

**SEARCH FOR RESISTANCE AND CHEMICAL CONTROL  
AGAINST STEMPHYLIUM BLIGHT DISEASE OF LENTIL**

**SHAH MD. ARIFUL ISLAM**



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

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**SEARCH FOR RESISTANCE AND CHEMICAL CONTROL  
AGAINST STEMPHYLIUM BLIGHT DISEASE OF LENTIL**

**BY**  
**SHAH MD. ARIFUL ISLAM**  
**Registration no. 13-05770**

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**Approved by:**

.....  
**Dr. Ashraf Uddin Ahmed**  
Principal 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)**

.....  
**Prof. Dr. F. M. Aminuzzaman**  
Chairman  
Examination Committee  
Department of Plant Pathology  
Sher-e-Bangla Agricultural University



**Plant Pathology Division**  
Bangladesh Agricultural Research Institute  
Joydebpur, Gazipur-1701, Bangladesh.

Ph: +880-2-9256405  
PABX: +880-2-9261501-06  
Fax: +880-2-9262713  
E-mail: csopath@bari.gov.bd

## Certificate

This is to certify that the thesis entitled "*SEARCH FOR RESISTANCE AND CHEMICAL CONTROL AGAINST STEM PHYLIUM BLIGHT DISEASE OF LENTIL*" 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 *Registration No. 13-05770*, 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 and study of the thesis have been approved and recommended for submission.

Dated: 25<sup>th</sup> May, 2015

Dhaka, Bangladesh

\_\_\_\_\_  
Dr. Ashraf Uddin Ahmed

Supervisor

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The Author

# **SEARCH FOR RESISTANCE AND CHEMICAL CONTROL AGAINST STEMPHYLIUM BLIGHT DISEASE OF LENTIL**

**BY**

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## **ABSTRACT**

Experiments were carried out to search for resistant source(s) and chemicals for controlling Stemphylium blight disease of lentil at Plant Pathology Division, Bangladesh Agriculture Research Institute, Joydebpur, Gazipur during the period of September 2013 to April 2014. The experimental design was RCB in field condition having three replications. Eleven lentil test entries along with 2 check variety BARI masur-1 and BARI masur-7 were evaluated. At maturity 4 lines showed Moderately Resistant (MR) and 7 lines showed Moderately Susceptible (MS) types of reaction. The line BLX-06004-12 gave the highest yield ( $1456 \text{ kg ha}^{-1}$ ) followed by BLX-06004-2 ( $1113.30 \text{ kg ha}^{-1}$ ) and BLX-05001-6 ( $1106.30 \text{ kg ha}^{-1}$ ) which were designated as moderately resistant to Stemphylium blight disease. The lowest yield ( $987.30 \text{ kg ha}^{-1}$ ) was recorded in BLX-05008-21 which was designated as moderately susceptible to Stemphylium blight disease. In case of chemical treatment, the minimum disease score (1.0) was recorded in Rovral 80 WP treated plot and the highest disease score (4.0) was counted in control plot. Among the six fungicides Rovral 80 WP from the iprodione group gave the best performance in respect of plant height (35.50 cm), number of branch per plant (8.33), number of pod per plant (36.67), number of seed per pod (1.98), thousand seed weight (18.50 g) and grain yield ( $1280 \text{ kg ha}^{-1}$ ).

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## ABBREVIATIONS AND ACRONYMS

ABBREVIATIONS	ACRONYMS
%	Percent
@	At the rate of
°C	Degree Celsius
ai	Active Ingredient
BARI	Bangladesh Agricultural Research Institute
CDC	Crop Development Centre
cm	Centimeter
DMRT	Duncan's Multiple Range Test
EC	Emulsifiable Concentration(s)
<i>et al.</i>	And Others
g	Gram(s)
h	Hour(s)
ha	Hectare(s)
HR	Highly Resistant
Kg	Kilogram(s)
M	Meter
mm	Millimeter
MR	Moderately Resistant
MS	Moderately Susceptible
ppm	Parts per million
R	Resistant
RCB	Randomized Complete Block
RH	Relative Humidity
S	Susceptible
<i>S. botryosum</i>	<i>Stemphylium botryosum</i>
<i>S. vesicarium</i>	<i>Stemphylium vesicarium</i>
t	Ton(s)
WG	Wettable Granule
WP	Wettable Powder

### CHAPTER 1

## INTRODUCTION

Lentil (*Lens culinaris* Medik.) is the most ancient cultivated crops among the legumes. Lentil is an important pulse crop in Bangladesh covering an area of 162 thousands hectares of land with an annual production of 211 thousands metric tons (Anonymous, 2012). Greater Faridpur, Jessore, Kushtia, Pabna and Rajshahi are the major lentil growing areas in Bangladesh. Lentil is the second most important pulse crop in terms of both area and production and rates of the highest consumers' preference. It contributes 25% of the total pulse production in the country (Anonymous, 2008). The production of the crop is decreasing every year due to several constraint factors such as low genetic potentials of the existing varieties, poor response to high input, susceptibility to disease, low acreage and delay sowing by the farmers (Anonymous, 1989). Among the factors susceptibility to disease is very important. As many as 15 pathogens causing 17 diseases of lentil crop have so far been recorded in Bangladesh (Ahmed, 1985). The major diseases affecting the crop are Stemphylium blight (*Stemphylium botryosum*), Rust (*Uromyces fabae* L.) and Foot rot (*Sclerotium rolfsii* Sacc.). Stemphylium blight of lentil was first recorded in Bangladesh by Bakr and Zahid (1986). Stemphylium blight of lentil is a defoliating fungal disease of lentil caused by *Stemphylium botryosum* and up to 62% yield losses have been reported in Bangladesh. The disease is a serious constraint in lentil cultivation and is widespread throughout the country with the highest severity in Jessore, Pabna, Kushtia, Faridpur, Madaripur and Dhaka (Bakr and Ahmed, 1992). In the recent years, the disease is also a threat to lentil cultivation in the southern parts of the country like Barisal and Bhola districts.

The disease is a serious concern not only in Bangladesh but also in northeast India and Nepal causing up to 100% yield losses under epidemic conditions. The climatic conditions in Bangladesh are favorable for the rapid development and growth of various plant pathogens. Thus, due to the importance of lentil diseases attention needs to be paid to combat these diseases. Among the plant disease management options, use of disease-resistant cultivars is considered as the most economical and best option for management of *Stemphylium* blight disease. Chemical management is a quick, easy and effective option against plant diseases.

In view of the above facts research works have been undertaken with the following objectives:

1. To screen out the resistant/tolerant source(s) of lentil germplasms against *Stemphylium* blight disease.
2. To find out effective fungicide(s) against *Stemphylium botryosum* causing *Stemphylium* blight disease in lentil.

## CHAPTER 2

### REVIEW OF LITERATURE

Lentil (*Lens culinaris* Medik) is one of the most important food legumes crops of Bangladesh. It ranks second in respect of acreage a production in the country. The most important diseases of lentil in Bangladesh are Stemphylium blight, rust and foot and root rot. Among them Stemphylium blight caused by *stemphylium botryosum* has created panic in the lentil growers as well as researchers in the country. Although some fungicides are available to manage the Stemphylium blight disease but it is necessary to develop alternate and more effective control measures with fungicides. Among the alternation means of disease management, development of resistant variety is the most widely preferred method. Relevant literature to the present study has been complied and presented below.

#### **2.1. The causal agent *Stemphylium spp.***

Stemphylium blight was not an age old disease of lentil which was first recorded during 1986 in Bangladesh (Bakr & Zahid 1986). Occurrence of the disease was also newly reported from Hungary (Simay, 1990). In Bangladesh the disease was widespread almost throughout the country but severity was highest in greater districts of Jessore, Kushtia, Faridpur, Dhaka and Pabna. Due to its increasing severity the disease has already gained much importance. The pathogen seems to be air-born. No report was available on its seed borne nature (externally or internally).

Bakr and Zahid (1986) observed during the cropping season 1986-87 the widespread occurrence of a new foliar disease on lentil. The diseases manifested if first appearance as



small pin-headed white spots on leaf-lets which enlarged rapidly covering the entire leaf surface within a few days. The foliage and twigs gradually turned dull yellow colour giving a blighted appearance of the affected crop. The infected leaves shed severely leaving only the terminal leaves on the twigs, the twig bended down dry up and gradually turn ashy white in colour. On careful observation white mycelial growth was seen in the infected twigs.

Sinha and Singh (1993) investigated the reason behind the periodic appearance, every 2-4 years of *Stemphylium* blight of lentil in Bihar, India. Surveys were conducted in several locations from December to February 1986-90. An average mean temperature of  $18\pm 2^{\circ}\text{C}$  and morning RH of 85-90% appeared to be favorable for the appearance, development and spread of the disease, while an afternoon RH >50% was essential. An average 7.7 hours of sunshine was favorable but >8 hours was unfavorable. The most important factor in determining the appearance and development of the disease was the number of cloudy and foggy days, which was between 30 and 40 days in the favorable years and between 17 and 23 days in unfavorable years.

Bakr (1993) reported that *Stemphylium* blight caused by *Stemphylium* sp. was becoming a serious threat to lentil cultivation in Bangladesh. Occurrence of the disease was recorded during 1981 (Anon., 1981) and was further confirmed by Bakr and Zahid in 1986. Since then the prevalence of the disease was being monitored in the farmer's field. Preliminary studies have indicated that the disease caused up to 62% yield reduction.

Mwakutuya *et al.* (2002) reported that the *Stemphylium* blight a defoliating fungal disease caused by *Stemphylium botryosum* was detected regularly in Saskatchewan lentil fields for many years, but was poorly understood. Up to 62% yield loss was reported from Bangladesh and eastern India. A research project was initiated to study the biology of the pathogen. Culture age and different light regimes did not effect on conidial germination.

High temperatures favoured the germination of *Stemphylium botryosum* and the optimum temperature for conidial germination was between 25°C and 30°C.

