradually with temperature ranged from 10-20°C. No growth was found at 5 and 35°C temperatures in all the isolates.

2.1.3. Effect of pH on *Botrytis cinerea*

Fungal pathogens can successfully grow and sporulate in acid conditions. The fungal pathogen *Botrytis cinerea* can thrive in pH range 4 to 7. Little literature has been found on pH in terms of *Botrytis cinerea* growth and sporulation.

Ahmed *et al.* (2007) reported that the *Botrytis cinerea* was acid loving and variation in isolates were observed in respect of colony growth and sporulation at different levels of pH. Suitable pH was 5.5 for growth and sporulation of the *Botrytis cinerea.*

2.2. Pathogenicity of *Botrytis cinerea*

Ahmed *et al.* (2007) conducted an experiment and found that 131 chickpea genotypes showed differential reaction to 4 isolates (PNR, BGMN, LDH and BGMP) of *Botrytis cinerea.*

Rewal and Grewal (1989b) categorized the *Botrytis cinerea* isolates to five pathotypes on the basis of their reaction on a set of five chickpea differential varieties/lines.

2.3. *In vitro* management of *Botrytis cinerea*

2.3.1. *In vitro* management of *Botrytis cinerea* through antagonist

A little research work has been done on biological control of *B. cinerea* using bio-agent in chickpea crops.

Haware (1998) conducted an experiment with *Trichoderma harzianum* against BGM of chickpea and found that *Trichoderma harzianum* can effectively controlled BGM in the field conditions.

Agarwal and Tripathi (1999) observed that *Trichodemra* sp. exhibited the antagonistic effect against the pathogen *Botrytis cinerea* causing Botrytis gray mold. Under dual culture the hyphal growth of pathogen was inhibited at the zone of contact with the hyphae of the antagonist. Microscopic examination revealed that the hyphal tips of *B. cinerea* swelled and become curved.

Pande (2006) reported that *T. harzianum* and *T. viridae* were highly antagonistic to *B. cinerea* and completely parasitized the pathogen on PDA medium. *Trichoderma viridae* inhibited the growth of *B. cinerea* causing the swelling of the hyphal tips.

2.3.2. *In vitro* management of *Botrytis cinerea* through fungicides

Madhu Meeta *et al.* (1986a) reported that four fungicidal sprays with Bavistin followed by seed treatment with the same chemical reduced BGM incidence and resulted in higher yields.

Agarwal and Tripathi (1999) conducted an experiment on the effect of eight fungicides namely Bavistin, Bayleton, Vitavax, Ronilan, Indofil M-45, Thiram, Captafol, Ridomil @ 10, 25, 50 and 100 μgml^{-1} respectively were tested against the pathogen. Among the non systemic fungicides Ronilan inhibited the radial growth of *Botrytis cinerea* at a lower concentration @ 10 μgml^{-1} (72 % inhibition). The rest of systemic fungicides Bavistin and Bayletan @ 10 μgml^{-1} completely inhibited of the test pathogen while Vitavax inhibited only 77 % mycelial growth at the same concentration.

CHAPTER 3

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

The experiment was conducted to record the cultural, morphological, physiological and pathogenic variability of ten *Botrytis cinerea* isolates collected from major chickpea growing areas of Bangladesh. The experiment was extended for the *in vitro* management of the most virulent isolates causing Botrytis gray mold (BGM) in chickpea.

3.1. Experimental site

The experiment was laid during July to December'07 at the Plant Pathology Laboratory, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur.

3.2. Collection of isolates

The *Botrytis cinerea* isolates used in this study were obtained from different major chickpea growing areas of Bangladesh (Table 1). These isolates were collected during the month of March, 2007 from the infected chickpea field. Then the specimens were taken to the Plant Pathology Laboratory, BARI and were subjected to the process of isolation.

3.3. Isolation and identification of the pathogens

The pathogens were isolated using tissue culture techniques. The surface of the working clean bench was sterilized with ethanol (70 %). Then the infected chickpea samples were taken into the clean bench and cut into small pieces (0.5-1.0 cm). The cut pieces were sterilized in $HgCl_2$ solution (1:1000) 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, 1996). 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 of ten *Botrytis cinerea* isolates with their locations

Isolates	Locations	
	District	Upazilla
AHI-1	Chuadanga	Chuadanga Sadar
AHI-2	Meherpur	Meherpur Sadar
AHI-3	Pabna	Atghoria
AHI-4	Pabna	Ishurdi
AHI-5	Faridpur	Faridpur Sadar
AHI-6	Rajbari	Rajbari Sadar
AHI-7	Kushtia	Bheramara
AHI-8	Kushtia	Bheramara
AHI-9	Pabna	Ishurdi
AHI-10	Rajshahi	Godagari

3.4. Purification and preservation of *Botrytis cinerea*

After identification of *Botrytis cinerea* organism was purified for further study. Purification of *Botrytis cinerea* 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.

3.5. Preparation of culture medium and culture plates

Extra pure dehydrated potato dextrose agar (PDA) manufactured by DIFCO 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: 3D Power, Rangs). The pH of the medium was adjusted with 0.1N HCl or NaOH solution and utilizing a pH metre (Hariba pH metre, Model D-12). 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.

3.6. Cultural and morphological variations of *Botrytis cinerea*

Cultural characteristics were noted on the chickpea dextrose agar (CDA) after three days of incubation at 20°C. Cultural features were observed both microscopically and naked eyes like colony color, shape, margin and texture of ten *Botrytis cinerea* isolates.

Morphological variations in terms of sclerotia color, shape and size, ability of sclerotia production and their arrangement were observed on PDA medium. Data on sclerotia production were recorded 18 days after incubation.

Length and breadth of conidia was measured using ocular micrometer and ocular micrometer is calibrated by comparing the ocular micrometer scale with a pre-calibrated stage micrometer (Model: Erma). To determine the conidial size each isolates of *Botrytis cinerea* was measured 10 times in length and breadth wise.

3.7. Scanning Electron Microscope (SEM), stereo and compound microscopic study of *Botrytis cinerea*

A double sided adhesive carbon cement tape was attached on an alluminium SEM stub. A loop full of *Botrytis cinerea* pure culture (sporulating plate, 15 days old culture) was taken out with the help of a tungsten loop and gently placed onto the adhesive carbon cement tape. Then aluminium SEM stubs were placed in a platinum coater (Model: JEOL JFC-1600, Auto fine coater) and provided 10 mA current flow and 5 ± 0.5 Pa pressure at 10 seconds to make the test samples conductive. After coating the samples then was placed into the SEM (Model: JEOL JSM-6490 LA, Analytical Scanning Electron Microscope) for obtaining the image. For getting a clear SEM image working distance, spot size and accelerating voltage was maintained (40, 12 and 10 Kv in high vacuum condition). The pathogen of *Botrytis cinerea* was also studied under stereo (Model: Olympus, SZ 61, Japan) and compound (Model: Olympus, CX 21 FSI, Tokyo, Japan) microscope.

3.8. Effect of different temperature levels on mycelial radial growth of ten *Botrytis cinerea* isolates

Four days old cultures of *Botrytis cinerea* were used in this study. Inoculated plates were incubated in an incubator (Model: DB-3153, Delux Automatic B.O. D. Incubator, ®Yorco) with seven different levels of temperature viz., 5, 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 four days old culture of *Botrytis cinerea* 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.

3.9. Effect of different pH levels on mycelial radial growth of 10 *B. cinerea* isolates

The isolates were inoculated onto PDA medium having 5 pH levels viz., 4.5, 5.0, 5.5, 6.0 and 6.5 in 90 mm diameter glass petriplates and incubated at 20 ± 0.5°C with alternating 12 hrs of light and 12 hrs of dark period in an incubator (Model: DB-3153, Delux Automatic B.O.D. Incubator, ®Yorco). The design of experiment was the same as mentioned under temperature studies. After 1 day of incubation, data on mycelial radial growth recording were started and continued till covering the whole glass petriplates of any isolates of *Botrytis cinerea*.

3.10. Effect of different nutrient media on mycelial radial growth, sporulation and days required for sclerotia formation of *B. cinerea* isolate (AHI-9)

Seven different non-synthetic culture media such as Potato dextrose agar (PDA), Chickpea dextrose agar (CDA), Barley dextrose media (BDA), Chickpea-barley dextrose agar (CBDA), Lentil dextrose agar (LDA), V-8 Juice agar (V-8 A) 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 *B. cinerea* after one day of incubation till covering the glass petriplates. The number of spores of *Botrytis cinerea* on different media were counted using haemacytometer after 15 days of incubation and the days taken of sclerotia formation also noted upto 7 days of incubation.

Table 2. Composition of nutrient media used in the experiment

Culture Media	Composition
PDA	Slice potato - 200 g, dextrose - 20 g, agar - 17 g and distilled water - 1000 ml
CDA	Chickpea (remove seed coat) - 200 g, dextrose - 20 g, agar - 17 g and distilled water - 1000 ml
BDA	Barley - 200 g, dextrose - 20 g, agar - 17 g and distilled water - 1000 ml
CBDA	Chickpea - 100 g, barley - 100 g, dextrose - 20 g, agar - 17g and distilled water - 1000 ml
LDA	Lentil (remove seed coat) - 200 g, dextrose - 20 g, agar - 17 g and distilled water - 1000 ml
V-8 A	V-8 juice - 100 g, $CaCO_3$ - 3 g, agar - 17 g and distilled water - 1000 ml
WA	Agar - 17 g and distilled water - 1000 ml

3.11. Pathogenicity test of *Botrytis cinerea*

3.11.1. Collection of chickpea seeds

For testing the virulence levels of *Botrytis cinerea* isolates, seeds were collected from Pulse Research Centre, Bangladesh Agricultural Research Institute (BARI). Chickpea variety such as BARI Chola 1, BARI Chola 2, BARI Chola 3, BARI Chola 4, BARI Chola 5, BARI Chola 6, BARI Chola 7, BARI Chola 8 and a BGM tolerant variety ICCL-87322 were collected.

3.11.2. Growing conditions for raising chickpea seedlings

Polypropylene (PP) bags ($5\frac{1}{2} \times 4$ inches) were used to grow the plants in the net house. The PP bags were filled with sterilized soil with well decomposed organic matter. Five seeds of each variety were sown in each bag having 3 replications. The net house temperature was $25 \pm 2°C$ without extra light and temperature provided. Seedlings were watered whenever necessary. After 15 days of sowing, the chickpea seedlings were shifted in a room.

3.11.3. Preparation of inoculums

In order to get huge amount of inocula of *Botrytis cinerea*, each isolate was sub-cultured onto marigold petals aided sucrose (3 g litre^{-1}) in a conical flask and incubated for at least 10 days in an open room temperature at $25 \pm 2°C$. Conidial suspension of different isolates of *Botrytis cinerea* was floated with sterile distilled water and was allowed to stand for 5 minutes. Then the flasks were thoroughly shaken well to dislodge the conidia in a solution. The conidial suspension was filtered through one layer of muslin cloth and the concentration of the conidial suspensions was estimated by a haemacytometer (Model: Tiefe Depth Profondeur, Precicolor, HBG, W. Germany). Conidial suspensions were diluted to get the required concentrations (6×10^4 conidia ml^{-1}) adding with sterilized distilled water.

From the freshly prepared inoculum, loops full of conidial suspensions were smeared out onto a PDA plate to test the viability of the conidia. After 24 hrs of incubation at 20°C in an incubator (Model: DB-3153, Delux Automatic B.O.D. Incubator, ®Yorco) the plates were checked under a microscope (Model: Olympus, CX 21 FSI, Tokyo, Japan) to confirm spore germination.

3.11.4. Foliar inoculation of the chickpea plants

Fifteen day old seedlings of chickpea were inoculated with spore suspension (6×10^4 conidia ml^{-1}) with a hand sprayer. The inoculated plants were covered with polyethylene sheet to maintain relative humidity (% RH) and also to prevent natural contamination with other fungal conidia/spores. The plants were kept in a room at 25 ± 2°C temperature and humid condition by gently spraying sterilized distilled water.

3.11.5. Confirmation of disease

To confirm the symptoms shown on inoculated plants that were caused by test pathogens, infected plant parts were collected and causal organisms were re-isolated, following the standard isolation procedure (Nene *et al.* 1981). The pathogens isolated were examined with the aid of a compound microscope (Model: Olympus, CX 21 FSI, Tokyo, Japan) (40X) to confirm the pathogen as *Botrytis cinerea*.

3.11.6. Assessment of disease severity

Disease score was recorded after 5 days of inoculation on chickpea cultivars using a 9 point scoring scale as described by Singh (1999) and are shown in Table 3.

Table 3. Description of 1-9 scoring scale as described by Singh (1999)

Scale levels[1]	Assessment of disease severity
1	No infection on any part of the plant
2	Minute lesions on lower leaves, flowers and pods covered under dense plant canopy, usually not visible
3	Lesions on less than 5 % of the leaves, flowers and pods covered and dense plant canopy,
4	Lesion and some fungal growth (conidiophores and conidia) can be seen on up to 15 % of the leaves, flowers and pods and branches covered under dense plant canopy
5	Lesions and slight fungal growth on up to 25 % of the leaves, flowers and pods, stems and branches covered under dense plant canopy
6	Lesions and fungal growth on up to 40 % of the leaves, flowers and pods, stems and branches defoliation, 25 % of the plant killed
7	Large lesions and good fungal growth on up to 60 % of the leaves, flowers and pods, stems and branches defoliation common, drying of branches and 50 % of the plants killed
8	Large lesions and profuse fungal growth on up to 80 % of the leaves, flowers and pods, stems and braches, severe defoliation, drying of branches and 75 % of the plants killed
9	Large lesions, very profuse fungal growth on up to 100 % of the flowers, pods, stems, braches, almost complete defoliation, drying of plants and 100 % of the plants killed

[1] 1= Immune or asymptomatic (I), 2-3 = Highly Resistant (HR), 4-5 = Resistant (R), 6-7 = Susceptible (S) and 8-9 = Highly Susceptible (HS)

3.12. *In vitro* management of *Botrytis cinerea*

3.12.1. *In vitro* management of *Botrytis cinerea* through bio-agent

The fungal antagonist *Trichoderma harzianum* used in the present investigation was obtained from Plant Pathology Division, BARI, Joydebpur, Gazipur, Bangladesh. Sixteen ml (16) sterilized melted PDA media was poured aseptically in a 90 mm diameter glass petriplates. Five (5) mm discs of four days old cultures of test pathogen and antagonist was cut separately from the periphery of the petriplates grown on PDA with the help of a sterilized cork borer. These discs were placed apart on solidified PDA in an equal distance having 3 replications and then incubated in a computerized incubator ((Model: DB-3153, Delux Automatic B.O.D. Incubator, ®Yorco) at 20±0.2°C alternating 12 hrs of light and 12 hrs of dark phase. Petriplates were observed regularly for the growth of the antagonist (*Trichoderma harzianum*) and the test pathogen (*Botrytis cinerea*). The radial growth of antagonist and its ability to colonize over the pathogen was recorded at 24 hrs intervals.

