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Studies on stemphylium blight of lentil (Lens culinaris) and its management practices

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University of Rajshahi

PhD Thesis

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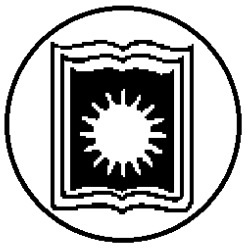

PhD Thesis

By

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Registration No. 0033, Roll No. 10609
Session: 2010-2011

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DEPARTMENT OF CROP SCIENCE AND TECHNOLOGY
FACULTY OF AGRICULTURE
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STUDIES ON STEMPHYLIUM BLIGHT OF LENTIL (*Lens culinaris*) AND ITS MANAGEMENT PRACTICES

A thesis submitted for the degree
of

Doctor of Philosophy

in the
Department of Crop Science and Technology
University of Rajshahi, Bangladesh

BY

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BScAg, MS in Crop Botany, MS in Plant Pathology

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RAJSHAHI-6205, BANGLADESH

DEDICATED

TO MY

BELOVED PARENTS

DECLARATION

I do hereby declare that the entire work now submitted as a thesis entitled "**STUDIES ON STEMPHYLIUM BLIGHT OF LENTIL (*Lens culinaris*) AND ITS MANAGEMENT PRACTICES**" in the Department of Crop Science and Technology, University of Rajshahi for the degree of Doctor of philosophy is the result of my own investigation. The thesis contains no materials which has been accepted for the award of any other degree or diploma elsewhere, and to the best of my knowledge, the thesis contains no material previously published or written by another person, except where due reference is made in the text.

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CERTIFICATE

We have pleasure in certifying the thesis entitled "**Studies on stemphylium blight of lentil (*Lens culinaris*) and its management practices**" submitted to the Department of Crop Science and Technology, Faculty of Agriculture, University of Rajshahi for the degree of Doctor of Philosophy.

We hereby certify that the candidate has fulfilled the requirements and the research work embodied in the thesis was carried out by the candidate. To the best of our knowledge, all the data and materials are genuine and original. No part of the research work has been submitted for any other degree.

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The Author

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ABSTRACT

A field survey was conducted to know the prevalence of stemphylium blight caused by *Stemphylium botryosum* of lentil (*Lens culinaris*) growing 11 districts *viz.* Jessore, Kushtia, Faridpur, Pabna, Rajshahi, Maherpur, Madaripur, Barisal, Jhalokathi, Khulna and Satkhira in Bangladesh during cropping season of 2012-13 in the farmers' field. Out of 11 districts the highest disease incidence was found in Jhalokathi (Sadar) (77.90%) and the lowest disease incidence was found in Pabna (Ishardi) (45.50%). The highest disease severity was found in Faridpur (Sadar) and the lowest disease severity was found Kushtia (Kumarkhali). Farmers used six different varieties in 11 districts. Local variety performed highly susceptible (HS), BARI Masur-3 and BARI Masur-4 showed moderately susceptible (MS) and BARI Masur-5, BARI Masur-6 and BARI Masur-7 showed moderately resistant (MR) disease reaction under field condition. Isolation and identification of the pathogen with proof of pathogenicity were carried out under laboratory condition. First appearances of stemphylium blight disease in lentil in the field were found at pre-flowering or flowering stages from another experiment. Out of 8 weed species, Bathua (*Chenopodium album*) was identified as an alternate host of *Stemphylium botryosum* and it was first report in Bangladesh. A total of 214 lentil lines/varieties were screened out against stemphylium blight in the field under artificial inoculated condition in three subsequent years of 2011-2014 in Barisal. In the first year screening program out of 214 lines/varieties 22 lines were selected as resistant to *Stemphylium botryosum*. In the second year out of 24 lines/varieties 3 lines namely BD-6002, BD-3837 and BD-3926 were selected. In the third year out of selected 5 lines/varieties including 2 check varieties BARI Masur-1 and BARI Masur-7 were screened at Barisal and Rajshahi and finally 2 lines *viz.* BD-6002 and BD-3837 showed the highest yield (1628 Kg/ha and 1447 Kg/ha). In case of effect of different dates of sowing and varieties on stemphylium blight, the highest yield and the lowest disease severity were recorded in November 08 and November 15 sowing with BARI Masur-7 and the lowest yield and the highest disease severity were recorded in October 25 and November 01 sowing. But late sowing (November 29 and December 06) performed the lowest disease severity as well as lower yield. Among the fungicides (Rovral 50WP, Compenion, Nativo, Amistar Top 325 SC and Secure 600 SC) tested for controlling stemphylium blight in Barisal and Rajshahi, 3 sequences foliar spray with Rovral (Iprodione) @ (0.2%) and Amistar Top (Azoxystrobin 20% + Difenoconazole 12.5%) @ (0.1%) at an interval of 12 days effectively controlled the disease and increased yield of lentil by 55.50% and 53.58 %, respectively. In another experiment, efficacy of different fungicides with foliar spray and seed treatment were evaluated against stemphylium blight of lentil in Rajshahi in 2014-15. Seed treatment with Bavistin or Provax and selected fungicide Rovral was sprayed as foliar spray were effectively controlled the stemphylium blight of lentil in a susceptible variety BARI Masur-1.

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CHAPTER 1

INTRODUCTION

The lentil plant (*Lens culinaris* Medikus) is the fourth most important pulse (legume) crop in the world after bean (*Phaseolus vulgaris* L.), pea (*Pisum sativum* L.) and chickpea (*Cicer arietinum* L.) (Szilagyi *et al*., 2011). Lentil is the second most important pulse crop in Bangladesh in terms of both area and production (KD, 2016). Lentil is considered one of the first domesticated crops in the world, the earliest evidence of lentil as carbonized remains was reported from Greece's Franchthi cave dated to 11,000 BC (Sandhu and Shing, 2007). Lentil was domesticated in West Asia and introduced into the Indo-Gangetic plain around 2000 B.C. (Cubero, 1981). Lentil is a self-pollinating plant, a member of the legume family, was grown globally as seeds for human diet and straw for animal feed and can improve soil nutrient status through symbiotic nitrogen fixation.

Lentil is an important pulse crop rich in protein and carbohydrate. In the developing world it is often referred to as "poor man's meat" because of its high protein content and easy accessibility by the lower economic class and its local name is '*masur dal*'. Like many other pulses, it is rich in cholesterol-lowering soluble fiber and high in folate, a valuable functional food in the human diet. It is also used as a meat substitute in gluten free diabetic low salt, low calorie, low cholesterol and high fiber contents due to its high protein contents and quality. Lentils contain approximately 25% protein (Langer and Hill, 1982) and this protein has a high apparent digestibility of 80 to 93% (Williams and Nakkoul, 1985). It is rich in phosphorus, calcium, iron, zinc and carotene. Due to presence of more protein, calcium and phosphorus it is preferred fodder for animals compared to wheat straw (Gupta *et al*., 2013).

Lentil is one of the most favorite pulse crops and food items on the Bangladeshi diet which is consumed everyday by almost every family. The production of lentil does not meet the current requirement for the people of Bangladesh. Oppositely, mean pulse intake for Bangladeshi population according to 2010 survey was 14.30 g/person/day and it was mostly from lentil (6.7 g) (HIES, 2010) but requirement was 50 g (DDP, 2013).

The average lentil cultivated area in the world considering last 5 years is estimated around 4.37 million hectares with average annual production 4.81 million tons and productivity 1099 Kg/ha. In the same time average lentil cultivated area in Bangladesh is 86749 ha with annual production 84575 ton and productivity 972 Kg/ha (FAOSTAT, 2015). Although the total cultivated area and production of lentil in our country have increased gradually over last 5 years but productivity is still very low compared to average yield of the world.

Among the factors responsible for low yield of lentil, diseases are considered to be the most serious one. Globally lentil is susceptible to more than 35 diseases (Wikipedia, 2016). So far, 15 pathogens causing 17 diseases have been recorded in Bangladesh, among them stemphylium blight caused by *Stemphylium botryosum* Walr. is considered as the most devastating one (Rashid *et al*., 2007). Stemphylium blight is a serious threat to lentil cultivation across the world particularly in South Asia (Bayaa and Erskine, 1998; Huq and Khan, 2008). In Bangladesh, the disease was first recorded during 1981 and was further confirmed in1986 (Bakr and Zahid, 1986). The disease was also observed as a major disease in 1990 decades to till date (Bakr *et al*., 2011). Since then it has gained importance due to its increased severity with reports of more than 80% crop loss (Bakr and Ahmed, 1992; Mwakutuya and Banniza, 2010). 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 Sarker, 1997).

Genus *Stemphylium* spp. was proposed by Wallroth in 1833 with *Stemphylium botryosum* (asexual stage) as the type species, whereas, *Pleospora tarda* is the sexual stage (Simmons, 1985). More than 50 species of *Stemphylium* (sexual state: *Pleospora*) have been described and they are commonly isolated from a range of plants (Farr and Rossman, 2014).

Disease symptoms have been well characterized in South Asia where *Stemphylium botryosum* has caused a 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 to 3 days. The symptoms differ 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 mycelia growth can also be observed on the infected stems. Bayaa and Erskine, 1998 reported that massive defoliation and stem bending was observed in leaf blight of lentil caused by *Stemphylium botryosum*.

Important sources of *Stemphylium* spp. inoculum were air, infected crop debris, infected seed and in the soil. But researchers around the world have emphasized the importance of plant debris as the primary source of inoculum. The ascospores from pseudothecia on overwintering asparagus debris on the soil surface were the only source of inoculum for infection of spears (Elmer *et al.*, 1996). *Stemphylium botryosum* was reported to cause in internal infection on spinach seed (du Toit and Derie, 2004). As with other pathogens, the air-borne ascospores are discharged into the air from the protruding pseudothecia (Prados-Ligero *et al.*, 2003). The surveys and field experiments carried out in India and Bangladesh on *Stemphylium botryosum*, have confirmed the importance of temperature and relative humidity for successful development of

stemphylium blight in lentil (Bakr, 1991; Sinha and Singh, 1993). Bakr (l993) has reported that *Stemphylium botryosum* commences infection when the ambient night temperature remains above 8^0C; and the mean day temperature exceeds 22^0C as well as the relative humidity inside the canopy must also reach 94%.

Stemphylium species are pathogenic to plants, humans and animals and are distributed throughout the world. *Stemphylium* spp. are also pathogenic on many horticultural cash crops and cause losses up to 100% yield loss in cotton in Brazil (Mehta, 1998). *Stemphylium botryosum* is pathogenic to lentil (Bakr and Ahmed, 1992), spinach (Koike *et al.*, 2001), onion (Aveling and Snyman, 1993), tomato (Bashi and Rotem, 1975), alfalfa (Cowling and Gilchrist, 1982a), clover (Graham, 1957) and Drummond phlox (*Phlox drummondii*) in Japan (Takeuchi and Horie, 1997).

The diverse host range of *Stemphylium botryosum* that includes leguminous and non-leguminous crops in different parts of the world indicate its adaptability to different environments (du Toit and Derie, 2004).Weeds are considered as a major constraint for lentil production in Bangladesh. Aly, 2010 reported that *Stemphylium botryosum* was isolated from leaves of Bathua weed (*Chenopodium album*) collected in Egypt.

Stemphylium blight, a damaging major disease of lentil, attacked the crop at any growing stage of damage depended upon how early was disease 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. International Center for Agricultural Research in Dry Areas (ICARDA) holds one of the largest collections of lentil with 11643 accessions. ICARDA has provided 103197 seed samples to scientists in 52 countries since its establishment (Kumar *et al.*, 2013).

Stemphylium blight may become a more serious problem in the future and there is little or no understanding of the host resistance against the disease and potential management practices for its control (Pearse, 2005). Only a few reports of resistance to stemphylium blight in lentil are available and these are limited to screening of cultivated germplasm from several parts of the world (Beare, 2002). Among the different approaches of disease management, development of resistant/tolerant variety is the most widely preferred method.

Early sowing of lentil resulted in more vegetative growth and crops prone to lodging, increasing the risk of disease infection and subsequent poor grain quality. Later sowings reduce disease risk but can result in lower yields due to the risk of dry conditions, high temperatures at flowering-pod fill and reduced crop height making harvest difficult (Hawthorne *et al*., 2016). Potential increase in lentil yield was found by changing sowing date in Ethiopia (Ghanem *et al*., 2015). Early sowing can increase the yield of lentils (Wang *et al*., 2013). In northern India, Singh and Saxena (1982) obtained the highest yield from lentil sown in the first fortnight of November, while later sowing resulted in lower yield. Sinha and Singh (1991) reported that early appearance of stemphylium blight caused alarming yield loss in lentil. It is reflected from the study that yield gradually reduced and PDI increased with delayed sowing (after second week of November). This is probably because of the change in environmental condition, which might be congenial for disease development.

The use of fungicides has been effective in reducing the economic losses due to *Stemphylium* spp. in a range of crops. Several fungicides (chlorothalonil, mancozeb, tebuconazole, procymidone and iprodione) have been found to provide effective control of diseases caused by *Stemphylium* spp. in various host species (Basallote-Ureba *et al.*, 1998; Menzies *et al.*, 1992; Meyer *et al.*, 2000). The foliar fungicide Bravo 500 (chlorothalonil) is known to control stemphylium blight of lentil in Canada (Hnatowich, 2000). Azoxystrobin is registered in Florida to manage stemphylium leaf spot of spinach (Raid and

Kucharek, 2003). However, not all chemical control has been effective in reducing the disease under weather conditions which favor the fungus (Basallote-Ureba *et al.*, 1998). In lentil, stemphylium leaf blight caused by *Stemphylium botryosum* was controlled most effectively by a foliar spray of Rovral 80WP (iprodione) at 0.2% (Bakr and Ahmed, 1992).

Research on lentil was initiated during the early 1950 decade, where efforts were confined to the collection and evaluation of local germplasm (Gowda and Kaul, 1982). Considering 50 years (1964-2014) of Bangladesh, lentil production gradually increased from 1964 to 1976 then steady up to 1998 and later gradually decreased to 2014 (FAOSTAT, 2015). The major yield gap contributing factors are insect pests, diseases, low yielding landraces grown by farmers, and the narrow genetic base (Asnake and Bejiga, 2003). Stemphylium blight is becoming a threat to lentil production in Bangladesh. The severity of the disease varies between locations within a season and between seasons within a location (Haque *et al.*, 2013) making it difficult for farmers to properly formulate financially-viable fungicide-based disease control measures. Few bench mark research on stemphylium blight have been carried out in Bangladesh. To ensure profitable cultivation of lentil, the prime importance has to be given for effective management strategy for the disease. In this views of point the study of research work have been undertaken with the following objectives:

i) To survey the prevalence of lentil diseases emphasized the stemphylium blight at different lentil growing area of Bangladesh
ii) To isolate and identify the causal pathogen of blight of lentil
iii) To search of alternate host of stemphylium blight of lentil in different weed species
iv) To find out the resistant/tolerant source of lentil germplasm against stemphylium blight
v) To determine management practices for disease control.

CHAPTER 2

REVIEW OF LITERATURE

The purpose of this chapter is to provide a selective review of the research works accomplished in relation to the present study. Literatures on lentil and stemphylium blight related study have been reviewed under the following headings.

2.1. Origin, taxonomy and domestication of lentil

Lentil (*Lens culinaris* Medik.) is a member of the Leguminosae family (Webb and Hawtin, 1981) and is characterized as herbaceous annuals with slender stems and branches. The Near East arc and Asia Minor are believed to be the center of origin of cultivated lentils. Although lentil originated the Mediterranean region, it is well adapted and grown in temperate and semi-arid regions on all continents (Muehlbauer, 2009). The crop is cultivated in West Asia and North Africa, the Indian subcontinent, North America, South America and Australia (Webb and Hawtin 1981, Erskine, 1997). Lentil is considered the oldest food crop that researchers have traced back to 7000-8000 BC (Ladizinsky, 1979). The earliest evidence of lentil as carbonized remains was reported from Greece's Franchthi cave dated to 11,000 BC (Sandhu and Shing, 2007). The wild ancestor of cultivated lentil (*Lens culinaris* subsp. *orientalis*) was found throughout the Fertile Crescent (Pearman, 2005).

The plant was given the scientific name *Lens culinaris* in 1787 by Medikus, a German botanist and physician (Cubero, 1981; Hanelt, 2001). The morphological characteristics of the *Lens* species as well as synonyms are given by Cubero (1981). The most detailed and complete study of the cultivated lentil was made by Barulina (1930). Davis and Plitmann (1970) describe *Lens* as intermediate between *Vicia* and *Lathyrus*, but closer to *Vicia*.

Lens is distinguished from *Vicia* by calyx morphology and stylar characters, and the shape of the pods and seeds (Muehlbauer *et al*., 1980).

Kingdom: Plantae, Division: Magnoliophyta, Class: Magnoliopsida, Order: Fabales, Family: Fabaceae, Subfamily: Faboideae, Tribe: Vicieae, Genus: *Lens*, Species: *L. culinaris*

Lentil is mostly consumed as dhal or soup, mainly in India, Bangladesh, Nepal, Pakistan, Sri Lanka, Turkey and Egypt. Canada was the largest producer of lentil, but India surpassed their production in 2014. India is the largest consumer of lentil while Canada is now the largest exporter in the world. Australia, Turkey, Syria, Nepal, the USA, Bangladesh and Morocco are other major producers of lentil (FAOSTAT, 2015).

The region for the domestication of lentil consists of Southeastern Turkey and Northern Syria, including the sources of the Tigris and the Euphrates rivers (Lev-Yadun *et al.*, 2000). *L. culinaris* is the putative ancestor of domesticated lentil (Cubero, 1981). After domestication in the cradle of agriculture, lentil spread to Cyprus in the Neolithic (Erskine *et al.*, 1994). Lentil disseminated from Southeastern Europe to Central Europe around the 5,000 years BC via the Danube. From Europe, it dispersed to the Nile Valley and from there to Ethiopia. However, in Georgia, lentil was propagated during the 5,000 and 4,000 years BC and in the Indian sub-continent around 2,500–2,000 years BC (Sonnante *et al.*, 2009). Lentil is now grown all over the world (Chahota *et al.*, 2007).

2.2. World Status about lentil cultivation

The year 2016 promises to be an important and exciting year for agriculture as the international community celebrates the International Year of Pulses, IYP 2016 and approved by the Food and Agriculture Organization of the United Nations (Agrawal and Majumdar, 2016). Lentil (*L. culinaris*) is an important

food legume with various uses as food and feed because of its protein-rich grains and straw. The total lentil cultivated area in the world is estimated around 4.34 million hectares with annual production and productivity of 4.95 million tons and 1260 Kg/ha, respectively (FAO, 2014). The major geographical regions of lentil production are South Asia and China (44.3%), North America (41%), Central and West Asia and North Africa i.e. CWANA (6.7%), Sub-Saharan Africa (3.5%) and Australia (2.5%). World lentil production has risen steadily by nearly four times (375 %) from an average of 0.92 million tons in 1961-63 to 3.59 million tons in 2008-10. This growth is primarily from an expanding harvested area from 1.64 million hectares in 1961-63 to 3.85 million hectares in 2008-10. Additionally, it also reflects an improvement in productivity from an average yield of 560 Kg/ha in 1961-63 to 930 Kg/ha by 2008-10 (Kumar *et al*., 2013). By 2030, the world lentil consumption is estimated at 5.5 million tons, being an increase of almost 2 million tons from the present level (Clancey, 2009).

2.2.1. Status of lentil production and stemphylium blight in India

In India, lentil was cultivated on 1.42 million hectares area in 2012-13 with a production of 1.13 million tons (DoAC, 2014). Lentil is generally grown as rain fed crop during rabi season after rice, maize, pearl millet or kharif fallow. It is also grown as intercrop with barley, linseed, mustard and occasionally with autumn planted sugarcane. In north-eastern parts of the India, lentil is also cultivated as sequential crop after rice, where seeds of lentil are broadcast in the standing crop of rice just before its harvest. Productivity of lentil is also limited by several biotic stresses such as diseases, pests and weeds (Gupta, 2014). India alone produces 20.84% of world production but yield is very low (0.5938 t/ha) compare to world average (1.0837 t/ha) which clearly indicates the lack of efficient agriculture practice and high yielding varieties. The major constrains to yield are low-yielding cultivated varieties and the narrow genetic base (Asnake and Bejiga, 2003).