Suheri and Price (2000) studied the infection of onion by *Alternaria porri* and *Stemphylium vesicarium* under a range of controlled temperatures (4-25°C) and wetness periods (0-24 h). Conidia of *Alternaria porri* and *Stemphylium vesicarium* germinated within 2 h when incubated at 4°C. Terminal and intercalary appressoria were produced at similar frequencies at or above 10°C. The maximum number of appressoria was produced after 24 h at 25°C. Penetration of leaves by both pathogens was via the epidermis and stomata, but the frequency of stomatal penetration exceeded that of epidermal penetration. There was a strong correlation ( $R^2 > 90\%$ ) between appressorium formation and total penetrations at all temperatures. Infection of onion leaves occurred after 16 h of leaf wetness at 15°C and 8 h of leaf wetness at 10-25°C, and infection increased with increasing leaf wetness duration to 24 h at all temperatures. Interruption of a single or double leaf wetness period by a dry period of 4-24 h had little effect on lesion numbers. Conidia of *Alternaria porri* and *Stemphylium vesicarium* separately or in mixtures caused similar numbers of lesions. *Alternaria porri* and *Stemphylium vesicarium* are both potentially important pathogens in winter-grown Allium crops and purple leaf blotch symptoms were considered to be a complex caused by both pathogens.

Dar *et al.* (1992) isolated *Stemphylium botryosum* from dark brown lesions on *Phaseolus vulgaris* leaves in several fields in the Kashmir Valley during July 1990 and 1991. Pathogenicity was confirmed on this local cultivar in inoculation tests.

Kiffer and Morelet (2000) reported that *Stemphylium botryosum* is the anamorph of *Pleospora herbarum*, an ascospore teleomorph. *Stemphylium* is a ubiquitous, dematiaceous filamentous fungus that belongs to the kingdom Fungi, phylum Ascomycota, class Ascomycetes, order Pleosporales, family Pleosporaceae. The fungus is commonly

referred to using the anamorph. Species of *Stemphylium* that have been documented as plant pathogens include *S. botryosum*, *S. vesicarium*, *S. radicum* and *S. solani*. *Stemphylium botryosum* is the causal organism for Stemphylium blight on lentil.

Basallote *et al.* (1993) surveyed 125 garlic fields in southern Spain during 1989-91 and observed 31%, irrespective of cultivar, were affected by dark purple and white leaf spots indistinctly produced mainly on older leaves. *Stemphylium vesicarium*, were isolated from these spots and pathogenicity was confirmed. Pseudothecia of *Pleospora sp.* the teleomorph of *Stemphylium vesicarium*, were observed in debris of overwintered garlic leaves affected by leaf spots. Field observations during the survey indicated that outbreaks of garlic leaf spots are favored by foggy and rainy weather in spring, followed by warm days.

Everts and Armentrout (2001) observed leaf spot symptoms were on spinach (*Spinacia oleracea* cv. Seven R) at the University of Maryland. Leaf spot lesions were small (0.2-0.7 cm), circular, tan and papery and lacked visual signs of fungal infection. *Stemphylium botryosum* (*Pleospora herbarum*) was consistently reisolated from leaf spot lesions in plants tested in the laboratory and greenhouse trials. The pathogenicity test was repeated and recorded similar results.

a. Aveling *et al.* (1993) inoculated onion leaves with conidia of *Stemphylium botryosum*, conidial germination; appressoria formation and penetration of the leaf surface were studied. Several germ-tubes developed from each conidium and grew in any direction across the leaf surface. Terminal (29.7%) or intercalary (18.8%) appressoria were formed above epidermal cells. Of these appressoria, 72.3 and 47.7%, respectively, successfully penetrated epidermal cells. Single, terminal (35.9%) and double (12.5%) appressoria formed above stomata and 96.5% and 89.4% of these appressoria respectively, successfully penetrated the leaf via stomata. Occasionally compound appressoria were formed above stomata (3.1%) with 100% successful penetration. Penetration of the leaf surface, whether

directly through the epidermis or via stomata, only occurred after the formation of appressoria.

Bello *et al.* (1989) studies *Stemphylium loti* and 4 other isolates of *Stemphylium sp.* obtained from necrotic leaf spots on plants in pasture fields. In inoculation tests, symptoms produced by *Stemphylium loti* were similar to those found in the field, but those of the *Stemphylium sp.* were more severe than when occurring naturally.

Outbreaks of a disease on asparagus in growing regions of the German Federal Republic in 1986 and 1987 were reported (Leuprecht 1988). Pale elliptical lesions (2-5 mm long), which appeared and spread rapidly to all aerial parts of the plants under moist, warm weather conditions leading to yellowing, drying and loss of side branches. Conidia of *Stemphylium botryosum* were isolated from infected material.

## **2.2. Epidemiological aspects of the fungus**

Mwakutuya (2006) reported that the duration of the latent period increased with decreasing temperatures and decreasing wetness duration. *S. botryosum* required warm temperatures (above 25°C) and a minimum wetness period of 8 h for optimal disease development. Low levels of infection were observed within the first 2 h of incubation at 10°C and increased with longer wetting periods up to 48 h and temperatures up to 30°C. The pathogen could maintain infectivity during interrupted wetness periods despite its requirement for prolonged wetness periods. Infection levels were not significantly affected by interrupting dry periods of 6 to 24 h although long dry periods (24 h) combined with higher temperatures (30°C) resulted in a decrease in *Stemphylium* blight severity.

Cova and Rodriguez (2003) reported that the leaf blight of onion is one of the most important diseases of the crop in Lara State. To determine the pathogens causing the

disease and their relative importance, 22 leaf samples from 16 localities in four counties were analyzed through plant pathological standard techniques. Collected leaves showed visual symptoms of leaf blight. *Stemphylium botryosum* (*Pleospora herbarum*), *Stemphylium vesicarium*, and *Alternaria alternata* were isolated from 93.7, 12.5 and 50.0% of the samples, respectively. *Alternaria alternata* was always associated with *Stemphylium botryosum*. Pathogenicity tests with *Stemphylium botryosum* and *Alternaria alternata*, either individually or combined, reproduced disease symptoms similar to those observed in the field.

Huq and Khan (2008) reported among the atmospheric factors maximum and minimum temperature, relative humidity ranging from 20.5-29.0°C (mean 23.7°C), 5.4-13.3°C (mean 9.4 °C) and 78.0-84.5% (mean 81.0%), 48.0-63.0% (mean 55.5%) respectively are the predisposing factors for disease initiation. Whereas the maximum and minimum temperature, relative humidity ranging from 20.5-35.0°C and 13.0-21.0°C, 78.0 -100.0% and 48.0-80.2% respectively and rainfall 1.2-14.6 mm are favorable for disease development. The trends of disease development among the sowing dates were similar. The lowest and the highest severity were noted on November 01 and December 30 respectively.

Mwakutuya and Banniza (2010) reported an experiment *Stemphylium* blight of lentil (*Lens culinaris* subsp. *culinaris*) caused by *Stemphylium botryosum* has become more prevalent in the Canadian prairies. Germination of conidia, appressorium formation, and infection of lentil plants were evaluated under controlled conditions at temperatures from 5 to 30°C and increasing incubation periods under wet conditions to elucidate the epidemiology of this disease. On glass slides, conidial germination increased steadily with temperature up to 25 and 30°C, and reached more than 80% after 20 h at these temperatures, compared with around 30% at 5°C. The response of germination on glass slides to temperature was nonlinear, as evident in significant linear, quadratic single factor,

and linear, quadratic, and cubic cross factor temperature effects in the model. On lentil leaves, 18% of conidia had germinated after 2 h of incubation at 25°C, and a few germ tubes penetrated into the tissue through stomata. Germination reached 89% after 12 h, and 12% of germ tubes had penetrated into the leaves. *Stemphylium* blight severity reached more than 80% at 25 and 30°C with leaf wetness periods of 48 h. A simple logistic model with linear temperature, leaf wetness period, and cross factor effects described disease development on lentil plants.

*Stemphylium botryosum* was reported to cause internal infection on spinach seed. Based on the results of a component seed assay, the pathogen was detected on 54% of the pericarps and 29% of the embryos. The internal infection rendered seed treatments ineffective (Du Toit and Derie, 2004).

Prados-Ligero *et al.* (2003) reported that environmental factors have been found to affect the spread and release of ascospores of *Stemphylium spp.* Studies on *Stemphylium vesicarium* on onion revealed that rainfall was directly related to the spread of ascospores and conidia. Relative humidity played a role in the absence of rainfall.

Sinha and Singh (1993) reported that an average mean temperature of 18±2°C and RH of 85 to 90% in the morning were favorable for the appearance, development and spread of *Stemphylium* blight of lentil in India, whereas RH of >50% in the afternoon was essential.

Basallote-Ureba *et al.* (1999) reported that the effects of environmental conditions have been well investigated in studies of *Stemphylium vesicarium* which causes leaf spot in garlic, onion and asparagus. These studies concluded that temperature, leaf wetness and relative humidity (RH) are the most important environmental factors affecting the development of the disease.

*Stemphylium* is assumed to be a diurnal sporulator; it requires an alternating light and dark cycle for spore development. In total darkness, it produces only a few spores and sterile conidiophores are formed under constant light (Warner, 2005).

Cultural methods represent an effective and environmentally friendly way of disease control. Little research has been conducted to determine the effect of cultural methods on the incidence, severity and economic impact of *Stemphylium* blight on lentil. Cultural control methods, which have been employed to combat *Stemphylium spp.* in other hosts, include crop rotation, residue incorporation, choosing the best planting and harvesting dates; and the use of resistant varieties (Bayaa and Erskine, 1998; Schwartz and Gent, 2005; Shanmugasundaram, 2001).

Factors associated with resistance to *Stemphylium* blight were studied in lentil (Chowdhury *et al.*, 1997). The resistant varieties were found to have a thicker cuticle, thicker epidermal cell layer, thicker cortical layers, fewer stomata and a large number of epidermal hairs compared to the susceptible lines.