3.12.2. *In vitro* management of *Botrytis cinerea* through fungicides

The experiment was carried out in the Plant Pathology Laboratory, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur during July to December, 2007. Desired quantity of selected fungicides namely Bavistin® DF 50WF (Carbendazim), CP-Zim 50WP (Carbendazim), Sunphanate 70WP (Thiophanate methyl), Rovral 50WP (Iprodione), Zhetalux 25WP (Metalaxyl 25WP), Kafa 80WP (Mancozeb) and Agromil 72WP (Metalaxyl + Mancozeb) were weighed using electronic balance (Model: Adamlab, MAW-300) and made the concentrations 0, 500, 1000, 1500, 2000 ppm having 3 replications, respectively. The test fungicides were evaluated *in vitro* against *Botrytis cinerea* following poison food technique using PDA as basal medium (Nene and Thapliyal, 1979).

Hundred (100) ml conical flasks were taken and poured 50 ml of double strength PDA (peeled slice potato - 400 g, dextrose - 40 g, agar - 34 g and distilled water -1000 ml) and sterilized in an autoclave at 121°C for 30 minutes. After autoclaved, 50 ml of sterilized distilled water including desired concentration (mg ml^{-1}) of the fungicides were poured into a 100 ml conical flask which previously containing 50 ml double strength PDA medium and thoroughly mixed in a laminar air flow bench (Model: VS-1400 LVN, Vision Scientific Co., Ltd.) then 20 ml melted media poured in a sterilized 9 cm glass petriplates. After settle down the media, 5 mm mycelial discs were cut from the periphery of 4 days old culture with the help of a sterilized cork borer and put in the center of the 9 cm petriplates. The glass petriplates were arranged in an incubator ((Model: DB-3153, Delux Automatic B.O.D. Incubator, ®Yorco) at 20±0.2°C following Completely Randomized Design (CRD) with three replications in alternating 12 hrs of light and 12 hrs of dark phase. Starting from 1 day of incubation, diameter of the colony was measured till covering the entire petriplates of control treatment and per cent growth inhibition was calculated. Per cent inhibition of the mycelial radial growth was calculated on the basis of the following formula-

$$I = \frac{C - T}{C} \times 100$$

Where,

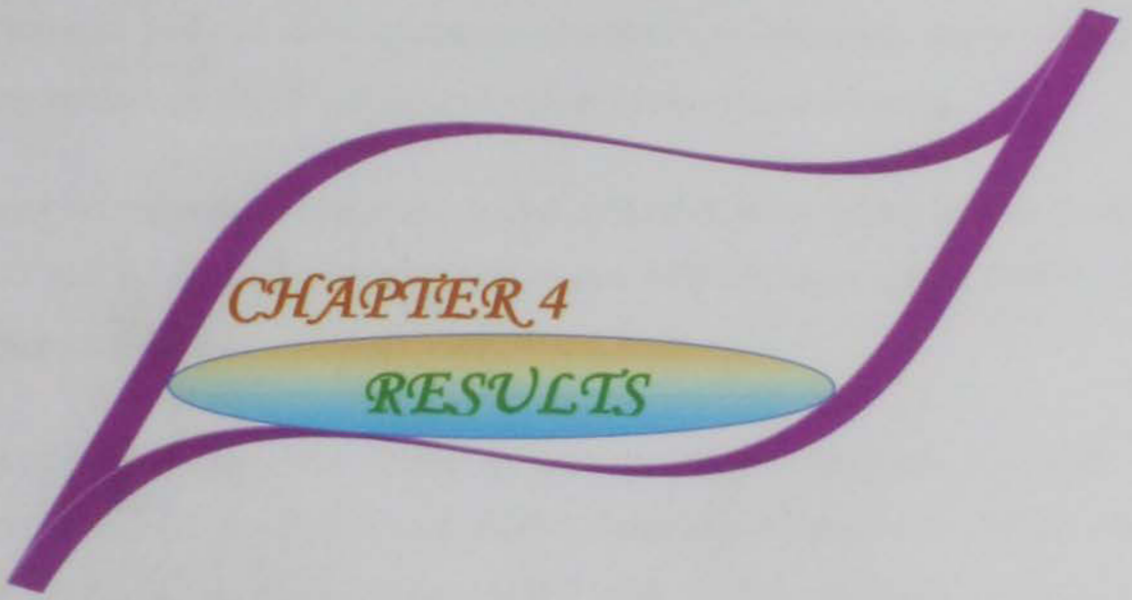
I = Inhibition per cent

C = Colony diameter in control (mm) and

T = Colony diameter in fungicides treated medium (mm)

3.13. Data analysis

All data were analyzed with the help of the statistical software, MSTAT-C computer package program. The data were subjected to an analysis of variance and LSD (Least Significance Difference) were used to separate means and compared with DMRT (Duncan's Multiple Range Test) when F values indicated significantly differences at 5 % level of probability.


CHAPTER 4
RESULTS

CHAPTER 4

RESULTS

4.1. Cultural and morphological variations of 10 *Botrytis cinerea* isolates

4.1.1. Cultural variations of *Botrytis cinerea* isolates

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, light ash and greenish white; colony shape irregular, regular with sector and regular without sector; colony margin was irregular, wavy and entire; colony texture as fluffy, effuse and velvet (Table 4 and Plate 1).

Cottony white colony color was found in AHI-1, ashy white in AHI-2, AHI-3, AHI-5 and AHI-6; off white in AHI-4 and AHI-10; light ash in AHI-7, AHI-9 and greenish white in AHI-8 (Table 4 and Plate 1).

Marked variability was found in colony shape. Irregular colonies were observed in isolates AHI-1 and AHI-7, whereas regular with sector colonies were observed in two isolates AHI-5 and AHI-8. Regular without sector colonies were found in isolates AHI-2, AHI-3, AHI-4, AHI-6, AHI-9, AHI-10 and (Table 4 and Plate 1).

Colony margin was observed irregular in isolates AHI-1, AHI-2, AHI-4 and entire margin in most of the isolates AHI-3, AHI-5, AHI-6, AHI-8, AHI-9, and AHI-10. Only wavy margin was found in isolate AHI-7 (Table 4 and Plate 1).

Distinct differences of the ten isolates were obtained in terms of colony texture. Fluffy texture was observed in isolates AHI-1, AHI-2, AHI-3, AHI-8, AHI-10 and velvet texture was found in isolates AHI-4, AHI-5, AHI-6, and AHI-9. Effuse type texture was noted incase of isolate AHI-7 (Table 4 and Plate 1).

Table 4. Colony characteristics of 10 *Botrytis cinerea* isolates on CDA

Isolates	Characteristics features
	Colony color
AHI-1	Cottony white
AHI-2, AHI-3, AHI-5, AHI-6	Ashy white
AHI-4, AHI-10	Off white
AHI-7, AHI-9	Light ash
AHI-8	Greenish white
	Colony shape
AHI-1, AHI-7	Irregular
AHI-5, AHI-8	Regular with sector
AHI-2, AHI-3, AHI-4, AHI-6, AHI-9 and AHI-10	Regular without sector
	Colony margin
AHI-1, AHI-2, AHI-4	Irregular
AHI-7	Wavy
AHI-3, AHI-5, AHI-6, AHI-8, AHI-9 and AHI-10	Entire
	Colony texture
AHI-1, AHI-2, AHI-3, AHI-8, AHI-10	Fluffy
AHI-7	effuse
AHI-4, AHI5, AHI-6 and AHI-9	Velvet

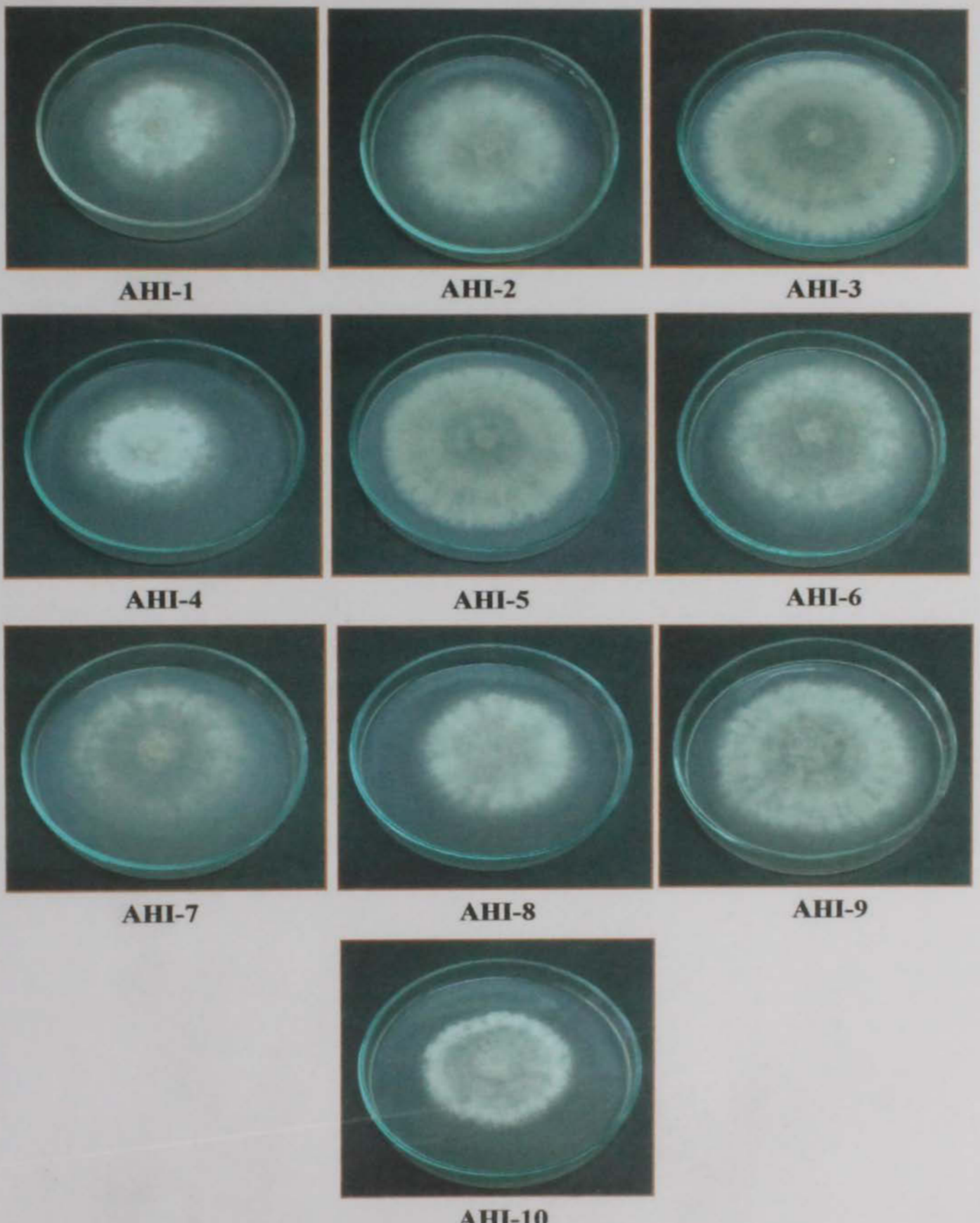


Plate 1. A cultural characteristic of 10 *Botrytis cinerea* isolates on chickpea dextrose agar (CDA) medium

4.1.2. Morphological variations of 10 *Botrytis cinerea* isolates on PDA medium

All the isolates of *Botrytis cinerea* collected from different chickpea growing locations were grouped into five group viz. BC-I, BC-II, BC-III, BC-IV and BC-V on the basis of their sclerotia production, color and arrangements. Of the 10 isolates, two belongs to group BC-I, four to BC-II, one to BC-III, BC-IV and two to BC-V (Table 5 and Plate 2). Almost all the sclerotia were blackish in color. Sclerotial production was very few to high and only group BC-V produced high amount of sclerotia and spread over the entire plates preferably in peripheral region. Incase of group BC-I, very few sclerotia were produced and arranged in the centre of the block and rest of them produced few to medium amount of sclerotia (Table 5 and Plate 2).

Distinct variations were observed in length and breadth of conidia of ten different isolates of *Botrytis cinerea* (Table 6). The length of conidia varied from 5.00 to 15.00 µm, whereas the highest mean length (12.00 µm) was recorded in isolate AHI-9 and minimum (7.50 µm) in isolate AHI-1. Breadth of conidia ranged from 5.00 to 10.00 µm. The highest mean breadth 8.25 µm was observed in isolate AHI-9 and the lowest 6.00 µm in isolate AHI-4.