During 2014, the out-scaling project in India reached 3452 farmers in 175 villages, providing information and training on how to store lentil seed, and when to sow seed and apply fertilizers and pesticides. Equipped with this knowledge, Indian farmers sowed lentil crops on 1067 ha of rice fallow land, reaping yields of up to 1.8 t/ha, compared to average yields of 0.85 t/ha from traditional varieties (ICARDA, 2015). India was the largest producer of the Lentil crop in the world until recently Canada took over the lead leaving India at the second place. Indian production of this crop covers around 10 lakh metric tons per year that is cultivated on about 14 lakh hectares of land (Singh and Singh, 2014).

Occurrence of stemphylium blight disease has been reported from northeast India (Sinha and Singh, 1991). Disease intensity as high as 83% was observed on an unsprayed local cultivar in Bihar of India causing above 90% yield loss (Sinha and Singh, 1993).

2.2.2. Status of lentil production and stemphylium blight in Canada

Canada is the leading lentil exporting nation, while India is the leading lentil consuming and producing nation. Commercial production of lentil in Western Canada began in 1970, when approximately 600 hectares (ha) were grown. Production has increased in Saskatchewan to as much as 960,000 ha in 2009. The 10-year average yield in Saskatchewan is 1475 Kg/ha. Stemphylium blight has been identified in a number of lentil fields in Saskatchewan. This foliar disease has similar leaflet drop symptoms as anthracnose and similar lesions on leaves as ascochyta blight. It has not yet been confirmed as causing significant yield losses, because the disease tends to show up later in the summer. The fungus thrives under warm (28^0C) and wet conditions. There have been differences noted between lentil varieties regarding their susceptibility to stemphylium blight (McVicar *et al*., 2010).

One of the major challenges of lentil production is the control of diseases. Diseases of lentil in Saskatchewan are ascochyta blight (*Ascochyta lentis*), anthracnose (*Colletotrichum truncatum*), botrytis grey mould (*Botrytis cinerea*), stemphylium blight (*S. botryosum*), and sclerotinia stem rot (*Sclerotinia sclerotiorum*). Ascochyta blight and anthracnose are presently the primary diseases and are being controlled by fungicide applications, crop rotation and varietal resistance. The production of the crop in more humid areas increases the risk of infestation by ascochyta blight and anthracnose (Campbell *et al.*, 2002).

Vandenberg and Morrall (2002) reported that stemphylium blight had become a more serious problem with the increase of lentil production and cultivation of new cultivars with resistance to ascochyta blight and anthracnose. In recent years, stemphylium blight has been observed with increasing frequency in lentil fields in Saskatchewan when seed tested for infection with *A. lentis* and other common lentil pathogens (Banniza *et al*., 2005). Seed infection with *S. botryosum* has been observed yearly since 2000, as reported in the Canadian Plant Disease Survey (Dykstra *et al.*, 2004).

2.2.3. Status of lentil production and stemphylium blight in Australia

Lentil (*Lens culinaris*) is an established, high value pulse crop, first grown commercially in Australia during the early 1990s. They are mainly grown in the semi-arid regions of Victoria and South Australia with winter dominant rainfall patterns. Lentil consumption in Australia is gradually increasing however it is widely grown and consumed throughout the Mediterranean, the Indian subcontinent, southern Asia and northern America. Lentil is a versatile and lucrative pulse crop in Australia that offers a number of rotational and financial benefits in many cropping systems (Raynes *et al*., 2015). Botrytis grey mould (BGM), caused by the pathogens *Botrytis cinerea* & *Botrytis fabae*, and ascochyta blight, caused by *Ascochyta lentis*, are the two major diseases

affecting the production of lentil in southern Australia (Hawthorne *et al*., 2012). Stemphylium blight disease caused by the fungus *S. botryosum* is occurring in Australia as a saprophyte but could be a potential threat for the Australian lentil industry (PBA, 2013).

2.2.4. Status of lentil production and stemphylium blight in Nepal

Lentil (*L. culinaris* Medik) is the most important and highly commercialized pulse among the grain legumes in terms of both area (206522 ha), production (226931 ton) and productivity (1099 Kg/ha) which shares almost 62% of total area and 65% of total production of pulses in Nepal (MOAD, 2013). Lentil was Nepal's third largest exportable commodity during the year 2009-10. Bangladesh has emerged as a major importer of Nepali lentils. Bangladesh was the major buyer importing 83% of lentils from Nepal (TEPC, 2011).

Stemphylium blight caused by *S. botryosum* Walr is one of the major diseases of lentil (*L. culinaris* Medik) in Nepal (Subedi *et al*., 2015). It was first reported during 1993 in Nepal and has become widespread throughout major lentil growing areas of the country (Bayaa *et al*., 1998). In recent years, *Stemphylium* has been observed increasingly in lentil fields in 9 districts of Nepal (Joshi, 2006).

2.2.5. Status of lentil production and stemphylium blight in Ethiopia

Ethiopia ranks first in Africa and tenth in the world in lentil production (Sarker *et al*., 2003). Currently lentil covers an area of about 90,000 hectares with an annual production of 125008 tones; the average national productivity being about 1.17 t/ha (CSA, 2013). Biotic and abiotic factors limit lentil productivity and seed quality. The major yield gap contributing factors are insect pests, diseases, low yielding landraces grown by farmers, and the narrow genetic base (Asnake and Bejiga, 2003). There are about ten important lentil diseases in

Ethiopia, among which rust, root rots and fusarium wilt are the major ones. Usually rust causes about 25% yield loss in the normal year while 100% crop loss seldom occurs (MOARD, 2003).

2.2.6. Status of lentil production and stemphylium blight in Germany

One of the most relevant challenges for lentil growing is increasing its yield, which in practical farming in Germany is currently 0.5-0.8 t/ha. This yield is much lower than the 1-2 t/ha, for example, Turkey, Canada and Australia, even when the crop is grown in sole cropping in these countries (Baird *et al.*, 2009). Germany has a history for cultivation of different pulse crops like dry bean, horse bean, lupin, pea and lentil. In 2012 about 200 hectare of land was under lentil cultivation in Germany. For several decades, little attention was paid to lentil cultivation and research in Germany. Nowadays, there is an increasing market for high priced lentil in Germany. Considering the various advantages like nitrogen fixation potential, high nutritive values and essentiality for mix cropping, more and more farmers are beginning to reintroduce lentil to German organic farming, and scientists are paying more attention to lentil research (Wang *et al.*, 2013).

2.2.7. Status of lentil production and stemphylium blight in Bangladesh

Lentil is the second most important pulse crop in area and production, but stands first in the consumer's preference in this country. Cultivation of lentil is mainly concentrated within the Gangetic floodplains in the northern and southern districts of the country. In Bangladesh, lentil purchased mostly from Australia, Nepal, Turkey and Canada (Uddin *et al*., 2008)

Legumes play an important role in agriculture and daily diet in Bangladesh. Around 5.2% of cultivable lands are subject to legume cultivation (Rahman *et al.*, 2009). Because poor people cannot afford expensive fish and meat, pulses have been known for a long time as the "meat of the poor people" in

Bangladesh. Although legumes have been grown in Bangladesh for a long time, farmers have largely been cultivating pulse legumes without applying major agricultural inputs like fertilizers, irrigation and plant protection. Lentil is the most popular and has been cultivated since ancient times. In 2014, total production of lentil in Bangladesh was 98210 tons from 97400 hectares land and productivity was 1001 Kg/ha (FAOSTAT, 2015). Preliminary studies in Bangladesh and India estimated yield losses of 62% and total crop failure have been reported in some cases where the stemphylium blight disease defoliated the crop in the early pod setting stage (Bakr, 1991; Erskine and Sarker, 1997).

2.3. Status of different diseases of Lentil

Lentil crop is attacked by a wide range of disease causing agents including fungi, bacteria, nematodes, viruses and phanerogramic parasites. It is mainly affected by alternaria blight (*Alternaria* spp.), ascochyta blight (*Ascochyta lentis*), anthracnose (*Colletotrichum truncatum*), botrytis stem and pod rot (*Botrytis cinerea*), rust (*Uromyces fabae*), sclerotinia stem and pod rot (*Sclerotinia sclerotiorum*), stemphylium blight (*S. botryosum*), wilts (*Pythium* spp., *Rhizoctonia* spp., *Fusarium oxysporum*), and downy mildew (*Peronospora lentis*) (Khare, 1981). Ascochyta blight, anthracnose, botrytis grey mould and sclerotinia stem and pod rot are major problems in Canada (Chongo *et al*., 2002). Stemphylium blight has started to appear in fields in Saskatchewan in recent years (Holzgang and Pearse, 2001). Botrytis grey mold and sclerotinia stem and pod rot, ascohyta blight, and anthracnose are major problems in lentil growing area of the world. Recently, powdery mildew has also been reported on lentil in Saskatchewan (Banniza *et al*., 2004). For instance, ascochyta blight (caused by *Ascochyta lentis* Vassilievsky) and fusarium wilt (caused by *Fusarium oxysporum* f. sp. *lentis*) are considered to be worldwide (Chen and Sharma, 2011).

The viral diseases are the second most important group of lentil pathogens and have appeared in different lentil growing areas of the world. Six viruses have been reported cause yellowing, stunting/necrosis and ten viruses for mosaic or mottling symptoms (Kumari *et al*., 2009). Three different types of broomrape, an obligate root parasitic angiosperm weed, are reported as a serious threat for lentil cultivation in Mediterranean region (Fernandez-Aparicio *et al*., 2009). *Orobanche crenata* is the most common lentil parasite in the Mediterranean Basin, Middle East, Andalusia and southern Spain.

Fifteen pathogens causing 17 diseases have so far been recorded in Bangladesh (Rashid *et al.*, 2007) but only few are severe causing severe losses in yield. These are stemphylium blight (*Stemphylium* sp.), Wilt (*Fusarium oxysporum*), foot rot (*Sclerotium rolfsii*) and rust (*Uromyces fabae*). Stemphylium blight is a new disease of lentil which was recorded during 1986 in Bangladesh (Bakr and Zahid, 1986). Stemphylium blight caused by *S. botryosum* has emerged as an important disease in Bangladesh and may cause up to 62% yield reduction (Bakr, 1993). Uddin *et al*. (2008) described that in lentil stemphylium blight (*S. botryosum*) causes 88%, collar rot (*Sclerotium rolfsii*) 44.4%, rust (*Uromyces fabae*) 34.4% yield loss in Bangladesh.

2.4. Symptoms of stemphylium blight disease

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 color 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 color. On careful observation white mycelial growth was seen in the infected twigs.

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 to 3 days. The symptoms differ 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 mycelia growth can also be observed on the infected stems.

Barker (2009) observed that the development of stemphylium blight on lentil first appears on lentil leaflets as small, light beige lesions. While the disease is most readily apparent when blighted leaves are noticed at the top of the canopy, it is likely present under the canopy as well. Eventually, smaller lesions merge to produce larger, irregularly shaped lesions that can kill entire leaflets and branches.

Symptoms of disease start as pinhead-sized light brown or colored spots on leaflets of plants in dense populations. The spots enlarge rapidly and within 2-3 days they cover the entire leaflet resulting in defoliation and death of young plants. In severe cases the crop may exhibit a blighted appearance causing large scale defoliation; however, the pods may remain green (Erskine and Sarker, 1997). Bayaa and Erskine, 1998 reported that massive defoliation and stem bending was observed in leaf blight of lentil caused by *S. botryosum*.

In Saskatchewan, it is suspected that stemphylium blight has not been correctly identified in the field, as the lesions closely resemble those of ascochyta blight (Morrall *et al*., 2004).

Prolonged moist periods promote further infections and give the upper canopy of lentil a grey-brown appearance. Infected leaflets may fall to the ground, and serve as a source of spores for future infections of a wide range of plants.

Fungal spores can be produced on older lesions and appear dark brown and fuzzy. Leaves may twist and roll due to desiccation caused by the pathogen. Stemphylium blight usually affects different crops from the flowering stage onwards. Infection can occur on all the aerial parts of lentil plants, such as leaflets, pedicels, flowers, and entire branches and results in a blighted appearance (Chen *et al*., 2009).

Stemphylium blight usually affects different crops from the flowering stage onwards. Defoliation of lower branches is very common in many host species (Polfliet, 2002). In alfalfa, *S. botryosum* causes leaf spot followed by chlorosis and leaf defoliation (Lucas *et al*., 1973). In spinach, symptoms start with round to oval, 2-5 mm leaf spots which later coalesce and form a necrotic leaf tissue (Koike *et al.*, 2001). Similar disease symptoms were caused by *S. vesicarium* on garlic leaves 8 to 10 days after inoculation in a greenhouse (Boiteux *et al*., 1994) and on onion and leek (*Allium ampeloprasum* L.) in field conditions with natural inoculum (Cho and Hun, 1998). du Toit and Derie (2002) reported that symptoms of stemphylium leaf spot developed within 80 hr of inoculation of spinach plants in the greenhouse. Spots on the leaves were initially small (1 to 2 mm in diameter) and sunken, circular, gray-green in color, and changed to a white color 24 to 48 hr later. The spots then became dry and bleached, light tan in color, papery, and typically showed no apparent sign of fungal infestation except in very humid conditions, and were more abundant on older leaves.

McGreevy (2013) reported that at disease onset, stems are healthy, but leaflets exhibit angular zones of light brown to tan discoloration. When relative humidity is high (early mornings and after rainfall events), *S. botryosum* sporulates and diseased leaves taken on a gray to black appearance. Under conditions favorable for the disease, lesions quickly coalesce to cover entire leaflets, leaf drop occurs, and plants are left defoliated. Often, only the terminal leaves at the tops of plants remain. As the disease progresses, tan to light brown stem lesions develop from the tips of diseased compound leaves and defoliated

branches. Along with leaves and stems, the pathogen also infects flowers, resulting in incomplete flower development. Besides reducing yield, stemphylium blight of lentil can result in small and discolored seeds.

2.5. Taxonomy, morphology and microscopic structure of *Stemphylium* sp.

Genus *Stemphylium* spp. was proposed by Wallroth in 1833 with *Stemphylium botryosum* Wallr. as the type species. More than 50 species of *Stemphylium* (sexual state: *Pleospora*) have been described, and they are commonly isolated from a range of plants (Farr and Rossman, 2014). Four new species of *Stemphylium* spp. were recently described in China as *S. gossypii*., *S. lactuci, S. momordi* and *S. alli-cepae* affecting cotton, lettuce (*Lactuca sativa*), bitter gourd (*Momordica charantia*) and onion (Zhang *et al*., 2003). In China, also three new species of *Stemphylium* spp. were recently identified by both morphological observations and molecular study in *Luffa cylindrica*, *Lycium chinense* and *Cucumis melo* and named as *Stemphylium luffae, S. lycii* and *S. cucumis*, respectively (Pei *et al*., 2011).

Stemphylium spp. is a ubiquitous, dematiaceous, filamentous fungus that belongs to the kingdom Fungi, phylum Ascomycota, class Ascomycetes, order Pleosporales, family Pleosporacea (Camara *et al*., 2002). The asexual stage (the anamorph, or imperfect stage) of the causal organism of stemphylium blight is *S. botryosum* Wallr.; whereas, *Pleospora tarda* is the sexual stage (the teleomorph or perfect stage). Previously, it seems to be *Pleospora herbarum*is the sexual stage of *S. botryosum* but that was wrong. *Pleospora herbarum* is the sexual stage of *S. herbarum* (Simmons, 1985).

Species of *Stemphylium* were dematiacious hyphomycetes with muriform, septate, usually pigmented conidia produced by a conidiophore that proliferates percurrently. The percurrently proliferating conidiophore is the principal morphological characteristic that distinguishes this genus from other genera with muriform conidia such as *Ulocladium* and *Alternaria* (Simmons, 1969).

Morphological and developmental characters such as size and shape of the conidia, conidiophores & ascospores and the size & time of maturation of pseudothecia were useful for diagnosing species variation (Camara *et al*., 2002). However, many of these characters overlap among species, making species determinations difficult. Other morphological characters such as septum development and small variations in conidial wall ornamentation were not reliable.

The macroscopic and microscopic features of *S. botryosum* on lentil have not been studied in detail. However, several studies on the fungus have been conducted in medical science, where the same fungus is considered an allergen (Larone, 2002). *Stemphylium* spp. can be compared to *Alternaria, Pithomyces and Ulocladium* in terms of microscopic and macroscopic features as they are closely related. Nevertheless, *Stemphylium* is differentiated from *Pithomyces* and *Ulocladium* by producing percurrent conidiophores (Sutton *et al*., 1998).

Conidiophores of *Stemphylium* are short, arise singly or in groups and are aseptate and swollen at the apex. After a conidium is produced, the end of the conidiophore grows out and produces a new cell and a new conidium. The conidiophore may grow to a considerable length and have a nodulose appearance. Conidia are olive brown, muriform and echinulate measuring 24-40 µm X 14-25 µm. Conidia are oblong with three to four septae and often constricted at the center by a medium cross walls. Perithecia are globose, membranous and black, and sometimes have a slender neck. Asci (183-267 µm X 27-37µm) are oblong to clavate with outer and inner walls. Ascospores (32-48 µm X 12-21 µm) are elongate to ovate, characteristically with seven cross walls and three to five longitudinal septa, and yellowish to brown in color and muriform when mature (Bayaa and Erskine, 1998). *S. botryosum* developing from the holotype produces conidia 33 to 35 µm in length and 24 to 26 µm wide. A majority of the conidia are almost as broad as they are long (length/width ratio is 1.0 to 1.5) (Simmons, 1985).

2.6. Media for sporulation

Seven different media: 25% Potato Dextrose Agar (PDA), Water Lentil Seed Agar (LSA), 25% Ground Lentil Stem and Leaf Agar (SLA), 25% Ground Lentil Stem, Leaf and Seed Agar (SLSA), 25% Ground Wheat Straw Agar (GWSA), V8 Juice Agar (V8), V8 Juice with PDA (V8P) had been tested for sporulation ability with 23 *Stemphylium* sp. isolates collected from Saskatchewan in preliminary experiments (Hashemi *et al*., 2004). *S. botryosum* colonies grow rapidly on a variety of media. They mature in 15 days at 25°C on potato dextrose agar (Hashemi *et al*., 2005). On most media, the colonies are velvety to cottony in texture with a gray, brown or brownish-black color and black pigmentation on the colony reverse (Larone, 2002). The production of conidia in abundance under laboratory conditions is difficult, even when it is grown on PDA or V8 juice agar under alternate cycle of 12 hr light and 12 hr darkness (Mehta, 1998). *Stemphylium* spp. were grown on V8 juice agar media (Camara *et al*., 2002).

Koike *et al*. (2005) studied a new disease of spinach (*Spinacia oleracea*) crops in the Yuma region of Arizona a foliar disease that previously had not been diagnosed in this geographic area. The disease caused by fungus *S. botryosum* was identified based on the morphological characteristics of isolates onto V8 juice agar media. Mycelial growth of *S. botryosum* onto V8 juice agar media was found dark green-to-brown in color.

Kumar (2007) conducted an experiment at the University of Saskatchewan to identify a suitable culture medium for the sporulation of *S. botryosum*. V8 (V8 Juice Agar Medium), V8P (V8 Juice Potato Dextrose Agar Medium), V8P TD1 (V8P + 2% Tamarind Juice Medium), V8P TD2 (V8P + 4% Tamarind Juice Medium) respectively, were tested for their suitability for increasing conidia production by isolate SB-19. All the media used allowed the fungus to grow to sporulate profusely.

Usually higher concentration (e.g. $1x10^5$ spores m/l, $2X10^5$ spores m/l) was found to better estimate disease severity. But for breeding purposes, Banniza *et al*., (2005) suggested that intermediate disease severity is desirable to identify subtle differences between genotypes, since stemphylium blight is considered to be a quantitative trait. So, $2x10^4$ spores m/l or $1x10^5$ spores m/l was used for inoculation.

2.7. Sources of Inoculum

Important sources of *Stemphylium* spp. inoculum are air, infected crop debris, infected seed and in the soil.

