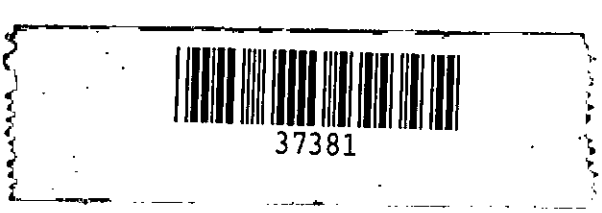
STUDY ON SOME MORPHOLOGICAL AND PHYSIOLOGICAL FEATURES OF *STEMPHYLIUM BOTRYOSUM* CAUSING STEMPHYLIUM BLIGHT DISEASE OF LENTIL AND ITS CONTROL

By

TANJINA RAHMAN
Registration No. 07-02615

DEPARTMENT OF PLANT PATHOLOGY
SHER-E-BANGLA AGRICULTURAL UNIVERSITY
DHAKA-1207



DECEMBER, 2008

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Approved By:

Ashraf Uddin Ahmed
Senior Scientific officer
Bangladesh Agricultural Research Institute
Joydebpur, Gazipur
Supervisor

Prof. Dr. Md. Rafiqul Islam
Department of Plant Pathology
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207
Co-supervisor

Dr. M. Salahuddin M. Chowdhury
Chairman
Examination Committee
Department of Plant Pathology
Sher-e-Bangla Agricultural University

উদ্ভিদ রোগতত্ত্ব বিভাগ
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Plant Pathology Division
Bangladesh Agricultural Research Institute
Joydebpur, Gazipur-1701, Bangladesh

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CERTIFICATE

This is to certify that the thesis entitled, *"STUDY ON SOME MORPHOLOGICAL AND PHYSIOLOGICAL FEATURES OF STEMPHYLIUM BOTRYOSUM CAUSING STEMPHYLIUM BLIGHT OF LENTIL AND ITS CONTROL"* 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 **TANJINA RAHMAN**, *Registration No. 07-02615,* under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma in anywhere.

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

Dated: 31-12-2008
Dhaka, Bangladesh

Ashraf Uddin Ahmed
Supervisor
Senior Scientific officer
Bangladesh Agricultural Research Institute
Joydebpur, Gazipur

Dedicated to
My Beloved Parents

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ABBREVIATIONS AND ACRONYMS

ABBREVIATIONS	ACRONYMS
%	Per cent
@	At the rate of
°C	Degree Celsius
ai	Active Ingredient
BARI	Bangladesh Agricultural Research Institute
BBS	Bangladesh Bureau of Statistics
cm	Centimeter
Conc.	Concentration
CRD	Completely Randomized Design
CV	Coefficient of Variance
DMRT	Duncan's Multiple Range Test
EC	Emulsifier Concentration(s)
et al.	And Others
g	Gram(s)
h	Hour(s)
ha	Hectare(s)
HR	Highly Resistant
HS	Highly Susceptible
kg	Kilogram(s)
ml	Milliliter
mm	Millimeter
MR	Moderately Resistant
MS	Moderately Susceptible
N	Normal
NaOH	Sodium Hydroxide
PDA	Potato Dextrose Agar
psi	Per Square Inch
R	Resistant
S	Susceptible
S. botryosum	*Stemphylium botryosum*
SAU	Sher-e-Bangla Agricultural University
SEI	Secondary Electron Image
SEM	Scanning Electron Microscope
t	Ton(s)
T. harzianum	*Trichoderma hazianum*
USA	United States of America
WA	Water Agar
WP	Wettable Powder

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ABSTRACT

Experiments were carried out to study on some morphological and physiological features of *Stemphylium botryosum* causing Stemphylium blight and its control with fungicides and antagonist both *in vitro* and *in vivo* at Plant Pathology Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur during the period of September'07 to April'08. The experimental design was CRD in lab condition and RCBD in field condition having five and four replications, respectively. The colony color of *S. botryosum* was greenish brown, irregular margin and velvety texture on PDA medium. The suitable temperature and pH for the maximum colony growth was 25°C and 6.0, respectively. The maximum (100%) germination of conidia was found at 25°C after 6 h of incubation. In the *in vitro* test, six fungicides viz. Iprosun 50WP, Edcuzeb 80WP, Proud 25EC, Rovral 50WP, Emivit 50WP and Agrimyl had the potentiality to inhibit the radial mycelial growth even at a lower (500 ppm) concentration except Agrimyl (Mancozeb + Metalaxyl) that inhibited radial colony growth at higher (2000 ppm) concentration. In field condition the minimum disease score (1.0) was recorded in Iprosan 50 WP treated plot and the highest (4.75) was found in control plot. Among the six fungicides Iprosan 50WP from the iprodione group gave the best performance and yielded the highest root length (9.48 cm), shoot length (44.6 cm), number of branches plant^{-1} (9.25), number of pods plant^{-1} (39.10), thousand grain weight (21.08 g) and grain yield (1271.00 kg ha^{-1}).

CHAPTER 1
INTRODUCTION

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INTRODUCTION

Lentil (*Lens culinaris* Medik.) is one of the oldest and second most important pulse grain legume crops in terms of both area (154,000 ha) and production (116,000 t), the highest consumer preference and the total consumption (BBS, 2002). This crop has-been grown mainly as an inexpensive source of high quality protein in human diets, especially in West Asia. Lentil contributes 25% of the total pulse production (Anonymous, 1991). Lentil is best adapted to the cooler temperate zones of the world, with more recent introductions to Australia, Canada, New Zealand and in the USA (Bayaa and Erskine, 1998). Lentil has been cultivating in Bangladesh since long back and the crop is found to grow everywhere.

Now a days the production of the crop is decreasing every year due to some biotic and abiotic factors. Among the two groups, diseases under biotic factors are the major constraint for lentil production all over the country. So far 15 pathogens causing 17 diseases of lentil have been reported in Bangladesh (Ahmed, 1985). Among the diseases stemphylium blight caused by *Stemphylium botryosum* is a serious threat to lentil cultivation. Stemphylium blight disease of lentil has been reported in Bangladesh, Egypt, Syria and the USA (Bayaa and Erskine, 1998).

Disease symptoms have been well defined in South Asia where *S. botryosum* has caused great devastation to the lentil crop. Bakr (1991) reported that the symptoms of the disease in Bangladesh include the appearance of small pin-headed light brown to tan colored spots on the leaflets which later enlarge, covering the leaf surface within 2 - 3 days.

In Bangladesh, it is a major disease causing large scale defoliation (Erskine and Sarkar, 1997). Preliminary studies in Bangladesh and India estimated yield losses of 62% and total crop failure have been reported in some cases where the disease defoliated the crop in the early pod setting stage (Bakr, 1991; Erskine and Sarkar, 1997). The disease was first documented in Bangladesh by Bakr and Zahid (1986), Nene *et al.*, (1984) in India, Kaiser (1972) in Iran. and Simay (1990) in Hungary.

The disease is widespread throughout the country with the highest incidence and severity in the southern region of Bangladesh especially in Jessore, Pabna, Kushtia, Faridpur, Madaripur and Dhaka (Bakr and Ahmed, 1992). Stemphylium blight disease is increasing tremendously in the last decades and great hamper to the successful production of lentil. The disease already gained much more importance and 80-92.35% crop loss has been reported by Bakr and Ahmed (1992). In neighboring country India, the intensity of the disease was 82.55% and the loss was recorded as 93.4% (Singh *et al.*, 1990).

Temperature plays an important role to develop this disease. The prevalence of warm temperature (>25°C) and wetness duration longer than 24 h favors the appearance, development and spread of the Stemphylium blight disease in South East Asia (Erskine and Sarkar, 1997). A wide range of pH (6-8) also favors the radial growth of *S. botryosum* (Huq, 2003). However, few studies have been investigated for the detail study of the pathogen *Stemphylium botryosum*.

Hence, due to importance of this disease concentration need to be paid and try to shed new focus on the causal agent of this disease especially of its physiological requirements for the growth and development which will help to manage the disease efficiently.