Hashemi *et al.* (2005) reported that *Stemphylium botryosum* colonies grow rapidly on a variety of media. They mature in 5 days at 25°C on potato dextrose agar. The use of mycelial suspensions in disease screening has been found to be as efficient and reliable as spore suspensions.

Earlier studies on *Stemphylium botryosum.sp.lycopersici* of tomatoes indicated that sporulation was optimal in continuous darkness, with the highest spore yield occurring when a 12 h light period was followed by a 12 h dark period (Bashi and Rotem, 1975).

Jakhar *et al.* (1996) reported that *S. vesicarium* required at least 16 h in a saturated atmosphere for initiation of disease development on onion. When moisture was available,

76% of *S. vesicarium*'s conidia germinated after 32h. The availability of long periods of moisture is important for infection of most *Stemphylium spp.*

### **2.3. Resistant cultivar**

Stemphylium blight, a damaging major disease of lentil, attacked the crop at any growing stage of damage depending upon how early the disease was appeared. Chemical control measure of this disease was to some extent costly and cumbersome. Growing of resistant cultivar was therefore, easy, cheap and environment friendly.

Sarker *et al.* (1992) reported that the 'Utfala' was the first improved *Lens culinaris* variety in Bangladesh. A selection among land varieties, it showed consistently higher yield over years across locations and exhibited yield potential of up to 3.45 ton/ha in favorable climatic conditions at Ishurdi during 1983-84. However, averaged over 21 trials from 1981-82 to 1986-87 at different agro-ecological zones, 'Utfala' yielded more than 1.3 ton/ha against a national average of 760 kg/ha. 'Utfala' was an early maturing semi-dwarf type of variety with good podding intensity. It was susceptible to rust and Stemphylium blight than the local check.

Sarker and Erskine (1998) reported that the BARI Musur-2 was derived the cross ILL4353 × ILL353 and was released in Bangladesh in 1993. It produced average seed yields of 1800 kg/ha compared to 1500 kg/ha for control variety 'Utfala'. It was also highly rust (*Uromyces viciae-fabae*) resistant. BARI Masur-4 was selected from the cross ILL5888 × FLIP84-112L in 1995 and produced an average seed yield of 2300 kg/ha. Barimasur-4 has an erect growth habit and was suitable for intercropping with sugarcane and mixed cropping with mustard. It had combined resistance to rust and Stemphylium blight (*Stemphylium botryosum*).



Beare (2002) reported that resistant varieties provide a more effective and more consistent method of control. However, preliminary screening of lentil varieties at the Crop Development Centre has indicated that Crimson and Eston have moderate resistance to *Stemphylium* blight while CDC Glamis and CDC Milestone were susceptible.

Rashid *et al.* (2009) screened and found that 21 entries viz. 10/P8406-122, FLIP-92-52LX, LR-9-135, LR-9-130, LR-9-179, LR-9-69, LR-9-69, LR-9-100, LR-9-118, LR-9-28, LR-9-25, ILL-4605 Procoz, LR-9-57, LR-9-107, LR-9-105, LR-9-48, LR-9-62, LR-9-25, 10/P11X955-135, 10/P2 FLIP-92-52LX955-167(4) and 10/P8405-23 were Resistant (R) to *Stemphylium* blight. The yield varied significantly from 656.25 to 1882.82 kg ha<sup>-1</sup>. The highest yield (1882.82 kg ha<sup>-1</sup>) was observed in BCX-980012-5 followed by BCX-98006-6 followed by BCX-98006-7 (757.82 kg ha<sup>-1</sup>) was recorded from BCX-98006-6 followed by BCX-98006-7 (757.82 kg ha<sup>-1</sup>).

Bisht and Thomas (1992) reported that out of 900 accessions of garlic germplasm under natural and artificial epiphytotic conditions, 39 and 18 were resistant to *Stemphylium vesicarium* and *Alternaria porri*, respectively, while 9 lines were resistant to both pathogens (IC-32320, -35286, -43398, -48157, -48875, -49415, -EC-158250, T84/13 & C-1525).

Podder (2012) reported that three experiments were conducted to evaluate disease resistance of germplasm accessions selected from seven *Lens* spp. and in intraspecific and interspecific RIL population. Growth chamber, greenhouse and field trials in Saskatoon and Bangladesh were conducted. Seventy accessions selected from all wild species of the *Lens* genus were screened for *Stemphylium* blight (SB) resistance. An F7-derived *Lens culinaris* intraspecific ('Eston' X PI 320937) RIL population (LR-39) with 96 lines along with checks was screened for SB resistance in the field at the University of Saskatchewan and at the field of Pulses Research Centre (PRC), Ishurdi, Bangladesh.

Pathak *et al.* (2001) screened five welsh onion (*Allium fistulosum*) and 106 *Allium cepa* lines in field and laboratory of Taiwan. They found all the *Allium fistulosum* lines as resistant or moderately resistant to *Stemphylium* blight (SLB), whereas all the *Allium cepa* lines were susceptible. Crosses were successfully made between five *Allium fistulosum* and 29 *Allium cepa* lines to introgress SLB resistance into onion lines. A total of 48 crosses thus produced and all F1 hybrids were resistant or moderately resistant to SLB.

Francovig *et al.* (1999) attempted to identify sources of resistance to *Stemphylium solani* in Brazilian cotton germplasm. A total of 14 single spore isolates of *Stemphylium solani* from different cultivars and locations in the state of Parana, were tested individually on 48 cultivars at the seedling stage under greenhouse conditions. Disease severity was rated 7 days after inoculation by measuring the percentage of leaf area infected (LAI). The reaction of 35 cultivars to a mixture of 5 aggressive isolates of *Stemphylium solani* was studied at the adult plant stage in 3 experiments conducted under controlled environmental conditions. At the seedling stage, significant differences between the degrees of resistance of some cultivars as well as between the aggressiveness of some *Stemphylium solani* isolates were observed. Isolate 11681 was the most aggressive. None of the cultivars was immune to all isolates of *Stemphylium solani*.

#### **2.4. Chemical control**

Bakr and Ahmed (1992) found Rovral 80 WP at 0.2% as effective foliar spray in controlling the *Stemphylium* blight disease of lentil. Plots sprayed with Rovral yielded 1506 kg/ha and harvest index was also (35.5%) in Rovral sprayed plots.

Huq and Khan (2007) reported in an experiment with seven different 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 % were tested in the field during 1998-2001 to control *Stemphylium* blight of lentil. Among the fungicides Rovral 50WP @ 0.2 % was noted as the most effective fungicide followed by Dithane M-45 @ 0.2 % and Tilt 250EC @ 0.05 %.

Gupta and Pandey (1986) observed that the *Stemphylium vesicarium* (Pleosporaallii) caused severe damage especially to the seed crop in Haryana and western Uttara Pradesh. Spraying with Dithane M-45 (mancozeb) at 2.5 g/litre + a sticker at fortnightly intervals from the beginning of February was recommended.

Grinestein *et al.* (1988) observed that the severe leaf spot disease on the flower stalks of seed onions in Israel was caused by *Stemphylium sp.* which attacked the seed stalk, weakening it and finally causing its collapse. Disease severity was reduced by 75%. The air-assisted placement spraying compared with 30% by cloud spraying. The results also indicated that the infection area was linearly proportional to the calculated seed yield.

Hossain (2012) conducted an experiment on among the treatments Tilt-250EC, Rovral, Rovral + Tilt-250EC performed better in respect of disease reduction and yield increase. The highest grain yield was observed in Tilt-250EC, which was statistically identical with Rovral and Rovral +Tilt-250EC.

Jong and Boshuizen (2004) carried out an investigation to control black leaf (*Stemphylium vesicarium*) of pear with different fungicides. Fungicides included Thiram, Score (difenoconazole) + Thiram, Flint (trifloxystrobin), and an unidentified fungicide from Bayer. Untreated trees showed 4.57% leaf infected, while preventive spraying with Flint+Thiram prevented infection.

Gupta and Srivastava (1988) studied 8 fungicides namely Copper Oxychloride, Mancozeb, Captafol, Thiram, Captan and Carboxin for the control of *Stemphylium vesicarium* in onions. Of the fungicides, 4 as sprays (Copper Oxychloride, Mancozeb, Carbendazim and Thiram) prevented the disease and all 8 fungicides applied as sprays after disease appearance cured the disease. The cost benefit ratio revealed that a preventive spray of 0.25% Mancozeb gave the highest net financial return.

Haque *et al.* (2013) conducted an experiment on Fungicides with different spray schedule showed a significant interaction in controlling *Stemphylium* blight and yield of lentil. Among the treatments lowest disease severity of *Stemphylium* blight was recorded with Rovral 50 WP at 7-days interval spray schedule at Gazipur and Ishurdi, followed by Secure-600 WG. But at Jessore Rovral and Nativo at 7 days and 15 days intervals spray showed the lowest disease severity and at 10 days interval Nativo again produced the lowest severity (2.68) but Rovral had the score of 3. So it appeared from the study that Rovral produced the lowest disease score at Ishurdi in all three spray schedule, Rovral and Secure were equally effective at Gazipur and Rovral and Nativo produced the lowest disease score at Jessore at 7 and 15 days interval but Nativo produced lowest disease score at 10 days interval followed by Rovral. So these chemicals may be used in different locations against *Stemphylium* blight at an interval of 7-10 days.

Ahmed *et al.* (2013) reported that the experiment was carried out to evaluate a bio-pesticide Keyunmycin against *Stemphylium* blight disease of lentil caused by *Stemphylium botryosum*. The investigation was designed in RCB with three replications. The minimum disease score (1.0) was recorded from Keyunmycin @ 2.25 ml L<sup>-1</sup> and Rovral treated plots and the highest (2.00) was found in control plot. Among the three doses of Keyunmycin higher (@ 2.25%) dose gave the best performance on number of pods plant<sup>-1</sup> (63.33) and grain yield (1174.33 kg ha<sup>-1</sup>).