2.7.1. Infected crop debris

Researchers have generally agreed that infected plant debris is normally the main source of primary inoculum and the pathogen can survive for long periods. Srivastava *et al*. (1996) reported that *S. vesicarium* remained viable for 4 months on diseased plant debris. The burial of the infected plant debris reduced the disease severity in the coming season. However, *S. botryosum* should be able to survive for more than 8 months in Saskatchewan for successful infection in the following spring.

Infection of *S. vesicarium* on pear increases every year as the inoculum accumulates in plant debris (Polfliet, 2002). Ascospores and conidia of *Pleospora herbarum* on debris from the previous year's fern growth serve as primary inoculum for asparagus (Johnson, 1990). Survival of pseudothecia of *Pleospora herbarum* through over-wintering in plant debris for a long period was reported in garlic (*Allium sativum)* (Basallote-Ureba *et al*., 1999). du Toit and Derie (2003a, 2003b) found pseudothecia of *P. herbarum* on spinach stem debris that was left on the soil surface through the winter in fields in which spinach seed crops had been grown the previous season. Isolates of *S. botryosum* generated from ascospores discharged from pseudothecia onto agar

in petri plates inverted over this debris were pathogenic on spinach in the greenhouse.

2.7.2. Infected seed with *Stemphylium botryosum*

Stemphylium botryosum infected the lentil seed, but seed-to-seedling transmission of the disease has not been demonstrated. Besides reducing yield, stemphylium blight of lentil can result in small and discolored seeds (McGreevy, 2013). Infected seed is an important means of transmission of the disease from region to region and also serve as a source of initial inoculum early in the season (Agarwal and Sinclair, 1996).Seed infection of lentil with *S. botryosum* has been observed yearly since 2000, as reported in the Canadian Plant Disease Survey (Dykstra *et al*., 2004). It overwinters on seed and as mycelium on dead stems and leaves in many cropping systems. Limited information is available on whether the pathogen is seed-borne in nature on lentil (Bayaa and Erskine, 1998). Though seed infection by *S. botryosum* has been reported in previous studies, there is no clear understanding of the significance of seed borne *S. botryosum* inoculum on disease initiation of lentil (Mwakutuya, 2006).

S. botryosum was detected in commercial spinach seed grown in the USA as well as commercial seed lots from the European Union (EU), suggesting that *S. botryosum* may be prevalent in the global spinach seed industry. This possibility could explain the recent reports of the pathogen in spinach crops in other states (du Toit and Derie, 2001). *S. botryosum* can be seed borne in spinach. Therefore, infected seed may be a source of inoculum of the pathogen (du Toit and Derie, 2003b). *S. botryosum* was reported to cause in 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. (duToit and Derie, 2004).

2.7.3. Air and water borne of *Stemphylium botryosum*

Asexual spores (conidia) of the fungus are borne by air and water to the surfaces of leaves and stems, where infection begins. Disease development is favored by extended periods of high canopy humidity and temperatures above approximately 60^{o}F. In Canada and the US Northern Plains, disease outbreaks are observed when heavy rainfall and/or extended periods of high relative humidity occur late in the growing season, and they are most severe in closed lentil canopies, which trap humid air (McGreevy, 2013). In alfalfa, *S. botryosum* is spread by airborne and waterborne conidia (conidia and ascospores) (Malvick, 1998). Heavy rainfall events were common across much of North Dakota in late July and early August 2011, and outbreaks of stemphylium blight occurred in several of the regional variety trials of lentil. In the variety trials in which stemphylium blight developed, 'Morena', a Spanish brown type, consistently exhibited severe canopy defoliation due to stemphylium blight (McGreevy, 2013).

2.7.4. Soil borne of *Stemphylium botryosum*

The fungal pathogen of onion caused by *S. vesicarium* were isolated from soil and infected onion leaves by soil dilution and plate count method (Gaikwad *et al*., 2014). Srivastava *et al*. (1996) reported that *S. vesicarium* remained viable for 3 months at soil depths of 2.5, 5.0 and 7.5 cm and for 2 months at soil depths of 10 and 15 cm.

2.8. Factors Affecting Disease Development

The initiation and development of plant disease is affected by environmental factors through their influence on host susceptibility, pathogen infectivity and the host-pathogen interaction (Agrios, 2005). The surveys and field experiments carried out in India and Bangladesh on *S. botryosum*, have confirmed the importance of temperature and RH for successful development

of stemphylium blight in lentil (Bakr, 1991; Sinha and Singh, 1993). Infection of the host plant is a very complex process that is influenced by environmental interactions.

2.8.1. Influence of Temperature

Temperature is the important factor in disease development. The temperature requirements for optimum disease development seem to vary among populations of *S. botryosum* and from region to region. In Bangladesh, *S. botryosum* initiated infection on lentil when the night temperature remained above 8^0C with average day temperature above 22^0C and the relative humidity in the plant canopy exceeded 95% (Bakr, 1991; Erskine and Sarker, 1997). Among the atmospheric factors maximum and minimum temperature from 20.5-29.0^0C (mean 23.7^0C), 5.4-13.3^0C (mean 14.2^0C) and are the predisposing factors for disease initiation whereas the maximum and minimum temperature ranging from 20.5-35.0^0C and 13.0-21.0^0C are favorable for stemphylium blight disease development of lentil in Bangladesh (Huq and Khan, 2008).

In northeastern India, an average mean temperature of 18±2°C and relative humidity (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. Being based solely on meteorological data analysis, these predictions may have neglected other variables (Sinha and Singh, 1993). In New Zealand, *Stemphylium* sp. infection levels on asparagus were significantly higher at l4°C than at 20 or 26°C (Menzies *et al*., 1991). In surveys conducted in Spain, leaf spot of garlic outbreaks caused by *S. vesicarium* were favored by high RH followed by dry, warm weather (Basallote-Ureba *et al*., 1999). In the United States of America, *S. botryosum* causing stemphylium leaf spot of alfalfa is divided into two biotypes differing in their optimum temperature and symptoms. Cool temperature (18 to 20°C) favor the C-T biotype that is mainly a problem in spring and fall in California.

Warm temperature (23 to 27°C); favor the W-T biotype, which is mainly prevalent in the eastern U.S. (Cowling and Gilchrist, 1982b).

A study in onion showed that conidia of *S. vesicarium* germinated within 2 hr when incubated at 4°C (Suheri and Price, 2000). Terminal and intercalary appressoria were produced at higher frequency after 24 hr at 25°C. In another study, using *S. vesicarium*, a positive relationship was reported between conidial concentration in the air and the number of hours with temperatures in the range of 12-21°C (Prados-Ligero *et al*., 2003). In a recent study on the biology of *S. botryosum* in lentil, the minimum latent period was 48 hr and was observed at the ideal temperature of 25-30°C under controlled conditions. It increased with decreases in temperature and wetness period (Mwakutuya, 2006).

High temperature favored the germination of conidia of *S. botryosum* and under controlled conditions the optimum temperature for conidial germination was between 25°C and 30°C (Mwakutuya *et al.*, 2004).

2.8.2. Influence of Moisture

The availability of moisture is critical during the time of conidial germination. Under moist conditions, disease incidence increases rapidly. Excessive vegetative growth in combination with high humidity favors disease development.

Most plant pathogens require moisture for spore germination and penetration of the host by the germ tube (Agrios, 2005). In lentil, one of the important factors determining the appearance, development and spread of *S. botryosum* was the number of cloudy and foggy days during the November-February cropping season in the Indian sub-continent (Sinha and Singh, 1991). *S. botryosum* on lentil is known to require RH of more than 90% and prolonged periods of leaf wetness (Bakr, 1991). Splashing rain and running water promote the spread of most disease. As with most fungal diseases, the availability of moisture is important for successful infection by and development of *S. botryosum* on

lentil. The moisture requirements can be specific, just as those required for the appearance of stemphylium leaf spot in alfalfa (Emery and English, 1994)

Stemphylium spp. requires at least 8 hr of wetness at low temperatures (10°C) for successful infection and infection increases with increased leaf wetness for 24 hr (Mwakutuya, 2006). Reviewing the effect of leaf wetness periods on *Stemphylium* spp., Bradley *et al*. (2003) reported on an association of longer wetness periods with increased sporulation, infection and disease severity. Mwakutuya (2006) explored in detail optimum temperature and humidity (%) for higher stemphylium blight severity in controlled conditions after inoculation with *S. botryosum*. Sinha and Singh (1993) reported that high relative humidity, cloudy days and moderate to warm temperatures are the optimal conditions for stemphylium blight epidemics in lentil in Indian tropical or sub-tropical environment. Suheri and Price (2000) observed that the infection of onion leaves by *S. vesicarium* occurred after 16 hours of leaf wetness at 15°C and 8 hr of leaf wetness at 10 to 25°C. Infection increased with increasing leaf wetness duration to 24 hr. Similarly, 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 32 hr.

Desiccation of *Stemphylium* conidia for 3h or 14h reduced maximum germination to 20% and 11% respectively, compared to 92% germination for continuously wet conidia (Bradley *et al*., 2003). The effect of the interrupted leaf wetness on *Stemphylium* spp. is related to whether there is high or low RH during the dry interrupting periods. The prevalence of high RH during the dry interrupting periods is thought to enable the conidia of *S. vesicarium* to continue germ tube development (Llorente and Montensinos, 2002).

Prolonged periods of leaf wetness are favorable for the disease. Stemphylium leaf spot was enhanced during rainy periods and in fields irrigated with overhead sprinklers (Koike *et al*., 2001). *S. vesicarium* required wet conditions for growth on garlic (Aveling and Naude, 1992).

The role of relative humidity was critical in cases of low precipitation. Field observations during a survey indicated that outbreaks of garlic leaf spot were favored by foggy and rainy weather in spring, followed by dry warm days (Basallote *et al.*, 1993). Severe foliar damage with subsequent yield losses occurred when leaf wetness periods exceeded 24 continuous hours. Warm, humid conditions were conducive to the development of severe epidemics (Aveling and Naude, 1992). The leaf wetness requirements and response to interrupted leaf wetness are expected to vary from region to region as the pathogen adapts to prevailing environmental conditions (Jhorar *et al*., 1998).

2.8.3. Influence of Light

Fungi exhibit different responses to light, depending on the light intensity, quality, and duration of exposure as well as temperature (Alam *et al*., 2001). Light affects various processes in disease development including pathogen sporulation, germination and disease severity. However, light is not as important as temperature or moisture (Agrios, 2005). Studies in India with the aid of meteorological data concluded that an average of 7.7 or less sunshine hours favored the development of stemphylium blight on lentil. Eight or more sunshine hours per day were unfavorable for disease development (Sinha and Singh, 1993). *Stemphylium* spp. is assumed to be a diurnal sporulation; 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).

The effect of various light sequences and moisture periods on severity of stemphylium leaf spot was tested in a controlled environment chamber on a susceptible alfalfa clone inoculated with the cool-temperature biotype of *S. botryosum*. Plants inoculated after a 12 hr light period exhibited more disease than those inoculated after a 12 hr dark period, regardless of the post-inoculation light sequence. Plants inoculated before a 12 hr light period exhibited more disease than those inoculated before a 12 hr dark period, regardless of the pre-inoculation light treatment. No leaf spot symptoms

appeared if plants were exposed to continuous light after inoculation. Thus, high disease severity was achieved only when plants were exposed to light before and after inoculation, followed by alternating dark/light periods until leaf spot symptoms developed. Disease severity increased when the period of free moisture on the leaves was extended from 1 to 4 days. Only two day moist period produced symptoms most like those observed in the field in California (Cowling and Gilchrist, 1982b).

Earlier studies on *S. botryosum* f. sp. *lycopersici* of tomatoes indicated that sporulation was optimal in continuous darkness, with the highest spore yield occurring when a 12 hr light period was followed by a 12 hr dark period (Bashi and Rotem, 1975). Continuous light inhibits the sporulation of *S. solani* and conidiophores were formed but no conidia developed (Minussi *et al*., 1977). The release of *S. botryosum* spores borne singly or in chains on simple sporophores is favored by low RH in the presence of light (Leach, 1971).

2.9. Disease ratings and assessments

Selection or development of rating scales for different diseases depend on the nature of a particular disease, pathogen biology and host pathogen interaction. Various methods have been developed for stemphylium blight screening and assessments by different researchers.

Chen (2007) used a 0-9 scale to phenotype stemphylium blight disease reaction, taking into account leaf area infection during the late flowering stage. Banniza *et al*. (2005) reported that the Horsfall-Barratt scale was used to screen stemphylium blight. A field screening was done using a 0-10 scale for assessing the severity of stemphylium blight in lentil (Banniza and Vandenberg, 2009). During taking diseases data of stemphylium blight of lentil a 0-5 scoring scale was used by Bakr *et al*., 2000.

Horsfall-Barrat's logarithmic scale had unequal intervals in disease scores and is difficult to use for quantitatively inherited traits. To overcome this problem Hashemi *et al*. (2005) modified this scale to a 0-10 linear semi-quantitative

scale. This scale considered disease development pattern consisting of the appearance of chlorotic spots followed by gradual defoliation of plants (0= free of disease, 1=a few tiny tan spots, 2=few small to large chlorotic spots, 3=expanding lesions on leaves to defoliation started, 4=20% nodes on main stem showing necrotic symptoms and defoliation, 5=40% nodes on main stem showing necrotic symptoms and defoliation, 6=60% nodes on main stem showing necrotic symptoms and defoliation, 7=80% nodes on main stem showing necrotic symptoms and defoliation, 8=100% leaves defoliate but small green tip recovering, 9=100% leaves defoliate but stem still green, 10= Completely dead).

A descriptive scale from 1-5 was used for scoring leaf spot caused by *S. botryosum* in alfalfa (Salter and Leath, 1991). Koike *et al*. (2001) used a sign scale (- = no disease; + = small leaf spot <5mm; + + = medium leaf spot) to score leaf spot disease of spinach caused by *S. botryosum*. A qualitative scale (HR, R, MR, S and HS) and a semi-quantitative scale of 1-5 (1= no symptoms; 2= < 5% infection; 3=6-25% infection; 4=26-50% infection and 5= >50% infection) was used to score stemphylium blight caused by *S. vesicarium* in onion and garlic (Stares, 1999).

2.10. Pathogenicity

Koch's postulates are four criteria designed to establish a causal relationship between a causative microbe and a disease. The postulates were formulated by Robert Koch and Friedrich Loeffler in 1884 and refined and published by Koch in 1890. Koch applied the postulates to establish the etiology of anthrax and tuberculosis, but they have been generalized to other diseases (Agrios, 2005).

The suspected causal agent (bacterium or other microorganism) must be present in every diseased organism (e.g., a plant) examined. The suspected causal agent (bacterium, etc.) must be isolated from the diseased host organism (plant) and grown in pure culture. When a pure culture of the suspected causal agent is inoculated into a healthy susceptible host (plant), the host must reproduce the specific disease. The same causal agent must be recovered again

from the experimentally inoculated and infected host, i.e., the recovered agent must have the same characteristics as the organism in step 2. Despite the difficulties of carrying out Koch's postulates with some causal agents, they have been and continue to be applied, sometimes with certain modifications, in all cases of disease (Agrios, 2005).

2.11. Host range of *Stemphylium* spp.

Isolates of *S. botryosum* have been reported as pathogens of a broad range of plants, including alfalfa (*Medicago sativa* L.), bean (*Phasaeolus vulgaris* L.), carrot (*Daucus carota* L. sub sp. *sativus*), endive (*Cichorium endivia* L.), fava bean (*Vicia faba*), lettuce (*Lactuca sativa*), landino clover (*Trifolium repens*), lupine (*Lupinus* spp.), onion (*Allium cepa*), parsley (*Petroselinum crispum* Mill.), pea (*Pisum sativum*), radish (*Raphanus sativus*), yellow sweet clover (*Melilotus officinalis*), tall fescue (*Festuca arundinacea* Schreb.), tomato (*Lycopersicon esculentum* Miller), gladiolus (*Gladiolus italicus* Mill.), spinach (*Spinacia oleracea* L.) and sanfoin (*Onobrychis* spp.) (Koike *et al*., 2001). *S. botryosum* has been reported on a stem spot and needle blight on asparagus, first observed in Greece (Elana, 1996). In 1996, leaf spot of Drummond phlox (*Phlox drummondii*) caused by *S. botryosum* was observed in Japan (Takeuchi and Horie, 1997). *S. botryosum* also attacked on chickpea worldwide (Thakur *et al*., 2010)

2.12. Alternate host of *Stemphylium botryosum*

Weeds are one of the most significant agronomic problems that are considered as a major constraint for lentil production in Bangladesh. A study by Halila (1995) showed that the average yield loss on winter-sown lentils caused by weeds could be 60%-100%. Aly, 2010 reported that *S. botryosum* was isolated from leaves of Bathua weed (*Chenopodium album*) collected in Egypt. Hanse *et al*., 2015 also reported that in the Netherlands, during the summer of 2007, *C. album* was identified as hosts in an assay of plants grown and inoculated in climate rooms.

2.13. Diseases Management

Control of plant diseases becomes successful and economical when management approach contains several methods including chemical means (Bakr and Ahmed, 1992), cultural practices (Howlider *et al*., 1989) and use of resistant varieties (Ahmed, 1986). Use of fungicide is the most dependable method to control plant diseases.

2.13.1. Resistant cultivars

Limited efforts have been made to screen lentil germplasm against the pathogen. Genetic resistance is a cost effective and ecosystem friendly approach to disease management. Despite considerable progress in breeding for resistance to individual diseases, the example is cited to emphasize the need for more cultivars with multiple disease resistance that is durable. Resistant varieties provide a more effective and more consistent method of control.

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 t/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 *et al.* (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 to 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. BARI Masur-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 (*S. botryosum*).

BARI Masur-5, a released variety of lentil in Bangladesh developed from the line X95S-136. Single plant selection from this breeding line was done from F3 in Bangladesh. BARI Masur-6, a released variety of lentil in Bangladesh developed from the line X95S-167(5). Single plant selection was done from F3 of X95S-167(5) in Bangladesh to develop BARI Masur-6 (Uddin *et al*., 2008).

In early 2015, BARI released a micronutrient-rich variety of lentil in Bangladesh named BARI Masur-8, which is an outstanding lentil variety developed from a crossing made between the lines, ILL 5888 (BARIMasur-1, a Bangladeshi lentil cultivar) and ILL 6002 (an ICARDA breeding line). It was selected from among 412 lines supplied by ICARDA. It also provides combined resistance to stemphylium blight and rust (ICARDA, 2016).

Beare, 2002 reported that only a few reports of resistance to stemphylium blight in lentil are available and these are limited to screening of cultivated germplasm from several parts of the world. Stemphylium blight resistant lines in Bangladesh mainly comprise local land races, exotic genotypes and ICARDA lines (Sarker *et al*., 1991). Research between ICARDA and Bangladesh programs has resulted in the development of stemphylium blight resistant cultivars (Sarker *et al*., 2004). Genetics and inheritance of resistance to stemphylium blight of lentil have not been completely elucidated. Two studies have been conducted on genetic control of stemphylium blight resistance in lentil, both indicating that resistance to stemphylium blight is quantitatively inherited (Saha, 2009). Studies on defense structural factors such as epidermal hairs, thickness of epidermis and cortical layers revealed considerable variation for resistance to *S. botryosum* in lentil (Chowdhury *et al*., 1997). The lentil cultivar Precoz, also reported as ILL 4605, developed in Argentina (Riva, 1975), had also been reported to have resistance to *S. botryosum* (Erskine and Manners, 1996).

In Nepal, lentil varieties released selections of local landraces (Sindur); local selection of South Asian origin introduced either from India (Simrik. Sisir, Simal, Shital, Khajura Masuro-1) or from ICARDA (Sikhar, Khajura Masuro-2), however, the recently released varieties Sagun and Maheswor Bharati are from crosses made using lentils from South Asia and West Asia, specifically for Nepal. These varieties have 40-60% higher yield and 20-30% larger seed size as compared to released variety Shital/Simal, and resistant to moderately resistant to stemphylium blight and wilt disease (Shrestha *et al*., 2011).