In view of the above facts the present research work was undertaken with the following objectives-

1. To know the morphological growth pattern of *Stemphylium botryosum*
2. To know the physiological features of *Stemphylium botryosum*
3. To screen out of effective fungicides against *Stemphylium botryosum* causing Stemphylium blight of lentil

CHAPTER 2

REVIEW OF LITERATURE

CHAPTER 2
REVIEW OF LITERATURE

Stemphylium blight caused by *Stemphylium botryosum* is a defoliating fungal disease and has been reported as one of the notorious disease causing considerable economic loss to the farmers. The disease occurs every year all over the country especially lentil growing areas of Bangladesh. Although a good number of work have been done to manage the disease through chemicals but details study of causal agent are inadequate in the previous literatures. However the available literatures on Stemphylium blight caused by *Stemphylium botryosum* has been complied and presented below-

2.1. Symptomology of Stemphylium blight disease

Mwakutuya (2006) found that the symptom of Stemphylium blight of lentil was greatly differed from other foliar lesions by being larger and spreading across or along the entire leaflet. A blighted dull yellow appearance is observed with infected foliage and branches. Defoliation occurs rapidly, leaving the branches with terminal leaves. The stems and branches also bend down, dry up and gradually turn ashy white, but the pods remain green and white mycelial growth can be observed on the infected stems.

Huq (2003) conducted an experiment and noted that the disease symptoms of lentil blight usually appears on leaves and shoots. The characteristics feature of symptoms is the formation of small pinheaded gray spots on the leaflets which

rapidly spread over the shoots and twigs. In severe condition leaflets get blighted and plants become defoliated leaving a few green leaves and some immature fruits.

2.2. Cultural and morphological characteristics

Mwakutuya (2006) found that *Stemphylium botryosum* conidia are solitary, rough or smooth and conidia size ranged from 12-20 µm × 15-30 µm.

Hosen *et al.*, (2009) conducted an experiment and found variation in isolates of *Stemphylium botryosum* in respect of colony color, shape and texture. Colony color varied from greenish brown to dirty white, shape was regular to roughly irregular and velvety to fluffy type texture were observed. They also observed in variability in conidia size which was ranged from 10.00 to 25.00 µm in length wise and 5.00 to 15.00 µm in breadth wise. The average conidia size of *S. botryosum* was 13.33-16.04 µm × 6.46-9.17 µm.

2.3. Spore germination of *Stemphylium botryosum*

Mwakutuya *et al.*, (2002) reported that the Stemphylium blight caused by *Stemphylium botryosum* was detected regularly from Saskatchewan lentil fields in consecutive years. The culture age and different light regimes did not effect on conidia germination of *S. botryosum*. High temperatures (°C) favored the germination of conidia and optimum temperature for conidial germination was between 25 to 30°C.

According to Mwakutuya (2006) in the presence of free water, conidia of *S. botryosum* germinated over a wide range of temperatures (5 to 30°C). The conidia were polyspermic and produced up to six germ tubes depending on the temperature and the incubation time. The percentage of conidia that had germinated increased with temperature and incubation period. The highest rate of germination was in 30°C followed by 25°C after 20 h of incubation. The impact of the rate of germination increased as temperature increased above 15°C and generally the lowest and fasted response was at 5°C and 30°C, respectively.

2.4. Effect of temperature (°C)

Montesions *et al.*, (1995) conducted an experiment on *Stemphylium vesicarium* isolated from the lesion on pear fruit and found that optimum temperature for the radial mycelia growth and conidia germination were 15-25°C and 20-30°C, respectively.

Erskine and Sarkar (1997) studied on stemphylium blight disease of lentil and reported that the prevalence of warm temperature (>25°C) and wetness duration longer than 24 h favors the appearance, development and spread of this disease in South East Asia.

Sarker *et al.*, (2004) reported that in epidemic years, complete crop failure has been observed due to Stemphylium blight caused by *S. botryosum*. High

humidity, coupled with ambient night temperature around 8°C and mean day temperature above 22°C, promotes stemphylium blight disease in lentil.

Hosen *et al.*, (2009) conducted an experiment and they found that maximum radial colony growth of *Stemphylium botryosum* was in 25°C followed by 20°C and no growth was recorded at 40°C.

2.5. Effect of pH

Huq (2003) conducted an experiment with seven levels of pH viz. 4, 5, 6, 7, 8, 9 and 10 on radial growth and sporulation of *Stemphylium botryosum* and found that this pathogen grew well at a different pH level. The effect of pH 6, 7 and 8 were statistically similar and the best growth was observed in pH 6 (63.7 mm) followed by pH 7 (63.0 mm). Among the pH involved the lowest radial growth was found at pH 4 (51.0 mm). The pathogen sporulated at all the pH levels but it was scanty and rated as poor.

Rajani *et al.*, (1991) carried out an experiment and observed that pH 5.5 as an optimum pH for the highest radial mycelial growth for the *Stemphylium lycopersici.*

Raza *et al.*, (1991) reported that pH effect on the radial growth of *Stemphylium botryosum* the best growth was noted at pH 7.0.

Padhi and Synder (1954) reported that the pathogen *Stemphylium botryosum* grew in wide rage of pH 2.9 to 7.8. The optimum being 5.5 which gave the luxuriant mycelial dry weight and the highest sporulation was found at pH 5.4 but it was good between pH ranges 4.9 to 5.

2.6. Chemical control

The use of chemical to control the plant disease is an effective tool in order to get return of maximum amount of economic yield.

2.6.1. *In vitro* assay

Rahman *et al.*, (1988) evaluated six fungicides *in vitro* in different concentration of 250, 500, 1000, 2000 and 10000 ppm for their effect on radial mycelial growth and sporulation of necrotrophic fungus like *Stemphylium botryosum* using paper disc method. The diameter of the inhibition zone increased gradually with increasing concentration of Rovral 50WP and Dithane M-45. Inhibition zone developed at the lowest concentration of Rovral was significantly wider than that of the highest concentration of the other fungicides under trial condition.

Rajani *et al.*, (1992) tested nine (9) fungicides *in vitro* against *Stemphylium* sp. and found that Vitavax (Carboxin) and Blitox (Copper oxychloride) can control the radial mycelial growth at a lower concentration (250 ppm) while carboxin, Dithane M-45 group and Captan successfully inhibited the conidial germination.

Huq (2003) tested seven fungicides (Rovral 50WP, Dithane M-45, Tilt 250EC, Cupravit, Macuprax, Ridomil MZ-72 and Bavistin 50WP) and found that all fungicides inhibited radial mycelial growth significantly over the control at different concentrations. Rovral 50WP (2000 ppm) was the most effective and completely inhibited the mycelia growth among the others.

Sardar (2005) evaluated sixteen fungicides *in vitro* against *Stemphylium sarciniformis* on PDA medium. The highest (80 mm) radial colony growth was recorded from control plate where no fungicides were used and the lowest (35.2 mm) was recorded from the plate where both the fungicides Rovral 80WP and Tilt 250EC were applied and growth reduction was up to 56%. The fungicides Acrobat could not inhibit the radial growth on PDA at all whereas little effect on colony growth was found by applying Haymaxil 50WP, Metaplus 72WP, Ridomil Gold and Pipertox 50WP.

Hosen *et al.*, (2009) evaluated six fungicides and found that Rovral 50WP from the iprodione group was the best fungicides among the others even at a lower (500 ppm) concentration followed by CP-Zim 50WP (Carbendazim), Agromil 72WP (Mertalaxyl + Mancozeb) and Kafa 80WP (Mancozeb) and inhibited the radial mycelial growth of *S. botryosum* 22.3, 23.7 and 24.7 mm, respectively at higher concentration (2000 ppm).

2.6.2. *In vivo* condition

Gupta and Srivastava (1988) tested eight (8) fungicides group namely Copper oxychloride, Mancozeb, Captafol, Thiram, Carbendazim, Ziram, Captan and Carboxin for the management of *Stemphylium vesicarium* in onion and found that Copper oxychloride, Mancozeb, Carbendazim and Thiram effectively prevented the disease with 4 sprays after appearing the disease symptoms.

Rahman *et al.*, (1988) evaluated six fungicides in *vivo* to control the leaf blotch of onion caused by *Stemphylium botryosum* and *Alternaria porri* and found Rovral 50WP as the best fungicides in reducing the PDI and increasing the yield.

Bakr and Ahmed (1992) carried out an experiment and found that disease score was lowest in plots treated with Rovral 50WP @ 0.2% and it is indicating the highest disease reducing efficacy of Rovral and rest of three fungicides Uniflow TM sulfur, Antracol and Dithane were not statistically difference among the score of infection in the plot sprayed with them. Plots sprayed with Rovral produced the highest (1506 kg ha^{-1}) seed yield followed by others while the lowest yield found in control plots which were statistically significant with the others.