Table 5. Grouping of 10 *Botrytis cinerea* isolates on the basis of sclerotia production, color and arrangement of sclerotia on PDA medium after 17 days of incubation

Isolates	Sclerotial characteristics features	Grouping of isolates
AHI-1, AHI-4	Very few, blackish, moderate size, blackish, and grouped in the centre form of clots	BC-I
AHI-2,AHI-5, AHI-7 and AHI-10	Few, blackish, moderate to large size, blackish and few centre and some scattered entire plates in the form of clots	BC-II
AHI-3	Medium, blackish, moderate to large size and spread in the plate leaving periphery	BC-III
AHI-6	Medium, blackish, large size and spread all over the plates	BC-IV
AHI-8, AHI-9	High, blackish, comparatively large size and spread all over the plate preferably peripheral region	BC-V

Table 6. Length and breadth of conidia of 10 *Botrytis cinerea* isolates

Isolates	Conidia length[1] (µm)		Conidia breadth[1] (µm)	
	Range	Mean	Range	Mean
AHI-1	6.25-10.00	7.50	5.00-7.50	7.50
AHI-2	7.50-11.25	10.00	6.25-7.50	7.25
AHI-3	7.50-12.50	10.00	6.25-7.50	6.75
AHI-4	7.50-11.25	8.00	6.25-7.50	6.00
AHI-5	5.00-13.75	8.25	5.00-7.50	6.25
AHI-6	7.50-12.50	9.50	6.25-10.00	7.75
AHI-7	8.75-11.25	9.75	7.50-10.00	7.50
AHI-8	7.50-12.50	10.50	5.00-7.50	7.00
AHI-9	7.50-15.00	12.00	5.00-10.00	8.25
AHI-10	5.00-10.00	10.25	5.00-10.00	7.50

[1]Means of ten observations for each isolate

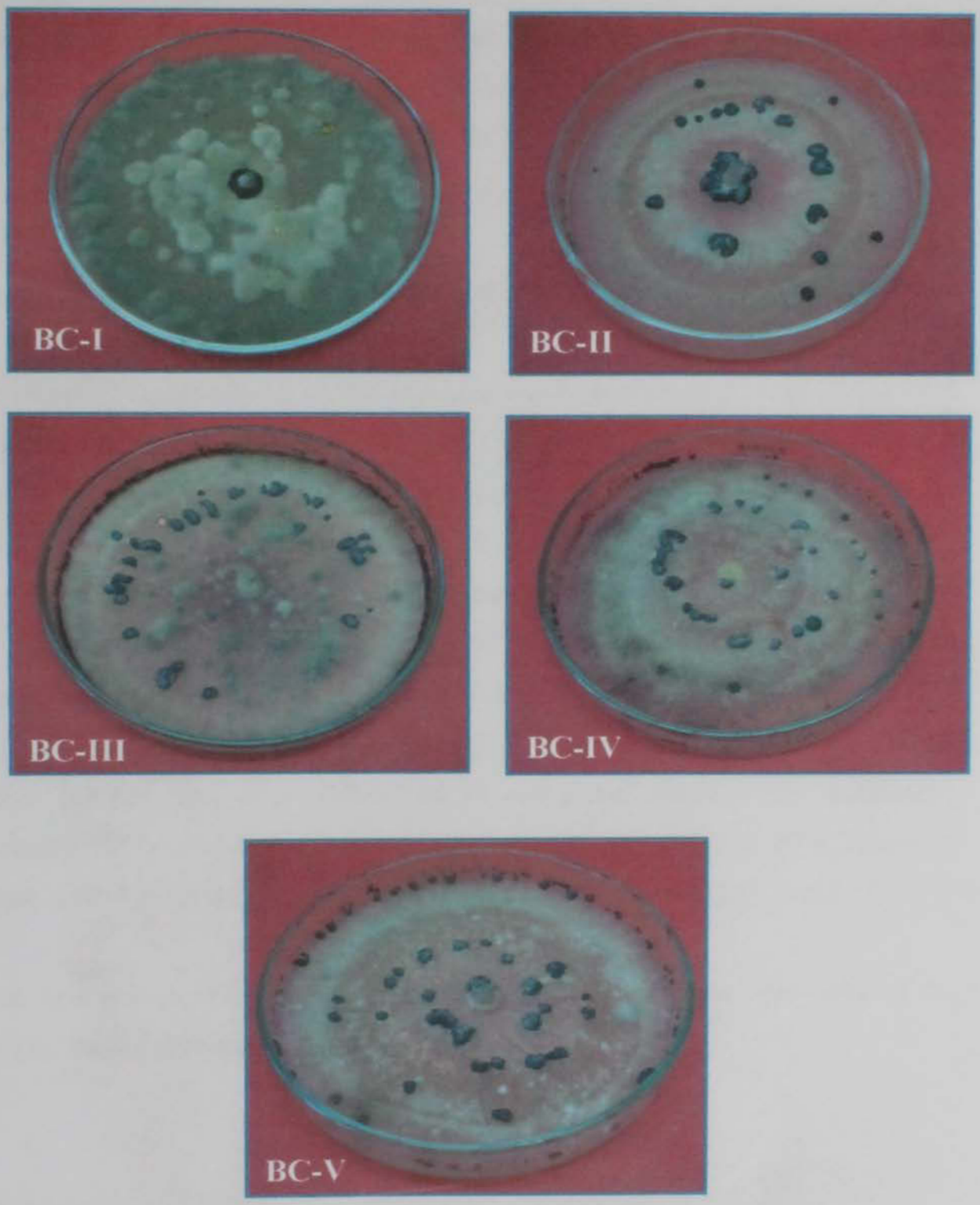


Plate 2. Grouping (BC-I, BC-II, BC-III, BC-IV and BC-V) of 10 *B. cinerea* isolates on the basis of their sclerotia production, color and arrangement on the PDA medium

4.1.3. Stereo, compound and SEM study of *Botrytis cinerea*

Characteristics features were investigated by studying *Botrytis cinerea* under stereo, compound and SEM microscope. Mycelium was dichotomous, twisted, ribbon like, conidiophore bears cluster of conidia; conidia were single celled, oval and often round shaped (Plate 3 and 4).

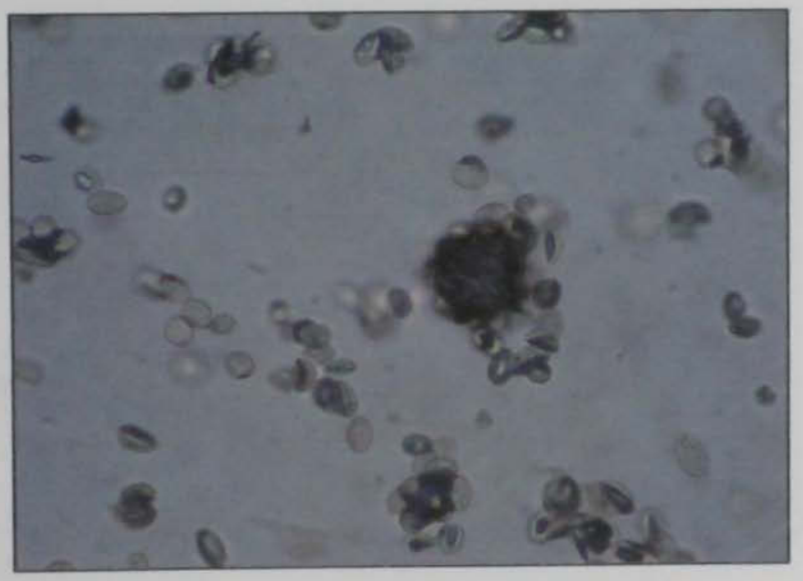
4.1.4. Mycelial radial growth of *Botrytis cinerea* at different temperature (°C) levels

The effect of temperatures on the mycelial radial growth of *B. cinerea* on PDA is presented in Table 7 and Plate 5. The growth of *Botrytis cinerea* was increased with the time for a certain period for each temperature. The mycelial radial growth gradually increased upto 20°C. No growth was obtained at 35°C temperature. The mycelial colony diameter was highest for all ten isolates at 20°C and gradually decreased by upto 30°C temperature. At 20°C maximum colony diameter (86.00 mm) was obtained in isolate AHI-9 followed by AHI-10 (85.33 mm), AHI-5 (84.50 mm), AHI-6 (84.17 mm), AHI-2 (83.83 mm), AHI-7 (83.83 mm) and AHI-8 (83.50 mm) and those were statistically identical. The lowest colony growth (78.00 mm) was noted at 20°C incase of isolate AHI-4 preceded by AHI-3 (81.00 mm) and AHI-1 (81.17 mm).

From this investigation, it appeared that 20°C temperature was suitable for mycelial radial growth of *Botrytis cinerea*.

(A)

(B)



(C)

Plate 3. Characteristics features of *Botrytis cinerea*: (A) profuse sporulation on PDA culture media seen under stereo microscope, (B) twisted mycelia and arrangement of spores on conidiophores in a cluster form seen under compound microscope and (C) numerous spores or conidia (oval, near round shaped) single form seen under compound microscope

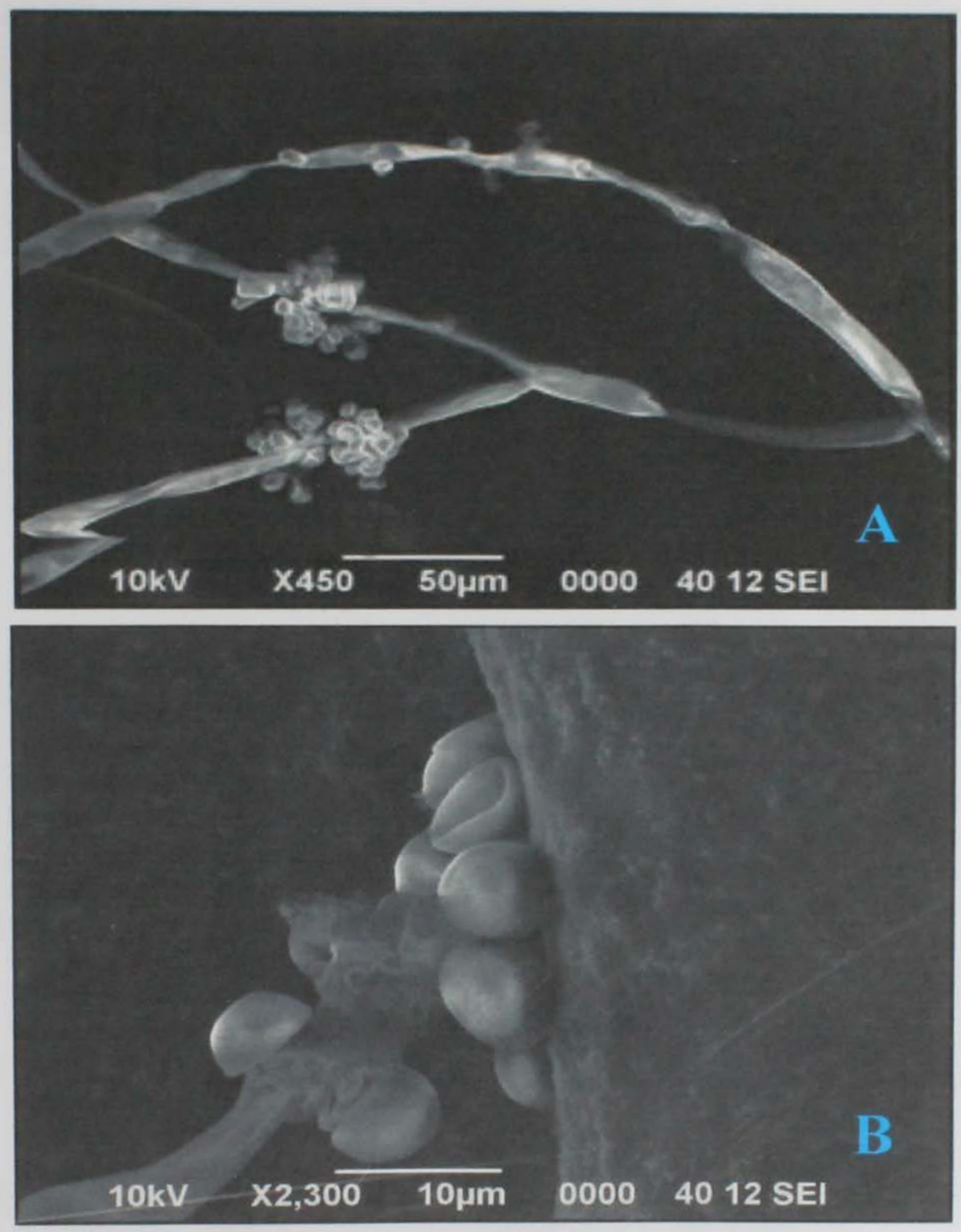


Plate 4. Secondary electron image of *Botrytis cinerea*: (A) Twisted and dichotomous mycelia, conidiophores with the botryose conidia and (B) Conidia on conidiophores at closer view

Table 7. Mycelial radial growth of *Botrytis cinerea* at different temperature (°C) levels

Isolates	Radial colony growth[1](mm)						
	5°C	10°C	15°C	20°C	25°C	30°C	35°C
AHI-1	11.33 d	32.50 f	52.17 e	81.17 c	40.50 d	5.83 g	0.00
AHI-2	18.10 a	55.00 a	63.67 bc	83.83 ab	63.83 b	16.33 d	0.00
AHI-3	14.17 b	53.50 b	62.00 d	81.00 c	64.33 b	17.83 c	0.00
AHI-4	11.17 d	37.67 d	51.83 e	78.00 d	35.00 e	8.50 f	0.00
AHI-5	12.00 cd	46.33 d	64.50 b	84.50 ab	58.17 c	19.00 b	0.00
AHI-6	19.50 a	55.83 a	65.00 b	84.17 ab	63.50 b	9.17 f	0.00
AHI-7	11.17 d	37.83 e	44.76 f	83.83 ab	25.00 f	14.17 e	0.00
AHI-8	12.00 cd	38.50 e	64.83 b	83.50 b	63.33 b	18.50 bc	0.00
AHI-9	18.90 a	56.17 a	66.50 a	86.00 a	66.00 a	20.33 a	0.00
AHI-10	13.17 bc	51.50 c	62.50 cd	85.33 ab	65.83 a	18.83 b	0.00
CV (%)	6.09	1.62	1.26	1.42	1.47	3.89	-

[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)

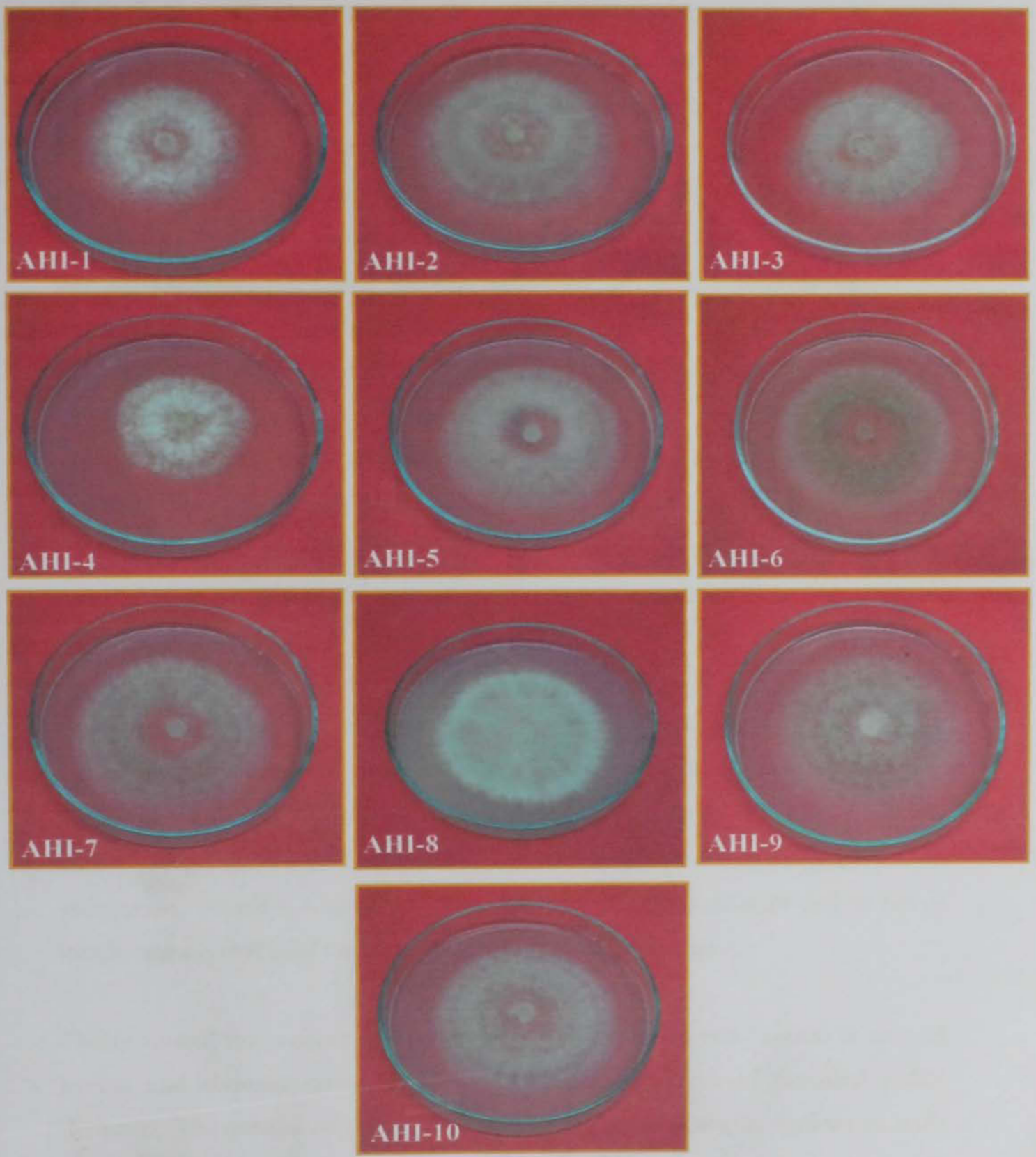


Plate 5. Maximum mycelial radial growth of *Botrytis cinerea* at 20°C temperature