Basallote *et al.* (1998) observed that Tebuconazole, Procymidone and Fosetyl sprays prior to artificial inoculation significantly reduced leaf spots in garlic caused by *Stemphylium vesicarium*. Results from field experiments in Spain indicated a good control of *Stemphylium* leaf spots when Tebuconazole or Procymidone (alone or alternated with Chlorothalonil) were applied at regular intervals during vegetative growth (total of 4-9 sprays) to garlic crops. A significant effect on garlic yield was observed in experiments conducted under environmental conditions conducive for disease development.

Rajani *et al.* (1992) tested 9 compounds against the pathogen *Stemphylium sp.* in vitro, Vivatex [carboxin] and Blitox [copper oxychloride] prevented mycelial growth even at the lower concentration (250 ppm) while carboxin, Dithane M-45 (mancozeb) and Captan inhibited germination.

Mehta and Oliveira (1998) reported that the effective results of 40% fenitrothion (200 g a.i./ha, 20% fenitrothion acetate (200 g a.i./ha), tebuconazole (250 g a.i./ha), difenconazole (37.5 g a.i./ha) + propiconazole (37.5 g a.i./ha) and mancozeb (2000 g a.i./ha) for the control of *Stemphylium solani* on cotton in Brazil.

Montenon and Vilardell (1992) reported that the influences of temperature on mycelial growth and also temperature and RH on germination of conidiophores were studied in 6 strains of *Stemphylium vesicarium* isolated from lesions on pear fruit grown in areas of Catalunya, Spain. Optimum temperatures were 15-25°C for mycelial growth and 20-30°C for germination of conidia.

## **2.5. Integrated management options**

Bakr and Ahmed (1993) conducted an experiment in an integrated management effort against *Stemphylium* blight (*Stemphylium sarciniformis* Cav.) of lentil. Fungicides, plant

genotypes and spacing were tested and found that disease severity was reduced significantly by the fungicide Rovral 80 WP and thereby increased seed yield considerably. Out of 110 genotypes tested, only one (L-80670) was found to be resistant to the pathogen and 11 genotypes were tolerant. Wider plant spacing did not reduce the disease severity significantly. Resistant genotype produced the highest seed yield (1157 kg/ha) followed by foliar spray of Rovral 80 WP to the same genotype at space-plated conditions (1106.3 kg/ha). The susceptible genotype (L- 81124) produced 934.8 kg/ha when Rovral 80 WP was sprayed three times. Integration of foliar spray with resistant genotype was found to be most effective for *Stemphylium* blight management.

Hussein *et al.* (2007) reported an experiment that *Bacillus subtilis*, *Pseudomonas fluorescens*, *Trichoderma harzianum*, *Gliocladium sp.* and *Saccharomyces cerevisiae* were evaluated for their efficacy in controlling *Stemphylium* blight on onion plants. *In vitro* study, the highest inhibition of *Stemphylium vesicarium* mycelia growth was achieved by *P. fluorescens*, *B. subtilis* and *T. harzianum*. In greenhouse experiment, application of Ridomil gold plus on onion plants resulted in significant reduction of disease severity percentage. The bioagents, i.e. *B. subtilis*, *Sacc.cerevisiae* and *P. fluorescens* exhibited the highest reduction in disease severity. On the other hand, *T. harzianum* gave the lowest reduction in the disease severity. All resistance inducers (i.e. Bion, K<sub>2</sub>HPO<sub>4</sub> and salicylic acid) treatments resulted in significant reduction in disease severity.

b. Aveling *et al.* (1993) tested Anilazine, benomyle, a Carbendazim/ Flusilazole mixture procymidone, tebuconazole and thiram for their efficacy in reducing the pathogen *Alternaria porri* and *Stemphylium vasicarium* of onion both on seed and in culture. An untreated control, hot water soaks (50°C for 20 min) and sodium hypochlorite treatments were also included for comparison. Treated seeds were rated for germination by the blotter method and by emergence and seedling growth in seedling trays in the greenhouse. None of

the treatments eradicated *Alternaria porri* and *Stemphylium vesicarium* from onion seeds. The hot water soak proved to be the best treatment for reducing these pathogens, although the percentages of germination and emergence of onion seeds were reduced compared with the control.

Zheng *et al.* (2010) reported an experiment Leaf blight caused by *Stemphylium solani* is a major fungal disease of garlic (*Allium sativum*) in central China. Garlic cultivars 'Qingganruanye', 'Ruanruanye' and 'Zixuan-2' were among the most resistant, but except for 'Zixuan-2', did not produce sufficient harvestable bolts as would be desirable for the local market. All fungicide treatments applied to cloves used as planting material seemed to promote seedling emergence, but significant effects ( $P = 0.05$ ) were observed only with fludioxonil ( $0.05 \text{ g kg}^{-1}$ ) and thiram ( $1.25 \text{ g kg}^{-1}$ ). Fungicide applications in the field were effective in controlling leaf blight and flusilazole ( $50 \text{ g ha}^{-1}$ ), flusilazole plus famoxadone ( $50 \text{ g plus } 104 \text{ g ha}^{-1}$ ) or mancozeb ( $350 \text{ g ha}^{-1}$ ) had the highest efficacy in reducing leaf blight severity.

Mishra and Gupta (2012) reported an experiment eight plant extracts, bio agents and fungicides were evaluated *in vitro* conditions against purple blotch and *Stemphylium* blight of onion caused by *Alternaria porri* and *Stemphylium vesicarium*. Among the plant extracts, clove extracts of *Allium sativum* at 10% resulted in maximum inhibition of growth (58.05 and 57.31%) of *A. porri* and *S. vesicarium*, respectively followed by Aloe vera at 10% (53.5 and 47.15%). Among the bioagents, *Trichoderma viride* was effective in inhibition of growth (53.17 and 56.15%). Out of eight fungicides evaluated, Mancozeb at 0.2% completely inhibited the growth of both the pathogens. Azoxystrobin (0.1%), propiconazole (0.1%) and antracal (0.2%) were the other effective fungicides.

Huq and Khan (2007) reported that lentils could escape the infection of Stemphylium blight by changing sowing dates. During the periods of studies the trends of disease development were similar. The lowest and the highest severity were noted on November 01 and December 30, respectively. The severity increased progressively with the advancement of sowing date from November 01 to December 30. In case of yield/ha November 01 gave the highest yield followed by November 10. In the sowing of November 01, 10 and 20 lentil could escape the infection of Stemphylium blight. The yield was maximum in the early sown seeds of lentil.



## **CHAPTER 3**

### **MATERIALS AND METHODS**

#### **3.1. Efficacy of fungicides against Stemphylium blight disease of lentil under field condition**

##### **3.1.1. Experimental site**

The experiment was conducted at the research field of Plant Pathology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur during September 2013 to April 2014.

##### **3.1.2. Soil type**

The experiment field was high land with soil of sandy loam texture belonging to the Madhupur tract under AEZ-28.

##### **3.1.3. Crop variety**

All varieties of lentil are not equally susceptible to the Stemphylium blight disease. Therefore, comparatively more susceptible variety BARI Masur-1 was used in this investigation.

##### **3.1.4. Land Preparation**

The experimental plot was nicely prepared mechanically in late October 2013. Weeds and other rubbishes were removed. Fertilizes were applied at the time of final land preparation as per recommendation.

##### **3.1.5. Experimental design**

The experiment was conducted in Randomized Complete Block (RCB) design with 3 replications. Each replication received 7 unit plots (3m X 2m). The space between the block was 1m and between the plots was 1m.

The treatments were assigned through a random selection of plots in each block.

### 3.1.6. Evaluation of fungicides

Altogether 6 fungicides were evaluated along with a control. The treatments were as follows:

**Table 1. Trade name, Chemical name and Concentration of the fungicides used against Stemphylium blight disease of lentil under field condition**

<b>Trade name of fungicides</b>	<b>Chemical name</b>	<b>Concentration of formulated product (%)</b>
Rovral 80WP	Iprodione	0.2
Secure 600 WG	Fenamidone+ Mancozeb	0.1
Nativo	Tebuconazole+Trifloxystobin	0.2
CM 75WP	Carbendazim+ Mancozeb	0.2
Dimox 35	Dimathomorph+Cymoxonil	0.05
Vita Flo 200 FF	Carboxin +Thiram	0.05
Control	-	-

### **3.1.7. Sowing of seeds**

A susceptible variety BARI Musur-1 (utfala) seeds were used in this experiment. Furrows were made with power tiller driven furrow making device maintaining a distance of 30 cm. The required amounts of seeds for each unit plot were sown in the furrow. The furrows were covered with soil soon after sowing. The line to line distance was maintained 30 cm with continuous sowing of seed in the lines. The seed were sown in the afternoon on November 21, 2013.

### **3.1.8. Application of the spray**

The experiment was monitored regularly to observe the on-set of Stemphylium blight disease from 55 days to 110 days. Spraying of fungicides was started when the disease appeared on 55 days. Altogether three sprays were applied at 10 days interval beginning from 55 days after sowing when the symptoms of the disease first appeared in the experimental plots. Spraying was done with the help of a Knapsack type sprayer and 3 liter of suspension of fungicide was used to spray in each unit plot.

### **3.1.9. Intercultural operation**

Intercultural operation was done in order to maintain the normal hygienic condition of crop growth. Weeding was done two times during

the growing period of the crop. One weeding was done at 20 days and another at 35 days after sowing. Light **irrigation** was provided after each weeding and excess water was drained out immediately to save the crop from stagnant water.

### **3.1.10. Data Recording on Stemphylium Blight Severity**

The severity of Stemphylium blight disease was recorded at 45, 60 and 75 days after sowing. The severity of Stemphylium blight of lentil was rated based on a (0-5) scoring scale described by Bakr *et al.*, 2000.