Characteristics of the resistance genes in lentil are not known. However, lentil cultivars in Bulgaria (Naslada and Stella) are said to possess complex resistance to stemphylium blight (Mihov and Stoyanova, 1998). In general, Bangladeshi lentils have narrow genetic base with respect to morphological, phenological and agronomic traits as well as biotic and abiotic stresses. Therefore, efforts were undertaken to study 110 accessions of lentil from home and abroad to identify diverse group of genotypes. Disease severity of stemphylium blight was recorded carefully in all exotic and native diverse lentil accessions under natural epiphytotic condition. Among them 6 accessions were highly resistant, 43 were moderately resistant and the rest were moderately susceptible, susceptible and highly susceptible. These precious genotypes could be used as breeding material for varietal development of lentil (Roy and Begum, 2012).

There is an urgent need to gain confidence that Australian lentil has resistance to this fungus. It has been studied the Australian isolates of *Stemphylium* sp. and developed screening methods to efficiently test resistance of lentil breeding lines. Evaluation has been conducted in controlled environment conditions and confirmed the presence of resistance in the breeding material. To find new sources of resistance against *Stemphylium* sp., screening of lentil landraces was done and found higher levels of resistance in some of the ICARDA germplasm. Furthermore, laboratory tests are being used to analyses the fungal response to different registered fungicides used in pulse crops (PBA, 2013).

2.13.2. Different dates of sowing as a cultural practice

Sowing on time (late April-May) is important to maximize yields in drier situations. In wetter situations in southern Australia, lentil can be sown much later (to mid spring) without significantly affecting yield. Increase the sowing rate if sowing is very late (Raynes *et al*., 2015). Manipulation of sowing time has some effect on the incidence and severity of many diseases. Many field crops can escape various diseases with the shifting of sowing time (Sud and Singh, 1984). Effects of sowing date on yield of lentil on November 01 showed significantly the lowest PDI and the highest yield followed by November 10 and November 20. Sowing on early November in Barisal region of Bangladesh could avoid disease significantly and increase yield. It is recommended that lentil may be sown before November 20 for maximum yield by the reduction of disease severity significantly (Huq and Khan, 2008). Effect of sowing date on yield of lentil on November 01 showed significantly the lowest PDI and the highest yield followed by November 10 and November 20. Sowing on early November could avoid disease significantly and increase yield. This was confirmed by Jain *et al*. (1987).

In northern India, Singh and Saxena (1982) obtained the highest yield from lentil sown in the first fortnight of November, while later sowing resulted in lower yield. In Syria, seed yield was maximized by sowing in December, while delaying sowing to January and February reduced seed yield by 25% (Saxena *et al*., 1983). In Ethiopia, Bejiga (1991) found that early lentil sown between the last week of June and the second week of July increased yield.

Many field crops can escape various diseases with the shifting of sowing time (Sud and Singh, 1984 and Singh and Agrawal, 1986). Time of sowing had marked effect upon level of disease incidence of mustard and thus manipulating the sowing time infection may be avoided (Hedge and Anahosur, 1994). In mustard progressive increase in infection rate and decrease in yield was found in delay sowing (Howlider *et al.*, 1989) which is in close agreement of the present findings. Delayed sowing greatly increased the incidence of

anthracnose of French bean (Sindhan and Bose, 1981). Changing sowing dates in wheat could be an option for increasing yields in future climates (Ludwig and Asseng, 2010).

Lentil cultivation time is different in the world and it varies location to location on the basis of environment. Range of sowing date of lentil in Bangladesh is October to December and range of harvested date is February to March. Global lentil production calendar (Raynes *et al*., 2015) was presented in the Appendix-3.

2.13.3. Chemical control

A field experiment was carried out during Rabi seasons of 2011-12 and 2012-13 in Bangladesh to evaluate the efficacy of fungicides in controlling stemphylium blight (*S. botryosum*) of lentil. Five fungicides were evaluated under higher disease pressure (10^6 m/l) of stemphylium blight. Results revealed that foliar spray (4 sequences) with Rovral 50WP (Iprodione) @ (0.2%) and Secure 600WG (Fenamidione+Mancozeb) @ (0.2%) at an interval of 7 days effectively controlled the disease and increased yield of lentil by 31.99% and 28.20%, respectively. The fungicides may be selected for control of the disease (Shahiduzzaman *et al*., 2015).

Gupta and Srivastava (1988) studied 8 fungicides namely Copper Oxychloride, Mancozeb, Captafol, Thiram, Captan and Carboxin for the control of *Stemphylium vesicarium* in onion. The cost benefit ratio revealed that a preventive spray of 0.25% Mancozeb gave the highest net financial return.

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.

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 lowest concentration (250 ppm) while carboxin, Dithane M-45 (mancozeb) and Captan inhibited germination.

Basallote-Ureba *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 Chlorathalonil) 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 conductive for disease development.

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.

Currently, azoxystrobin (e.g., Amistar, Syngenta Crop Protection, Inc.), is registered in Washington to manage leaf spot in spinach (PICOL, 2004). duToit and Derie (2003a) and du Toit *et al.* (2004 and 2005) documented that fungicides in the strobilurin family as well as iprodione were highly efficacious against stemphylium leaf spot. Azoxystrobin (Quadris 2.08 FL) is registered in Florida to manage stemphylium leaf spot of spinach (Raid and Kucharek, 2003). Foliar applications of azoxystrobin, acibenzolar-S-methyl (Actigard 50 WG, Syngenta Crop Protection, Inc.), or azoxystrobin + acibenzolar-S-methyl significantly reduced severity of stemphylium leaf spot in Maryland (Everts and Armentrout, 2002).

Experiments were carried out to evaluate five different fungicides against lentil stemphylium blight in Nepal during two winter seasons of 2011/12 and 2012/13. All tested fungicides at lower doses inhibited radial mycelia growth of *S. botryosum* significantly at different concentrations under in vitro test. Fungicides Krilaxyl (Metalaxyl 8% + Mancozeb 64% WP) and Blitox-50

(Copper oxychloride 50% WP) had positive response in checking the growth of pathogen completely even in the lowest dose (500 ppm) while SAAF (Carbendazim 12%+ mancozeb 63% WP) had better results with the increase in concentrations. The mycelial growth inhibition percent of SAAF, Mancozeb and Bavistin at 2000 ppm were 68.7, 55.9 and 47.1, respectively. Results of field experiment (during 2011/12) revealed that all fungicides had significant effect on disease severity and crop yield compared the control. The highest percent yield increase (PYI) was obtained from Mancozeb treated treatment (40.20%) followed by Krilaxyl (22.46%) and SAAF (21.63%) over the control. During 2012/13, in field condition, all tested fungicides had significant effect on percent disease index (PDI), yield and hundred seed weight as compared to control. The lowest PDI was observed in the Krilaxyl (36.00%) followed by Mancozeb (37.35%), and the highest PDI was recorded in control (72.00%). The highest crop yield was recorded from the plot treated with Krilaxyl (1008.00 Kg/ha) followed by Mancozeb (914.30 Kg/ha) and SAAF (853.80 Kg/ha). Over years, among the fungicides, the performance of Krilaxyl was noted as the most effective fungicide followed by mancozeb to manage stemphylium blight under both in-vitro and in-vivo (Subedi *et al*., 2015). Two sprays of Mancozeb 75WP or Carbendazim 50WP reduce severity of stemphylium blight disease of lentil, (Shrestha *et al*., 2011).

S. botryosum were more aggressive on spinach in the presence of pollen (du Toit and Derie, 2002). Therefore, fungicide applications should be initiated just prior to pollen shed in spinach seed crops (du Toit and Derie, 2002) and repeated at appropriate intervals if conditions are conducive for this disease (e.g., wet). To avoid development of resistance to fungicides by *S. botryosum*, fungicides with different modes of action should be alternated or mixed to manage this disease for the long term (du Toit and Derie, 2003a). Treatment of infected seed with chlorine (1.2% NaOCl) for up to 40 minutes reduced the incidence of *S. botryosum* from 54.8% for the non-treated seed to18.3% (du Toit and Derie, 2003a). Marja-Leena, 2003 reported that using a *Streptomyces*-based bio fungicide was found to be effective against seed borne *Stemphylium* sp.

2.13.4. Integration of management options

Bakr and Ahmed (1993) conducted an experiment in an integrated management effort against Stemphylium blight (*Stemphylium sarciniformis* (Cav.) Willr. 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 (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 the most effective for stemphylium blight management.

Aveling *et al.* (1993) tested Anilazine, benomyle, a Carbendazim/Flusilazole mixture procymidone, tebuconazale and thiram for their efficacy in reducing pathogen. *Alternaria porri* and *Stemphylium vesicarium* of onion were 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.

CHAPTER 3

MATERIALS AND METHODS

A set of 8 laboratory and field experiments were conducted at Regional Agricultural Research Station, Bangladesh Agricultural Research Institute (BARI), Rahmatpur, Barisal and Fruit Research Station, BARI, Binodpur, Rajshahi, Bangladesh which site is geographically located at 22.42^0 North latitude and 90.23^0 East longitude and at 24.35^0 North latitude and 88.16^0 East longitude, respectively. These experiments were conducted four subsequent years of 2011-12, 2012-13, 2013-14 and 2014-15. Field survey for the prevalence of stemphylium blight of lentil (*Lens culinaris*) was done at 11 districts (pulse growing area) of Bangladesh. The experimental field, Barisal was medium high land in south-west of the country belonging to the Ganges Tidal Flood Plain (Agro-Ecological Zone, AEZ 13) and Rajshahi was medium high land under the Active Ganges Flood Plain (AEZ 10).

3.1. Experiment-I: Survey of stemphylium blight of lentil at farmer's field indifferent lentil growing areas of Bangladesh (2012-13)

3.1.1. Study location

A survey was conducted during cropping season of 2012-2013 throughout the major lentil growing area of Bangladesh to record the disease incidence and severity of stemphylium blight of lentil at farmer's field. Eleven lentil growing districts (Table 3.1.1.) of Bangladesh were surveyed in this study.

3.1.2. Survey procedure

This survey work was done following random sampling technique. Eleven districts were selected from lentil growing area of Bangladesh and one upozila were selected in each district. Ten farmers field were selected randomly from

Table 3.1.1: List of surveyed upazila and districts of lentil growing areas in Bangladesh

Sl. No.	Name of Districts	Name of Upazila
1	Barisal	Babuganj
2	Madaripur	Sadar
3	Jhalokathi	Sadar
4	Khulna	Paikgasa
5	Satkhira	Tala
6	Maherpur	Gangni
7	Jessor	Sadar
8	Kushtia	Kumarkhali
9	Faridpur	Sadar
10	Pabna	Ishardi
11	Rajshahi	Puthia

in each upozila. A total number of 110 fields were investigated. Randomly three places were considered in each lentil field and data on incidence and severity of stemphylium blight were recorded from one square meter in each field. At the same time's diseased samples were collected in an air tied polyethylene bag and placed in an ice box. A total of 330 (110x3) samples were collected for laboratory study.

3.1.3. Data recording of disease incidence, severity and disease reaction of stemphylium blight

Diseases Incidence: The percent disease incidence of lentil was calculated by the following formula:

$$\text{Incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants counted}} \text{X } 100$$

Disease severity scale: Disease severity data was recorded described below on the basis of 0-5 scoring scale adopted from Bakr *et al*. (2000).

0 = no infection

1= few scattered leaf infection but no twig blighted

2 = 5-10% leaflets infected and/or few scattered twigs blighted

3 = 11-20% leaflets infected and/or 1-5% twigs blighted

4 = 21-50% leaflets infected and/or 6-10% twigs blighted

5 = ≧ 51% leaflets infected and/or ≧11% twigs blighted.

Disease reaction:

0 =Highly Resistant=HR

1= Resistant=R

2= Moderately Resistant=MR

3= Moderately Susceptible=MS

4= Susceptible =S

5= Highly Susceptible=HS

0 **1** **2** **3** **4** **5**

Plate 3.1.1: Chronological photography of diseases infected lentil plants as 0-5 scoring scale by *Stemphylium botryosum*

3.1.4. Laboratory study

Collected diseased leaf samples were investigated at the Plant Pathology Laboratory of Regional Agricultural Research Station, Bangladesh Agricultural Research Institute (BARI), Rahmatpur, Barisal for confirmation of stemphylium blight of lentil caused by *Stemphylium botryosum*. A glass slide was prepared directly from infected lentil leaflet i.e. diseased specimen from freshly collected materials and scraped with the help of a sharp blade and needle. The glass slide was mounted in a drop of glycerin and covered with a cover slip and examined under compound microscope. *S. botryosum* and other fungus were recorded under compound microscope.

3.1.5. Statistical analysis of data

The collected data from the study were analyzed statistically by using MSTAT-C computer package program. Mean comparisons for treatment parameters were compared using Duncan's Multiple Range Test (DMRT) at 5% or 1% level of significance.

3.2. Experiment-II: Isolation, identification and morphology of *Stemphylium botryosum* in lentil (2013)

3.2.1. Experimental site

The experiment was conducted at Plant Pathology Division, Regional Agricultural Research Station (RARS), Bangladesh Agricultural Research Institute (BARI), Rahmatpur, Barisal, Bangladesh.

3.2.2. Preparation of culture media

V-6 juice agar media was used in this experiment for culture the *S. botryosum*. V-6 juice agar media was made by extract of six vegetables that media

basically modified of V-8 juice agar media. List of the used materials for preparation of V-6 media were presented in Table 3.2.1.

Procedure:

1. Equal amount (34 g) of six vegetable i.e. 200 g vegetable was blended with blender machine.
2. Extract of six vegetable was made by boiling blended vegetable in water. Sieving the extract with mark in cloth.
3. Then the extract was taken in a conical flask (1000 ml) and adjusted the volume up to 1000ml by adding distilled water.
4. 20 g dextrose and 20 g agar were dissolved in the extract.
5. In order to overcome the problem of overgrowth by other fast growing fungi, Rose Bengal 0.3 g was added as a growth inhibitor that can slowdown the radial growth of the fungal colony.
6. The extract was autoclaved at 121^0C under15 psi for 30 minutes. After autoclaving the medium was kept few minutes for cooling. Then 20 ml medium was poured into each sterile petridish for use.

Table 3.2.1: List of the used materials for preparation of V-6 media

Name of media	Composition for one liter	
	Ingredient	Amount (g)
V-6 juice agar	Tomato	34
	Carrot	34
	Potato	34
	Lettuce leaves	34
	Cabbages leaves	34
	Indian Spinach	34
	Dextrose	20
	Agar	20
	Rose Bengal	0.3

3.2.3. Isolation of the pathogen

S. botryosum was isolated by tissue planting methods from diseased samples. Then the samples were cut into small pieces (0.5-1.0cm). Infected leaflets were surface sterilized with 1% sodium hypochlorite solutions for two minute and then rinsed in sterile distilled water for three times. After washing, cut pieces were transferred to the surface of the medium aseptically. The inoculated petri dishes were then incubated at 25^0C under 12 hr darkness alternate with 12 hr florescence light or near ultra-violate light (NUV light) for 10 days for growing or sporulating the pathogen.

3.2.4. Purification and Identification of the pathogen

Purification was carried out using single spore isolation technique (Toussoun and Nelson, 1976). Spore suspension of 10 day old culture was prepared in the test tube (15x1cm) containing 5 ml of sterilized water. The spore density in suspension was observed under compound microscope taking a drop of suspension on a glass slide. The spore density was adjusted with the addition of sterile water to get 1-5 spores per microscopic field (10x). One drop of the suspension was then poured on water agar medium (2%) in petri dishes and spread over the medium thoroughly with a sterilized needle and the petri dishes were then incubated for 16-20 hr at 25 ± 1^0C and allowed the spores to germinate. A germinated spore was then picked up under a microscope and transferred to a slant culture in the tube and incubated for 10 days at 25 ± 1^0C under 12 hr darkness alternate with 12 hr florescence light or near ultra-violate light (NUV light) for 10 days for growth and sporulation. The pure culture of the pathogen thus obtained was then preserved at 5^0C in the refrigerator. For identification, conidia were taken from mature colonies and examined for size, shape and color. Details of the cultural characters and microscopic details were noted and the fungus was identified following Ellis (1971).

3.2.5. Pathogenicity test of Stemphylium blight of lentil

Pathogenicity test of Stemphylium blight of lentil was followed by Koch's Postulates (Agrios, 2005).

1. The suspected causal agent i.e. *Stemphylium botryosum* was presented in every diseased lentil plant examined.

2. The suspected causal agent i.e. *S. botryosum* was isolated from the diseased lentil plant and grown in pure culture.

3. When a pure culture of *S. botryosum* is inoculated into a healthy lentil host, the host plant reproduces the specific disease symptom.

4. The same causal agent i.e. *S. botryosum* was recovered again from the experimentally inoculated and infected lentil plant. The recovered pathogen had the same characteristics as the lentil plant in step 2.

3.3. Experiment-III: Searching of the time of first appearance of stemphylium blight in lentil (2013-2014)

3.3.1. Experimental site

The experiment was conducted at the research field of Plant Pathology Division, Regional Agricultural Research Station (RARS), Bangladesh Agricultural Research Institute, Rahmatpur, Barisal.

3.3.2. Crop variety

A susceptible variety BARI Masur-1 and registrant variety BARI Masur-7 were used in this investigation.

3.3.3. 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 (FRG, 2012).

3.3.4. Experimental design

The experiment was conducted in Randomized Complete Block Design (RCBD) two factors with three replications. The unit plot size was 5m x 3m with spacing 30cm x 10cm. Seeds were sown at the rate of 30 Kg/ha on 7 different sowing dates were given below:

T_1=25-10-2013
T_2=01-11-2013
T_3=08-11-2013
T_4=15-11-2013
T_5=22-11-2013
T_6=29-11-2013
T_7=06-12-2013

Intercultural operations were done as per needed and to maintain the hygienic condition of crop in the field.

3.3.5. Data recorded

Initiation of first disease symptom on plant was observed minutely from seedling to flowering stages. Infected plants (5) were tagged in each plot for data recording i.e. on days to first flowering, days to first diseases record and name of the growth stage for first disease record. Diseases incidence and diseases severity were recorded following the same procedure as of the experiment-I (**3.1.3.**)

3.3.6. Statistical analysis of data

The collected data were analyzed statistically following the same procedure as of the experiment-I (**3.1.5.**)

3.4. Experiment-IV: Searching of the alternate host of *Stemphylium* spp. in different weed species in lentil field (2012-2013)

3.4.1. Investigation location

Sources of alternate host of *Stemphylium* spp. were investigated in the field during the surveyed period of 2012-2013 as follow as experiment-I (**3.1**). A total of 11 lentil growing areas i.e. Barisal, Madaripur, Jhalokathi, Khulna, Satkhira, Maherpur, Jessor, Kushtia, Faridpur, Pabna and Rajshahi were included in this study.

3.4.2. Collection of the diseased samples

Different type of weeds grown in the lentil fields in surveyed area was concentrated to infection of *S. botryosum*. When any leaf spot symptom developed in any weed species grown in the lentil fields were collected in the air tied polyethylene bag and then each polythene bag were taken to the Plant Pathology laboratory.

3.4.3. Isolation of the pathogen

The presence of *S. botryosum* was confirmed by preparing temporary slides and examined under the compound microscope. Preparing a slide directly from infected weed i.e. diseased specimen as scraped with the help of a sharp blade & needle and directly mounted in a drop of glycerin or in a drop of water on a clean glass slide and covered with a cover glass and which was then examined under compound microscope. If the sample infected by *S. botryosum*, conidia was found clearly under compound microscope.

S. botryosum was isolated by tissue planting methods from diseased samples. Then the samples were cut into small pieces (0.5-1.0cm). Infected leaves of weeds were surface sterilized with 1% sodium hypochlorite solutions for two

minutes and then rinsed in sterile distilled water for three times. After washing, cut pieces were transferred to the surface of the medium aseptically. The inoculated petri dishes were then incubated at 25 ± 1^0C under 12 hr darkness alternate with 12 hr florescence light or near ultra-violate light (NUV light) for 10 days for growth and sporulation.

3.4.4. Preparation of culture media

V-6 media was used in this experiment as of the experiment-II (**3.2.2**)**.**

3.4.5. Purification and identification of the pathogen

Purification and identification was done as of the experiment-II (**3.2.4).**

3.4.6. Pathogenicity test of stemphylium blight of Bathua

Pathogenicity test was done as of the experiment-II (**3.2.5**).