4.1.5. Radial growth of *Botrytis cinerea* at different pH levels on PDA

The pH of the culture medium had a significant role on colony growth of *Botrytis cinerea*. The fungus grew well on PDA medium with a wide range of pH 4.5 to 6.5 in this study (Table 8 and Figure 1). Deviating from this range caused gradual retardation of radial colony growth of different isolates. Profound growth was recorded at pH 4.5 than the other pH levels. Among the pH levels, luxuriant radial growth exhibited all of the isolates at pH 4.5 followed by 5.0 and radial growth decreased for the rest of other pH. The highest colony diameter was noted for the isolate AHI-6 (81.00 mm) followed by AHI-9 (80.00 mm) and AHI-8 (79.33 mm) at pH 4.5. The lowest radial growth was recorded in isolate AHI-4 (7.50 mm) preceded by AHI-1 (9.33 mm) at pH 6.5. But sharp incase of radial growth 81.00, 66.33, 53.8 and 26.00 mm were recorded at 4.5, 5.0, 5.5 and 6.0 pH levels for the isolate AHI-6, respectively which was grown well in all pH levels except pH 6.5 (16.17 mm) level.

In general, mycelial radial growth increases with days after of incubation. After 24 hrs of incubation successive radial growth was increased gradually upto 72hrs of incubation (Figure 1). Isolates AHI-1, AHI-4, AHI-7 at pH 6.0 and AHI-7 at pH 5.5 and 6.0 no radial growth was obtained after 24 hrs of incubation. At pH 6.5 isolates AHI-1, AHI-4 and AHI-7 after 24 and 48 hrs of incubation no mycelial radial growth was observed (Figure 1).

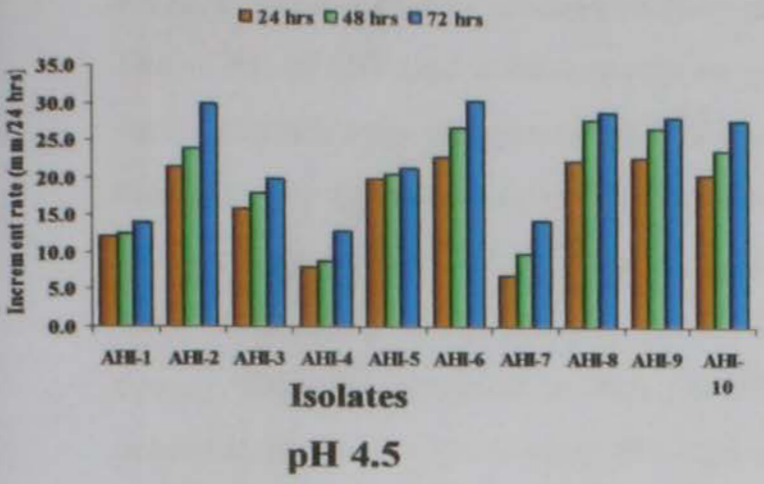
The results of the present investigation showed that *Botrytis cinerea* is an acid loving and showed variability in all isolates in respect of mycelial radial diameter. The results of this study on *Botrytis cinerea* are in agreement with Ahmed *et al.* (2007) who worked on Indian BGM isolates but conflict with definite pH levels in Bangladeshi isolates.

Table 8. Mycelial radial growth of *Botrytis cinerea* at different pH levels

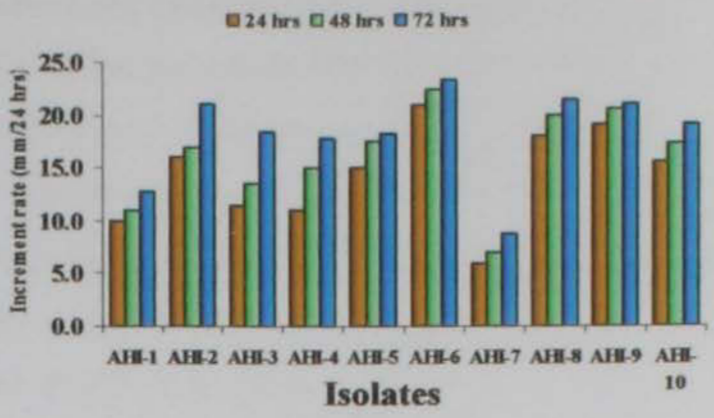
Isolates	Radial colony growth[1](mm)				
	pH 4.5	pH 5.0	pH 5.5	pH 6.0	pH 6.5
AHI-1	38.83 f	33.83 f	21.50 g	16.00 d	9.33 d
AHI-2	75.33 b	54.17 c	39.20 e	24.33 b	19.83 a
AHI-3	54.67 e	43.50 e	40.80 d	26.17 a	18.50 b
AHI-4	31.33 g	43.83 e	24.30 f	13.50 e	7.50 e
AHI-5	63.17 d	50.83 d	41.70 d	20.83 c	18.67 b
AHI-6	81.00 a	66.33 a	53.80 a	26.00 a	16.17 c
AHI-7	22.00 h	21.83 g	16.20 h	12.17 e	9.50 d
AHI-8	79.33 a	59.50 b	46.20 b	27.33 a	17.83 b
AHI-9	80.00 a	60.50 b	43.00 c	26.83 a	18.17 b
AHI-10	71.50 c	51.67 cd	38.70 e	20.33 c	15.83 c
CV (%)	1.52	3.07	1.92	3.87	4.22

[1]Means of three replications for each isolate

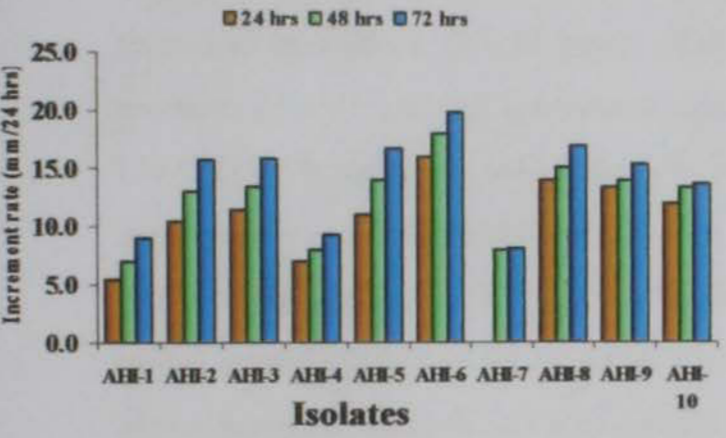
Numbers with similar letter do not differ significantly at 5 % level according to Duncan's Multiple Range Test (DMRT)



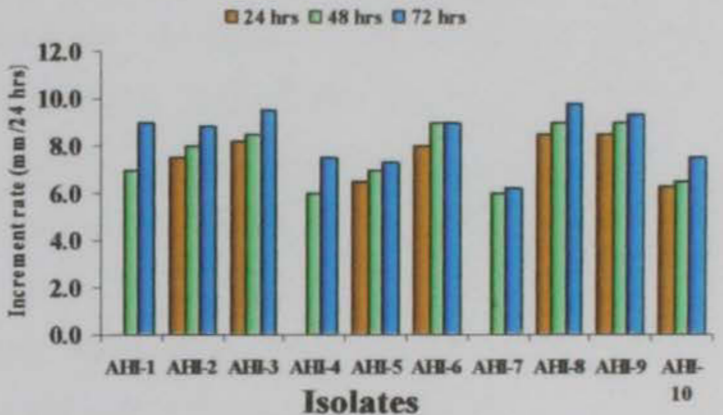
pH 4.5



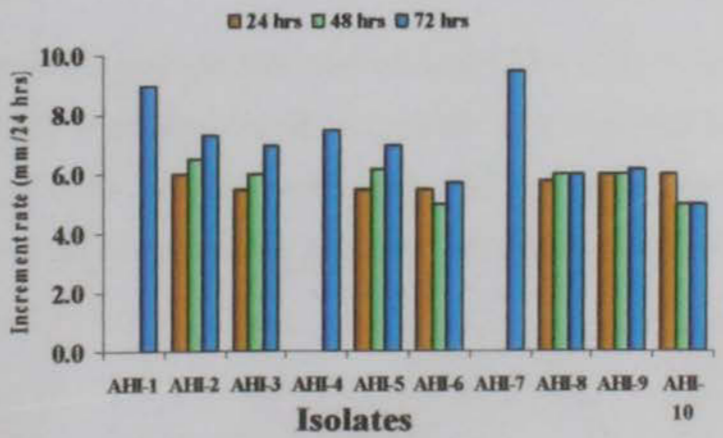
pH 5.0



pH 5.5



pH 6.0



pH 6.5

Figure 1. Rate of increment of mycelial radial growth (24, 48 and 72 hrs after incubation) of *Botrytis cinerea* at different pH levels

4.1.6. Mycelial radial growth of *Botrytis cinerea* on different culture media

The effect of different culture media on mycelial radial growth of *Botrytis cinerea* varied significantly and presented in Table 9 and Plate 6. After 24hrs of incubation maximal rate of increment of colony diameter was observed on CDA (24.50 mm) followed by CBDA (20.50 mm), PDA (20.50 mm) and LDA (20.30 mm) those were statistically identical. The lowest (10.00 mm) rate of increment mycelial colony diameter obtained in WA (10.00 mm) and V-8 A those were identical preceded by BDA (18.20 mm). But rate of increment of mycelial radial growth at 48 and 72 hrs of incubations differed from that of 24 hrs of incubations. In 48 hrs, significantly the highest rate of increment mycelial colony diameters were recorded in CBDA (27.50 mm), PDA (27.00 mm) and BDA (27.00 mm) and medium growth rate of increment was found on CDA (25.50 mm) followed by LDA (19.50 mm) in comparison to others culture media. The lowest rate of increment mycelial radial growth was recorded on WA (8.00 mm) and V-8 A (9.00 mm) media. In 72 hrs of incubation, maximum increment rate mycelial radial growth was noted in LDA (39.00 mm) followed by BDA (31.00 mm). Mycelial radial growth in CDA (29.20 mm), CBDA (27.70 mm) and PDA (28.50 mm) followed by V-8 A (25.27 mm) media were statistically identical.

It was revealed that the best growth was recorded in CDA (79.17 mm) followed by LDA (78.83 mm) which were statistically identical. The mycelial radial growth in CBDA (76.67 mm), BDA (76.17 mm) and PDA (76.00 mm) media also statistically identical. The lowest radial growth was obtained on WA (34.83 mm) preceded by V-8 A (44.17 mm).

Table 9. Rate of increment mycelial radial growth of *Botrytis cinerea* (AHI-9) at 24, 48 and 7 2 hrs of incubation on different culture media

Culture Media	Rate of increment mycelial radial growth[1] (mm/24 hrs)			Colony growth* (mm)
	24 hrs	48 hrs	72 hrs	Total colony growth
PDA	20.50 b	27.00 a	28.50 c	76.00 b
CDA	24.50 a	25.50 b	29.20 c	79.17 a
BDA	18.20 c	27.00 a	31.00 b	76.17 b
CBDA	20.50 b	27.50 a	28.70 c	76.67 b
LDA	20.30 b	19.50 c	39.00 a	78.83 a
WA	10.00 d	8.00 d	16.80 e	34.83 d
V-8 A	10.00 d	9.00 d	25.27 d	44.17 c
CV (%)	4.40	3.75	2.66	1.14

* Means of three replications for each culture medium

[1]Determined by measuring the rate of increment (mm/24hrs) of AHI-9 isolate on each culture medium over a 3 days of incubation

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

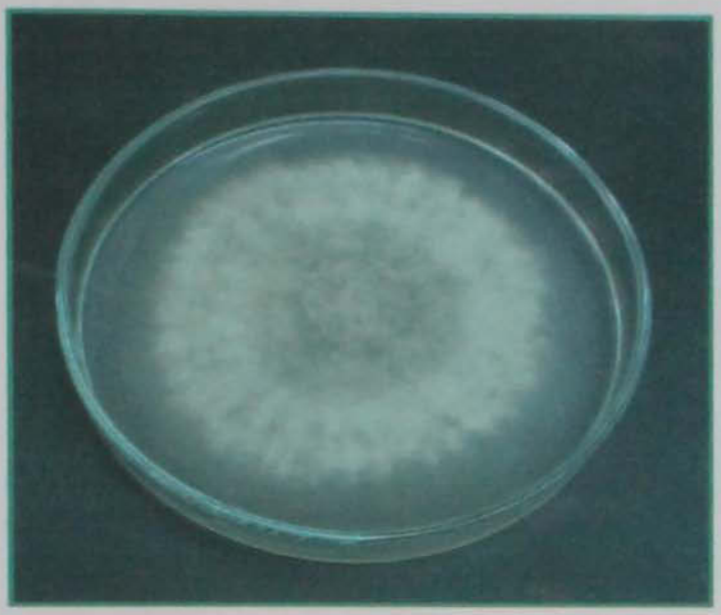
4.1.6. Sporulation and sclerotia formation of *Botrytis cinerea* at different culture media

The sporulation of *Botrytis cinerea* on different culture media is presented in Table 10. The maximum sporulation was found on LDA (2.5×10^4 ml^{-1}) followed by CDA (2.3×10^4 ml^{-1}) and BDA (2.2×10^4 ml^{-1}) media respectively. The lowest number (1.9×10^4 ml^{-1}) of spores were obtained on PDA preceded by CBDA (2.0×10^4 ml^{-1}) medium. No sporulation was observed on WA and V-8 A media. Time of sclerotia formation also noted on different media. The earliest (5 days) and excellent sclerotia formation was observed both on CDA and LDA. In PDA medium comparatively more days (7 days) required for sclerotia formation and the grade was good. No sclerotia were formation on WA and V-8 media upto 7 days of incubation.