Bakr and Ahmed (1993) conducted an experiment with integrated management effort against Stemphylium blight of lentil. Fungicide Rovral 80WP increased seed yield considerably. Resistant genotype (L-80670) produced the highest

seed yield (1157 kg ha^{-1}) followed by foliar spray of Rovral 80WP to the same genotype at space planted conditions (1106.3 kg ha^{-1}).

Kamalesh *et al.*, (1993) carried out an experiment with some systemic and non systemic fungicides to control the gray leaf spot of tomato caused by *Stemphylium botryosum* for two successive years. Fungicides Chlothanil was the best to reduce the diseases and gave the highest yield which was statistically similar with Captafol (0.2%). The systemic fungicides were found less effective than non systemic fungicides. It was also revealed that fungicides from the systemic group though reduced disease severity to some extent but were not at all economic as compared to non systemic fungicides.

According to Gupta *et al.*, (1996) to control Stemphylium blight of onion caused by *Stemphylium botryosum* Indofil M-45 (0.25%) was the best fungicides which was statistically identical with Rovral 50WP (0.25%). Rovral was not economical than Indofil because of its high price.

Basallote-Ureba *et al.*, (1998) studied chlorothalonil, in addition to the application of tebuconazole or procymidone (alone or alternated with chlorothalonil) provided effective control for *Stemphylium vesicarium* on garlic.

Huq (2003) tested seven fungicides such as Rovral 50WP @ 0.2%, Dithane M-45 @0.2%, Tilt 250EC @ 0.05%, Cupravit @ 0.3%, Macuprax @ 0.25%,

Ridomil MZ-72 @ 0.2% and Bavistin 50WP @ 0.15% in the field during 1998-2001 to manage of Stemphylium blight disease of lentil. Rovral 50WP @ 0.2% was the most effective fungicide followed by Dithane M-45 @ 0.2% and Tilt 250EC @ 0.05%.

Davis *et al.*, (2005) recommended Mancozeb, Iprodione and Chlorothalonil for the control of Stemphylium leaf blight and stalk rot caused by *Stemphylium vesicarium* and *Stemphylium botryosum* respectively in onion and lentil.

Sardar (2005) reported that the severity of Stemphylium blight disease was statistically different among the counting period as well as different fungicides application. Control plot showed the highest disease severity and the lowest disease was obtained from the Rovral 80WP and Tilt 250EC treated plot. Applying fungicides against Stemphylium blight of lentil individual or in combination of Rovral 80WP and Tilt 250EC gave the higher amount of seed plant^{-1}. The individual application of Rovral gave the yield of 1362.50 kg ha^{-1}. The maximum crop yield (1412.50 kg ha^{-1}) of lentil was recorded from Rovral 80WP + Tilt 250EC treated plot and minimum (700 kg ha^{-1}) from the control plot.

CHAPTER 3

MATERIALS AND METHODS

CHAPTER 3

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3.1. Experimental site

All the research experiment was carried out at laboratory and field of Plant Pathology Division, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur during September 2007 to April 2008.

3.2. Collection, isolation and identification of *Stemphylium botryosum*

The plant samples showing Stemphylium blight infection were collected from the field and isolation, identification and sensitivity to fungicides were done before going to set the field experiment. Infected plant samples were cut into small pieces about 0.5 cm and surface sterilized with 1% chlorox solution for 1 and half minutes and rinsed thrice in water then placed into the petriplates containing PDA medium. Inoculated plates were kept in an incubator at 25°C temperature and the petriplates were observed from the following day of incubation up to 9 days to form colony of *S. botryosum*. After taken out the plates from the incubator then examined under stereo, compound and scanning electron microscope (SEM) and identified following the characteristic features of *Stemphylium botryosum*. Isolation and identification was also done for the isolates collected from the field experiment confirm the stemphylium blight disease causing *Stemphylium botryosum* in lentil.

3.3. Purification of *S. botryosum*

Purification was done using single spore isolation technique. Spore suspension was prepared from the 15 days old culture of *S. botryosum*. One or two drops of spore suspension were poured onto a petriplate containing water agar (WA) the drop of suspension was rubbed onto WA with the help of a sterilized glass rod then the plate was observed under a compound microscope with 10 x magnification. After locating a single spore it was focused into centre. One of the microscope objectives was replaced with a specially made spore cutter. Then the spore was cut with the spore cutter. Cut agar block was taken and placed onto a petriplate containing PDA medium and incubated at 25°C for five days then observed confirmed. The purified spore was kept in a refrigerator at 4°C as a slant culture for further use.

3.4. Cultural and morphological characteristics of *S. botryosum*

The cultural characteristics of *S. botryosum* were observed on PDA medium after 6 days of incubation on the basis of colony color, texture, margin, conidial color, size and color of conidiophores.

3.5. Scanning Electron Microscope (SEM) study

A double sided adhesive carbon cement tape was attached on an aluminum SEM stub. A loop full of *S. botryosum* pure culture (sporulating plate, 12 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 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).

3.6. Physiological studies of *Stemphylium botryosum*

3.6.1. Effect of temperature (°C)

Seven different levels of temperature viz., 5, 10, 15, 20, 25, 30 and 35°C were studied for its impact on radial colony growth of *S. botryosum*. Sixteen (16) ml of PDA medium were poured into the Petri plates using media dispenser having 5 replications for each temperature and autoclaved at 121°C for 30 minutes at 15psi and then taken out and shifted into the clean bench for solidification. Five (5) mm diameter mycelial disc were cut from the periphery of 5 days old culture of *S. botryosum* and inoculation was done and the plates were placed in an incubator in respective temperature level. The radial mycelial growth in each Petri-plate was noted as an average of two diameters measured at right angles to one another after 2 days of incubation.

3.6.2. pH

Seven different pH levels viz., 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5 were studied in this experiment. The different level of pH were maintained using 0.1 N NaOH or 0.1N HCl and the other protocol was same as stated previously in 3.6.1.

3.6.3. Effect of incubation time and temperature (°C) on conidia germination of *Stemphylium botryosum*

For the test of conidia germination of *S. botryosum,* conidia were harvested from 12 day old culture. Conidial suspension (at least 50 conidia per low microscopic field) was prepared using glass slide in sterilized distilled water. Conidial suspension was evenly spread over each of six well slides. At least three blotter papers placed on each glass Petri dishes and it made moistened using sterile water. Then the petriplates were incubated in each temperature level viz. 5, 10, 15, 20, 25, 30 and 35°C. The slides were placed into centre of the labeled six glass Petri-dishes. Observations were made after 2, 4, 6, 8, 12 and 24 h of incubation. The number of germinated conidia was counted as percentage of the total number of conidia observed. The conidia were considered as a germinated when the germ tube was developed at least half of the width of the conidia. Conidia germination was determined by evaluating at least 50 conidia. Observed was taken in a 10x microscopic field under compound microscope.

3.7. *In vitro* management of *Stemphylium botryosum*

For the test of efficacy test of six different fungicides namely, Iprosun 50WP, Edcuzeb 80WP, Proud 25 EC, Rovral 50WP, Emivit 50WP and Agrimyl were studied *in vitro*. The required amount of fungicides was weighed using electric balance to get the proposed concentration such as 500, 1000, 1500 and 2000 ppm. The required concentrations of tested fungicides and sterilized distilled water were added to the conical flasks containing double strength PDA

medium to achieve the proposed concentration with 5 replications. The other method of this experiment was same as mentioned earlier under temperature experiment. Inoculated plates were incubated at 25°C with 12 h light and dark phase alternatively and then examined after 7 days of incubation.

Per cent inhibition of the radial mycelial growth of *Stemphylium botryosum* was calculated on the basis of the following formula-

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

Where, I = Per cent of growth inhibition

C = Average diameter (mm) of fungal colony in control treatment

T = Average diameter (mm) of fungal colony in fungicides treated PDA medium

3.8. Field Experiment

3.8.1. *In vivo* management of *Stemphylium botryosum*

Efficacy of 6 fungicides and an antagonist (*Trichoderma harzianum*) against stemphylium blight of lentil caused by *Stemphylium botryosum* were evaluated in the field. Trade name, active ingredient (ai) and applied concentration is shown in the Table 1.