3.5. Experiment-V: Screening of lines for resistant to stemphylium blight of lentil (2011-2014)

This experiment was conducted three subsequent years of 2011-12, 2012-13 and 2013-14. In the first year and second year different lines/varieties were screened in the field under artificial inoculated condition at Barisal. In the third year selected lines/varieties were screened out under natural epiphytotic condition at Barisal and Rajshahi.

3.5.A. Screening of 214 lines/varieties for resistant to stemphylium blight of lentil in 2011-12 (1st year)

3.5.A.1. Experimental site

The experiment was conducted at the research field of Plant Pathology Division, Regional Agricultural Research Station (RARS), Bangladesh Agricultural Research Institute, Rahmatpur, Barisal.

3.5.A.2. Collection of seeds

A total of 200 lentil lines were collected from Plant Genetic Resource Centre (PGRC), Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur, 10 lines were collected from International Centre for Agricultural Research in the Dry Areas (ICARDA), Syria and 4 check varieties (BARI Masur-1, BARI Masur-4, BARI Masur-6, BARI Masur-7) were collected from Pulses Research Centre, Regional Agricultural Research Station, BARI, Ishurdi, Pabna for conducting this experiment.

3.5.A.3. Preservation of seeds

The collected seeds received from BARI, ICARDA and PRC were immediately stored in a well-ventilated room at room temperature. Special care was taken of the seeds and they were duly registered. After registration seeds were preserved in a refrigerator in the laboratory of RARS. Rahmatpur, Barisal till it were used for field experiments.

3.5.A.4. Preparation of working sample

The seeds were divided into two parts. One portion was used for the field experiments. Another portion preserved in the refrigerator for various experiments

Table 3.5.A.1: List of lentil lines/varieties collected from ICARDA, Syria and BARI, Gazipur

BD-3804	BD-3833	BD-3859	BD-3887	BD-3916	BD-3963	BD-4023	BD-4127
BD-3806	BD-3834	BD-3860	BD-3888	BD-3917	BD-3964	BD-4024	BD-4130
BD-3807	BD-3835	BD-3861	BD-3889	BD-3918	BD-3965	BD-4026	BD-4134
BD-3808	BD-3836	BD-3863	BD-3890	BD-3920	BD-3966	BD-4028	BD-5976
BD-3809	BD-3837	BD-3864	BD-3891	BD-3921	BD-3970	BD-4046	BD-5982
BD-3810	BD-3838	BD-3866	BD-3892	BD-3922	BD-3972	BD-4047	BD-5983
BD-3811	BD-3839	BD-3867	BD-3893	BD-3924	BD-3974	BD-4049	BD-5986
BD-3812	BD-3840	BD-3868	BD-3894	BD-3925	BD-3975	BD-4050	BD-5989
BD-3815	BD-3841	BD-3870	BD-3895	BD-3926	BD-3977	BD-4051	BD-5991
BD-3817	BD-3842	BD-3871	BD-3896	BD-3927	BD-3978	BD-4053	BD-5992
BD-3818	BD-3843	BD-3872	BD-3897	BD-3928	BD-3979	BD-4054	BD-5993
BD-3819	BD-3844	BD-3873	BD-3898	BD-3929	BD-3980	BD-4062	BD-5994
BD-3820	BD-3845	BD-3874	BD-3899	BD-3930	BD-3981	BD-4069	BD-5996
BD-3821	BD-3846	BD-3875	BD-3900	BD-3931	BD-3983	BD-4087	BD-5997
BD-3822	BD-3848	BD-3876	BD-3901	BD-3932	BD-3984	BD-4088	BD-5998
BD-3823	BD-3849	BD-3877	BD-3902	BD-3936	BD-3985	BD-4090	BD-6002
BD-3824	BD-3850	BD-3878	BD-3905	BD-3938	BD-3986	BD-4091	BD-6007
BD-3825	BD-3851	BD-3879	BD-3907	BD-3940	BD-3987	BD-4093	BD-6008
BD-3826	BD-3852	BD-3880	BD-3908	BD-3941	BD-3988	BD-4094	BD-6010
BD-3827	BD-3853	BD-3881	BD-3910	BD-3943	BD-3989	BD-4095	BD-6017
BD-3828	BD-3854	BD-3882	BD-3911	BD-3945	BD-3990	BD-4097	BD-6018
BD-3829	BD-3855	BD-3883	BD-3912	BD-3948	BD-3995	BD-4102	BD-6019
BD-3830	BD-3856	BD-3884	BD-3913	BD-3950	BD-4009	BD-4105	BD-6020
BD-3831	BD-3857	BD-3885	BD-3914	BD-3961	BD-4010	BD-4115	BD-6021
BD-3832	BD-3858	BD-3886	BD-3915	BD-3962	BD-4013	BD-4122	BD-6022
LIRL-22-1751-1-1-0		LIRL-22-211-1-1-1-0		LIRL-22-21-1-1-1-0		LIRL-22-172-1-1-1-0	
LIRL-22-156-1-1-1-0		LIRL-22-178-1-1-1-0		LIRL-22-36-1-1-1-0		LIRL-22-51-1-1-1-0	
LIRL-22-158-1-1-1-0		LIRL-22-54-1-1-1-0					
BARI Masur-1		BARI Masur-4		BARI Masur-6		BARI Masur-7	

.3.5.A.5. Experimental design

The experiment was laid out in Randomized Complete Block Design (RCBD) one factor with two replications, due to fewer amounts of collected seeds and considering volume of experimental size. The line to line distance was maintained 30 cm and plant to plant spacing 10 cm. After every ten lines, four check varieties (BARI Masur-1, BARI Masur-4, BARI Masur-6 and BARI Masur-7) were sown.

3.5.A.6. Land preparation and sowing of seeds

The experimental plot was nicely prepared mechanically in early November 2011. Weeds and other rubbishes were removed from the field. Fertilizes were applied at the time of final land preparation as per recommendation (FRG, 2009).

Before sowing of lentil seeds furrows were made with hand plough. The required amounts of seeds for each plot (i.e. single row) were taken in polyethylene bags and seeds are broadcasted in the furrows immediately. Seeds were sown on 26 November 2011. The furrows were covered with soil soon after sowing. The crop was harvested within March 12 but different lines harvested in different times.

3.5.A.7. 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.

3.5.A.8. Data recorded

Data were recorded on diseases incidence and diseases severity and yield (Kg/ha). Disease data and grain yield was recorded from the whole plot (i.e. single row). Diseases incidence and diseases severity were recorded following the same procedure as done in experiment-I (**3.1.3.**)

3.5.A.9. Statistical analysis of data

The collected data were analyzed statistically following the same procedure as of the experiment-I (**3.1.5.**)

3.5.B. Screening of selected 24 lines/varieties for resistant to stemphylium blight of lentil in 2012-13 (2nd year)

3.5.B.1. Experimental site

The experiment was conducted at the research field of Plant Pathology Division, Regional Agricultural Research Station (RARS), Bangladesh Agricultural Research Institute, Rahmatpur, Barisal.

3.5.B.2. Collection of seeds

Selected 22 entries of lentil (previously selected from 210 lines in the 1st year) and two check variety BARI Masur-1 and BARI Masur-7 were used in this experiment.

3.5.B.3. Experimental design

The experiment was laid out in Randomized Complete Block Design (RCBD) one factor with three replications. The line to line distance was maintained 30 cm and plant to plant spacing 10 cm. After every eleven lines, two check varieties (BARI Masur-1 and BARI Masur-7) were sown.

3.5.B.4. Land preparation and sowing of seeds

The experimental plot was nicely prepared mechanically in early November 2012. Weeds and other rubbishes were removed. Fertilizes were applied at the time of final land preparation as per recommendation (FRG, 2012).

Before sowing of lentil seeds furrows were made with hand plough. The required amounts of seeds for each plot (i.e. single row) were taken in polyethylene bags and seeds are broadcasted in the furrows immediately. Seeds were sown on 28 November 2012. The furrows were covered with soil soon after sowing. The crop was harvested within 14 March 2013 but different lines harvested in different times.

3.5.B.5. Intercultural operation

Intercultural operation was done as of the experiment-III (**3.5.A.7.**).

3.5.B.6. Data recorded

After completion of the sowing, the experiment was kept under constant watch and from sowing up to harvest. Data were recorded on Days to 1st flowering, Days to 50% flowering, Days to maturity, Plant height (cm), No. of branch/plant, No. of pod/plant, No. of seed/pod, 100 seed weight (g), Yield (kg/h).Diseases incidence and diseases severity were recorded at three different growth stages (Flowering, pod setting and Pre-maturity). Data on yield contributing characters were recorded from 10 randomly selected plants of each plot (i.e. single row). Disease data and grain yield was recorded from the whole plot (i.e. single row). Diseases incidence and diseases severity were recorded following the same procedure as done in experiment-I (**3.1.3.**)

3.5.B.7. Statistical analysis of data

The collected data were analyzed statistically following the same procedure as of the experiment-I (**3.1.5.**)

3.5.C. Screening of 5 lines/varieties for resistance to stemphylium blight of lentil in 2013-14 (3rd year)

3.5.C.1. Experimental site

The experiment was conducted at two locations. One was at the research field of Plant Pathology Division, RARS, BARI, Rahmatpur, Barisal and another one was Fruit Research Station, BARI, Binodpur, Rajshahi during the period of winter season 2013-14.

3.5.C.2. Collection of seeds

Three entries of lentil (previously selected from 22 lines in the 2nd year) and two check varieties BARI Masur-1 and BARI Masur-7 were evaluated. Two check variety like BARI Masur-1 (susceptible check) and BARI Masur-7 (registrants check) were collected from Pulses Research Centre, Regional Agricultural Research Station, BARI, Ishurdi, Pabna for conducting this experiment.

3.5.C.3. Experimental design

The experiment was laid out in Randomized Complete Block Design (RCBD) one factor with three replications. The field was divided into three blocks (replication). Each block was divided into five experimental units. The size of each experimental unit was 4m×2.4m. Three lines (BD-3926, BD-6002 and BD-3837) and two check varieties (BARI Masur-1 and BARI Masur-7) were used as treatment. The treatments were assigned in each block at random. Line to line distance was maintained 30 cm and plant to plant spacing 10 cm.

3.5.C.4. Land preparation and sowing of seeds

Two experimental plots were nicely prepared mechanically, first one in early November 2013 and second one in early November 2013. Weeds and other rubbishes were removed. Fertilizes were applied at the time of final land preparation as per recommendation (FRG, 2012).

Before sowing of lentil seeds furrows were made with hand plough. The required amounts of seeds for each plot were taken in polyethylene bags and seeds are broadcasted in the furrows immediately. Seeds were sown on 22 November 2013 at Barisal and on 12 November 2013 at Rajshahi. The furrows were covered with soil soon after sowing. The crop was harvested within 1-7 March 2014.

3.5.C.5. Intercultural operation

Intercultural operation was done as of the experiment-III (**3.5.A.7.**).

3.5.C.6. Data recorded

Data were recorded on Days to 1st flowering, Days to 50% flowering, Plant height (cm), No. of branches/plant, No. of pods/plant, 100 seed weight (g), Yield (kg/h). Diseases incidence and diseases severity were recorded at pre-maturity stage. Data on yield contributing characters were recorded from 10 randomly selected plants of each plot. Disease data and grain yield was recorded from the whole plot. Diseases incidence and diseases severity were recorded following the same procedure as done in experiment-I (**3.1.3.**)

3.5.C.7. Statistical analysis of data

The collected data were analyzed statistically following the same procedure as of the experiment-I (**3.1.5.**)

3.6. Experiment-VI: Effect of different dates of sowing and varieties on stemphylium blight of lentil (2012-2013)

3.6.1. Experimental site

The experiment was conducted at the research field of Plant Pathology Division, Regional Agricultural Research Station (RARS), Bangladesh Agricultural Research Institute, Rahmatpur, Barisal.

3.6.2. Crop variety

All varieties of lentil are not equally susceptible to the stemphylium blight disease. Therefore, comparatively more susceptible variety BARI Masur-1 and moderately registrant variety BARI Masur-7 were used in this investigation.

3.6.3. Land Preparation

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

3.6.4. Experimental design

The experiment was conducted in Randomized Complete Block Design (RCBD) two factors with three replications. The unit plot size was 5m x 3m with spacing 30cm x 10cm. Seven different dates were:

T_1=25-10-2012
T_2=01-11-2012
T_3=08-11-2012
T_4=15-11-2012
T_5=22-11-2012
T_6=29-11-2012
T_7=06-12-2012

3.6.5. Seed rate

Seeds were sown at the rate of 30 Kg/ha.

3.6.6. Intercultural operation

Intercultural operation was done as of the experiment-V (**3.5.A.7.**).

3.6.7. Data recorded

After completion of the sowing, the experiment was kept under constant watch and from sowing up to harvest. Data were recorded on days to first flowering, days to maturity, plant height (cm), no. of branches/plant, no. of pods/plant, yield (Kg/h), diseases incidence and diseases severity. Diseases incidence and diseases severity were recorded following the same procedure as done in experiment-I (**3.1.3.**)

3.6.8. Statistical analysis of data

The collected data were analyzed statistically following the same procedure as of the experiment-I (**3.1.5.**)

3.7. Experiment-VII: Efficacy of different fungicides in controlling stemphylium blight of lentil (2013-2014)

3.7.1. Experimental site

The experiment was laid out during 2013-14 cropping season at two locations. First one was laid out at the research field of Plant Pathology Division, Regional Agricultural Research Station, Bangladesh Agricultural Research Institute (BARI), Rahmatpur, Barisal and second one was laid out at the research field of Plant Pathology Division, Fruit Research Station, BARI, Binodpur, Rajshahi.

3.7.2. 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 experiment.

3.7.3. Land preparation and seed sowing

The experimental plots were nicely prepared mechanically in early November 2013 and early November 2014. The soil was prepared into good tilth by cross plough followed by laddering. The soil of the field was leveled before seed sowing. Weeds and other rubbishes were removed from the experimental field. Fertilizes were applied at the time of final land preparation as per recommendation (FRG, 2012).

Before sowing of lentil seeds furrows were made with hand plough. 30cm distance was maintained between the furrows. In Barisal experiment, seeds were sown at 15 November 2013 and in Rajshahi experiment, seeds were sown at 12 November 2013. The required amounts of seeds for each plot were taken in polyethylene bags and seeds are broadcasted at a rate of 30 Kg/ha in the furrows immediately. The furrows were covered with soil soon after sowing.

3.7.4. Experimental design

The experiment was conducted in Randomized Complete Block Design (RCBD) one factor with three replications. The field was divided into three blocks (replication). Each block was divided into six experimental units. The size of each experimental unit was 4m×2.4m. The treatments were assigned in each block at randomly. Five fungicides were used as treatment with one control (Table 3.7.1.).

There were 5 treatments. The treatments are as follows:

T_1 =Rovral (0.2%)
T_2 =Companion (0.2%)
T_3 =Nativo (0.05%)
T_4 =Amister Top (0.1%)
T_5 =Secure (0.2%)
T_6 =Control

Table 3.7.1. List of fungicides with active ingredients, mode of action, name of company and dose used as a foliar spray

Sl. No.	Trade name	Active ingredients	Mode of action	Name of company	Dose
1	Rovral 50WP	Iprodione	Contact	Auto Crop Care Ltd.	0.2%
2	Compenion	Carbendazim12% +Mancozeb 63% (w/w)	Contact +Systemic	Auto Crop Care Ltd.	0.2%
3	Nativo 75WG	Tebuconazole 50% +Trifloxystrobin25% (w/w)	Contact +Systemic	Bayer Crop Science Ltd.	0.05%
4	Amistar Top 325 SC	Azoxystrobin20% + Difenoconazole12.5%	Systemic	Syngenta BD Ltd.	0.1%
5	Secure 600WG	Fenamidone 10% + Mancozeb 50% (w/w)	Contact +Systemic	Bayer Crop Science Ltd.	0.2%

3.7.5. Fungicides spray

Spray solution of each fungicide was prepared respectively in non-metal containers. Spraying was done with the help of a hand sprayer. Two liters of spray solution was sprayed in each experiment unit. Spraying was started at 50 days after sowing (DAS) and continued up to three sprays at 12 days interval.

3.7.6. Intercultural operation

Intercultural operation was done as of the experiment-III (**3.5.A.7.**).

3.7.7. Data recorded

Data were recorded on 100 seed weight (g), plant height (cm), no. of pod/ plant, no. of branch/ plant, yield (kg/h), diseases incidence and diseases severity. Diseases incidence and diseases severity were recorded following the same procedure as done in experiment-I (**3.1.3.**).

3.7.8. Statistical analysis of data

The collected data were analyzed statistically following the same procedure as of the experiment-I (**3.1.5.**).

3.8. Experiment-VIII: Efficacy of different fungicides with foliar spray and seed treatment in controlling stemphylium blight of lentil (2014-2015)

3.8.1. Experimental site

The experiment was conducted at the research field of Plant Pathology Division, Fruit Research Station, BARI, Binodpur, Rajshahi.

3.8.2. 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.

3.8.3. Land Preparation

The experimental plots were nicely prepared mechanically in early November 2014. The soil was prepared into good tilth by cross plough followed by laddering. The soil of the field was leveled before seed sowing. Weeds and other rubbishes were removed. Fertilizes were applied at the time of final land preparation as per recommendation (FRG, 2012). Before sowing of lentil seeds

furrows were made with hand plough. 30cm distance was maintained between the furrows.

3.8.4. Seed sowing

Seeds were sown at 12 November 2014. The required amounts of seeds for each plot were taken in polyethylene bags and seeds are broadcasted at a rate of 30 Kg/ha in the furrows immediately. The furrows were covered with soil soon after sowing.

3.8.5. Experimental design

The experiment was conducted in Randomized Complete Block Design (RCBD) one factor with three replications. The field was divided into three blocks (replication). Each block was divided into four experimental units. The size of each experimental unit was 4m×2.4m. The treatments were assigned in each block at randomly. One fungicide (Table 3.8.1.) used as a foliar spray and another one used as a seed treatment. Four treatment combinations were used in the experiment as follows:

T_1 =Seed treatment with Provax + Foliar spray with Rovral

T_2 =Seed treatment with Bavistin + Foliar spray with Rovral

T_3 =No seed treatment +Foliar spray with Rovral

T_4 =No seed treatment +No foliar spray with Rovral

3.7.6. Fungicides spray and intercultural operation

Spray solution of each fungicide was prepared respectively in non-metal containers. Spraying was done with the help of a hand sprayer. Two liters of spray solution was sprayed in each experiment unit. Spraying was started at 50 days after sowing (DAS) and continued up to three sprays at 12 days interval.

Table 3.8.1: List of fungicides with active ingredients, mode of action, name of company and doses

Sl. No.	Trade name	Active ingredients	Mode of action	Name of the Company	Dose	Use of Fungicides
1	Rovral 50WP	Iprodione	Contact	Auto Crop Care Ltd.	0.2%	used as a foliar spray
2	Provax 200WP	Carboxin 37.50% +Thirum37.50%	Contact +Systemic	Hossain Enterprise C.C. Ltd.	0.25%	used as a seed treatments
3	Bavistin DF	Carbendazim 50%	Systemic	Auto Crop Care Ltd.	0.2%	

3.7.7. Intercultural operation

Intercultural operation was done as of the experiment-III (**3.5.A.7.**).

3.7.8. Data recorded

After completion of the seed sowing, the experiment was kept under constant watch and from sowing up to harvest. Data were recorded on yield (kg/h), diseases incidence and diseases severity. Diseases incidence and diseases severity were recorded following the same procedure as done in experiment-I (**3.1.3.**).