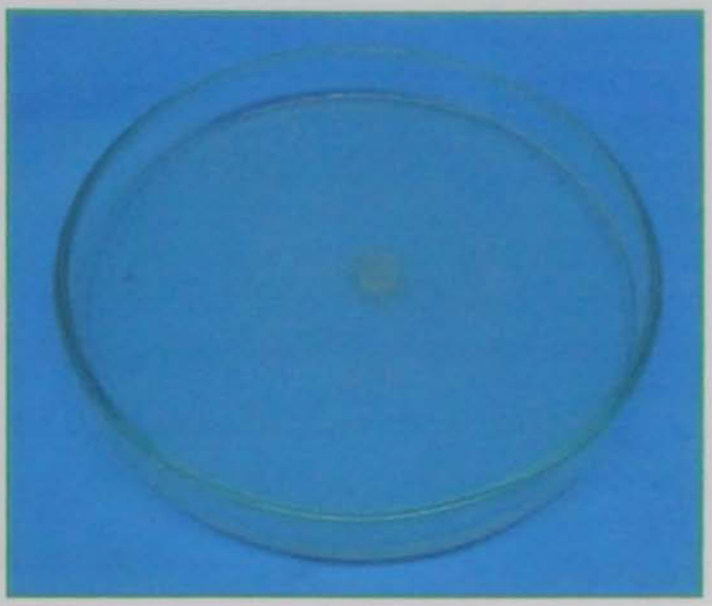
Table 10. Sporulation and days taken for sclerotia formation of *Botrytis cinerea* on different culture media

Culture media	Spores (ml^{-1})	Days taken sclerotia initiation
PDA	1.9×10^4	7 days +++
CDA	2.3×10^4	5 days ++++
BDA	2.2×10^4	6 days ++
CBDA	2.0×10^4	6 days +++
LDA	2.5×10^4	5 days ++++
WA	-	absent
V-8 A	-	absent

Grade: + = poor, ++ = fair, +++ = good and ++++ = excellent



A). Maximum mycelial radial growth of *B. cinerea* on CDA culture media after 3 days of incubation



B). Lowest mycelial radial growth of *B. cinerea* on WA culture media after 3 days of incubation

C). Maximum sporulation of *B. cinerea* on LDA medium after 11 days of incubation

D). Comparatively low sporulation of *B. cinerea* on PDA medium after 11 days of incubation

Plate 6. *Botrytis cinerea* mycelial radial growth (A&B) and sporulation (C&D)

4.2. Pathogenic variation

All of the 10 isolates showed differential reaction on chickpea varieties (Table 11, Figure 2 and Plate 7). Among the isolates only two (AH-9 and AHI-10) was found to be the most virulent and infected all varieties at an equal level (Table 11 and Figure 2).

Isolate AHI-1 induced susceptible reaction on four varieties (BARI Chola 1, 2 and 4) including ICCL-87322 and highly susceptible on 5 varieties (BARI Chola 3, 5, 6, 7 and 8) shown in Table 11. The lowest (6) disease score was observed in varieties BARI Chola-1, 2, and 4 and the highest (9) disease score was noted in BARI Chola-5, 7 and 8 shown in Figure 2.

Isolate AHI-2 showed susceptible reaction on two varieties (BARI Chola 5 and 6) and highly susceptible were noted on seven varieties (BARI Chola 1, 2, 3, 4, 7, 8, and ICCL-87322) shown in Table 11. The maximum (9) disease score was noted in varieties BARI Chola-3 and 4 and the lowest (7) disease score was observed in BARI Chola-5 and 6 shown in Figure 2.

Isolate AHI-3 showed susceptible reaction on three varieties (BARI Chola 6 , 7 and ICCL-87322), highly susceptible reaction on four varieties (BARI Chola 1, 2, 5 and 8) and resistant reaction showed on two varieties (BARI Chola 3 and 4) shown in Table 11. The disease score reached upto the highest level of the scale (9). The highest (9) disease score was observed in BARI Chola 2, 5 and 8 and the lowest (4) disease score was obtained in BARI Chola 4 shown in Figure 2.

Isolate AHI-4 induced susceptible reaction on three chickpea cultivars (BARI Chola 3, 4 and ICCL-87322) and highly susceptible on six cultivars (BARI Chola 1, 2, 5, 6, 7 and 8) shown in Table 11. The maximum (9) disease score was observed in BARI Chola-1, 2, 6, 8 and ICCL-87322 and the lowest (6) disease score was found in BARI Chola-3 shown in Figure 2.

Isolate AHI-5 induced highly susceptible reaction on five chickpea cultivars (BARI Chola 2, 6, 7 and 8) and resistant on four cultivars (BARI Chola 3, 4, 5 and ICCL-87322) shown in Table 11. The maximum (9) disease score was observed in BARI Chola-2, 6, 7, and 8 and the lowest (3) score was noted in BARI Chola-3 shown in Figure 2.

Isolate AHI-6 induced highly susceptible reaction on six chickpea cultivars (BARI Chola 2, 5, 6, 7, 8 and ICCL-87322) and susceptible on three cultivars (BARI Chola 1, 3 and 4) shown in Table 11. The maximum (9) disease score were observed in BARI Chola-2, 6, 7, 8 and ICCL-87322 and the lowest (6) score were noted in BARI Chola-1shown in Figure 2.

Isolate AHI-7 induced highly susceptible reaction on three chickpea cultivars (BARI Chola 4, 6 and ICCL-87322), susceptible reaction on three cultivars (BARI Chola 1, 2 and 8) and resistant on two cultivars (BARI Chola 3, 5 and 7) shown in Table 11. The maximum (9) disease score were observed in BARI Chola-4 followed by BARI Chola 6 and ICCL-87322 (8) and the lowest (4) score were noted in BARI Chola-3 shown in Figure 2.

Isolate AHI-8 induced highly susceptible reaction on three chickpea cultivars (BARI Chola 2, 6, 7 and 8), susceptible reaction on four cultivars (BARI Chola 2, 6, 7 and 8) and resistant on two cultivars (BARI Chola 1 and 3) shown in Table 11. The maximum (9) disease score were observed in BARI Chola 8 followed by BARI Chola 5 and ICCL-87322 (8) and the lowest (3) disease score was noted in BARI Chola 4 shown in Figure 2.

Isolate AHI- 9 and 10 both induced highly susceptible reaction on all chickpea cultivars (BARI Chola 1 to 8 and ICCL-87322) and disease score was 9 for all the chickpea cultivars except BARI Chola 7 in isolate AHI-10 where disease score was 7 shown in Table 11 and Figure 2.

Table 11. Resistant-susceptibility pattern of 10 *Botrytis cinerea* isolates on 8 chickpea cultivars and one tolerant genotype under artificial inoculation condition

Isolates	BARI Chola-1	BARI Chola-2	BARI Chola-3	BARI Chola-4	BARI Chola-5	BARI Chola-6	BARI Chola-7	BARI Chola-8	ICCL 87322
AHI-1	S	S	HS	S	HS	HS	HS	HS	S
AHI-2	HS	HS	HS	HS	S	S	HS	HS	HS
AHI-3	HS	HS	R	R	HS	S	S	HS	S
AHI-4	HS	HS	S	S	HS	HS	HS	HS	S
AHI-5	R	HS	R	R	R	HS	HS	HS	R
AHI-6	S	HS	S	S	HS	HS	HS	HS	HS
AHI-7	S	S	R	HS	R	HS	R	S	HS
AHI-8	R	S	R	HS	HS	S	S	S	HS
AHI-9	HS	HS	HS	HS	HS	HS	HS	HS	HS
AHI-10	HS	HS	HS	HS	HS	HS	HS	HS	HS

isease score (1-9 scale): 1 = Immune or asymptomatic (I), 2-3 = Highly Resistant (HR), 4-5 = Resistant (R), 6-7 = Susceptible (S) and 8-9 = ighly Susceptible (HS)

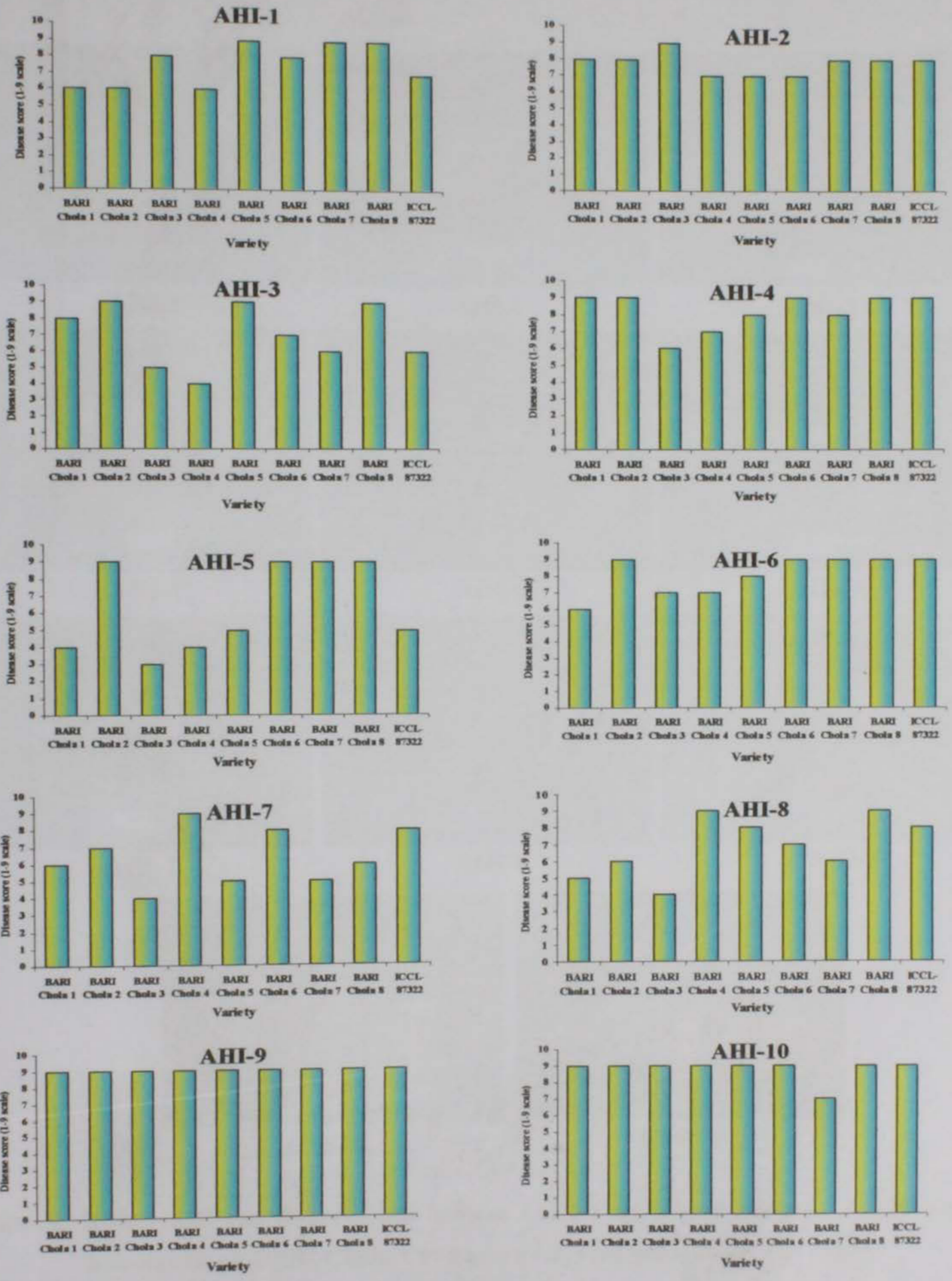


Figure 2. Reaction of 10 *Botrytis cinerea* isolates to nine chickpea cultivars including one tolerant genotype ICCL-87322