Table 1. List of fungicides with their trade name, active ingredient and applied concentration to the experimental field of lentil

Trade name of fungicides	Active ingredient (ai)	Concentration of applied product (%)
Iprosun 50WP	Iprodione	0.2
Edcuzeb 80WP	Mancozeb 80WP	0.2
Proud 25 EC	Propiconazole 25EC	0.2
Rovral 50WP	Iprodione	0.2
Emivit 50WP	Copper oxychloride 50WP	0.2
Agrimyl	Mancozeb + Metalaxyl	0.2

3.8.2. Duration of experiment

The research experiment was undertaken during the period from mid October, 2007 to April 2008.

3.8.3. Land Preparation

The land was well prepared mechanically in mid October, 2007. Unexpected plants and others material were removed from the experimental field. Fertilizers were applied during final land preparation as per recommendation.

3.8.4. Experimental design

The experiment was carried out in Randomized Block Design (RBD) having four replications in field condition and Completely Randomized Design (CRD) having 5 replications for each treatment in lab condition. The treatments were applied to the plots in a random selection in each block.

- Total area : 192 m^2
- No. of plot : 32
- Plot size : (2 × 3) m^2

3.8.5. Experimental treatments

Eight treatments including control were used in this experiment. The treatments are as follows-

- Iprosun (Iprodione)
- Edcuzeb 80WP (Mancozeb 80WP)
- Proud 25 EC ((Propiconazole 25EC))
- Rovral 50WP (Iprodione)
- Emivit 50WP (Copper oxychloride 50WP)
- Agrimyl (Mancozeb + Metalaxyl)
- Antagonist (*Trichoderma harzianum*)
- Control (Sterile water)

3.8.6. Sowing of lentil seeds

BARI Moshur-1 (Utfala) seeds were used in this experiment. The lentil seeds were sown in furrows maintaining 20 cm distance to another. The furrows were covered with the soil after sowing of seeds. The line to line distance also maintained as 20 cm with continuous sowing of seeds between the lines. The lentil seeds were sown in the afternoon on November 12, 2007.

3.8.7. Fungicides and antagonist application

The experimental plots were monitored regularly to notice any symptoms of stemphylium blight disease infection. Spraying of tested fungicides was applied when first disease was appeared. First disease was observed on 45 days after

sowing. At least three sprays were done at 10 days of interval. The experimental plots were sprayed through Knapsack sprayer as required volume. Spore suspension of 10^6 spore ml^{-1} was prepared by adding sterile water and sprayed into the concerned plot.

3.8.8. Intercultural operations

Intercultural practices were done to get the experimental field in hygienic condition for the crop growth of lentil with less competition. Weeding was done two times during the critical stage of lentil plant growth. Simply light irrigation was provided after each weeding and excess water was removed through well drainage system from the research plot to make safe the crop safe from stagnant water.

3.8.9. Data recording on disease score of Stemphylium blight

The disease score of stemphylium blight of lentil was recorded at 65 days after sowing of lentil. The plants were selected randomly and at least 10 plants were taken in each plot for recording the disease score following a rating scale (0-5 scale) and which were designated by Bakr and Ahmed, 1992. The rating scale is given bellow-

0 = No infection (HR)

1 = Few scattered leaf but no twig blighted (R)

2 = 5-10% leaflets infected and/or few scattered twig blighted (MR)

3 = 11-20% leaflets infected and/or 1% twig blighted (MS)

4 = 21-50% leaflets infected and/or 6-10% twig blighted (S)

5 = above 51% leaflets and/or more than 10% twig blighted (HS)

3.8.10. Data recording on yield and yield contributing parameters

Data were taken on-

i. Root length

ii. Shoot length

iii. Number of branches plant^{-1}

iv. Number of pods plant^{-1}

v. 1000 seed weight

vi. Yield kg ha^{-1}

3.8.11. Root length

Root length of lentil plant was measured in centimeter (cm) with a centimeter scale. Data were recorded as the average of 10 plants selected at random from the inner rows of each treated plot.

3.8.12. Shoot length

Shoot length of lentil plants was measured following same procedure as mention in root length.

3.8.13. Number of branches plant^{-1}

The number of branches plant^{-1} was counted from the average of 10 plants selected at random from the each plot.

3.8.14. Number of pods plant^{-1}

Ten plant selected unbiased from the inner rows of lentil plot and the number of pods plant^{-1} was calculated manually.

3.8.15. Weight of 1000 seed

Thousand seeds were counted by a seed counter and weight taken through digital balance (0.001 g).

3.8.16. Grain yield kg ha^{-1}

Grain yield of lentil kg ha^{-1} was calculated by converting the weight of plot yield into hectare and was expressed in kg.

3.9. Data analysis

All data were analyzed statistically using MSTAT-C computer package program. Treatment means were compared using Duncan's Multiple Range Test (DMRT) at 5% levels of significance.

CHAPTER 4
RESULTS

CHAPTER 4

RESULTS

4.1. Symptomology of stemphylium blight disease

The disease symptom usually appears on leaves and shoots. The characteristic feature of symptom was the formation of small pinhead gray spots on the leaflets, which rapidly spread over the shoots and twigs. In severe condition leaflets got blighted and plants become defoliated leaving a few green leaves and some immature pods. Sometimes the disease appeared with appearance of small pin headed light brown to tan colored spots on the leaflets, which later enlarge, covering the leaf surface within 2 to 3 days. The symptoms differed from other foliar lesions by being larger and spreading across or along the entire leaflet. A blighted dull yellow appearance is observed with infected foliage and branches. Defoliation occurs rapidly, leaving the branches with terminal leaves. The stems and branches also bend down, dry up and gradually turn ashy white, but the pods remain green. White mycelial growth also be observed on the infected stems.

4.2. Laboratory experiment

4.2.1. Cultural and morphological features of *S. botryosum*

Greenish brown colony color was observed in *S. botryosum.* Irregular shape of margin and velvety type texture was also found in culture plate. Conidia were brown in color and most of them are oblong round at the ends, muriform and constriction at the middle of the conidia, conidial surface is dotted. Length and breadth of conidia varied from 8-15µm and 3-8 µm, respectively. The mean length 10.48 µm and breadth 4.78 µm was observed. The conidiophores were brown in color and the terminal swollen. Conidia and conidiophores of *S. botryosum* are shown in Plate 1.