0-5 scoring scale:

- 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 1-5% twig blighted (MS),
- 4 = 21-50% leaflet infected and/or 6-10% twig blighted (S) and
- 5 = above 51% leaflet infected and/or more than 10% twig blighted (HS)

### **3.1.11. Data recording on yield and yield contributing parameters**

#### **3.1.12. Plant height:**

Ten plants of each unit plots were randomly selected at maximum growth stage for recording the data on plant height.

### **3.1.13. Number of branch plant<sup>-1</sup>:**

Ten plants of each unit plots were randomly selected at maximum growth stage for recording the data on number of branch per plant.

### **3.1.14. Number of pod plant<sup>-1</sup>:**

Ten plants of each unit plots were randomly selected at maturity stage for recording the data on total number of pods per plant.

### **3.1.15. Number of seed pod<sup>-1</sup>:**

Ten plants of each unit plots were randomly selected at maturity stage for recording the data on total number of seed per pod after harvest.

### **3.1.16. 1000-seed weight:**

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

### **3.1.17. Grain yield kg ha<sup>-1</sup> :**

Grain yield of lentil kg ha<sup>-1</sup> was calculated by converting the weight of plot yield into hectare and was expressed in kg.

### **3.1.18. Analysis of data:**

*The collected data were analyzed statistically. The experimental data were analyzed by MSTAT-C software. Mean comparisons for treatment parameters were compared using Duncan's Multiple Range Test (DMRT) at 5% level of significance.*

## **3.2. Screening of lentil varieties/lines resistant to Stemphylium blight**

### **3.2.1. Experimental site**

The experiment was conducted at the research field of Plant Pathology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur during September 2013 to April 2014.

### **3.2.2. Soil type**

The experimental field was high land with highly sandy loam texture belonging to the Madhupur tract under AEZ-28.

### **3.2.3. Collection of Seeds**

Seeds of 11 lentil lines and two check varieties were collected from Pulses Research Sub Station (PRSS), Gazipur for conducting this experiment (Table 2).

**Table 2. List of lentil entries collected from PRSS, BARI, Gazipur**

<b>Sl. No.</b>	<b>Entry name</b>
1.	BLX-05001-6
2.	BLX-05002-3
3.	BLX-05002-6
4.	BLX-05008-2
5.	BLX-05008-5
6.	BLX-05008-15

7.	BLX-05008-21
8.	BLX-05009-7
9.	BLX-06004-2
10.	BLX-06004-12
11.	FLIP-95-12
12	BARI masur-1 (Susceptible check)
13	BARI masur-7 (Resistant check)

#### **3.2.4. Preservation of Seeds**

The collected seeds received from PRSS were immediately stored in a well-ventilated room at room temperature. Special care was taken and the seeds were duly registered. After registration, seeds were preserved in a deep fridge in the Plant Pathology laboratory of BARI till they were used for field experiment.

#### **3.2.5. Preparation of working sample**

The seeds were divided into two parts. One portion was used for the field experiments and the other portion was preserved in the refrigerator for future uses.

### **3.2.6. Seed treatment**

To minimize the seed borne pathogen, seeds were treated with Provax-200 @ 2.5g/kg seed.

### **3.2.7. Land Preparation**

The experimental plot was nicely prepared mechanically in late October 2013. Weeds and other rubbishes were removed. Fertilizers were applied at the time of final land preparation as per recommendation. (FRG. 2012).

### **3.2.8. Experimental design and layout**

The experiment was conducted in Randomized Complete Block (RCB) design with three replications. The row length was 5m and width was 30 cm. The distance between the block was 1m. Susceptible check variety BARI masur-1 was sown after every two test lines. Resistant check variety BARI masur-7 was sown at the beginning and end of each block/replication.

### **3.2.9. Sowing of seeds**

Furrows were made with power tiller driven furrows maintaining a distance of 30 cm. The required amounts of seeds for each row were sown in the furrow. The furrows were covered with soil soon after sowing. The length of line was 5m with continuous



sowing of seed in the lines. The seed were sown in the morning on 21 November, 2013.

### **3.2.10. Intercultural operation**

Intercultural operation was done in order to maintain the normal hygienic condition of crop growth. Weeding was done three times during the growing period of the crop at 20, 35 and 50 days after sowing. Insecticide 'Karate' (0.2%) was applied for controlling pod borer and aphid of lentil.

### **3.2.11. Data Recording on Stemphylium Blight Severity**

The severity of Stemphylium blight disease was recorded at 45, 60 and 75 days after sowing. The severity of Stemphylium blight of lentil was rated based on a (0-5) scoring scale described by Bakr *et al.*, 2000.

0-5 scoring scale:

- 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 1-5% twig blighted (MS),
- 4 = 21-50% leaflet infected and/or 6-10% twig blighted (S) and
- 5 = above 51% leaflet infected and/or more than 10% twig blighted (HS)

### **3.2.12. Data recording on yield and yield contributing parameters**

### **3.2.13. Days to 1st Flowering:**

Days to 1st flowering was recorded in number of days after sowing when 1st flowering is open.

**3.2.14. Days to 50% Flowering (DFLR):**

DFLR was recorded in number of days after sowing when 50% plants in the row sets the first flower.

**3.2.15. Days to maturity (DMAT):**

DMAT was recorded in number of days after sowing when 90% of the row is ready for harvest.

**3.2.16. Plant height:**

Ten plants of each unit plots were randomly selected at maximum growth stage for recording the data on plant height.

**3.2.17. Number of branch plant<sup>-1</sup>:**

Ten plants of each unit plots were randomly selected at maximum growth stage for recording the data on number of branch per plant.

**3.2.18. Number of pod plant<sup>-1</sup>:**

Ten plants of each unit plots were randomly selected at maturity stage for recording the data on total number of pods per plant.

**3.2.19. Number of seed pod<sup>-1</sup>:**

Ten plants of each unit plots were randomly selected at maturity stage for recording the data on total number of seed per pod after harvest.

**3.2.20. 1000-seed weight:**

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

**3.2.21. Grain yield kg ha<sup>-1</sup>:**

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

### **3.2.22. Analysis of data:**

The collected data were analyzed statistically. The experimental data were analyzed by Statistix10.0 software at 5% level of significance. Treatment means were compared by DMRT.

## CHAPTER 4

### RESULT

#### **4.1. Efficacy of fungicides against Stemphylium blight disease of lentil under field condition**

##### **4.1.1. Fungicidal effect on disease severity and yield attributes of lentil**

All the tested fungicides reduced the disease and significantly increased plant growth parameters and yield of lentil compared to control (Table 3 and Table 4).

##### **4.1.2. Disease score**

The severity of Stemphylium blight disease was statistically different in respect of counting period and different treatments. The lowest disease score was observed in plots sprayed with Rovral 80 WP followed by Secure 600 WG indicating their higher disease reducing capability. The highest disease score was recorded in control plot (Table 3).

##### **4.1.3. Plant height**

The plant height differed significantly among the treatments due to the application of fungicides (Table 3). Plot sprayed with Rovral, Secure and Nativo produced the highest plant height 35.50 cm, 34.40 cm and 34.00 cm, respectively and the lowest plant height was in control plot (22.70 cm).

#### **4.1.4. Number of branches plant<sup>-1</sup>**

The number of branches per plant varied significantly due to application of fungicides over control. The highest number of branches per plant was recorded in the plot sprayed with Rovral (8.33) followed by Secure (7.33) and Nativo (7.00) and the lowest branches were recorded in control (2.67) preceded by Dimox (5.00) (Table 3).

#### **4.1.5. Number of pod plant<sup>-1</sup>**

The maximum number of pod per plant was obtained from the plot sprayed with **Rovral (36.67)** followed by **Secure (34.33)** and these are significantly differed from each other (**Table 3**). The minimum pod per plant was recorded from the control plot (25.00) preceded by **Dimox (29.33)**. The comparatively moderate number of pod plant<sup>-1</sup> was obtained from the **Nativo, CM and Vita Flo** treated plots.

**Table 3: Performance of fungicides in controlling Stemphylium blight disease and plant growth parameters of lentil**

Means denoted by same letter does not differ significantly.

<b>Treatments</b>	<b>Disease score (0-5 scale)</b>	<b>Plant height (cm)</b>	<b>Number of branches plant<sup>-1</sup></b>	<b>Number of pod plant<sup>-1</sup></b>
<b>Rovral 80WP</b>	1.00 f	35.50 a	8.33 a	36.67 a
CM 75WP	2.25 c	30.00 c	5.33 c	31.67 cd
Nativo	1.75 d	34.00 b	7.00 b	33.33 bc
Dimox 35	2.75 b	27.60 d	5.00 c	29.33 e
Vita Flo 200 FF	2.25 c	28.20 d	5.33 c	30.33 de
Secure 600 WG	1.50 e	34.40 b	7.33 b	34.33 b
Control	4.00 a	22.70 e	2.67 d	25.00 f
<b>LSD (5%)</b>	0.2468	0.8465	0.8980	2.194
<b>CV (%)</b>	6.50	1.64	9.00	4.09



#### **4.1.6. Number of seed pod<sup>-1</sup>**

Number of seed per pod ranged from 1.40 to 1.98 and all the treatments differed significantly (Table 4). The highest number of seed per pod was recorded in **Rovral (1.98)** followed by **Secure (1.83)** treated plot and the lowest in control plot (1.40).

#### **4.1.7. 1000-seed weight (g)**

Thousand seed weight also influenced by the application of fungicides and weight was increased over control (Table 4). The fungicides **Rovral (18.5 g)** and **Secure (18.0 g)** influenced equally on the thousand grain weight. The lowest thousand seed weight was recorded from control plot (12.80 g).

The remaining plots treated with other fungicides also gave the statistically different weight.

#### **4.1.8. Grain yield kg ha<sup>-1</sup>**

Remarkable effect of fungicides was noticed on the grain yield of lentil and yield was increased considerably compared to control (Table 4). Among the seven fungicides Rovral sprayed plot produced highest grain yield ( $1280 \text{ kg ha}^{-1}$ ) followed by Secure ( $1110 \text{ kg ha}^{-1}$ ) and Nativo ( $1100 \text{ kg ha}^{-1}$ ) and the lowest grain yield was obtained from control plot ( $700 \text{ kg ha}^{-1}$ ). The application of three fungicides CM, Vita Flo and Dimox also produced significantly higher yield over control.