3.7.9. Statistical analysis of data

The collected data were analyzed statistically following the same procedure as of the experiment-I (**3.1.5.**)

CHAPTER 4

RESULTS

A total of 8 experiments were conducted during October 2011 to March 2015. Experiment wise results are described below:

4.1. Experiment-I: Survey of stemphylium blight of lentil at farmer's field indifferent lentil growing areas of Bangladesh (2012-13)

4.1.1. Survey of stemphylium blight in some selected areas of Bangladesh

A survey was conducted to assess the percent disease incidence and severity of stemphylium blight of lentil at 11 lentil growing districts of Bangladesh during cropping season of 2012-13. The incidence and severity were calculated and presented in Table 4.1.1. The incidence and severity were varied from location to locations. The disease incidence (%) ranged from 45.50-77.90. Highest disease incidence was found in Jhalokathi (Sadar) (77.90%) followed by Faridpur (Sadar) (71.60%), Khulna (Paikgasa) (70.60%) and Satkhira (Tala) (61.40%) which was statistically similar with each other. Lowest disease incidence was found in Pabna (Ishardi) (45.50%) followed by Barisal (Babuganj) (48.90%), Kushtia (Kumarkhali) (54.10%) and Rajshahi (Puthia) (58.00%). Disease severity data was recorded 0-5 scoring scale. Lowest disease severity was found Kushtia (Kumarkhali), Pabna (Ishardi) and Barisal (Babuganj) and the highest was found in Faridpur (Sadar), Jhalokathi (Sadar) and Khulna (Paikgasa). Out of 11 districts disease severity graded as 2, 3 and 4 scoring scale in the number of 3, 5 and 3 districts, respectively (Table 4.1.1.).

Table 4.1.1: Incidence and severity of stemphylium blight disease of lentil at 11 lentil growing districts of Bangladesh in 2012-13

Sl. No.	Name of Districts	Disease Incidence (%)	Disease Severity (0-5 scale)
1	Jessore (Sadar)	60.90 a-c	3
2	Kushtia (Kumarkhali)	54.10 a-c	2
3	Faridpur (Sadar)	71.60 ab	4
4	Pabna (Ishardi)	45.50 c	2
5	Rajshahi (Puthia)	58.00 a-c	3
6	Maherpur (Gangni)	58.10 a-c	3
7	Madaripur (Sadar)	59.20 a-c	3
8	Barisal (Babuganj)	48.90 bc	2
9	Jhalokathi (Sadar)	77.90 a	4
10	Khulna (Paikgasa)	70.60 ab	4
11	Satkhira (Tala)	61.40 a-c	3
	Level of significance	*	-
	CV (%)	39.40	-

Means followed the same letter/letters do not statistically differ at 5% level tested by DMRT

* = Significant at 5% level of probability, ** = Significant at 1% level of probability, NS=Not significant

4.1.2 Survey of stemphylium blight on 5 varieties of lentil released by BARI

An observation was undertaken during rabi season of 2012-13 at 11 lentil growing districts of Bangladesh to know the assessment of disease incidence, severity and disease reaction of stemphylium blight disease of lentil. A wide variation was observed during the surveyed period (Table 4.1.2.). The highest disease incidence was found in local variety (74.33 %) followed by BARI Masur-3 (68.67%) and BARI Masur-4 (68.33%) which was not significantly difference with each other but significantly difference with BARI Masur-6 (41.00 %) and BARI Masur-7 (31.00 %). The lowest disease incidence was found in BARI Masur-7.

Disease severity data was recorded 0-5 scoring scale. Out of 6 varieties the lowest disease severity was observed in BARI Masur-5, BARI Masur-6 and BARI Masur-7 and the highest disease severity was observed in local variety. BARI Masur-3 and BARI Masur-4 showed 3 scoring scale in the farmers field. In case of disease reaction, local variety performed highly susceptible (HS), BARI Masur-3 and BARI Masur-4 showed moderately susceptible (MS) and BARI Masur-5, BARI Masur-6 and BARI Masur-7 showed moderately resistant (MR) disease reaction under field condition.

Table 4.1.2: Incidence and severity of stemphylium blight disease of different lentil varieties at 11 districts in 2012-13

Sl. No.	Name of Varieties	Disease Incidence (%)	Disease Severity (0-5 Scale)	Disease Reaction
1	BARI Masur-3	68.67 a	3	MS
2	BARI Masur-4	68.33 a	3	MS
3	BARI Masur-5	52.35 b	2	MR
4	BARI Masur-6	41.00 b	2	MR
5	BARI Masur-7	31.00 c	2	MR
6	Local variety	74.33 a	5	HS
	Level of significance	**	-	-
	CV (%)	18.15	-	-

Means followed the same letter/letters do not statistically differ at 1% level tested by DMRT

* = Significant at 5% level of probability, ** = Significant at 1% level of probability, NS=Not significant

4.1.3 Survey at different growth stages

During field survey disease incidence and severity was observed in different growth stages of lentil and found highly significantly difference within the growth stages. In most of the cases stemphylium blight disease was started at flowering stage. Lower amount of disease incidence (17%) was observed at flowering stage. Moderately disease incidence was recorded at pod setting

stage (54%) and severely incidence was recorded (76%) at pre-maturity stage. Same trend occurred in case of disease severity. Resistant (R) reaction was found in flowering stage, moderately resistant (MR) in pod setting stage and moderately susceptible (MS) in pre-maturity stage (4.1.3.).

Table 4.1.3: Incidence and severity of stemphylium blight disease of lentil at different growth stages in 11 districts in 2012-13

Sl. No.	Growth Stages	Disease Incidence (%)	Disease Severity (0-5 Scale)	Disease Reaction
1	Flowering	17 c	1	R
2	Pod setting	54 b	2	MR
3	Pre-Maturity	76 a	3	MS
	Level of significance	*	-	-
	CV (%)	22.15	-	-

Means followed the same letter/letters do not statistically differ at 5% level tested by DMRT

* = Significant at 5% level of probability, ** = Significant at 1% level of probability, NS=Not significant

4.1.4. Survey in relation to other diseases found in lentil field

A total of 330 diseased samples were collected from 110 fields from 11 districts. All samples were observed under compound microscope. Alternaria blight, rust (Plate 4.1.1), botrytis gray mold (BGM) (Plate 4.1.2) and leaf rot (Plate 4.2.3.d) were found as an associated with stemphylium blight infected lentil leaves.

4.1.5. Survey in relation to rust diseases

A total of 330 diseased samples were collected from 110 fields from 11 districts. A total of 110 fields were visited in 11 districts during survey period. In this study, out of 11 districts only 3 districts named Faridpur (sadar), Madaripur (sadar) and Khulna (Paikgasa) were infected by rust and rest of the districts were not found rust disease in any field. The highest 40% field was

infected by rust in Faridpur (sadar) district and followed by Madaripur (sadar) (15%) and Khulna (Paikgasa) (10%) (Fig. 4.1.1).

Plate 4.1.1: Lentil plant showing rust symptoms

Plate 4.1.2: Lentil plant showing BGM symptoms

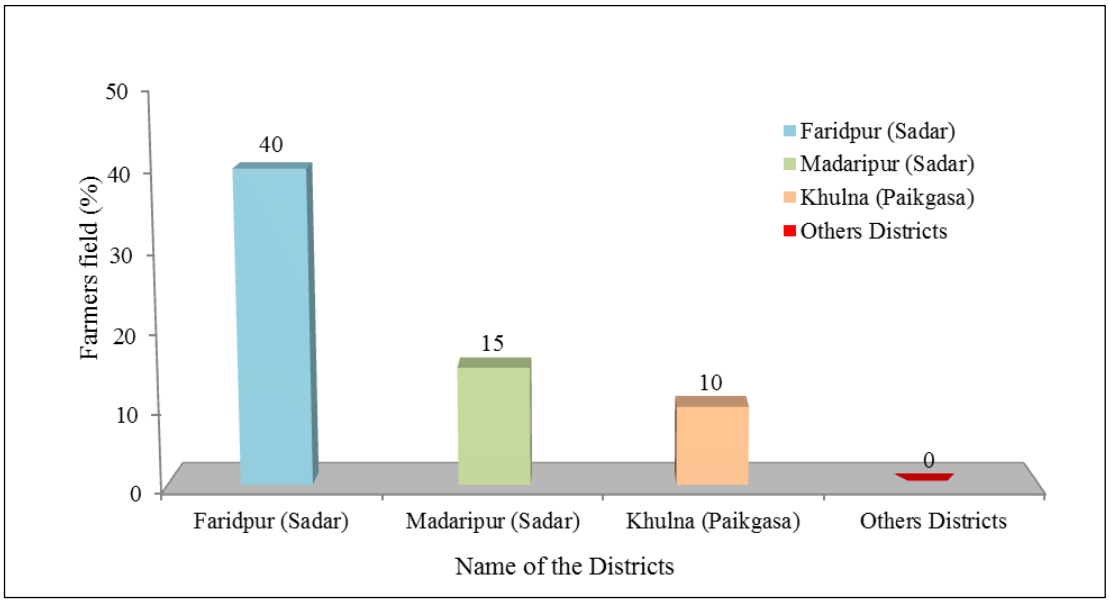


Fig. 4.1.1: Percentage of farmer's field regarding rust infection of lentil in 11 districts of Bangladesh in 2012-13

4.2. Experiment-II: Isolation, identification and morphology of *Stemphylium botryosum* in lentil (2013)

4.2.1. Symptoms of the disease

Stemphylium blight symptoms were started at flowering stage in most of the cases. Spore of *Stemphylium botryosum* landing and infected the leaflet and formed of small pinhead gray spots or light brown on the leaflets (Plate 4.2.1.a) which smaller lesions later irregularly enlarged, covering the surface of the leaflet within 2 to 3 days and killed single leaflet (Plate 4.2.1.b) or in presence of more inoculums more than one leaflet infected (Plate 4.2.1.c). The disease gradually increased by showing symptoms on shoots (Plate 4.2.1.d) and twigs which showed special type symptoms like fishing hook (Plate 4.2.1.e). Later rapidly spread over the leaf, shoots or twigs infection but pod remained green (Plate 4.2.2.a). The above symptoms clearly differ from other foliar lesions of lentil. These findings were recorded by frequently field visit with closed observation and collected samples were examined under microscope.

During favorable environment of the pathogen, plant became in severe condition, leaflet blighted and plant become defoliated leaving a few green leaves and some immature fruits (Plate 4.2.2.b). During susceptible condition of the disease within 7 to 10 days farmers' field might be possible to reach brownish color and looking just burn by fire (Plate 4.2.2.c). When plants were dense populated, infection occurred lower parts of the plants (Plate 4.2.2.d) but it should not confused with healthy mature plants at later growth stage (Plate 4.2.3.e). Stemphylium blight could not occur at seedling stages and should not confuse with foot rot occurring at seedling stages, foot rot shown brown or light yellow colored leaf looking stemphylium blight (Plate 4.2.3.a). Several symptoms collected from seedling stage and isolated following standard procedure but there was no *S. botryosum* conidia found. Some other symptoms were observed on lentil plant at different growth stages but after isolation it was confirmed that were not stemphylium blight (Plate 4.2.3.b, c, d and e).

(a) Small pinhead gray spot

(b) Single leaflet infected

(c) More than one leaflet infected

(d) Infected shoot

(e) Twig infected

Plate 4.2.1: Stemphylium blight infected lentil plant showing different kind of symptoms

(a) Leaf infected but pod remain green

(b) Severely infected plants

(c) Severely infected farmers field

(d) Infected the lower parts of the plant

Plate 4.2.2: Stemphylium blight infected lentil plant showing different kind of symptoms

(a) Foot rot infected at seedling stage

(b) Nutrient deficiency

(c) Red color leaf

(d) Leaf rot

(e) Crop maturity stage

Plate 4.2.3: Lentil plant showing different kinds of symptoms but that were not infected by *Stemphylium botryosum*

4.2.2. Isolation of the pathogen

Conidia of *S. botryosum* were isolated directly from leaf by scraping with needle and then prepared slide and later observed under compound microscope. Without scraping and without prepared slide an infected leaflet of lentil were shown huge amount of conidia directly under microscope (Plate 4.2.4.b). After isolation conidia and conidiophore were shown in the plate 4.2.4.c, d and e. On the other hand *S. botryosum* were isolated by using V-6 media (modified of V-8 media) and found pure culture.

4.2.3. Effect of light

Pure cultures were found at 25^0C under 12hr darkness alternating with 12 hr light (Fluorescence light or Near Ultra Violate, NUV light) (Plate 4.2.5.). But pure culture also found without alternating darkness and light i.e. using continuous light or continuous darkness.

4.2.4. Morphology of the pathogen

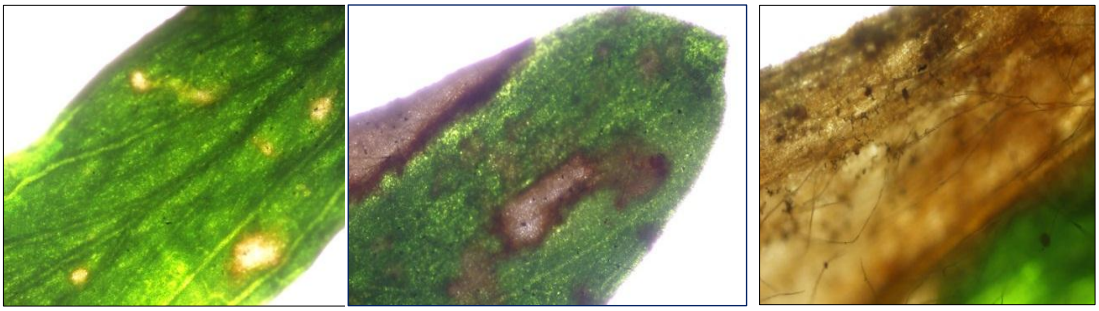
Conidia

Morphological characters were found under compound microscope:

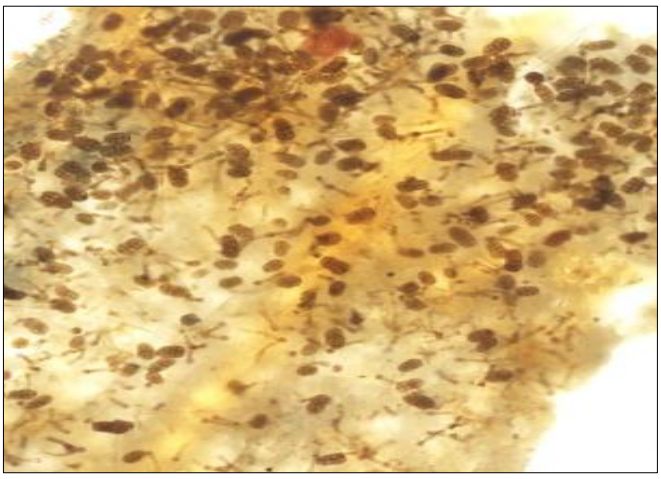
- Conidia oblong rounded at the ends, broadly ellipsoidal or sub-spherical, with usually 3 transverse and 1-3 longitudinal septa
- Constricted at the median transverse septum
- Pale to mid dark brown or olivaceous brown
- Minutely verrucos or echinulate and muriform
- Average size of conidia 18-28 × 11-17 μm
- Length to breadth ratio (L/B) was about 1.0 to 1.5

Conidiophores

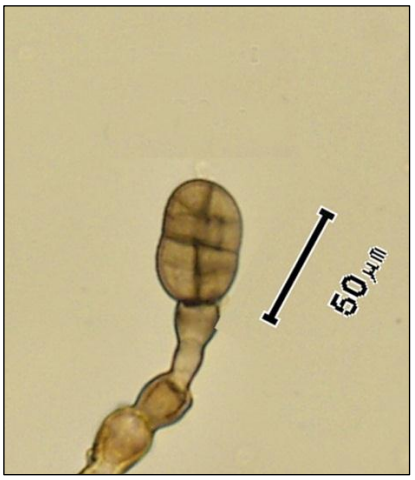
Conidiophores were brown with terminal swellings which become through percurrent proliferation intercalary 7-11μ diam, dark verrucose band a little way below the apex.



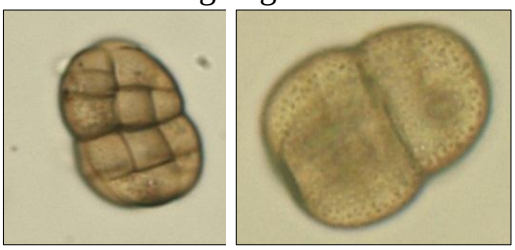
(a) dotted symptoms on leaflet with just little enlarged view under microscope



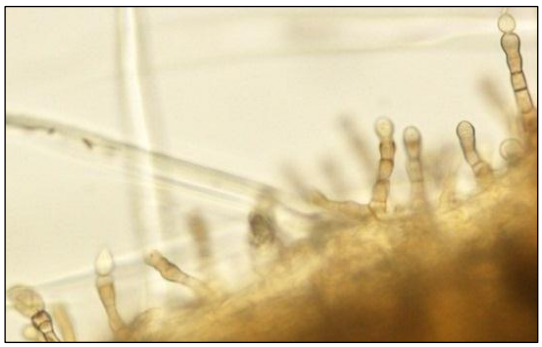
(b) enlarged view of leaflet under microscope showing huge amount of conidia



(c) Conidia with conidiophore



(d) Conidia with different view

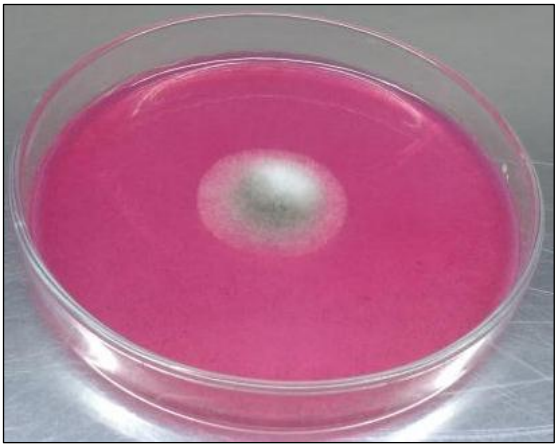


(e) Conidiophore

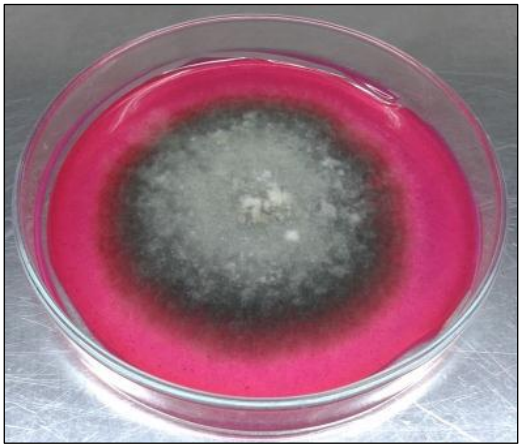
Plate 4.2.4: Enlarged view of lentil leaf and also showing conidia with conidiophore

Colony characteristics

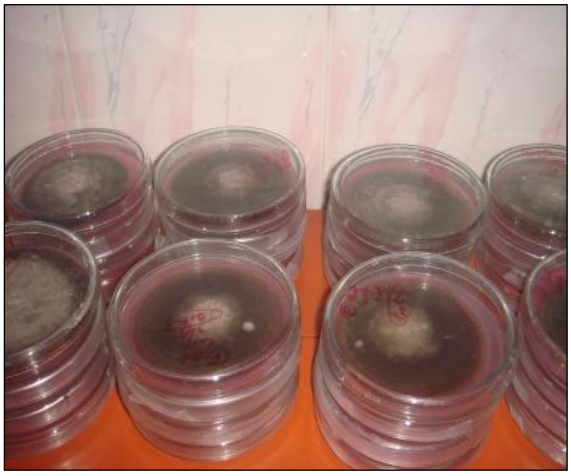
S. botryosum colonies were grown rapidly on V-6 media. The colonies were velvety to cottony in texture with a grey, brown or brownish-black color and black pigmentation on the colony.



(a) Pure culture on five days



(b) Pure culture on fifteen days



(c) Pure culture under fluorescence light

(d) Pure culture under NUV light

Plate 4.2.5: Different types of pure culture of *S. botryosum* from lentil

4.2.5. Pathogenicity test

Samples were collected from the stemphylium blight infected plants and isolation and purification were done following standard procedure. From the pure culture spore suspension were prepared and inoculated the healthy plant by using small spray machine. After few days same symptoms were developed

as like as previously collected lentil plant. Finally infected leaves were collected and isolated again from the infected leaves and found *S. botryosum* spores (Plate 4.2.6.).