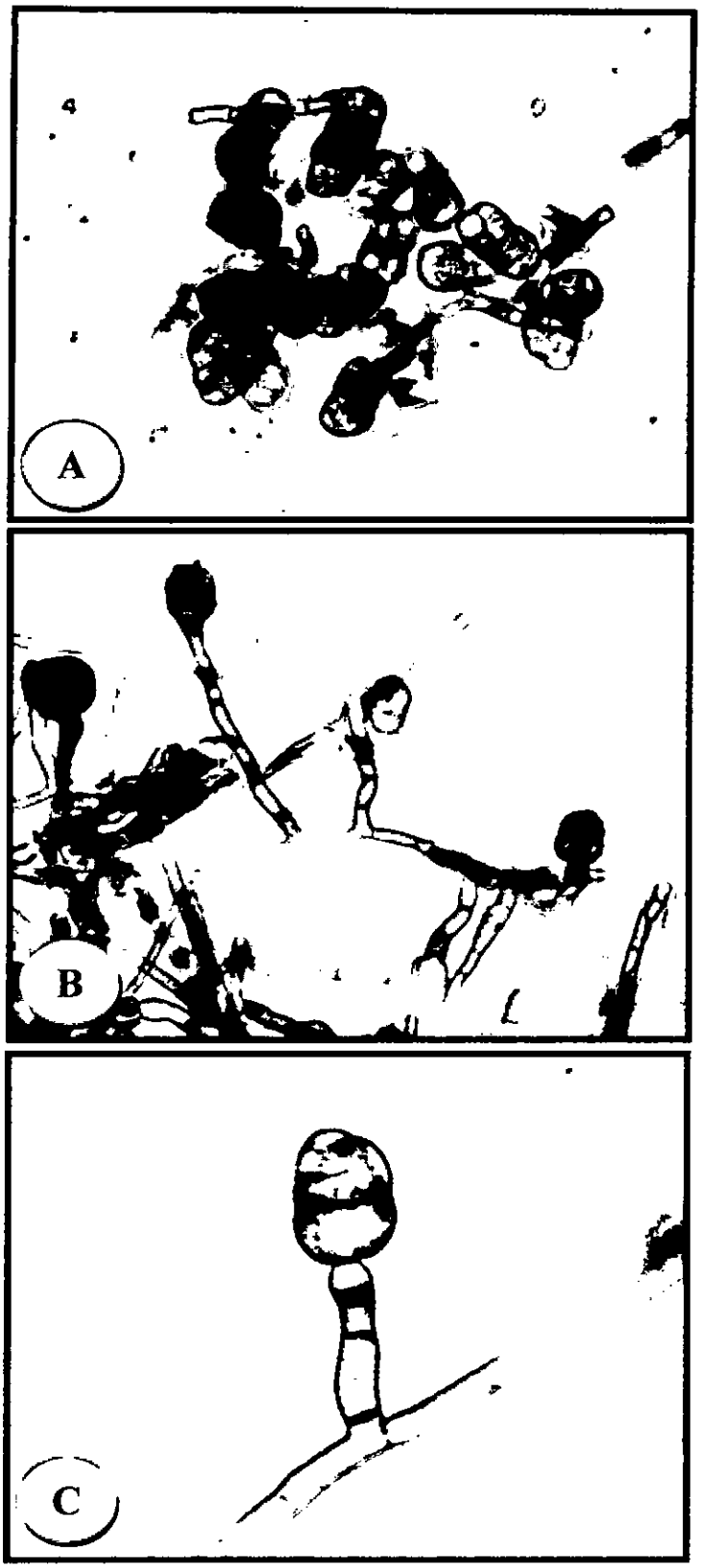

Plate 1. *Stemphylium botryosum.* (A) Conidia, (B) Conidia, on vesicular tip of conidiophores (C) Conidia develop singly on conidiophore

4.2.2. Electron microscopic study of *S. botryosum*

Single conidia, form on single conidiophore. New conidia borne by swelling the tip of conidiophore. Conidia polyspermic, it contain dotted structure on the whole surface and shown in Plate 2.

Plate 2 a: Single conidia form on a single conidiophore

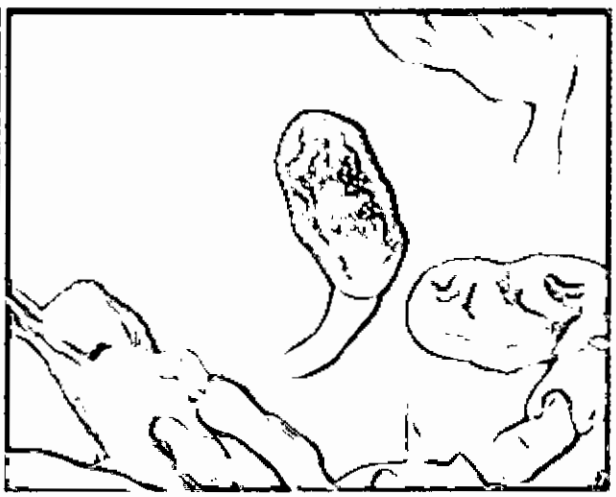

Plate 2 b: New conidia form by swollen the tip of the conidiophore

Plate 2c: Conidia polyspermic

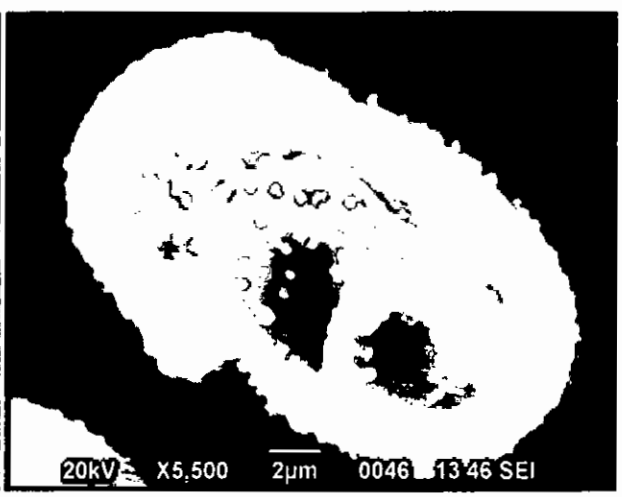

Plate 2d: Conidia surface is dotted

Plate 2. Scanning electron microscopic view of *S. botryosum*

4.2.3. Effect of physiological requirements

4.2.3.1. Effect of temperature (°C)

S. botryosum was grown in all the temperature level and ranging 5-35°C. The effect of temperature on radial mycelial growth of *Stemphylium botryosum* is presented in Table 2. The maximum radial mycelial growth was found at 25°C followed by 20°C which is statistically similar with 15°C and the lowest radial growth was found at 5°C preceded by 10°C. From this experiment it is clear that with the increasing of temperature level radial growth increased up to 25°C onward. So the suitable temperature for the fungal pathogen *S. botryosum* is 25°C.

4.2.3.2. Effect of pH

Results on effect of pH on the radial colony growth of *S. botryosum* are presented in Table 3 and Plate 3. The pathogen grew well at a wide range of pH. In the present research work excellent radial mycelia growth was observed at pH range 6.0 to 7.5. Though effect of pH 6.0 and 6.5 were statistically identical but the best growth (31.50 mm) was noted at pH 6.0 followed by 31.25 mm at pH 6.5. Among the pH level the lowest (16.50 mm) radial mycelia growth was recorded at pH 4.5 preceded by 18.00 mm at pH 5.0. From the test of different pH level against *S. botryosum* increasing trend of radial growth was observed up to pH 6.5 and the declined.

Table 2. Effect of temperature on radial mycelial growth of *Stemphylium botryosum*

Temperatures (°C)	Radial mycelial growth (mm)
5	10.7 e
10	18.0 d
15	33.8 bc
20	35.4 b
25	48.2 a
30	30.0 c
35	20.0 d
Lsd value (5%)	0.4674
CV (%)	11.26

Table 3. Effect of pH on radial mycelial growth and sporulation of *Stemphylium botryosum*

pH	Radial mycelial growth (mm)
4.5	16.50 e
5.0	18.00 de
5.5	19.00 d
6.0	31.50 a
6.5	31.25 a
7.0	27.50 b
7.5	25.00 c
Lsd value (5%)	0.1758
CV (%)	4.84