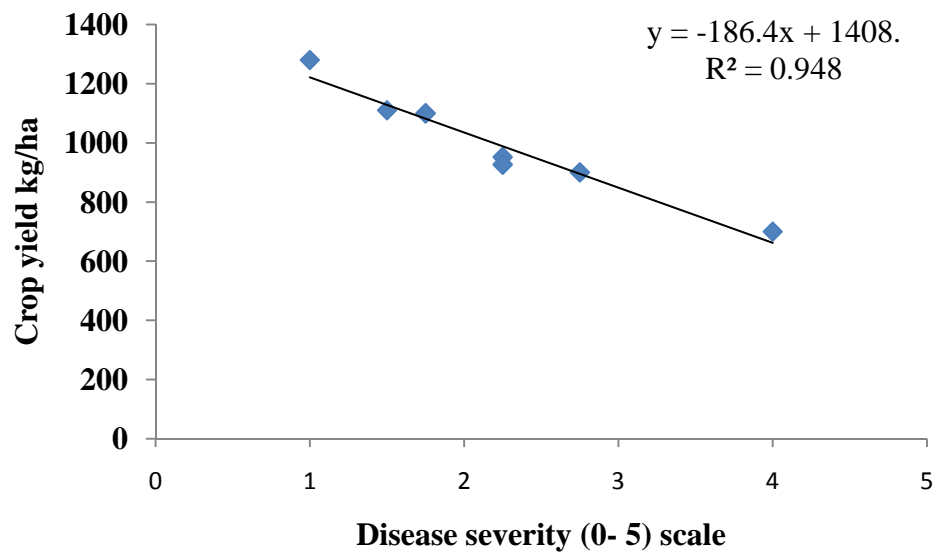
**Table 4: Performance of fungicides in controlling *Stemphylium* blight on grain yield and yield attributes of lentil**

Treatments	Number of seed/ pod	1000 seed weight (g)	Grain yield ( kg/ha )
<b>Rovral 80WP</b>	1.98 a	18.50 a	1280.00 a
CM 75WP	1.63 bcd	15.73 c	951.70 c
Nativo	1.80 abc	17.47 b	1100.00 b
Dimox 35	1.57 cd	14.43 e	900.00 e
Vita Flo 200 FF	1.60 bcd	15.10 d	926.70 d
Secure 600 WG	1.83 ab	18.00 a	1110.00 b
Control	1.40 d	12.80 f	700.00 f
<b>LSD (5%)</b>	0.2409	0.5250	20.79
<b>CV (%)</b>	8.40	1.93	1.23

Means denoted by same letter does not differ significantly.

#### **4.1.9. Relationship between disease severity and grain yield**

The crop yield of lentil was found to have significant negative correlation ( $r = -0.974$ ) with the severity of Stemphylium blight disease caused by *Stemphylium botryosum*. The predicted linear regression line was also displayed downward slope i.e.  $y = -186.4x + 1408$ , where 'y' denoted predicted crop yield of lentil and 'x' stood for disease severity of Stemphylium blight of the crop (Figure 1). The estimated regression line indicated that the unit rise in the severity of Stemphylium blight disease (within 0-5 scale) there existed possibilities of yield reduction by 186.4 kg/ha. The regression equation indicated the necessity of controlling Stemphylium blight of lentil.



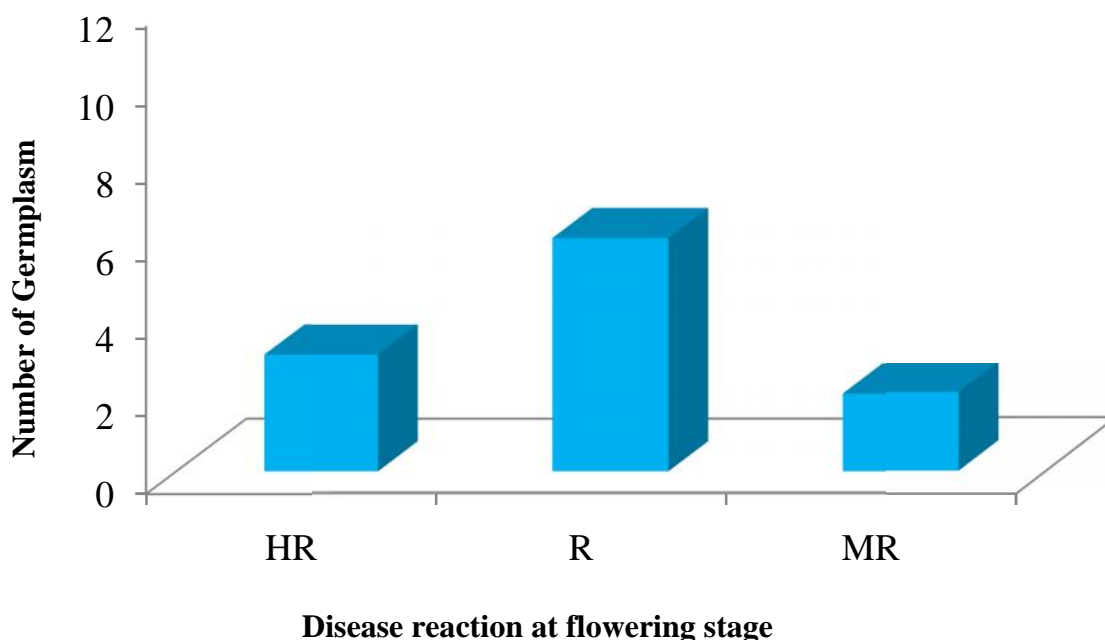
**Figure 1.** The relationship between crop yield (y) and disease severity (x) of *Stemphylium* blight of lentil caused by *Stemphylium botryosum*.

## 4.2. Screening of lentil varieties/lines resistant to *Stemphylium* blight

### 4.2.1. Disease Reaction of *Stemphylium* Blight of lentil during rabi season of 2013- 2014

#### 4.2.1.1. Disease reaction of flowering stage

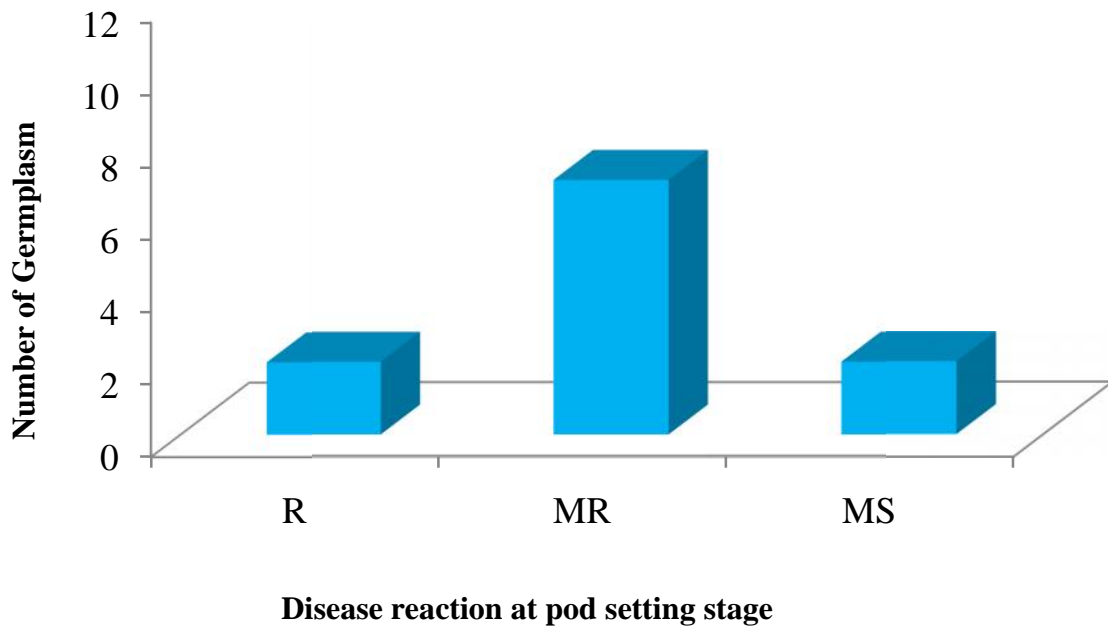
The lentil lines were evaluated for their reaction to *Stemphylium* blight under natural epiphytotic condition during the winter season of 2013-14 and showed significant difference in reaction to *Stemphylium botryosum*. In flowering stage i.e. at 45 days after sowing out of 11 lines 3, 6 and 2 lines showed Highly Resistant (HR), Resistant (R) and Moderately Resistant (MR) reaction, respectively. The 3 highly resistance lines were BLX-05002-3, BLX-06004-12 and BLX-06004-2 in the flowering stage (Figure 2).