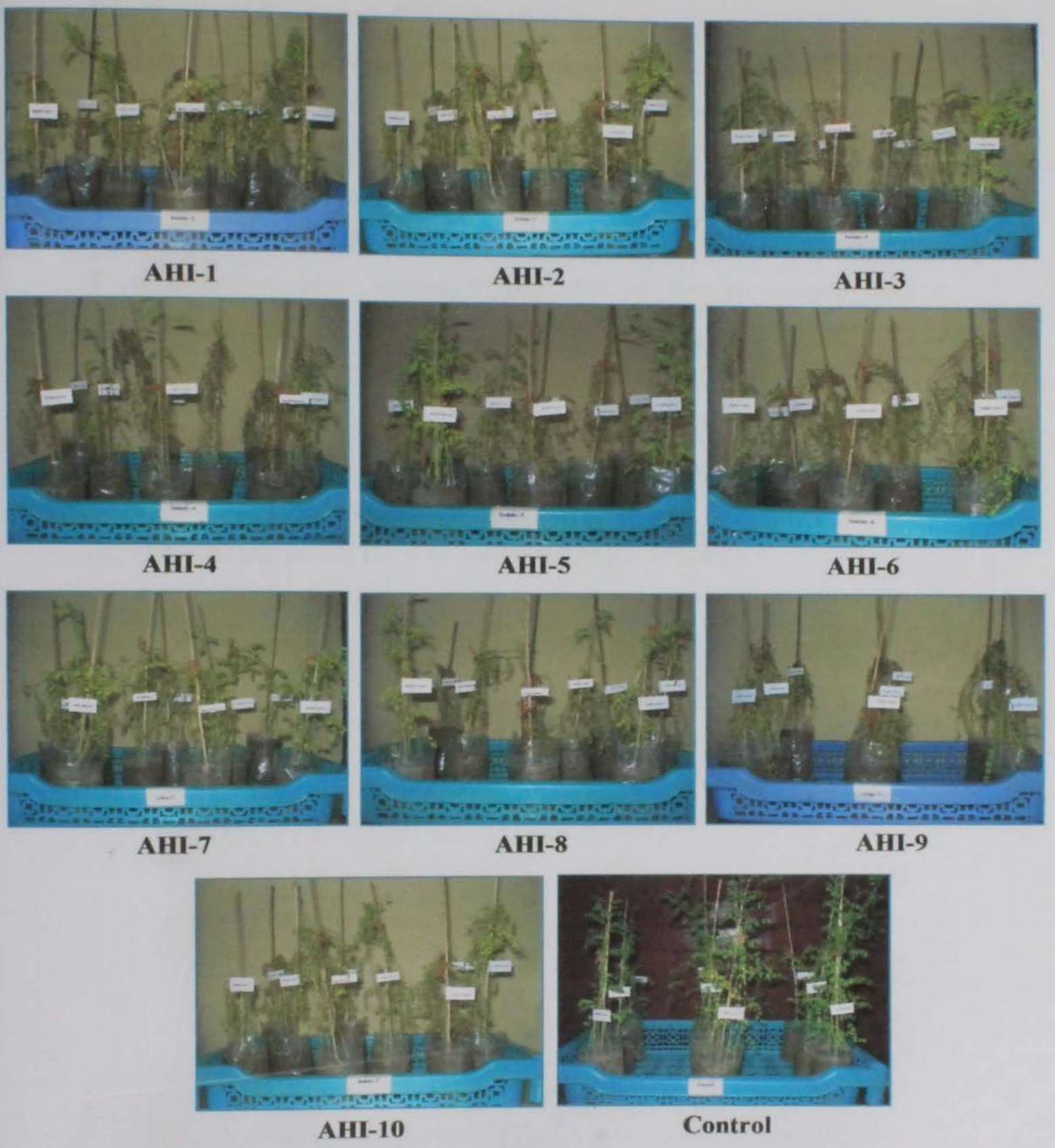
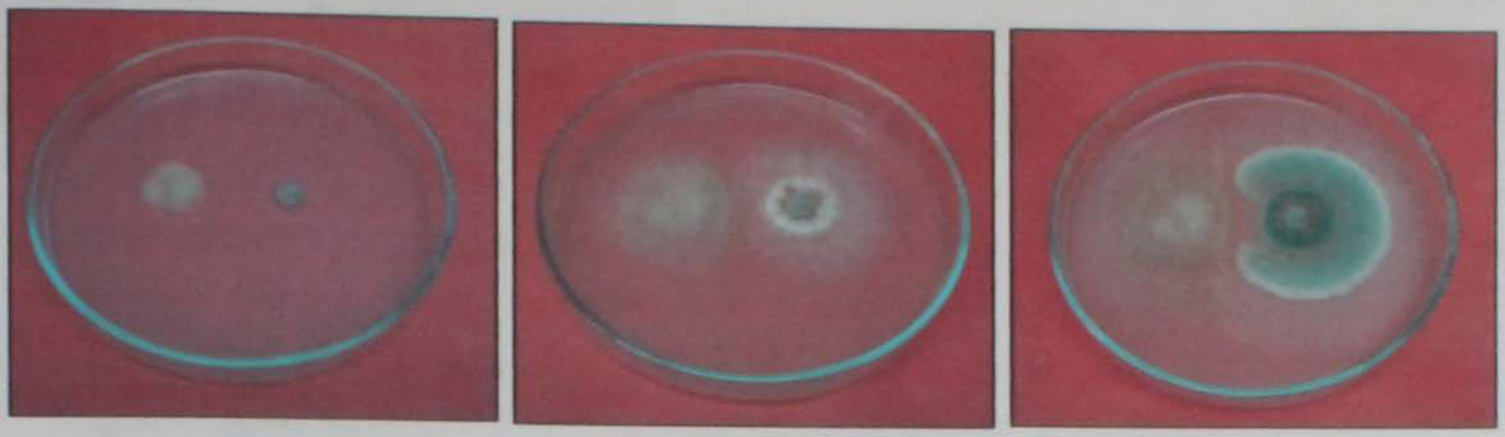


Plate 7. Differential reactions of ten isolates (AHI-1 to 10) of *Botrytis cinerea* after inoculation on BARI Chola 1 to 8 and a tolerant genotype ICCL 87322

4.3. *In vitro* management of *Botrytis cinerea*

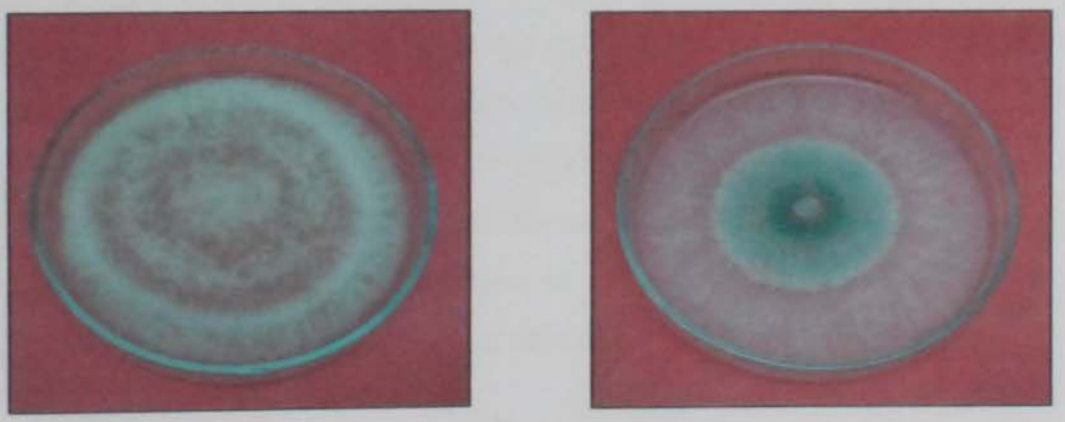
4.3.1. *In vitro* management of *Botrytis cinerea* through antagonist

Trichoderma harzianum revealed to be effective against *Botrytis cinerea* in dual culture experiment in the laboratory. After 48hrs of incubation the radial mycelial growth of the both cultures came in contact. The hyphal growth of *B. cinerea* was inhibited to some extent at the zone of contact with *Trichoderma harzianum* hyphae. Hyphal tips of *Botrytis cinerea* become swelled and curved at the point of contact that was observed under microscopic studies. A thick band of over growing antagonist mycelia was observed within 6 days of incubation, the advancing *T. harzianum* hyphae covered the entire petriplates suppressing the growth of *Botrytis cinerea*. The growth of test pathogen become dark green after 6 days of incubation and it could not be re-isolated from any part of the over grown petriplates (Plate 8).



(a). After 24 hrs of incubation (b). After 48 hrs of incubation (c). After 72 hrs of incubation

(d). After 96 hrs of incubation (e). After 120 hrs of incubation (f). After 144 hrs of incubation



(g). Pure culture of *Botrytis cinerea* (h). Pure culture of *T. harzianum*

Plate 8. Growth inhibition of *Botrytis cinerea* by antagonist *T. harzianum* after (a) 24, (b) 48, (c) 72, (d) 96, (e) 120 and (f) 144hrs of incubation and (g) pure culture of *Botrytis cinerea* (4 days old) and (h) Pure culture of *T. harzianum* (3 days old)

4.3.2. *In vitro* management of *Botrytis cinerea* through fungicides

The seven test fungicides at five concentrations viz. 0 (control), 500, 1000, 1500 and 2000 ppm significantly inhibited the mycelial growth of *Botrytis cinerea* except control (Table 12, Plate 9 and Figure 3). Among the seven tested fungicides Bavistin® DF 50WP, CP-Zim 50WP, Sunphanate 70WP and Rovral 50WP completely inhibited the mycelial growth at all concentrations except control (0 ppm) concentration. Incase of Zhetalux 25WP @ 1500 and 2000 ppm completely inhibited the radial mycelial growth and @ 500, 1000 ppm concentrations resulted in 32.40, 84.20 % inhibition of mycelial radial growth, respectively. Incase of Kafa 80WP, the colony growth inhibition was 55.09, 58.50, 62.88 and 64.63 % applied at all concentrations except 0 ppm. Incase of Agromil 72WP, 81.5 mm (0 ppm), 31.5 mm (500 ppm), 26.2 mm (1000 ppm), 20.0 mm (1500 ppm) and 12.7 mm (2000 ppm) mycelial radial growth was obtained that caused 61.35, 67.85, 75.46 and 84.42 % inhibition of radial growth over control.

Results from the *in vitro* test of seven fungicides showed that Bavistin® DF 50WP, CP-Zim 50WP, Sunphanate 70WP and Rovral 50WP were the most effective fungicides to inhibit the colony growth of *Botrytis cinerea* followed by Agomil 72WP. The lowest inhibition of *Botrytis cinerea* growth was noted in Zhetalux 25WP at 500 ppm (32.40 mm) preceded by Kafa 80WP at 500 (55.09 %) and 1000 ppm (58.50 %) but Zhetalux 25WP at 1000 ppm (84.20 %) concentration was the best concentration to inhibit the mycelial radial growth of the fungus than Agromil and Kafa at all concentrations except 0 ppm.

Radial growth rate of increment mycelial growth was also observed in this experiment. Mycelial radial growth rate of *Botrytis cinerea* increases gradually with days of intervals. Incase of 0 ppm growth rate increases significantly higher than rest of the concentrations (500, 1000, 1500 and 2000 ppm). After 24 hrs of incubation, growth rate slightly increased upto 72 hrs of incubation. Rate of increment mycelial radial growth was zero (0) incase of Bavistin, Sunphanate, CP-Zim, Rovral at lower (500 ppm) concentration and Zhetalux comparatively at higher (1000 ppm) concentration (Figure 3).

Table 12. Radial colony growth and per cent inhibition of *Botrytis cinerea* at different concentration of seven fungicides

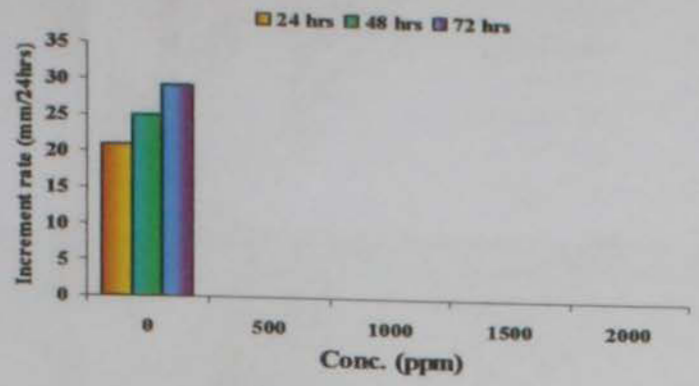
Fungicides	Concentrations (ppm)	Radial colony growth* (mm)	Per cent (%) inhibition of colony growth
Bavistin® DF 50WP (Carbendazim)	0	75.3 b (8.68)	-
	500	0 j (0.71)	100
	1000	0 j (0.71)	100
	1500	0 (0.71)	100
	2000	0 j (0.71)	100
Sunphanate 70WP (Thiophanate methyl)	0	75.3 b (8.68)	-
	500	0 j (0.71)	100
	1000	0 j (0.71)	100
	1500	0 j (0.71)	100
	2000	0 j (0.71)	100
Agromil 72WP (Metalaxyl+Mancozeb)	0	81.5 a (9.03)	-
	500	31.5 e (5.61)	61.35
	1000	26.2 g (5.12)	67.85
	1500	20.0 h (4.47)	75.46
	2000	12.7 i (3.56)	84.42
CP-Zim 50WP (Carbendazim)	0	75.2 b (8.67)	-
	500	0 j (0.71)	100
	1000	0 j (0.71)	100
	1500	0 j (0.71)	100
	2000	0 j (0.71)	100

Cont'd (Table 12....)

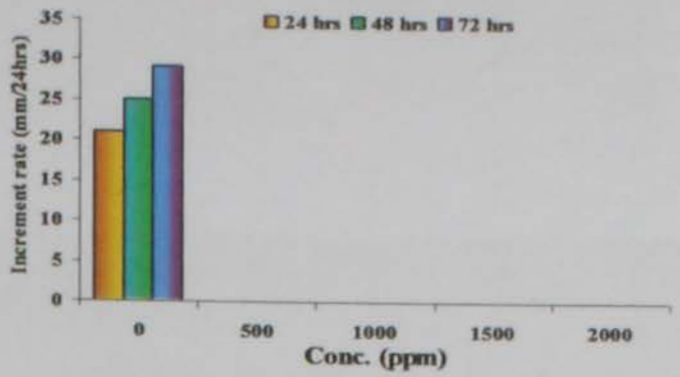
Fungicides	Concentrations (ppm)	Mycelial radial growth[1] (mm)	Per cent (%) inhibition of mycelial radial growth
Kafa 80WP (Mancozeb)	0	80.0 a (8.94)	-
	500	35.93 d (5.99)	55.09
	1000	33.2 e (5.76)	58.50
	1500	29.7 f (5.45)	62.88
	2000	28.3 f (5.32)	64.63
Rovral 50WP (Iprodione)	0	75.3 b (8.68)	-
	500	0.00 j (0.71)	100
	1000	0.00 j (0.71)	100
	1500	0.00 j (0.71)	100
	2000	0.00 j (0.71)	100
Zhetalux 25WP (Metalaxyl 25WP)	0	74.7 b (8.62)	-
	500	50.5 c (7.12)	32.40
	1000	0 j (0.71)	100
	1500	0 j (0.71)	100
	2000	0 j (0.71)	100
CV (%)		1.63	

[1] Mean of three replications for each concentration

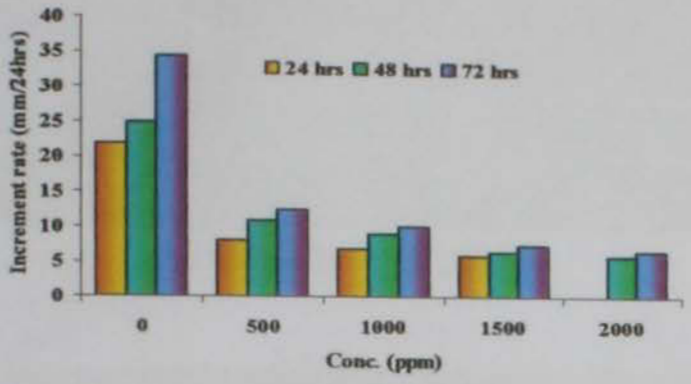
Figures within the parenthesis are square root transformed values. Numbers with similar letter do not differ significantly at 5% level according to Duncan's Multiple Range Test (DMRT)