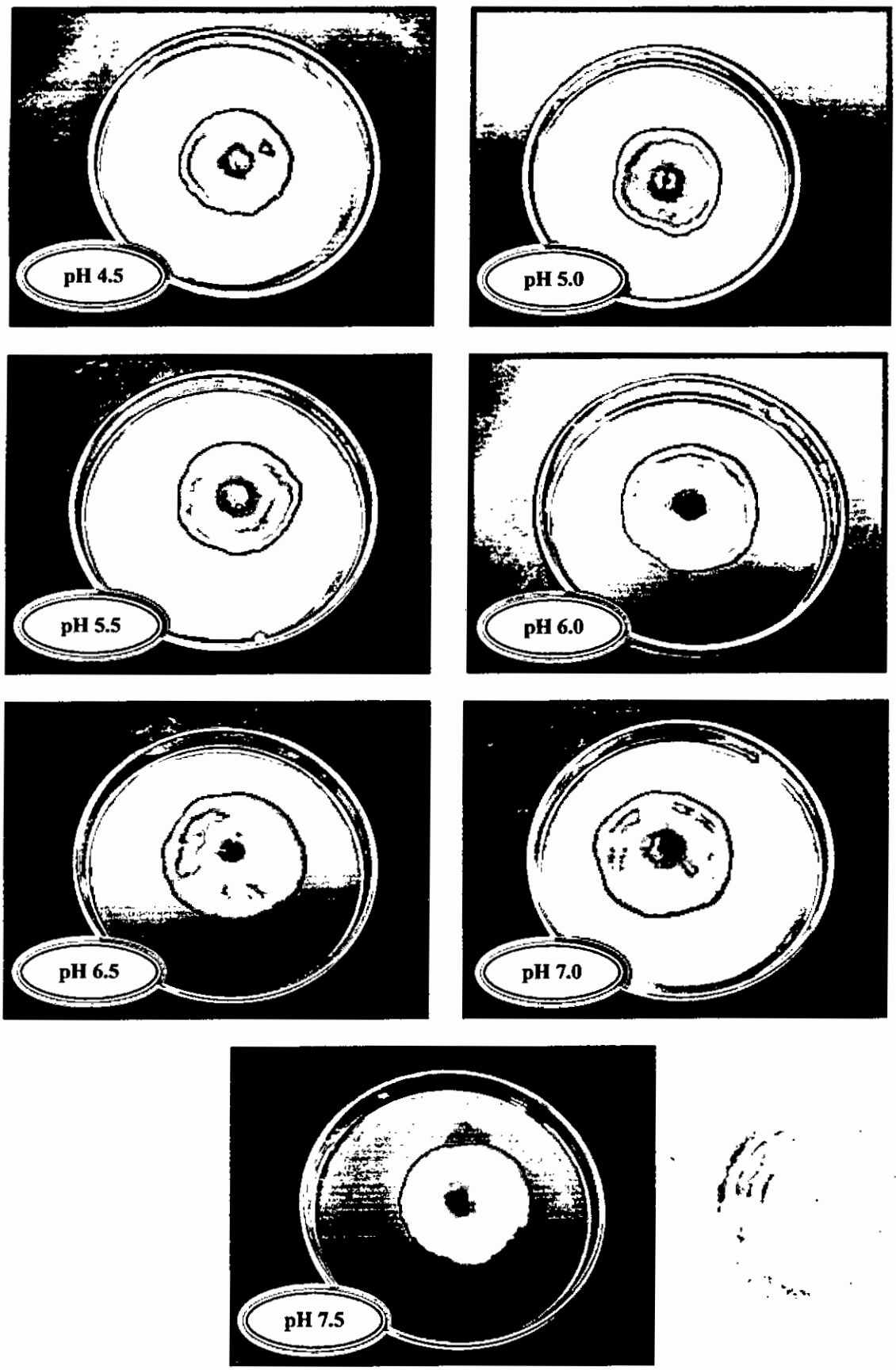

Plate 3: Effect of different pH levels on radial mycelial growth of *S. botryosum*

4.2.3.3. Effect of incubation period and temperature on conidia germination of *S. botryosum*

The conidia of *Stemphylium botryosum* germinated over a wide range of temperatures (5 to 35°C) and presented in Figure 1 and Plate 3. The conidia were polyspermic and produced several germ tubes depending on incubation period and temperature. Generally it was observed that per cent germination of conidia increased with the increased of temperature level from 5°C and incubation period from 5 hours. After 24 h of incubation maximum number of germinated conidia were observed for all the temperature levels. At least 12% conidia germinated after 2 h of incubation at 25°C whereas no germination was recorded at 5 and 10°C from 2 hours. The rate of germination of conidia increased with the temperature upto 30°C. However, the slowest and fasted germination response was observed at 5°C and 25°C. There were differences in number of germinated conidia after 4 h of incubation between 25°C (86%) and 30°C (19%) but after 6 h of incubation all conidia were germinated. The quickest 100% conidia germination was noted after 6 h of incubation at 25°C and 30°C. Although the number of germinated conidia differed at 4 h of incubation between 25°C and 30°C but there was no differences after 6 h of incubation between them. However it can be concluded that the optimum temperature for the germination of *Stemphylium botryosum* conidia is 25°C or close by rather than 30°C after 6 h of incubation period.

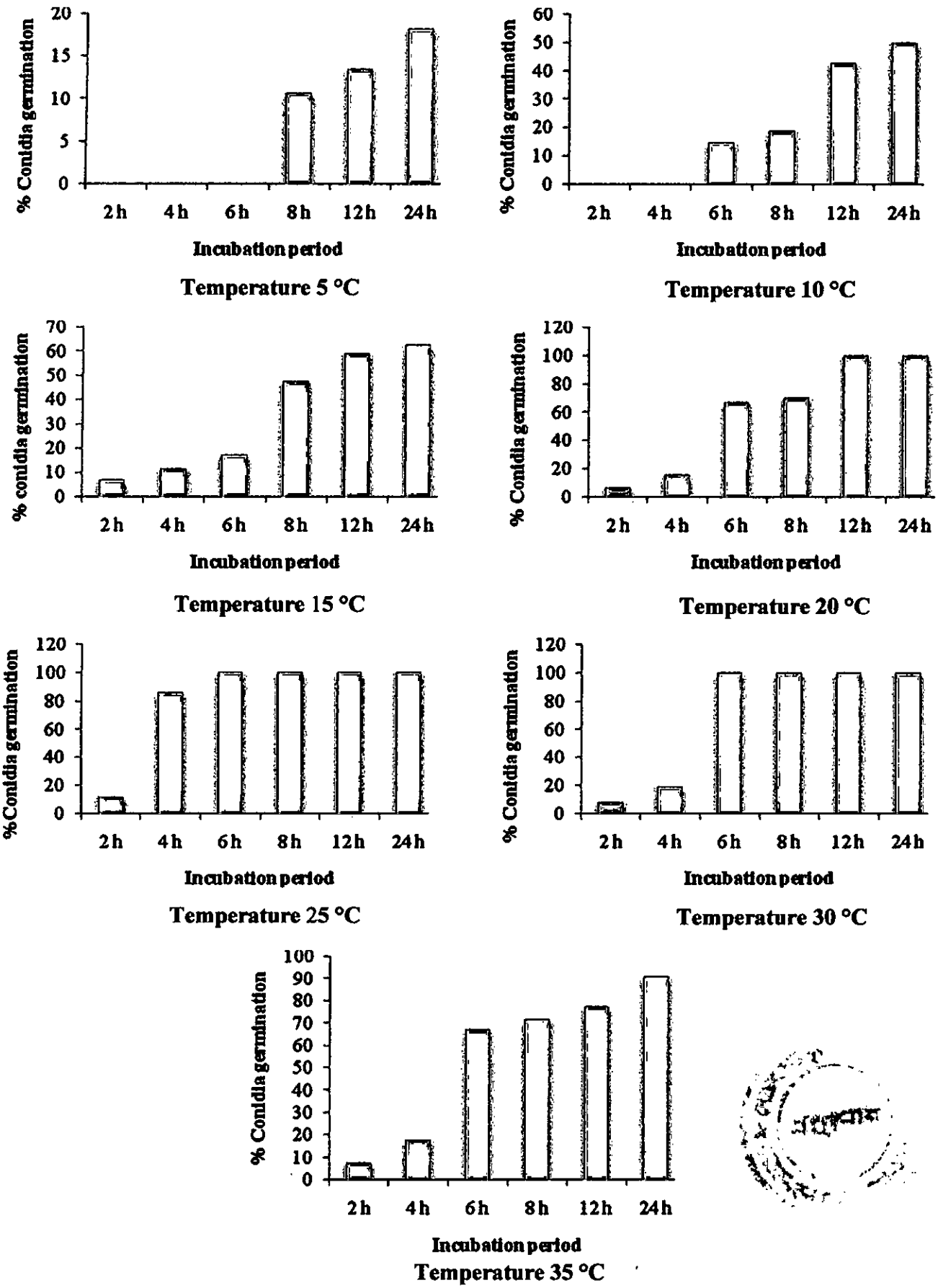

Figure 1: Effect of incubation period and temperature on conidia germination of *Stemphylium botryosum*