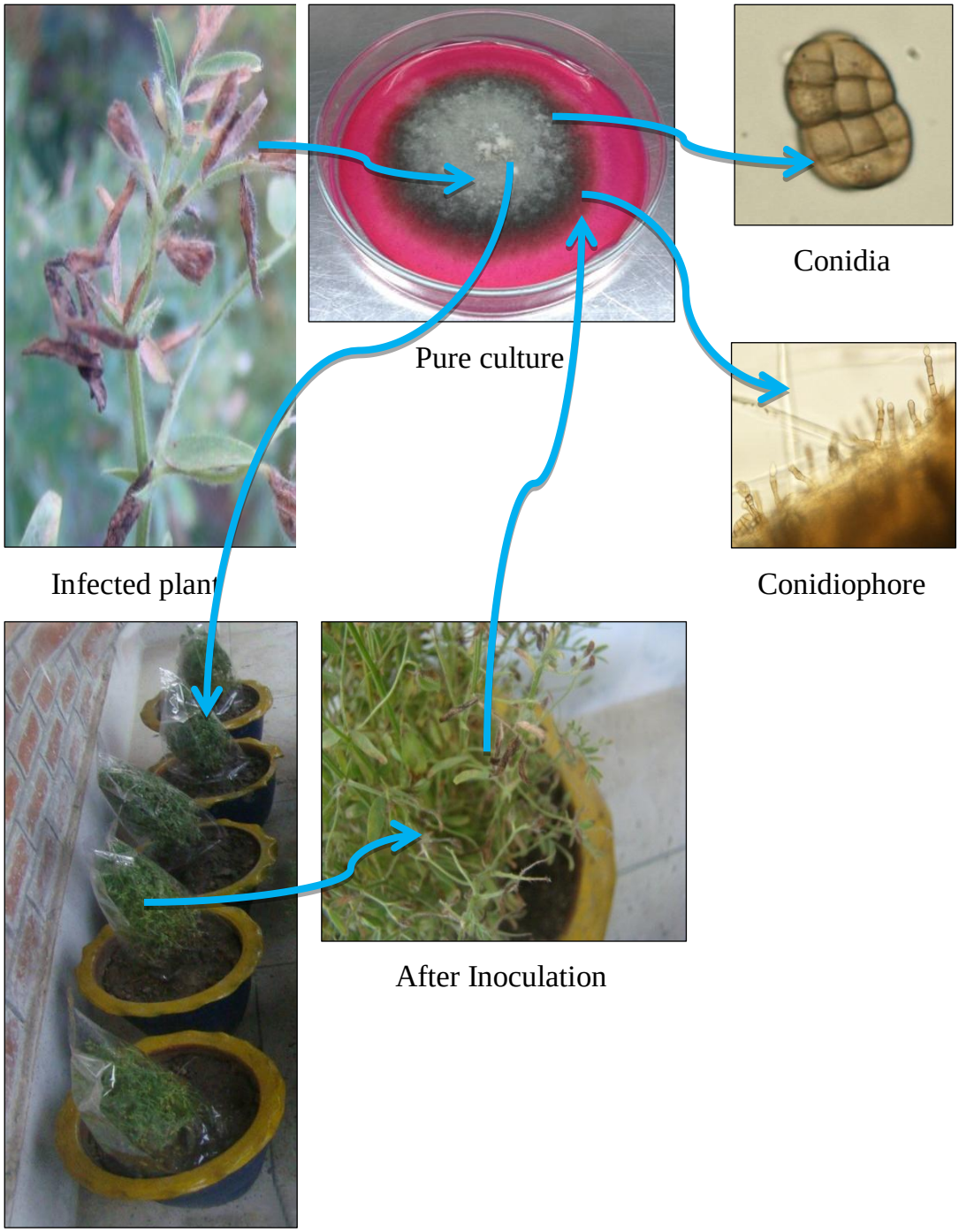


Plate 4.2.6: Koch's postulates for proof of pathogenicity

4.3. Experiment-III: Searching of the time of first appearance of stemphylium blight in lentil (2013-2014)

For successful management appropriate time of first appearance of the disease in the field should be known. From time to time field visit with close observation was done and found that growth stage of lentil was the major point for stemphylium blight diseases development, although favorable environmental condition including presence of inoculum might be essential. Out of 7 different dates of sowing stemphylium blight of lentil was searched minutely and recorded first appearance of the disease in the field at pre-flowering or flowering stages. Two lentil varieties named BARI Masur-1 (susceptible variety) and BARI Masur-7 (resistant variety) were investigated for searching the appropriate time of disease at Barisal, Bangladesh during 2013-14.

4.3.1. First disease recording in BARI Masur-1

In the present investigation, first flower initiation of BARI Masur-1 ranged from 44 to 52 days and appearance of first disease development ranged from 45 to 56 days after sowing (Table 4.3.1.).

In case of October 25 sowing, appearance of first disease was recorded 56 days after sowing but first flower initiation was found before four days i.e. 52 days after sowing. During appearance of first disease recorded in that sowing date the plants were flowering stage. Same trend was found November 01, 08, 29 and December 06 sowing plots.

In case of November 15 sowing, appearance of first disease was recorded 47 days after sowing but first flower initiation was found after two days i.e. 49 days after sowing. In case of November 22 sowing, appearance of first disease was recorded 45 days after sowing but first flower initiation was found after

one day i.e. 46 days after sowing. During appearance of first disease recorded in that sowing dates the plants were pre-flowering stage.

Table 4.3.1: Effect of sowing dates on1st stemphylium blight diseases initiation in lentil variety BARI Masur-1 in 2013-14

Sl. No.	Sowing dates	Days to first flowering	Days to first disease recording	Name of the growth stage for first disease recording
1	25-10-13	52 (16-12-2013)	56 (20-12-2013)	Flowering
2	01-11-13	50 (21-12-2013)	54 (24-12-2013)	Flowering
3	08-11-13	49 (27-12-2013)	55 (02-01-2013)	Flowering
4	15-11-13	49 (03-01-2014)	47 (01-01-2014)	Pre-Flowering
5	22-11-13	46 (07-01-2014)	45 (06-01-2014)	Pre-Flowering
6	29-11-13	44 (12-01-2014)	45 (13-01-2014)	Flowering
7	06-12-13	44 (19-01-2014)	46 (21-01-2014)	Flowering

4.3.2. First disease recording in BARI Masur-7

In the present investigation, first flower initiation of BARI Masur-7 ranged from 47 to 53 days and appearance of first disease development ranged from 47 to 57 days after sowing (Table 4.3.2). In case of October 25 sowing, appearance of first disease was recorded 57 days after sowing but first flower initiation was found before four days i.e. 53 days after sowing. During appearance of first disease recorded in that sowing date the plants were flowering stage. Same trend was found November 01, 08, 15, 29 and December 06 sowing plots. In case of November 22 sowing, appearance of first disease was recorded 47 days after sowing but first flower initiation was found after one day i.e. 48 days after sowing. During appearance of first disease recorded in that sowing dates the plants were pre-flowering stage.

Table 4.3.2: Effect of sowing dates on 1st stemphylium blight diseases initiation in lentil variety BARI Masur-7 in 2013-14

Sl. No.	Sowing dates	Days to first flowering	Days to first diseases recording	Name of the growth stage for first disease recording
1	25-10-13	53 (17-12-2013)	57 (21-12-2013)	Flowering
2	01-11-13	51 (22-12-2013)	56 (26-12-2013)	Flowering
3	08-11-13	52 (30-12-2013)	55 (02-01-2013)	Flowering
4	15-11-13	51 (05-01-2014)	55 (09-01-2014)	Flowering
5	22-11-13	48 (09-01-2014)	47 (08-01-2014)	Pre-Flowering
6	29-11-13	48 (16-01-2014)	49 (17-01-2014)	Flowering
7	06-12-13	47 (22-01-2014)	49 (24-01-2014)	Flowering

4.3.3. Incidence, disease severity and disease reaction

A wide variation of percent disease incidence and disease severity were measured at 7 different dates of sowing. Both disease incidence and severity were higher in susceptible variety BARI Masur-1 compare to resistant variety BARI Masur-7. In case of late sowing (November 29 and December 06) disease incidence and severity was recorded lower compare to other sowing dates (Fig. 4.3.1 and Fig. 4.3.2).

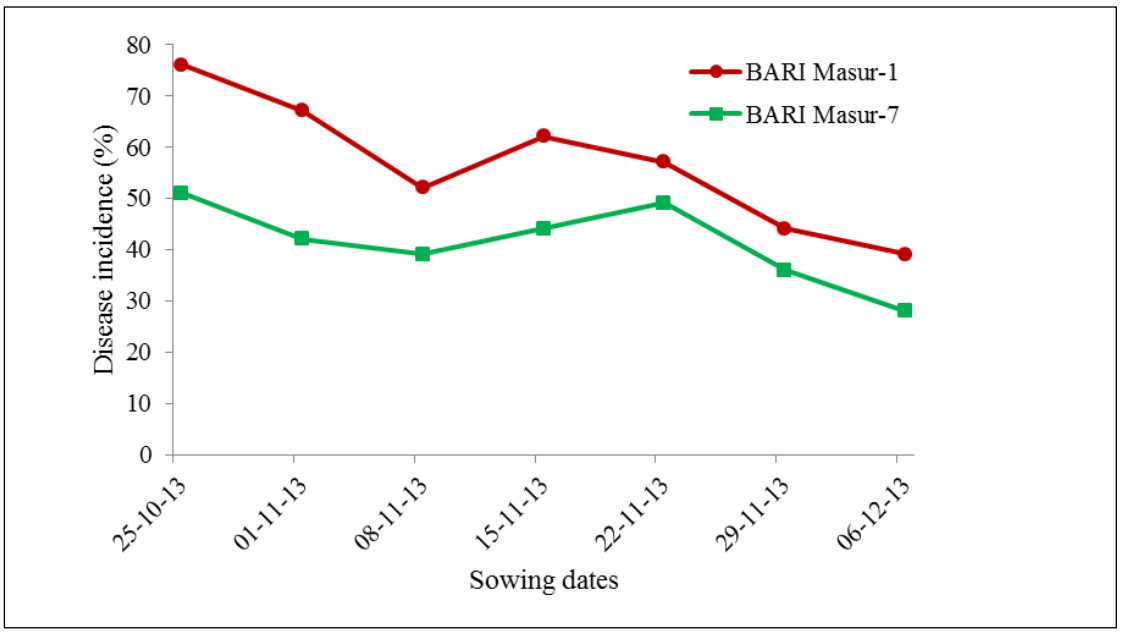


Fig. 4.3.1: Percent disease incidence of stemphylium blight in two varieties of lentil at different date of sowing in 2013-14

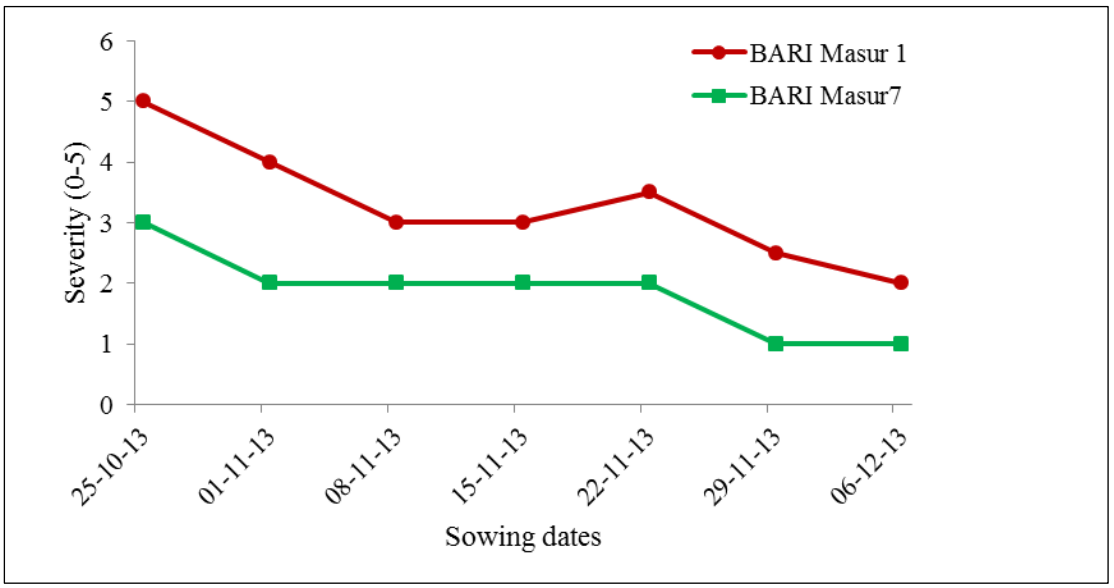


Fig. 4.3.2: Severity of stemphylium blight in two varieties of lentil at different date of sowing in 2013-14

In case of disease reaction in BARI Masur-1, HS, S, MS, MS, S, MS and MR were recorded at October 25 sowing to December 6. Highly susceptible (HS) disease reaction was found in October 25; susceptible (S) in November 01, 22;

moderately susceptible (MS) in November 08, 15 and moderately resistant (MR) in December 6. In case of BARI Masur-7, MS, MR, MR, MR, MR, R and R were recorded at October 25 sowing to December 6 (Table 4.3.3). Moderately susceptible (MS) disease reaction was found in October 25; moderately resistant (MR) in November 01, 08, 15, 22 and resistant (R) in November 29 and December 6.

Table 4.3.3: Diseases Reaction in two varieties of lentil at different dates of sowing

Sl. No.	Sowing dates	Diseases Reaction	
		BARI Masur-1	BARI Masur-7
1	25-10-13	HS	MS
2	01-11-13	S	MR
3	08-11-13	MS	MR
4	15-11-13	MS	MR
5	22-11-13	S	MR
6	29-11-13	MS	R
7	06-12-13	MR	R

4.4. Experiment-IV: Searching of the alternate host of *Stemphylium* spp. in different weed species in lentil field (2012-2013)

4.4.1. Different weed species

A total of 8 weed species *viz*. Bermuda grass, Goose grass, Crabgrass, Lambs quarter, Clammy ground cherry, Purple nut sedge, Smart weed and Wild lentil were recorded grown in lentil fields during survey period (2012-2013) at 11 districts. The weed species are listed in the table 4.4.1.

Table 4.4.1: List of different weed species grown in the lentil field in Bangladesh

Sl. No.	Common name	English name	Scientific name	Family
1	Durba	Bermuda grass	*Cynodon dactylon*	Poaceae
2	Chapra	Goose grass	*Eleusine indica*	Poaceae
3	Anguli	Crabgrass	*Digitaria sanguinalis*	Poaceae
4	Bathua	Lambs quarter	*Chenopodium album*	Chenopodiaceae
5	Foska begun	Clammy ground cherry	*Physalis heterophylla*	Solanaceae
6	Mutha	Purple nut sedge	*Cyperus rotundus*	Cyperacae
7	Bishkatali	Smart weed	*Polygonum hydropiper*	Polygonaceae
8	Ban masur	Wild lentil	*Vicia sativa*	Leguminosae

4.4.2. Isolation of the pathogen from weed

Conidium of *S. botryosum* was isolated from the collected leaf samples of Bathua weed (*Chenopodium album*). Other weed leaf spot samples were tested but *S. botryosum* was not found.

4.4.3. Pathogenicity test

Diseased leaf samples of Bathua were collected from lentil field. Healthy Bathua plants were grown in an earthen pot. Pathogenicity test was done at vegetative stage following as of the experiment-II (**4.2.5.**). Conidia of *S. botryosum* were found in the Bathua leaf (Plate 4.4.1.).

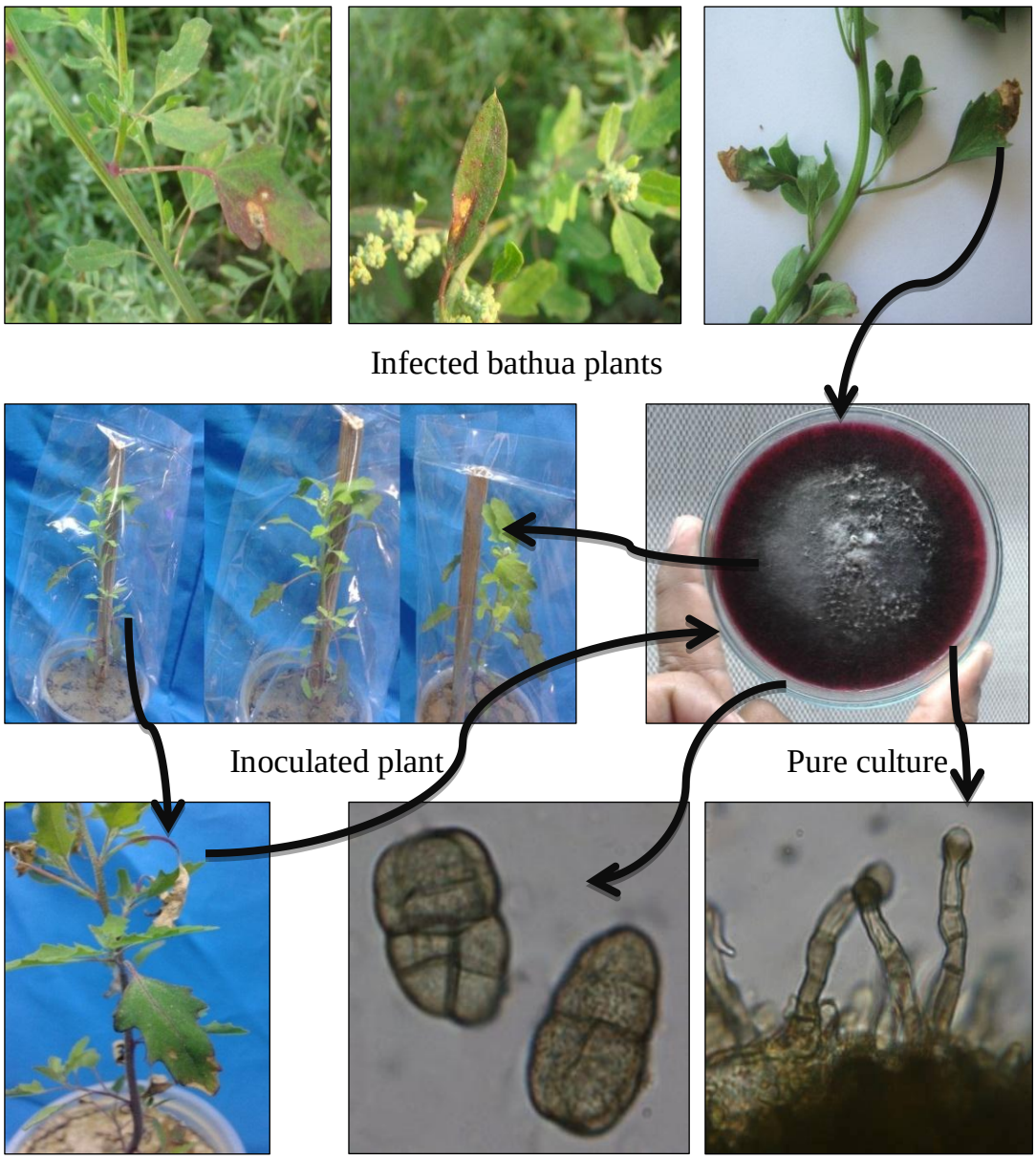


Plate 4.4.1: Koch's Postulates for proof of pathogenicity test

4.5. Experiment-V: Screening of lines for resistant to stemphylium blight of lentil (2011-2014)

This experiment was conducted three subsequent years of 2011-12, 2012-13 and 2013-14. In first year 214 lines/varieties were screened in the field under artificial inoculated condition and 22 lines were selected. In the second year out of 24 lines/varieties (22 lines and 2 check varieties) 3 lines were selected under artificial inoculated condition. In the third year, 5 lines/varieties (3 lines and 2 check varieties) were screened out and finally 2 lines were selected under natural epiphytotic condition at Barisal and Rajshahi.

4.5.A. Screening of 214 lines/varieties for resistant to stemphylium blight of lentil in 2011-12 (1st year)

A total of 210 lentil lines and four released varieties BARI Masur-1, BARI Masur-4, BARI Masur-6 and BARI Masur-7 were tested against stemphylium blight of lentil in Barisal during 2011-12.