**Figure 2.** Reaction on *Stemphylium* blight disease of 11 lentil lines at flowering stage

#### 4.2.1.2. Disease reaction at Pod setting stage

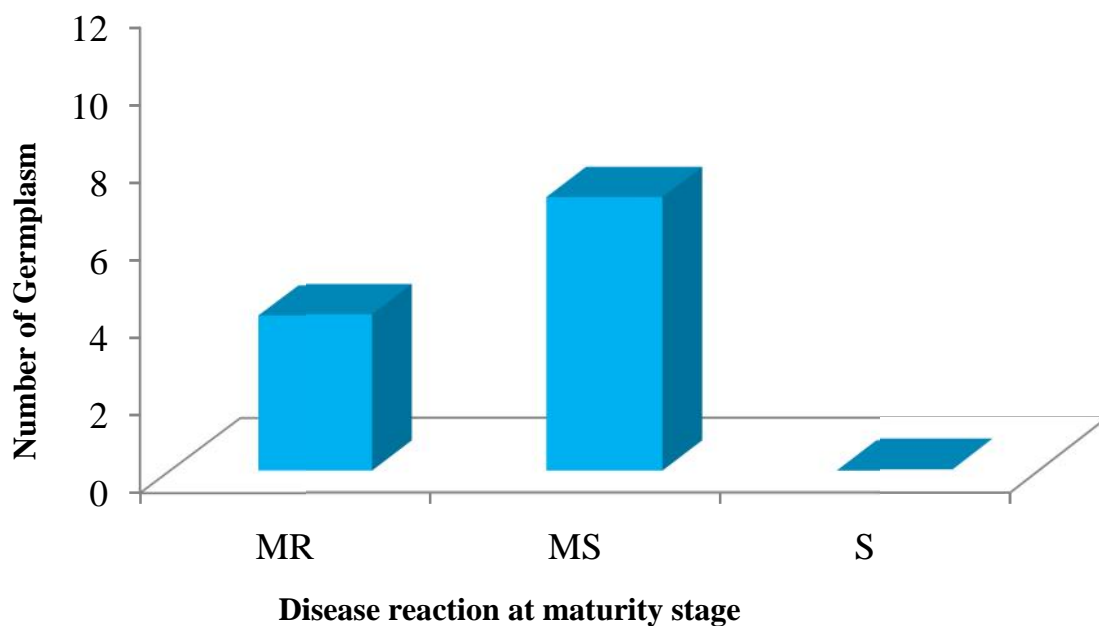
The lentil lines showed wide variation in reaction to *S. botryosum* at pod formation stage. Out of 11 lines 2, 7 and 2 lines showed Resistant (R), Moderately Resistant (MR) and Moderately Susceptible (MS) reaction, respectively in the pod setting stage (Figure 3). The resistant 2 lines were BLX-06004-12 and BLX-06004-2. The data was taken 60 days after sowing.



**Figure 3.** Reaction on Stemphylium blight disease of 11 lentil lines at pod setting stage

#### 4.2.1.3. Disease reaction at Maturity stage

At maturity stage, 4 lines showed Moderately Resistant (MR) and 7 lines showed Moderately Susceptible (MS) type of reaction (Figure 4). None of the 11 entries showed either resistant or susceptible reaction against the disease. The Moderately Resistant (MR) lines were BLX-06004-12, BLX-06004-2, BLX-05002-3 and BLX-05001-6. The data was taken 75 days after sowing.



**Figure 4.** Reaction on Stemphylium blight disease of 11 lentil lines at maturity stage



## **4.2.2. Performance of test lines/varieties in yield and yield contributing characteristics during Rabi season of 2013-14**

### **4.2.2.1. Days to 1st Flowering**

There was no variation regarding disease reaction at 1st flowering among different lentil lines/varieties. Days to the 1st flowering ranged from 49.93 days to 47.77 days. It was observed that BARI masur-7 started flowering 2 days later than the other lines. The highest (49.33) and the lowest (47.77) days to start flowering were recorded in BARI masur-7 and BLX-05001-6 line (Table 5).

### **4.2.2.2. Days to 50% Flowering (DFLR)**

The Days to 50% Flowering (DFLR) was recorded in 11 lines and two check varieties and is presented in Table 5. It showed variation among the lines/varieties. It ranged from 55.63 to 53.17 while the highest was recorded in BLX-05008-5 and the lowest was recorded in BARI masur-1.

### **4.2.2.3. Days to maturity (DMAT)**

The days to maturity of all the test and check lines/varieties ranged from 99.03-95.97 (Table 5). The highest days of maturity was observed in FLIP-95-12 followed by BLX-05002-3 (98.37), BLX-05001-6(97.70) and the lowest (95.97) in BLX-05009-7 followed by BARI masur-7 (96.40), BLX-05008-21(96.40), BARI masur-1(96.53).

**Table 5. Days to 1st flowering, 50% flowering and maturity of 11 selected lentil germplasms and two check varieties**

Sl. No.	Name of lines/varieties	Days to 1 <sup>st</sup> Flowering	Days to 50% Flowering	Days to Maturity
1	FLIP-95-12	48.80 ab	55.07 ab	99.03 a
2	BLX-05002-3	47.80 b	55.10 ab	98.37 ab
3	BLX-06004-12	47.93 b	54.87 ab	96.63 bc
4	BLX-05009-7	49.20 ab	54.83 ab	95.97 c
5	BLX-06004-2	48.10 b	54.33 bc	97.03 abc
6	BLX-05001-6	47.77 b	54.60 ab	97.70 abc
7	BLX-05002-6	48.30 ab	55.10 ab	97.60 abc
8	BLX-05008-21	47.83 b	54.57 ab	96.40 bc
9	BLX-05008-15	48.10 b	54.80 ab	97.07 abc
10	BLX-05008-2	47.97 b	55.03 ab	97.33 abc
11	BLX-05008-5	49.37 ab	55.63 a	97.10 abc
12	BARI masur-1	48.54 ab	53.17 c	96.53 bc
13	BARI masur-7	49.93 a	53.87 bc	96.40 bc
	CV (%)	2.03	1.37	1.34
	LSD (0.05)	1.65	1.25	2.20

Means denoted by same letter does not differ significantly.

#### **4.2.2.4. Plant height (cm)**

Plant height was taken as average height of 10 plants from plot at maximum growth of plant. This data was recorded at maturity stage of plant. The tested 11 lines and two check varieties showed significant difference to each other (Table 6) in the field condition. The plant height ranged from 33.40 cm to 43.40 cm while the tallest plant (43.40 cm) was found in BLX-06004-12 line and the shortest plant (33.40 cm) was recorded in BLX-06004-2 line. Plants in some of the lines were taller than the check variety.

#### **4.2.2.5. Number of branch plant<sup>-1</sup>**

Number of branch per plant was counted as the primary branch of plant that is the first branching of the plant. It was found that most of the lines/varieties gave two primary branches yet only a few lines gave about three primary branches. The highest (2.98) Number of branch per plant was observed in BLX-06004-12 and lowest (2.53) in BLX-05008-5 (Table 6).

#### **4.2.2.6. Number of pod plant<sup>-1</sup>**

Number of pod per plant was recorded after harvesting of plants. Pods were counted from ten (10) plants in every lines/varieties and showed significant difference from one to others (Table 6). Number of pod ranged from 58.23 to 86.57 while minimum number of pod was recorded in BLX-05008-21(58.23) followed by BLX-05009-7(58.33) and maximum number of pod was recorded in BLX-06004-12(86.57) followed by BARI masur-7(85.37).

**Table 6. Plant height, Number of branch plant<sup>-1</sup> and Number of pod plant<sup>-1</sup> of selected 11 lentil germplasms and two check varieties**

Sl. No.	Name of lines/varieties	Plant height (cm)	No. of branch plant <sup>-1</sup>	No. of pod plant <sup>-1</sup>
1	FLIP-95-12	37.80 c	2.77 abc	68.47 b
2	BLX-05002-3	35.63 def	2.63 bcd	64.07 c
3	BLX-06004-12	43.40 a	2.98 a	86.57 a
4	BLX-05009-7	34.10 gh	2.57 cd	58.33 e
5	BLX-06004-2	33.40 h	2.60 bcd	58.53 de
6	BLX-05001-6	35.30 efg	2.63 bcd	59.87 de
7	BLX-05002-6	34.90 fg	2.80 ab	69.10 b
8	BLX-05008-21	36.93 cd	2.60 bcd	58.23 e
9	BLX-05008-15	35.00 fg	2.57 cd	59.60 de
10	BLX-05008-2	36.63 cde	2.57 cb	61.27 cd
11	BLX-05008-5	34.30 fgh	2.53 d	59.03 de
12	BARI masur-1	34.57 fgh	2.67 bcd	71.30 b
13	BARI masur-7	39.87 b	2.93 a	85.37 a
CV (%)		2.43	5.05	2.55
LSD (0.05)		1.49	0.23	2.84

Means denoted by same letter does not differ significantly.

#### **4.2.2.7. Number of seed pod<sup>-1</sup>**

The number of seed per pod was recorded after harvesting of the plant by counting seed in each pod from ten (10) plants in every lines/varieties. Number of seed per pod ranged from 1.80 to 2.00 (Table 7). The highest number of seed per pod was recorded in BARI masur-7 and BLX-06004-12 and lowest in BLX-05009-7.

#### **4.2.2.8. 1000-seed weight**

In respect of 1000 seed weight marked variation has been found. The thousand seed weight under different lines/ varieties ranged from 15.50 to 21.10 g (Table 7). The highest weight was recorded in BLX-06004-12 followed by BARI masur-7(20.73 g) and lowest (15.5 g) 1000 seed weight was recorded in BLX-05008-2 followed by BLX-05002-6 (15.53 g).

#### **4.2.2.9. Grain yield kg ha<sup>-1</sup>**

The grain yield per hectare differed significantly among the test entries under field condition (Table 7). The yield ranged from 987.30 to 1456.00 kg ha<sup>-1</sup> while the highest (1456.00 kg ha<sup>-1</sup>) grain yield was recorded in BLX-06004-12 followed by BARI masur-7 (1451.00 kg ha<sup>-1</sup>) and BARI masur-1 (1125.00 kg ha<sup>-1</sup>) and lowest (987.3 kg ha<sup>-1</sup>) yield was recorded in BLX-05008-21 followed by BLX-05008-2 (995.00 kg ha<sup>-1</sup>) and BLX-05008-5 (996.30 kg ha<sup>-1</sup>).