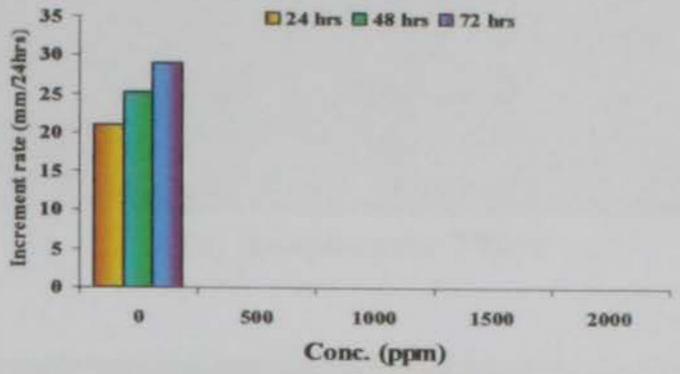
Bavistin 50WP



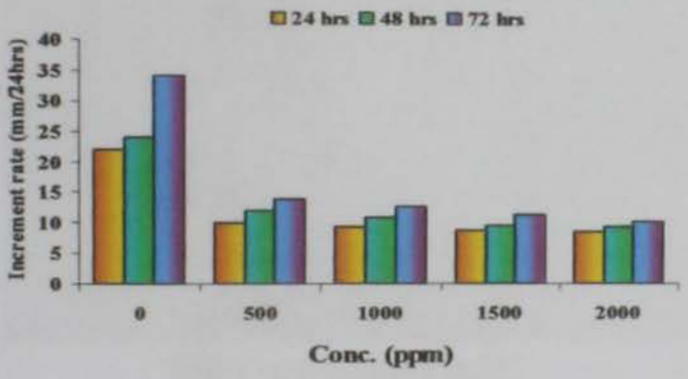
Sunphanate 70WP



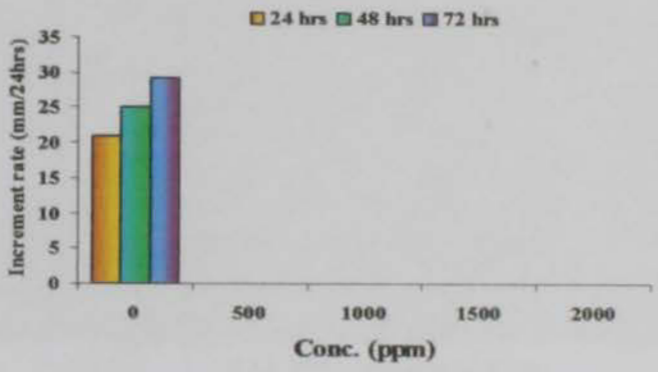
Agromil 72WP



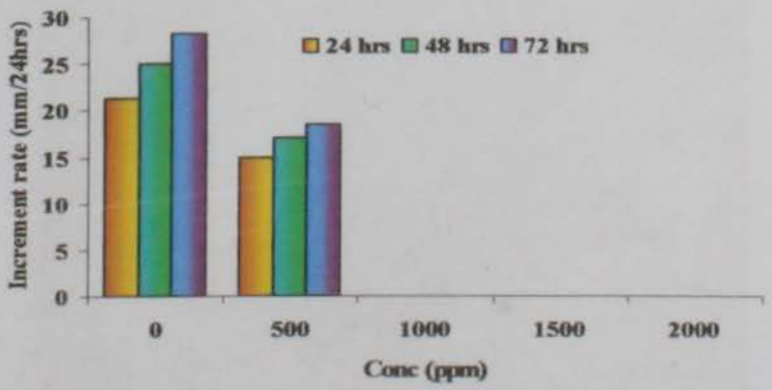
CP-Zim 50WP



Kafa 80WP

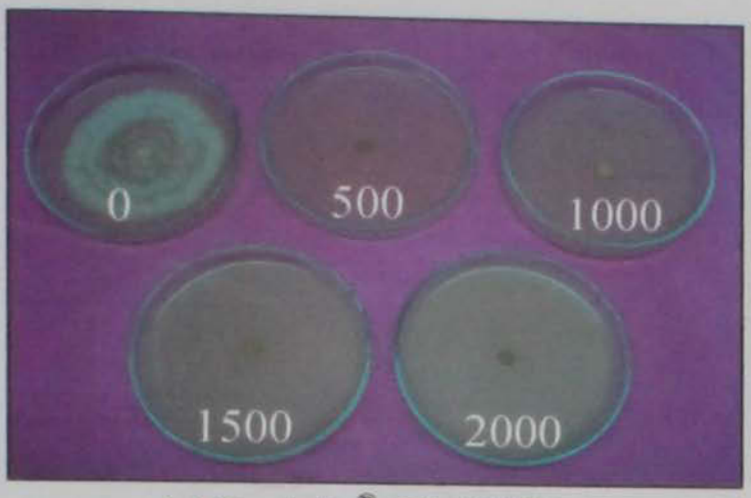


Rovral 50WP



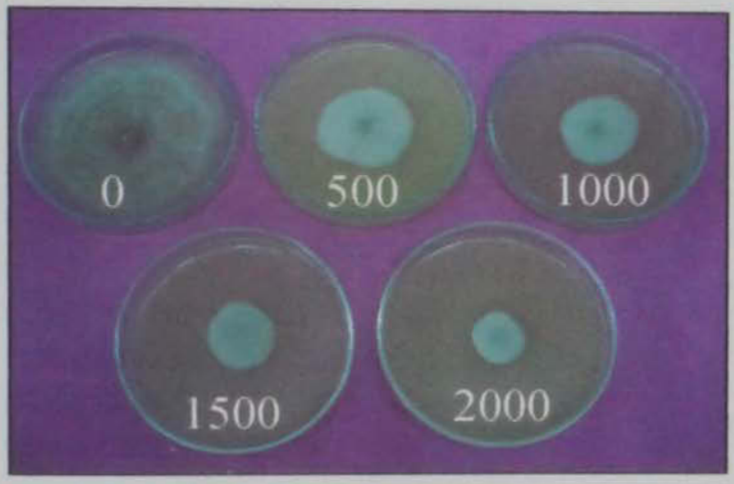
Zhetalux 25WP

Figure 3. Increment rate of mycelial growth of *Botrytis cinerea* at 24, 48 and 7 2hrs of incubation with different fungicides



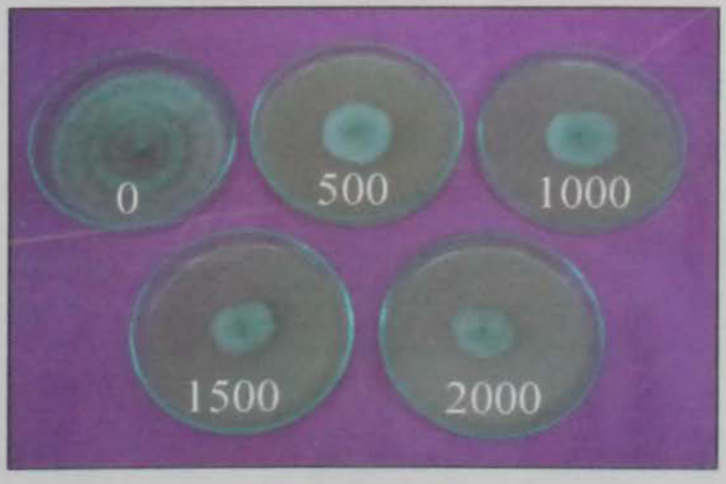
(a) Bavistin® DF 50WP

(b) Sunphanate 70WP



(c) Agromil72WP

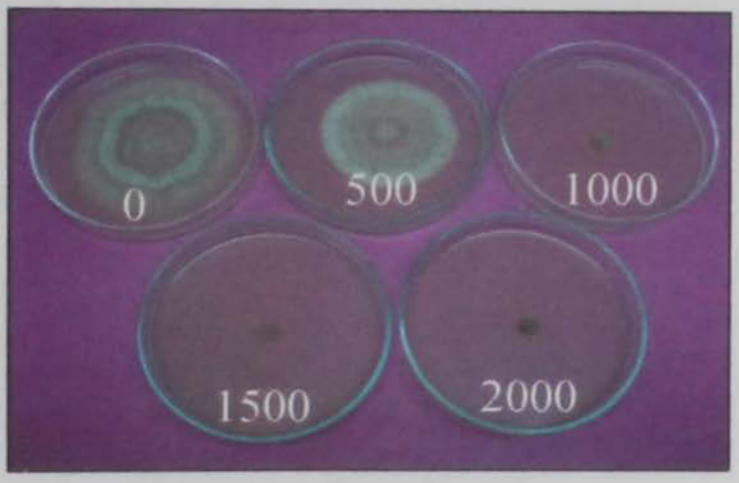
(d) CP-Zim 50WP



(e) Kafa 80WP

Cont'd .....

(f) Rovral 50WP



(g) Zhetalux 25WP

Plate 9. Colony growth of *Botrytis cinerea* inhibited by seven tested fungicides (a) Bavistin® DF 50WP, (b) Sunphanate 70WP, (c) Agromil 72WP, (d) CP-Zim 50WP, (e) Kafa 80WP, (f) Rovral 50WP and (g) Zhetalux 25WP at different concentrations (ppm)


CHAPTER 5
DISCUSSION

CHAPTER 5

DISCUSSION

In the present research work, all ten isolates (AHI-1 to 10) showed variations in respect of their cultural, morphological, physiological and pathogenic characteristics. In respect of cultural characteristics, the isolates of *B. cinerea* showed variation in colony color, shape, margin and textures. Remarkable variations found in colony color and shape. Colony color was cottony white and greenish white profound in AHI-1, AHI-8 respectively. Others colony colors were ashy white off white and light ash. Colony margin were irregular, wavy and entire. Colony textures were fluffy and velvet in most of the isolates but isolates AHI-8 posses effuses type of texture. The current findings were well supported by Ahmed *et al.* 2007 who reported different colony color and margin of 4 Indian isolates.

Botrytis cinerea showed variations in terms of sclerotia formation, color and conidia production. The pathogen produces sclerotia on culture media and the sclerotia formation was very much peculiar and varied with time, media, temperature and pH. On the basis of sclerotia production the isolates of *Botrytis cinerea* are grouped into 5 classes (BC-I to BC-V) in this investigation. Significant variation also observed in conidia size. The length and breadth of conidia ranged from 5.00 to 15.00 µm and 5.00 to 10.00 µm, respectively. Maximum mean length of conidia was observed in isolate AHI-9 (12.00 µm) and minimum in AHI-1 (7.50 µm). The highest mean breadth was noted in isolate AHI-9 (8.25 µm) and the lowest in AHI-4 (6.00 µm). The present findings agreed with the report of Joshi and Singh (1969) measured the conidia of *Botrytis cinerea* as 4-16×4-10 µm. Bakr *et al.* (2002) reported that variation exists in morphological and cultural characteristics of different isolates of *Botrytis cinerea*.

Stereo, compound and SEM study of *Botrytis cinerea* revealed that mycelium was dichotomous, twisted, ribbon, hyaline, botryose and single cell conidia borne on conidiophore, conidia were oval and often round in shape. This finding collaborate with the reports of other worker (Pande, *et al.* 2006) who found that conidiophores are light brown, pseudodichotomically with slightly enlarged tips bearing small pointed sterigmata with single celled, hyaline, oval conidia in cluster form.

Profound influences of temperature have been observed on colony growth of *Botrytis cinerea.* Increment rate of mycelial radial growth gradually increases with time of intervals for a certain period and maximum growth was found at 20°C. All the collected isolates (AHI-1 to AHI-10) grew well at varied range of temperatures (5 to 30°C) and gradually increased within the range of 5 to 20°C temperatures but gradually decrease after 20 to 30°C. At temperature 35°C growth was completely stopped. The highest (86.00 mm) colony diameter was noted in isolate AHI-9 and the lowest (78.00 mm) was obtained in isolate AHI-4 both at temperature 20°C. From this investigation, it appears that 20°C temperature is suitable for mycelial radial growth of *Botrytis cinerea.* The present findings are in agreement with other researchers (Ahmed, *et al.* 2007; Bakr and Ahmed, 1992; Bakr *et al.* 1997) who found that 20°C was the optimum temperature for maximum colony diameter of *B. cinerea*. They also observed that the fungal growth was completely inhibited at 5°C and 35°C. They also reported that the maximum disease severity caused by *Botrytis cinerea* was noticed around 20°C temperature.

Regarding mycelial radial colony growth of *Botrytis cinerea* under different pH levels, it was observed that the pathogen grew well in wide range of pH 4.5 to 6.5 levels. The maximum and minimum radial mycelial growth was observed at pH 4.5 and pH 6.5, respectively for all ten isolates.

With the increases of pH levels from pH 4.5 colony diameters gradually decreases upto pH 6.5. At pH 4.5 highest (81.00 mm) radial growth was obtained in isolates AHI-6 followed by AHI-9 (80.00 mm) and the lowest mycelial radial growth was obtained in isolates AHI-7 (22.00 mm) preceded by AHI-4 (31.33 mm). The results of the present investigation showed that *Botrytis cinerea* is an extremely acid loving fungus. Ahmed *et al.* (2007) reported that *Botrytis cinerea* is acid loving and pH 5.5 is suitable for its growth and sporulation. He also observed that different isolates of *B. cinerea* behaved differently in response to varied pH levels.

The remarkable effect of different culture media on mycelial radial colony growth, sclerotia formation and number of spores production were observed in *Botrytis cinerea* (Isolate AHI-9). After 24 hrs of incubation maximum (24.50 mm) colony diameter was recorded on CDA medium followed by CBDA (20.50 mm), PDA (20.50 mm), and LDA (20.30 mm) media those were statistically identical. It was also found that rate of increment of *Botrytis cinerea* mycelial radial growth increases with the increases of incubation time for a definite period. The highest colony diameter (79.17 mm) was found on Chickpea dextrose agar (CDA) media and the lowest (34.83 mm) on Water agar (WA) media.

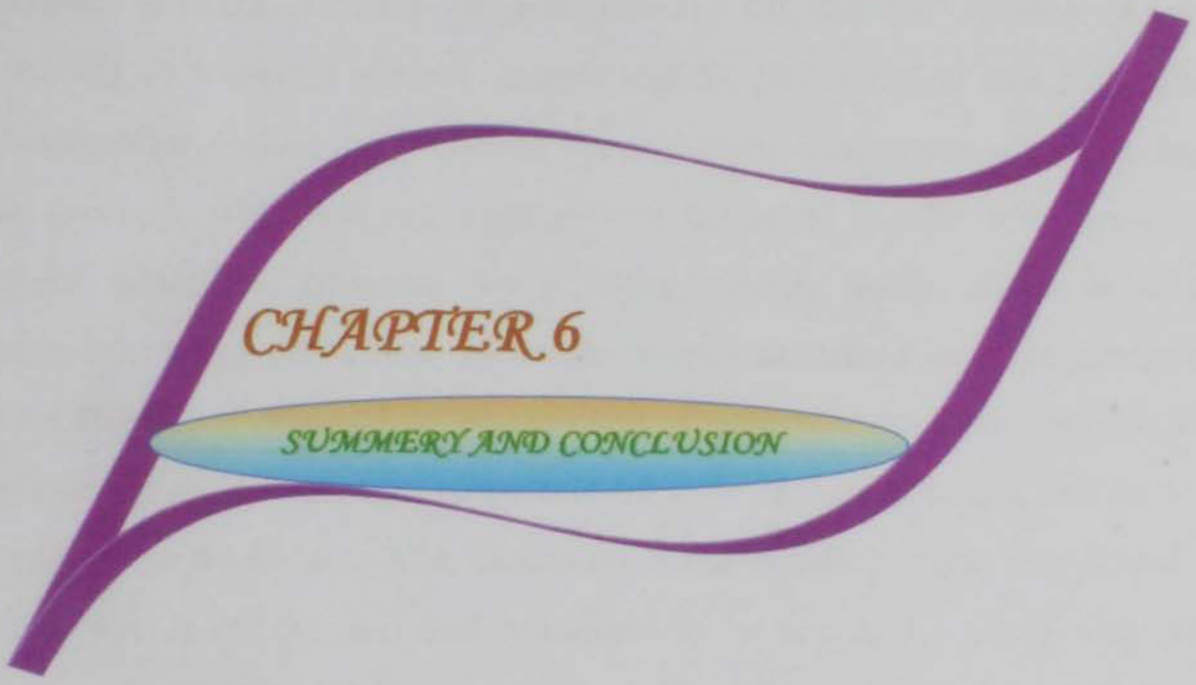
The maximum number (2.5×10^4 ml^{-1}) of spores was found on LDA and the minimum (1.9×10^4 ml^{-1}) on PDA in comparison to other culture media. No sporulation was observed on WA and V-8 A culture media. The earliest and good number of sclerotia was observed on CDA, LDA media on fifth day followed by BDA, CBDA on sixth day. Incase of WA and V-8 A nutrient media no sclerotia was produced. The present investigation clearly pointed that culture media showed profound effect on mycelial radial growth, rate of increment and sporulation of *Botrytis cinerea.* The maximum sporulation occurred on LDA medium and no sporulation was observed on V-8 A and WA media.