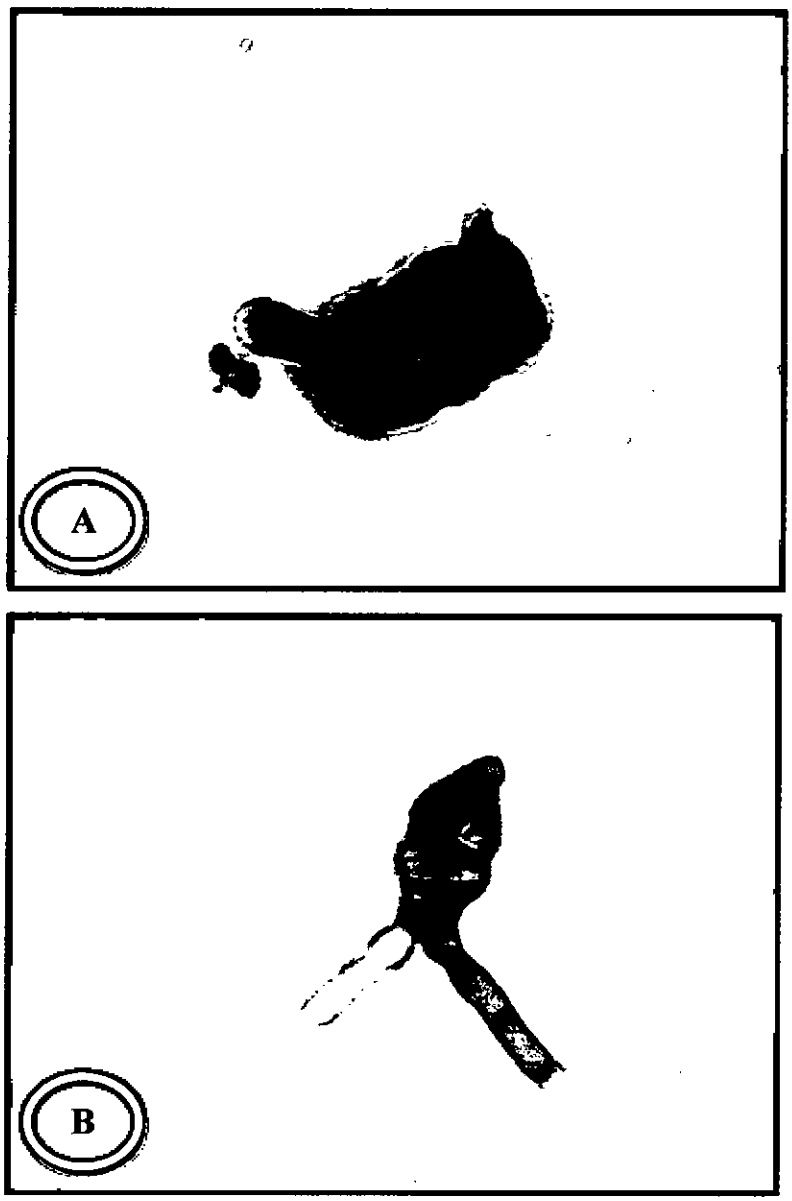

Plate 3: Germinating conidia of *Stemphylium botryosum*: (A) Germ tube initiation (B) Polyspermic germ tube

4.3. Bio assay of fungicides

All the fungicides inhibited radial mycelial growth significantly over the control at different concentrations and are shown in Table 4. All the fungicides and at lower concentration (500 ppm) inhibit the mycelial growth 100% except Agrimyl. The fungicides Agrimyl also inhibit the colony growth over control at higher concentration. The colony growth cumulatively decreases with the increases of Agrimyl concentrations. The highest colony growth (45.50 mm) was observed from the control preceded by Agrimyl (32.75 mm) at lower concentration (500 ppm). From the *in vitro* test of all fungicides showed the equal performance over control except Agrimyl.

Table 4. Bio assay of fungicides against radial mycelial growth and per cent inhibition of *Stemphylium botryosum*

Treatments	Concentrations (ppm)	Radial mycelial growth (mm)	Per cent (%) inhibition of radial mycelial growth
Iprosun 50WP (Iprodione)	500	0.0 (0.71) e	100
	1000	0.0 (0.71) e	100
	1500	0.0 (0.71) e	100
	2000	0.00 (0.71) e	100
Edcuzeb 80WP (Mancozeb)	500	0.0 (0.71) e	100
	1000	0.0 (0.71) e	100
	1500	0.0 (0.71) e	100
	2000	0.00 (0.71) e	100
Proud 25 EC (Propiconazole)	500	0.0 (0.71) e	100
	1000	0.0 (0.71) e	100
	1500	0.0 (0.71) e	100
	2000	0.00 (0.71) e	100

Table 4 (continued)

Treatments	Concentrations (ppm)	Radial mycelial growth (mm)	Per cent (%) inhibition of radial mycelial growth
Rovral 50WP (Iprodione)	500	0.00 (0.71) e	100
	1000	0.0 (0.71) e	100
	1500	0.0 (0.71) e	100
	2000	0.00 (0.71) e	100
Emivit 50WP (Copper oxychloride)	500	0.0 (0.71) e	100
	1000	0.0 (0.71) e	100
	1500	0.0 (0.71) e	100
	2000	0.00 (0.71) e	100
Agrimyl (Mancozeb+Metalaxyl)	500	32.75 b (5.72) b	28.02
	1000	23.50 (4.85) c	48.35
	1500	22.75 (4.77) c	50.00
	2000	16.75 (4.09) d	63.19
Control		45.50 (6.75) a	-
Lsd value (5%)			0.1807
CV (%)			3.05

Number having the same letters does not differ significantly at 5% level of significance according to DMRT
Figures within the parenthesis are square root transformed values

4.4. Field experiment

4.4.1. Fungicidal effect on yield attributes of lentil

All the tested fungicides reduced the disease score and significantly increased plant growth parameters and yield of lentil compared to control and are presented in Table 5 and 6.

4.4.2. Disease score

The lowest disease score was observed in plots sprayed with Iprosun 50WP followed by Edcuzeb 80WP indicating their higher disease reducing capability. There were not statistically different among the disease score in the plots treated with the other three fungicides of Proud 25EC, Rovral 50WP and Emivit 50WP, respectively. The other two treated plot spraying with Agrimyl and antagonist had no significant difference between them (Table 5).

4.4.3. Root length (cm)

The highest (9.48 cm) root length was produced in the plots treated with Iprosun and the lowest (4.44 cm) in control plot. Fungicides Rovral, Emivit, Agrimyl and *T. harzianum* had no significant different from each other but root length was significantly increased over control plot (Table 5).

4.4.4. Shoot length (cm)

Plot sprayed with Iprosun and Edcuzeb produced in the the highest shoot length 44.61 and 42.55 cm, respectively and the lowest in control and antagonist

treated plot, while shoot length in plots treated with Proud, Rovral and Emivit did not differ significantly from each other (Table 5).

4.4.5. Number of branches plant^{-1}

The number of branches plant^{-1} varied significantly due to application of fungicides over control. The highest number of branches plnat^{-1} was recorded in plot sprayed with Iprosun (9.25) followed by Edcuzeb (7.90) and Proud (6.75) and the lowest in control (3.00) preceded by antagonist sprayed (4.00) plot (Table 5).

4.4.6. Number of pod plant^{-1}

The number of pods plant^{-1} as influenced by the different fungicides application is presented in Table 5. The maximum pods plant^{-1} was obtained from the plot sprayed with Iprosun (39.10) followed by Edcuzeb (32.95) and Proud (29.60) but both were statistically identical. The minimum pods plant^{-1} was recorded from the control plot (12.75) preceded by antagonist (16.00) and these are significantly not differed from each other. The comparatively moderate number of pods plant^{-1} was obtained from the Rovral, Emivit and Agrimyl treated plots and all they were statistically similar.

Table 5. Effect of fungicides and antagonist on disease score and plant growth parameters of lentil

Treatments	Disease score	Root length (cm)	Shoot length (cm)	Number of branches plant^{-1}	Number of pod plant^{-1}
Iprosun 50WP	1.00 e	9.48 a	44.6 a	9.250 a	39.10 a
Edcuzeb 80WP	1.50 de	8.35 b	42.55 b	7.900 b	32.95 b
Proud 25 EC	1.75 cd	7.90 bc	40.55 c	6.750 c	29.60 b
Rovral 50WP	2.25 c	7.05 cd	40.22 cd	6.100 cd	23.20 c
Emivit 50WP	2.25 c	6.85 d	39.30 cd	5.250 de	19.90 c
Agrimyl	3.00 b	6.30 d	38.67 d	4.950 ef	19.60 c
*T. spore suspension	3.00 b	6.00 d	36.43 e	4.000 fg	16.00 d
T_8 = Control	4.75 a	4.44 e	36.15 e	3.30 g	12.75 d
Lsd value (5%)	0.6325	0.9930	1.603	1.036	3.546
CV (%)	17.62%	9.59%	2.74%	11.86%	9.99%

**Trichoderma* spore suspension (10^6 spore ml^{-1})

4.4.7. Thousand grain weight (g)

Thousand seed weight also influenced by the application of fungicides and weight was increased over control (Table 6). The fungicides Iprosun and Edcuzeb influenced equally on the thousand grain weight were 21.08 and 20.02 g, respectively. The lowest seed weight was obtained from control plot and the remaining plots treated with other fungicides and antagonist gave the statistically similar results.