**Table 7. Number of seed pod<sup>-1</sup>, 1000-seed weight and Grain yield kg ha<sup>-1</sup> of selected 11 lentil germplasms and two check varieties**

<b>Sl. No.</b>	<b>Name of lines/varieties</b>	<b>Number of seed/pod<sup>-1</sup></b>	<b>1000 seed weight (g)</b>	<b>Grain yield (kg ha<sup>-1</sup>)</b>
1	FLIP-95-12	1.82 bc	16.27 cd	1011.00 ef
2	BLX-05002-3	1.81 c	15.67 de	1020.30 e
3	BLX-06004-12	2.00 a	21.10 a	1456.00 a
4	BLX-05009-7	1.80 c	15.80 de	1005.00 fg
5	BLX-06004-2	1.83 bc	18.27 b	1113.30 bc
6	BLX-05001-6	1.82 bc	16.33 cd	1106.30 cd
7	BLX-05002-6	1.83 bc	15.53 e	1101.00 d
8	BLX-05008-21	1.82 bc	16.50 c	987.30 h
9	BLX-05008-15	1.85 bc	15.90 cde	1006.00 fg
10	BLX-05008-2	1.84 bc	15.50 e	995.00 gh
11	BLX-05008-5	1.82 bc	16.33 cd	996.30 gh
12	BARI masur-1	1.88 b	20.73 a	1125.00 b
13	BARI masur-7	2.00 a	20.47 a	1451.00 a
	CV (%)	2.17	2.30	0.64
	LSD(0.05)	0.07	0.67	11.20

Means denoted by same letter does not differ significantly.

## CHAPTER 5

### DISCUSSION

In the present investigation *Stemphylium* blight of lentil caused by *Stemphylium botryosum* showed typical symptoms on lentil plants. Successful management of the disease was achieved through application of chemical fungicides. All the tested fungicides reduced the disease score and increase the plant growth parameters and yield of lentil as compare to control plot. The lowest disease score was counted in plots sprayed with Rovral 80 WP followed by Secure 600 WG and the highest in control plot. Plant height, number of branch plant<sup>-1</sup>, number of pod plant<sup>-1</sup>, number of seed pod<sup>-1</sup> and 1000-seed weight were found maximum in the plot treated with Rovral 80 WP followed by Secure 600 WG and Nativo. The highest grain yield was recorded from the Rovral 80 WP treated plot followed by Secure 600 WG. The highest grain yield of lentil was recorded from the Rovral 80 WP and the lowest in the untreated control plot.

Bakr and Ahmed (1992) reported that disease score was the lowest in plots treated with Rovral 50 WP @ 0.2% indicating its highest disease reducing capability than rest of three fungicides and they also found that plots sprayed with Rovral produced the highest seed yield.

Huq (2007) also reported that the lowest disease was obtained from the Rovral 50 WP treated plot. From the other findings of several other researchers, Rovral 80 WP was the most effective fungicides in reducing the disease score and increasing the yield of lentil against *Stemphylium* blight. These findings are similar to our result.

So it is clear that Rovral 80 WP was most effective fungicides in controlling the disease severity and increasing the seed yield of lentil.

Another study was carried out to investigate the performance of different lentil lines/varieties under natural condition. The lentil lines/varieties were evaluated for their resistance to Stemphylium blight disease caused by *stemphylium botryosum*. This study was done during September 2013 to April 2014. The tested lentil lines/varieties showed wide variation in reaction to Stemphylium blight under field condition at different growth stages. The sensitivity of the tested lentil lines/varieties increased with the increase in age of the plants. The prevalence of Stemphylium was as follows: vegetative stage > flowering stage > pod setting stage. But this tendency may not be always a regular pattern to all the lines/varieties.

From this research, the tested lentil variety/genotypes differed significantly from one to another in respect of disease, yield and yield contributing characters under field condition. In flowering stage, out of 11 lines 3, 6 and 2 lines showed Highly Resistant (HR), Resistant (R) and Moderately Resistant (MR) type of reaction respectively. In the pod setting stage the scenario was changed and 2, 7 and 2 lines showed Resistant (R), Moderately Resistant (MR) and Moderately Susceptible (MS) reaction respectively. At maturity stage actual scenario was observed, only 4 and 7 lines showed Moderately Resistant (MR) and Moderately Susceptible (MS) type of reaction respectively. The Moderately Resistant (MR) lines were BLX-06004-12, BLX-06004-2, BLX-05002-3 and BLX-05001-6. None of the lines showed resistant type of reaction against the disease.



Bakr and Ahmed (1993) studied on 110 genotypes and found only one genotype resistant to *Stemphylium* blight and 11 genotypes were tolerant. Beare (2002) screened lentil lines/varieties against *Stemphylium* blight under natural condition and obtained some lines/varieties as moderately resistant and susceptible. The finding of the present study revealed that the tested lentil lines/varieties showed different types of reaction to *Stemphylium* blight under field condition. Rashid *et al.* (2009) screened lentil lines and found that 21 entries were Resistant (R) to *Stemphylium* blight.

From this research, it was observed that the tested lentil lines/varieties differed significantly in respect of plant height, number of pod per plant, number of branch per plant and yield. Plant height was found maximum in the BLX-06004-12 lines followed by BARI masur-7, FLIP-95-12, BLX-05008-21 and BLX-05008-2 and the minimum in the BLX-06004-2. Number of pod per plant was maximum in the BLX-06004-12 lines followed by BARI masur-7, BARI masur-1, BLX-05002-6 and FLIP-95-12 and the minimum in the BLX-05008-21. The highest grain yield was recorded from the BLX-06004-12 lines followed by BARI masur-7 and both were statistically identical. Considering the yield performance, it was observed seven lines/varieties produced more than 1 ton yield with a range from 1456 to 1020.30 (kg ha<sup>-1</sup>). The lines/varieties are: BLX-06004-12, BARI masur-7, BARI masur-1, BLX-06004-2, BLX-05001-6, BLX-05002-6 and BLX-05002-3.

The findings of the study is closely related with the study of Sarker *et al.* (1992), Rashid *et al.* (2009), Podder (2012) and Sarker *et al.* (1998). They reported that the lentil lines differed significantly in respect of agronomic traits and yield parameters. The variation in yield of lentil was mainly due to *Stemphylium* blight disease.

This variation may be due to i) Variations of genetic makeup of lentil lines/varieties and ii) growing conditions of plants.

Bakr (1993) and Mwakutuya *et al.* (2002) reported yield reduction of lentil due to Stemphylium blight. They were described that yield reduction of lentil increased with the increasing of Stemphylium blight disease severity.

With the findings of the present study it may be concluded that the lentil lines showed appreciable difference in reaction to *S. botryosum* which need to be tested further for more confirmation of the result of this study.

## CHAPTER 6

### SUMMARY AND CONCLUSION

Stemphylium blight of lentil caused by *Stemphylium botryosum* is considered now a day as one of the most important constrains of lentil production in Bangladesh. Six fungicides namely Rovral 80 WP, CM 75 WP, Nativo, Dimox 35, Vita Flo 200 FF and Secure 600 WG along with a control were used to reduce Stemphylium blight disease of lentil. All the fungicides were able to reduce the disease. The yield of lentil was enhanced significantly through the application of fungicides. Among the fungicides Rovral 80 WP was the most effective fungicides in reducing the disease score and increasing the yield of lentil. The maximum (4.00) disease score was recorded in control plot and the lowest (1.00) in Rovral treated plot. The highest (1280 kg ha<sup>-1</sup>) yield of lentil was recorded when sprayed with Rovral from the iprodione group and the lowest (700 kg ha<sup>-1</sup>) in control plot. The finding of present study revealed that Rovral 80 WP 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. This study confirms the earlier report of controlling Stemphylium blight with chemical fungicide Rovral 80 WP.

Another experiment was designed to screen resistant/tolerant lentil varieties/lines against Stemphylium blight disease. Eleven lentil lines along with a susceptible and a resistant check was evaluated and it was observed that the line BLX-06004-12 gave the highest yield (1456 kg ha<sup>-1</sup>) followed by BLX-06004-2 (1113.30 kg ha<sup>-1</sup>) and BLX-05001-6 (1106.30 kg ha<sup>-1</sup>) and

were moderately resistant to *Stemphylium* blight disease. The lowest yield (987.30 kg ha<sup>-1</sup>) was recorded in BLX-05008-21 followed by BLX-05008-2 and BLX-05008-5. So, the line BLX-06004-12, BLX-06004-2 and BLX-05001-6 may be selected for next year up-scaling due to synchronous maturity, higher yield and low disease severity.

From the above study it can be concluded that

- ❖ Rovral 80 WP is still best fungicide among the treated ones and can be sprayed @ 2 g/L, 3 times at an interval of 10 days against *Stemphylium* blight disease of lentil.
- ❖ Secure and Nativo can also be used as an alternative to Rovral.
- ❖ Three lentil lines viz. BLX-06004-12, BLX-06004-2 and BLX-05001-6 showed moderately resistant reaction against *Stemphylium* blight disease.
- ❖ These three lines can be used in the resistant breeding program as a *Stemphylium* blight disease resistant source.

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APPENDIX 1: Experimental plot of lentil



APPENDIX 2: Healthy lentil plant



APPENDIX 3: Stemphylium blight diseased lentil plant



APPENDIX 4: Rovral treated plot of lentil



APPENDIX 5: Control plot of lentil



APPENDIX 6: Maximum flowering stage of lentil



APPENDIX 7: Primary symptom of Stemphylium blight disease of lentil



APPENDIX 8: Severely affected lentil plant by Stemphylium blight disease

APPENDIX 9: The minimum and the maximum temperature and relative humidity as well as mean rainfall from November 2013 to April 2014 at Bangladesh Agricultural Research Institute (BARI), Gazipur.

Month	Temperature (°C)		Relative Humidity (%)		Rainfall (mm)
	max	min	max	min	
November 2013	30.38	18.63	92.50	60.36	0.00
December 2013	26.62	17.52	90.02	62.63	0.03
January 2014	22.81	14.58	88.77	57.06	0.10
February 2014	27.17	15.62	85.60	40.00	0.00
March 2014	31.45	19.81	83.00	37.21	0.00
April 2014	33.37	22.37	78.40	33.67	28.00

**Source:** Metrological center, BARI, Gazipur.