Marked variations were observed in all isolates in terms of reactions of *Botrytis cinerea* on different chickpea cultivars. All the isolates were not equally reacted on the chickpea cultivars but the isolates showed reactions in all cultivars. All the variety (BARI-Chola 1 to 8) and a tolerant chickpea genotype (ICCL-87322) showed highly susceptible reaction to isolate AHI-9 and 10. However, isolate AHI-9 and AHI-10 can be selected as different biotypes of the fungus *Botrytis cinerea.* The findings agreed with the findings of Rewal and Grewal (1989b) and they categorized the *Botrytis cinerea* isolates to five pathotypes on the basis of their reaction on a set of five chickpea differential varieties/lines.

The antagonist *Trichoderma harzianum* played an important role to restrict the growth of *Botrytis cinerea* causing BGM disease in chickpea. After 48hrs, growing mycelial of *Trichoderma harzianum* and the target pathogen came in contact and hyphal growth of *Botrytis cinerea* was profoundly inhibited at the zone of contact with the antagonist hyphae. Microscopic study showed that the hyphal tips of *Botrytis cinerea* become swelled and curved at the point of contact. On 6 days of incubation, antagonist hyphae covered the entire plates suppressing the growth of *Botrytis cinerea*. The result of dual culture assay indicates that *Trichoderma harzianum* has inhibitory effect on *Botrytis cinerea* and this antagonist could be use in controlling Botrytis gray mold of chickpea. Similar findings have been reported by Pande *et al.* (2006) who found that *T. harzianum* is highly antagonistic against *Botrytis cinerea* and parasitized mycelium at the point of contact.

From the result of the present piece of work, it was well exposed that among the seven fungicides four of them viz. Bavistin 50WP, CP-Zim 50WP, Sunphanate 70WP and Rovral 50WP completely inhibited the hyphal growth of *Botrytis cinerea.* The fungicide Zhetalux 25WP also completely retarded the mycelial growth at higher concentration from 1000 to 2000 ppm. Fungicide Kafa 80WP restricted 64.63 % mycelial growth when applied @ 2000 ppm concentration. Agromil 72WP retarded 84.42 % hyphal growth @ 2000 ppm concentration.

From the results of the *in vitro* test, it may be noted that Bavistin® DF 50WP, CP-Zim 50WP, Sunphanate 70WP and Rovral 50WP were the most effective fungicides and potential to inhibit the mycelial radial growth of *B. cinerea* at lower concentration (500 ppm) followed by Zhetalux 25WP, Kafa 80WP and Agromil 72WP at higher concentrations. The present findings are agreed with Agarwal and Tripathi (1999), Madhu Meeta *et al.* (1986a) and Rewal and Grewal (1989b) who's reported that Bavistin (10 μgml^{-1}) completely inhibited the growth of *Botrytis cinerea* while working with eight fungicides.


CHAPTER 6
SUMMERY AND CONCLUSION

CHAPTER 6

SUMMERY AND CONCLUSION

Botrytis cinerea causing Botrytis gray mold (BGM) in chickpea is considered the most damaging disease in Bangladesh. In Bangladesh, there has been sharp decline in chickpea area and production due to serious outbreaks of BGM disease. Ten isolates of *Botrytis cinerea* were collected from seven different chickpea growing districts of Bangladesh. All the 10 isolates showed variability in terms of cultural, morphological, physiological and pathogenic characteristics. Colony of *B. cinerea* cottony white, ashy white, off white, light ash, greenish white colored colony with irregular, regular with sector or without sector and irregular, wavy, entire margin; fluffy, effuse or velvet texture was observed. All the 10 isolates were categorized into five groups on the basis of their sclerotia production and arrangement on PDA medium. The conidia size varied within a range of 5.00 to 15.00 µm in length and breadth from 5.00 to 10.00 µm. The maximum mean conidial length was found in isolate AHI-9 (12.00 µm) and minimum in isolate AHI-1 (7.50 µm). The maximum mean breadth (8.25 µm) of conidia was found in isolate AHI-9 and the lowest (6.00 µm) was found in isolate AHI-4. The *Botrytis cinerea* mycelium was dichotomous and conidial arrangement on conidiophore in a cluster form, conidia were oval or nearly round shaped. The fungus grew well in a varied range of temperature, pH and nutrient media. The maximum radial colony diameter was found at 20°C, pH 4.5 and CDA media for all the isolates. No growth was observed at 35°C temperature. The highest number of spores (2.5×10^4 ml^{-1}) were noted from culture on LDA and no sporulation were observed in WA and V-8 A media. Invariably isolate AHI-9 formed sclerotia within 5 days on CDA and LDA media.

The isolates were varied markedly in their test of pathogenicity. All the isolates (AHI-1 to 10) were able to infect all the chickpea cultivars. The maximum disease reaction were observed incase of isolate AHI-9 and AHI-10 and all the cultivars were highly susceptible to these two isolates.

Trichoderma harzianum was found to be a good antagonist against *B. cinerea* pathogen and completely inhibited the growth and parasitized the pathogen on PDA medium.

The fungicides of carbendazim, thiophanate methyl and iprodione group completely inhibited the mycelial radial growth of *Botrytis cinerea* at lower concentration of 500 ppm. Fungicides from metalaxyl group can inhibit the mycelial radial growth comparatively at higher concentration (1000 ppm). Fungicides from mancozeb group have some effect on restricting mycelial radial growth over control at higher concentration.

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

- Variability exist in BGM pathogen (*Botrytis cinerea*) prevailing in the chickpea growing areas of Bangladesh.
- All the isolates were not virulent equally to all the tested chickpea cultivars.
- Bio-agent *Thrichoderma harzianum* was found effective against *Botrytis cinerea.*
- Fungicides from carbendazim, thiophanate methyl and iprodione group was found effective to inhibit mycelial radial growth of *Botrytis cinerea.*
- BARI Chola 3 can be suggested to cultivate in three major chickpea growing (Faridpur, Kushtia and Pabna) districts.
- Further study should be conducted in the field level to confirm effectivity of the recommended fungicides.
- Molecular characterization should be done to confirm the variability exist in the present BGM isolates.

CHAPTER 7

LITERATURE CITED

CHAPTER 7

LITERATURE CITED

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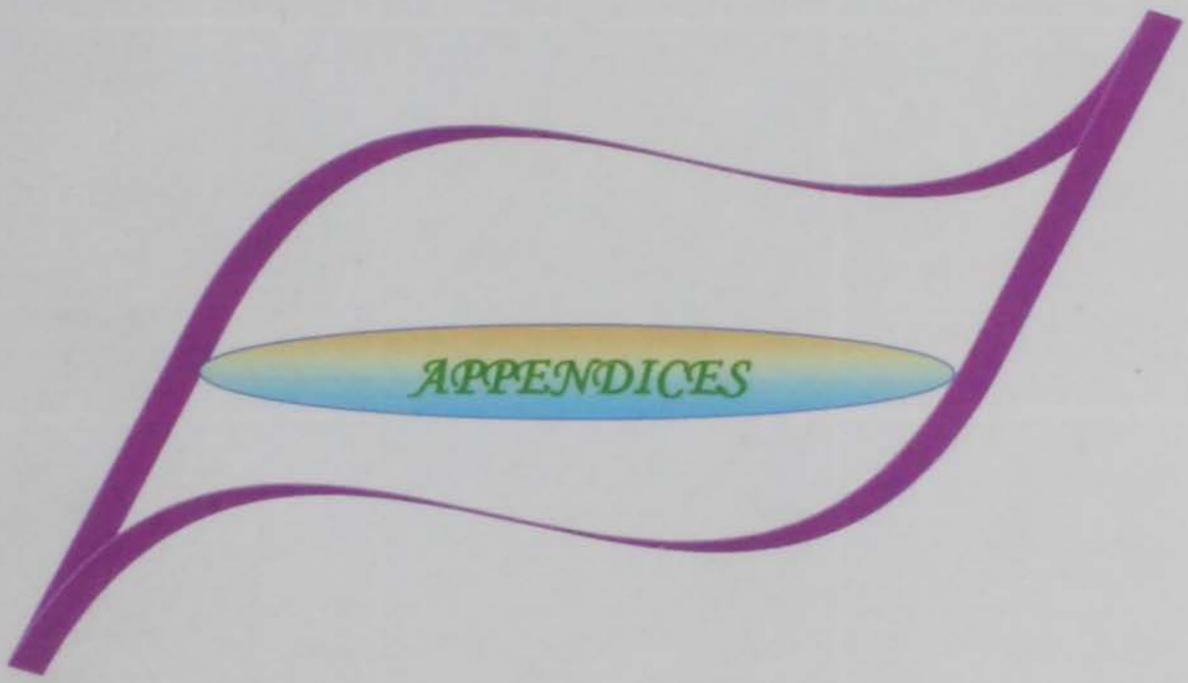
APPENDICES

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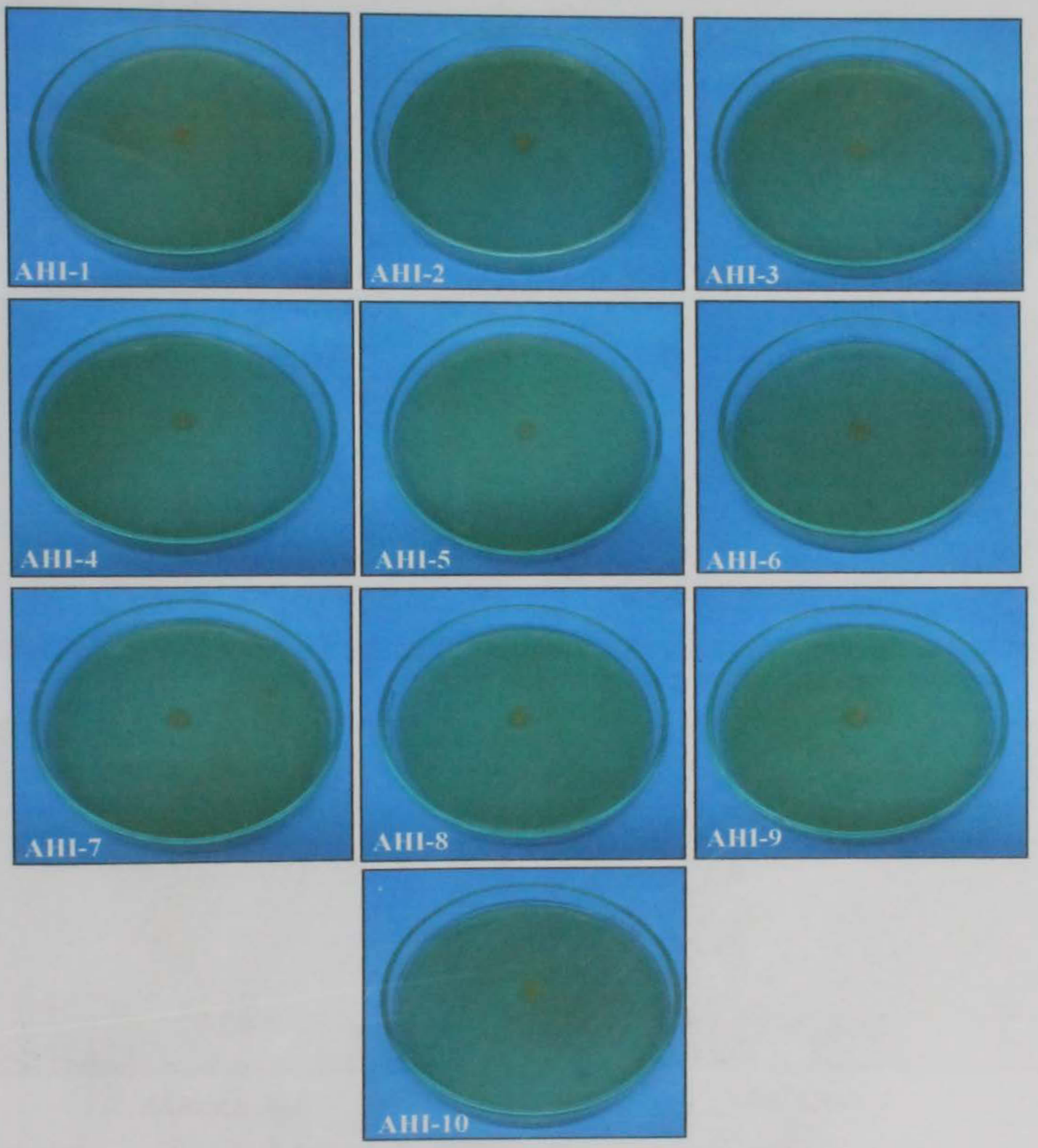

Appendix I. Completely retarded radial colony growth of isolates AHI-1 to 10 at 35°C temperature

BARI Chola-1

BARI Chola-2

BARI Chola-3

BARI Chola-4

BARI Chola-5

BARI Chola-6

Appendix II. Nine chickpea cultivars (BARI Chola-1 to 8) released by BARI and a tolerant genotype ICCL-87322

Cont'd (Appendix II)

BARI Chola-7

BARI Chola-8

ICCL-87322

Appendix III. Fifteen day old seedlings of 9 chickpea cultivars just immediate after inoculation with 10 isolates of *Botrytis cinerea*

Appendix IV. (A) Progressive symptoms of *Botrytis cinerea* infection and profuse mycelia, spores were found on BARI Chola-8 at 5th days after inoculation) and (B) covered with polyethylene sheet ensuring >90% RH for the disease development