4.4.8. Yield kg ha^{-1}

Tremendous effect of fungicides was noticed on the crop yield of lentil and yield was considerably increased compared to control (Table 6). The maximum grain yield (1271.00 kg ha^{-1}) was obtained from the plot treated with Iprosun and minimum from the control plot while yield was 666.20 kg ha^{-1}. The application of four fungicides Edcuzeb, Proud, Rovral and Emivit gave the statistically similar results.

Table 6. Effect of fungicides and antagonist on grain yield and yield attributes of lentil

Treatments	Thousand grain wt. (g)	Grain yield (kg ha^{-1})
Iprosun 50WP	21.08 a	1271.00 a
Edcuzeb 80WP	20.02 a	1108. 00 b
Proud 25 EC	17.77 b	1080. 00 b
Rovral 50WP	16.38 c	1028. 00 bc
Emivit 50WP	16.05 c	1018. 00 bc
Agrimyl	15.80 c	930.00 cd
Trichoderma spore suspension	15.15 cd	892.40 d
Control	14.23 d	666.20 e
Lsd value (5%)	1.149	98.98
CV (%)	4.58%	6.74%

CHAPTER 5
DISCUSSION

CHAPTER 5
DISCUSSION

In the present investigation stemphylium blight of lentil caused by *Stemphylium botryosum* showed typical symptoms on lentil plants. The pathogen *S. botryosum* was grown with greenish colony color, irregular shape and velvety texture on PDA medium. The dimension of conidia measured 10.48 × 4.78 µm. The present findings are well supported by Hosen *et al.*, (2009) who found that *Stemphylium botryosum* varied in their colony color, texture, margin, shape and also size of conidia on PDA medium while worked on four isolates (MIH -1 to MIH -4). They measured the conidia of *S. botryosum* as 13.33 – 16.04 × 6.46 – 9.17 µm.

Stemphylium botryosum was greatly influenced by physiological factors such as temperature (°C) and pH. The pathogen grew well at a wide range of temperature and pH. The highest radial colony growth was found at 25°C followed by 20°C and the lowest at 5°C. Radial growth increases upto 25°C and decreases after 25°C. The findings agreed with Montensions *et al.*, (1995) and Hosen *et al.*, (2009). They found that optimum temperature for the radial mycelial growth was 25°C.

Incubation time and temperature had a significant role in conidia germination. The conidia germinated with a wide range of temperature (5 to 35°C). After 4 h of incubation no germination was observed upto 6 h of incubation at

temperature 5°C and upto 4 h of incubation at 10°C whereas little germination was recorded in rest of temperatures upto 6 h of incubation. A maximum (85.71%) percentage germination of conidia was noted at 25°C after a short period (4 h) of incubation followed by 30°C. Hundred (100%) per cent germination of conidia germination was obtained from the temperature 25 and 30°C after 6 h of incubation. Mwakutuya (2006) was observed that the percentage of conidia germination increased with temperature and incubation period increased and noted that the maximum rate of germination were in 30°C followed by 25°C after 20 h of incubation and the impact of the rate of conidia germination increased as temperature above 15°C and generally the lowest and fated response was at 5°C and 20°C, respectively. From the results it is clearly noted that suitable temperature for germination of conidia of *S. botryosum* lies between 25-30°C.

The luxuriant radial growth was noted at pH 6.0 followed by pH 6.5. The lowest radial growth was recorded at pH 4.5. It was appeared that the higher range of pH is required for the radial mycelial growth of *Stemphylium botryosum*. Huq (2003) reported that the best growth was observed in pH 6.0 followed by pH 7.0 but Rajani (1991) found that optimum pH was 5.5 while working on *Stemphylium lycopersici*. Padhi and Synder (1954) reported that the optimum pH being 5.5 which gave the luxuriant mycelial dry weight and the maximum sporulation was recorded at pH 5.4.

From the *in vitro* test of fungicides radial mycelial growth inhibited significantly over the control. All the fungicides retarded radial colony growth of *Stemphylium botryosum* and no growth was observed at all concentration except Agrimyl. The maximum growth was noted in control plates followed by Agrimyl at lower concentration (500 ppm). Huq (2003) reported that Rovral 50WP was the most effective fungicides against *Stemphylium* spp. and no growth was recorded at higher concentration (2000 ppm). Hosen *et al.* (2009) evaluated six fungicides and found that Rovral 50WP from the iprodione group was the best fungicides in respect of reducing the radial colony growth of *S. botryosum* among the others even at a lower concentration (500 ppm).

Successfully management of the disease successfully achieved through application of chemical fungicides. All the tested fungicides reduced the disease score and remarkable increase of plant growth parameters and yield of lentil in comparison to control plot. The lowest disease score was counted in plots sprayed with Iprosun 50WP followed by Edcuzeb 80WP and the highest in control plot preceded by *Trichoderma harzianum* treated plot. Root length, shoot length, number of branches plnat^{-1}, and numbers of pod plant^{-1} were found maximum in the plot treated with Iprosun 50WP followed by Edcuzeb 80WP and Rovral 50WP. The highest grain yield was recorded from the Iprosun 50WP treated plot followed by Edcuzeb 80WP and both were statistically identical. The highest grain yield of lentil was recorded from the

Iprosun 50WP and the lowest in the untreated of with control plots. Bakr and Ahmed (1992) reported that disease score was the lowest in plots treated with Rovral 50WP @ 0.2% indicating of its highest disease reducing capability than the rest of three fungicides and they also found that plots sprayed with Rovral produced the highest seed yield. Sardar (2005) also reported that the lowest disease was obtained from the Rovral 80WP + Tilt 250EC treated plots. From the finding of several other researchers, Rovral 50WP was the most effective fungicides in reducing the disease score and increasing the yield of lentil. In the present research work, Iposan 50WP from the same group (iprodione) was most effective fungicides in controlling the disease severity increasing the seed yield of lentil.

CHAPTER 6

SUMMARY AND CONCLUSION

CHAPTER 6
SUMMARY AND CONCLUSION

Lentil is one of the most important sources of protein for human diet in every day dish and occupied the top position with consumer's preference in Bangladesh. *Stemphylium botryosum* causing stemphylium blight of lentil is considered the most damaging disease in Bangladesh. *Stemphylium botryosum* colony was greenish brown in color and velvety type texture. The conidia size was 10.48 x 4.78 µm. The pathogen grew well with a wide range of temperatures and pH. The maximum colony diameter was found at 25°C and pH 6.0. The lowest radial colony diameter was noted at 5°C and pH 4.5. The suitable incubation period and temperature and for the germination of conidia were 25°C and 6 h followed by 30°C. The fungicides were evaluated both *in vitro* and *in vivo* conditions against the pathogen *S. botryosum*. All the fungicides except Agrimyl appeared to be excellent fungicides in terms of inhibition of the radial colony growth of *Stemphylium botryosum* at a lower concentration (500 ppm). In lab condition all the fungicides gave equal result but it differed in field condition. However, Iprosun 50WP was the most effective fungicides in reducing the disease score and increasing the yield of lentil. The maximum (4.75) disease score was recorded in control plot and the lowest (1.00) in Iprosun treated plot. The highest (1271.00 kg ha^{-1}) yield of lentil was recorded when sprayed with Iprosun from the iprodione group and the lowest (666.20 kg ha^{-1}) in control plot.

The yield of lentil was enhanced sharply through the application of fungicides. The finding of present investigation revealed that Iprosun 50WP from the iprodione group was able to combat the stemphylium blight disease of lentil caused by *Stemphylium botryosum* and thereby increased the seed yield of lentil.

From the results it may be concluded that-

- *Stemphylium botryosum* colony showed greenish brown color and velvety texture on PDA medium
- The suitable temperature and pH for the radial mycelial growth of *Stemphylium botryosum* was 25°C and 6.0, respectively
- The optimum temperature and incubation period for the spore germination of *Stemphylium botryosum* was 25°C and 6 h, respectively
- The fungicides Iprosun 50WP from the iprodione group could be applied in the field to combat Stemphylium blight of lentil and to get the maximum yield of lentil
- Further studies for consecutive years need to be conducted to validate the technology in the farmers' field

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