4.5.A.1. Percent disease incidence

Percent disease incidences were recorded at three growth stages *viz*. flowering, pod setting and pre mature stage. Seven groups were categorized as guoup-1 (0% disease incidence), guoup-2 (1-10% disease incidence), guoup-3 (11-20% disease incidence), guoup-4 (21-30% disease incidence), guoup-5 (31-50% disease incidence), guoup-6 (51-70% disease incidence) and guoup-7 (71-100% disease incidence). At flowering stage out of 214 lines/ varieties 76, 133 and 5 lines were recorded as group-1, group-2, and group-3, respectively. At pod setting stage out of 214 lines/varieties 1, 3, 11, 26, 122 and 51 lines were recorded as group-2, group-3, group-4, group-5, group-6 and group-7, respectively. At pre-maturity stage out of 214 lines/varieties 17, 44 and 153 lines were recorded as group-5, group-6 and group-7, respectively (Table 4.5.A.1).

Table 4.5.A.1: Percent diseases incidence of stemphylium blight at three growth stages of different lentil lines/varieties

Number of Group	Range of diseases incidence (%)	No. of lines/varieties		
		Flowering stage	Pod setting stage	Pre-maturing stage
1	0	76	0	0
2	1-10	133	1	0
3	11-20	5	3	0
4	21-30	0	11	0
5	31-50	0	26	17
6	51-70	0	122	44
7	71-100	0	51	153
Total lines/varieties		214	214	214

Percent disease incidence increased with increased of growth stage. Result reveled that at flowering stage percent disease incidence was lower and it gradually increased at pod setting to pre-maturity stage (Fig. 4.5.A.1).

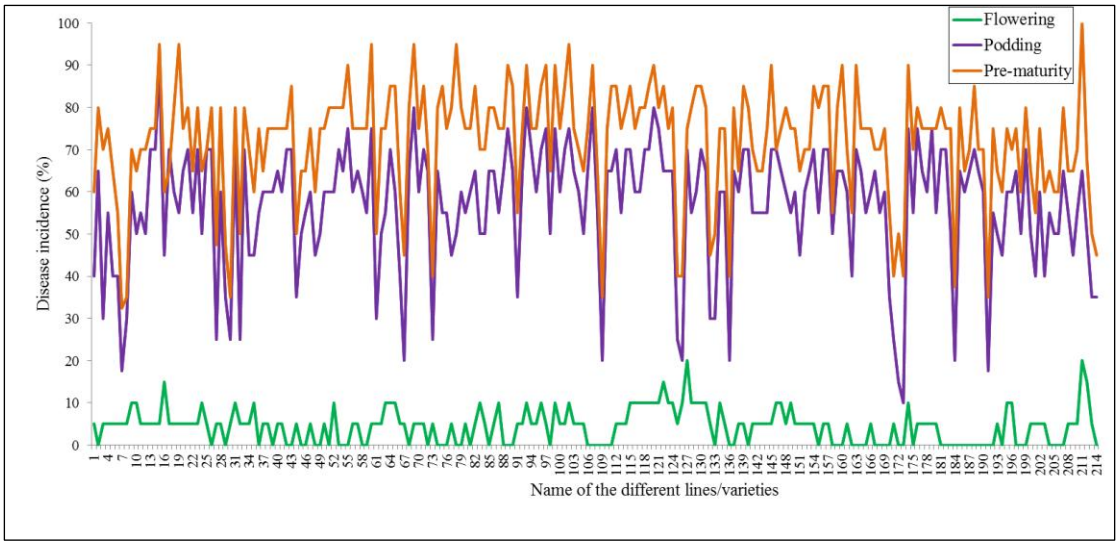


Fig. 4.5.A.1: Percent disease incidence of stemphylium blight at different growing stages of 214 lentil lines/varieties in Barisal in 2011-12

4.5.A.2. Disease severity

Disease severity increased with increased of growth stage. Result reveled that at flowering stage disease severity was lower and it gradually increased at pod setting to pre-maturity stage (Fig. 4.5.A.2).

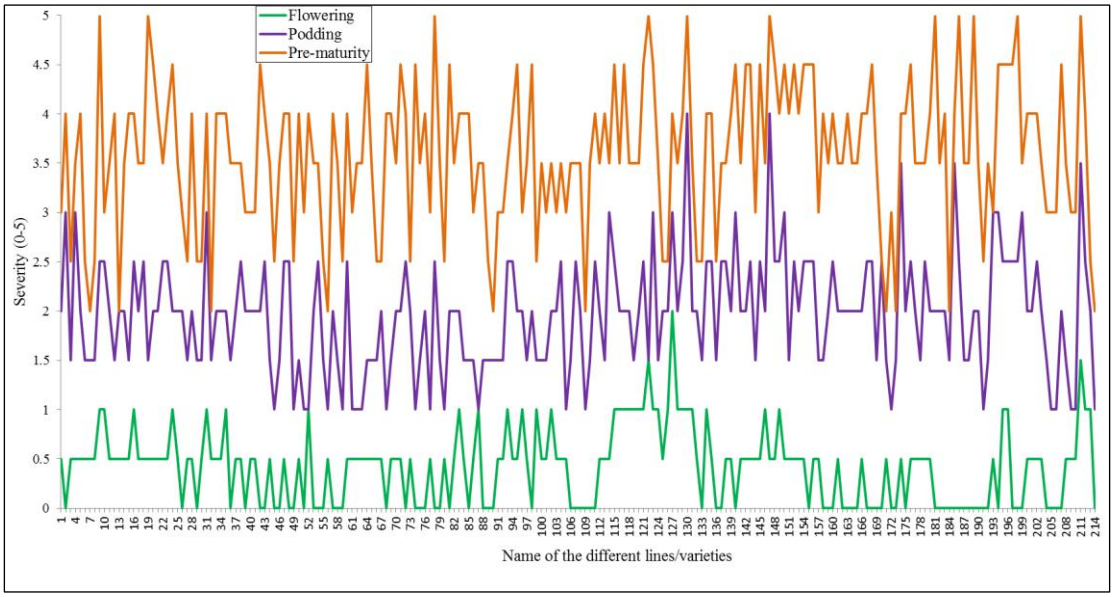


Fig. 4.5.A.2: Severity of stemphylium blight at different growing stages of 214 lentil lines/varieties in Barisal in 2011-12

4.5.A.3. Disease reaction

Disease reaction was recorded at three growth stages *viz*. flowering, pod setting and pre-mature stage. At flowering stage out of 214 lines/varieties 77, 134 and 3 lines were recorded as HR, R and MR, respectively. At pod setting stage out of 214 lines/varieties 23, 128, 58 and 5 lines were recorded as R, MR, MS, and S, respectively. At pre-maturity stage out of 214 lines/varieties 24, 41, 82 and 67 lines were recorded as MR, MS, S and HS, respectively (Table 4.5.A.2).

Table 4.5.A.2: Diseases reaction of stemphylium blight at three growth stages of different lentil lines/varieties

Sl. No.	Disease Reaction	No. of lines/varieties		
		Flowering stage	Pod setting stage	Pre-maturity stage
1	HR	77	0	0
2	R	134	23	0
3	MR	3	128	24
4	MS	0	58	41
5	S	0	5	82
6	HS	0	0	67
Total lines/varieties		214	214	214

4.5.A.4. Yield

According to yield performance all the lines/varieties were categorized as group-1, group-2, group-3, group-4, group-5 and group-6. Result reveled that four lines/varieties performed higher amount of grain yield and ranged from 1401 to 1700 Kg/ha (group-1). Eighteen lines/varieties produced medium ranged of grain yield and ranges from 1001 to 1400 Kg/ha (group-2). Fifty seven lines/varieties produce lower amount of grain yield and ranged from 801 to 1000 Kg/ha (group-3). Sixty seven lines/varieties produced very low grain yield and ranged from 601 to 800 Kg/ha (group-4). Forty six lines produced poor grain yield and ranged from 401 to 600 Kg/ha (group-5) and lastly twenty two lines produced very poor amount of grain yield and ranged from 100 to 400 Kg/ha (group-6) (Table 4.5.A.3).

A total of 22 lines were selected for next year trials considering yield and yield contributing performance including disease data.

Table 4.5.A.3: Yield performance of different lines/varieties of lentil at RARS, Rahmatpur, Barisal in 2011-12

No. of group	Range of Yield (Kg/ha)	Total Entry	Name of the Lines and Varieties
1	1401-1700	4	BD-6002, BD-3837, BD-3926, BARI Masur-7
2	1001-1400	18	BD-4102, BD-3962, BD-3979, BD-4105, BD-3836, BD-3884, BD-3812, BD-3825, BD-4134, BD-3975, BD-5991, BD-3834, BD-3877, BD-3811, BD-3963, BD-3846, BD-3839, BARI Masur-6
3	801-1000	57	BD-3908, BD-3872, BD-3974, BD-4095, BD-3941, BD-4097, BD-6021, BD-3864, BD-3878, BD-3922, BD-3857, BD-3896, BD-4062, BD-3892, BD-3911, BD-3893, BD-3924, BD-6022, BD-3855, BD-3881, BD-3833, BD-3890, BD-3945, BD-3838, BD-3950, BD-3886, BD-4069, BD-3912, BD-3988, BD-3806, BD-3888, BD-4091, BD-3874, BD-3870, BD-3948, BD-3832, BD-3880, BD-3972, BD-3895, BD-4088, BD-3848, BD-3876, BD-3844, BD-3905, BD-3889, BD-3916, BD-3841, BD-3929, BD-3860, BD-3804, BD-3861, BD-3820, BD-3901, BD-4051, BD-3882, BD-3910, BARI Masur-4
4	601-800	67	BD-6017, BD-4013, BD-3828, BD-3810, BD-4127, BD-3986, BD-3818, BD-3990, BD-3977, BD-4130, BD-3907, BD-3840, BD-3931, BD-3867, BD-3863, BD-4122, BD-5996, BD-3964, BD-4047, BD-4094, BD-5986, BD-3868, BD-3917, BD-3932, BD-4053, BD-3883, BD-3920, BD-4024, BD-3826, BD-4093, BD-3900, BD-3984, BD-3885, BD-3985, BD-5993, BD-3897, BD-3940, BD-4023, BD-4115, BD-3913, BD-5989, BD-3809, BD-3859, BD-3928, BD-5976, BD-3943, BD-4009, BD-3980, BD-3850, BD-3842, BD-3866, BD-4090, BD-3987, BD-3989, BD-4046, BD-4049, BD-3981, BD-3849, BD-3830, BD-3894, BD-3845, BD-3961, BD-3827, BD-4028, BD-3817, LIRL-22-172-1-1-1-0, BARI Masur-1
5	401-600	46	BD-3815, BD-4026, BD-3856, BD-5998, BD-6018, BD-3936, BD-3819, BD-3898, BD-4087, BD-3983, BD-3823, BD-3875, BD-5994, BD-3835, BD-3853, BD-6010, BD-3930, BD-3918, BD-3887, BD-6008, BD-3966, BD-3995, BD-6020, BD-4054, BD-5992, BD-3914, BD-6007, BD-3925, BD-3829, BD-3978, BD-3852, BD-3822, BD-3831, BD-5982, BD-3854, BD-4010, BD-3938, BD-3821, BD-3807, BD-3808, BD-3915, BD-3902, LIRL-22-178-1-1-1-0, LIRL-22-156-1-1-1-0, LIRL-22-36-1-1-1-0, LIRL-22-158-1-1-1-0
6	100-400	22	BD-3927, BD-3858, BD-3899, BD-3970, BD-6019, BD-3879, BD-3851, BD-3824, BD-3965, BD-5983, BD-3873, BD-4050, BD-3921, BD-3871, BD-3843, BD-5997, BD-3891,LIRL-22-211-1-1-1-0, LIRL-22-21-1-1-1-0, LIRL-22-175-1-1-1-0, LIRL-22-51-1-1-1-0, LIRL-22-54-1-1-1-0

4.5.B. Screening of selected 24 lines/varieties for resistant to stemphylium blight of lentil in 2012-13 (2nd year)

Selected 22 lines and two lentil varieties BARI Masur-1 (susceptible) and BARI Masur-7 (resistant) were evaluated under artificial inoculated condition against stemphylium blight in 2012-13.

4.5.B.1. Percent disease incidence

The percentage of disease incidence of 24 lentil lines/varieties is presented in Table 4.5.B.1. Significant difference was found in percent disease incidence at three growth stages of lentil. At flowering stage infection of the pathogen in most of the lines were very low. The data were converted square root transformation and analyze. Basically flowering stage was starting point of the disease. Disease incidence increased gradually flowering to pod setting stage and finally severely infected at pre-maturity stage. At flowering stage percent disease incidence ranged from 0.71-3.16. The highest disease incidence was observed in BARI Masur-1 (3.16%) followed by BD-3872, BD-3884, BD-3811, BD-3839, BD-3962 and BD-4102. The Lowest disease incidence was observed in BD-3812, BD-3963, BD-3837, BD-3834, BD-6002, BD-3979, BD-5991, BD-3975, BD-3836, BD-3974, BD-4005 and BARI Masur-7 (0.71%).

At pod setting stage percent disease incidence ranged from 10.80-32.67. The highest disease incidence was observed in BARI Masur-1 (32.67%) followed by BD-3878, BD-3877, BD-4095 and BD-4097. The lowest disease incidence was observed in BD-6002 (10.80%) followed by BD-3811, BD-3812, BD-38384, BD-3963, BD-3839, BD-3837, BD-3834, BD-5991, BD-3975, BD-3836 and BD-3974.

At pre-mature stage percent disease incidence ranged from 26.67-86.96. The highest disease incidence was observed in BARI Masur-1 (86.96%) followed by BD-3963, BD-3834 andBD-3836. The lowest disease incidence was observed in BD-3926 (26.67%) followed by BD-3811, BD-3812, BD-38384 and BD-6002.

Table 4.5.B.1: Percent disease incidence of stemphylium blight of 24 lentil lines/varieties in 2012-13

Sl. No.	Name of lines/varieties	Disease incidence (%)		
		Flowering stage	Pod setting stage	Pre-maturity stage
1	BD-3811	2.33 ab	16.00 bc	30.00 d-f
2	BD-3812	0.710 c	19.00 bc	30.00 d-f
3	BD-3884	2.44 ab	17.67 bc	30.17 c-f
4	BD-4097	1.53 bc	24.33 ab	45.00 b-d
5	BD-3963	0.710 c	16.00 bc	50.08 b
6	BD-3839	2.34 ab	14.33 bc	46.00 b-d
7	BD-3837	0.710 c	17.20 bc	35.67 e
8	BD-3878	1.53 bc	24.47 ab	43.33b-e
9	BD-3962	2.64 ab	21.00 a-c	47.67 bc
10	BD-3877	1.53 bc	24.60 ab	46.88 b-d
11	BD-4105	1.47 bc	21.00 a-c	42.33 b-e
12	BD-3834	0.710 c	17.67 bc	50.00 b
13	BD-3872	2.51 ab	23.10 a-c	46.00 b-d
14	BD-6002	0.710 c	10.80 c	27.72 ef
15	BD-3979	0.710 c	21.00 a-c	43.33 b-e
16	BD-5991	0.710 c	16.00 bc	46.86 b-d
17	BD-3975	0.710 c	17.53 bc	46.78 b-d
18	BD-3836	0.710 c	16.00 bc	50.00 b
19	BD-3974	0.710 c	14.33 bc	46.85 b-d
20	BD-4102	2.34 ab	21.00 a-c	45.00 b-d
21	BD-4095	0.710 c	24.33 ab	44.33 b-d
22	BD-3926	1.76 a-c	14.50 bc	26.67 ef
23	BARI Musur-1	3.16 a	32.67 a	86.96 a
24	BARI Musur-7	0.710 c	21.00 a-c	47.00 bc
	Level of significance	**	*	**
	CV (%)	57.11	32.80	21.61

Means followed the same letter/letters do not statistically differ at 1% and 5% level tested by DMRT

* = Significant at 5% level of probability, ** = Significant at 1% level of probability, NS=Not significant

4.5.B.2. Disease severity

The disease severity of 24 lentil lines/varieties is presented in Table 4.5.B.2. Wide variation was observed in disease severity at three growth stages of lentil. At flowering stage infection of the pathogen in most of the lines were very low. The data were recorded 0-5 scoring scale. Basically flowering stage was starting point of the disease. Disease severity increased gradually flowering to pod setting stage and finally severely infected at pre-maturity stage. At flowering stage, 12 lines/varieties were graded as 0 scoring scale and 12 lines/varieties showed 1 scoring scale. At pod setting stage out of tested lines/varieties 7 lines/varieties were graded as 0 scoring scale, 16 lines/varieties showed 1 scoring scale and 1 line showed 3 scoring scale. At pre-mature stage 13 lines/varieties were graded as 2 scoring scale, 10 lines/varieties showed 3 scoring scale and 1 line showed 4 scoring scale.

4.5.B.3. Disease reaction

The disease reaction of 24 lentil lines/varieties is presented in Table 4.5.B.3. Disease reaction of 24 lentil lines/varieties in the field were recorded and found wide variation at three growth stages. Considering flowering stage infection of the pathogen in most of the lines were very low. Basically flowering stage was starting point of the disease. Disease reaction increased gradually flowering to pod setting stage and finally severely infected at pre-maturity stage. At flowering stage Out of 24 accessions 12 lines showed highly resistant (HR) reaction and rest of them showed resistant (R) reaction. Considering pod setting stage 7, 16 and 01 lines/varieties were graded as resistant (R), moderately resistant (MR) and moderately susceptible (MS) reaction, respectably. Considering pre-maturity stage 13, 10 and 01 lines/varieties were recorded as moderately resistant (MR), moderately susceptible (MS) and susceptible (S) reaction, respectably.

Table 4.5.B.2: Disease severity of stemphylium blight of 24 lentil lines/ varieties in 2012-13

Sl. No.	Name of lines/varieties	Disease severity		
		Flowering stage	Pod setting stage	Pre-maturity stage
1	BD-3811	1	2	2
2	BD-3812	0	1	2
3	BD-3884	1	2	2
4	BD-4097	1	2	2
5	BD-3963	0	1	3
6	BD-3839	1	2	2
7	BD-3837	0	2	2
8	BD-3878	1	2	2
9	BD-3962	1	2	3
10	BD-3877	1	2	3
11	BD-4105	1	2	2
12	BD-3834	0	2	3
13	BD-3872	1	1	3
14	BD-6002	0	1	2
15	BD-3979	0	3	3
16	BD-5991	0	1	3
17	BD-3975	0	2	3
18	BD-3836	0	2	2
19	BD-3974	0	2	2
20	BD-4102	1	2	3
21	BD-4095	0	1	3
22	BD-3926	1	2	2
23	BARI Musur-1	1	2	4
24	BARI Musur-7	0	1	2

Table 4.5.B.3: Disease reaction of stemphylium blight of 24 lentil lines/ varieties in 2012-13

Sl. No.	Name of lines/varieties	Disease reaction		
		Flowering stage	Pod setting stage	Pre-maturity stage
1	BD-3811	R	MR	MR
2	BD-3812	HR	R	MR
3	BD-3884	R	MR	MR
4	BD-4097	R	MR	MR
5	BD-3963	HR	R	MS
6	BD-3839	R	MR	MR
	BD-3837	HR	MR	MR
8	BD-3878	R	MR	MR
9	BD-3962	R	MR	MS
10	BD-3877	R	MR	MS
11	BD-4105	R	MR	MR
12	BD-3834	HR	MR	MS
13	BD-3872	R	R	MS
14	BD-6002	HR	R	MR
15	BD-3979	HR	MS	MS
16	BD-5991	HR	R	MS
17	BD-3975	HR	MR	MS
18	BD-3836	HR	MR	MR
19	BD-3974	HR	MR	MR
20	BD-4102	R	MR	MS
21	BD-4095	HR	R	MS
22	BD-3926	R	MR	MR
23	BARI Musur-1	R	MR	S
24	BARI Musur-7	HR	R	MR

4.5.B.3. Growth and yield contributing characters and yield

Significant differences were found among lentil lines/varieties for days to first flowering, days to 50% flowering, days to maturity, plant height, number of branch per plant, number of pods per plant, number of seed per pod, 100-seed weight and yield were presented in Table 4.5.B.4, Table 4.5.B.5 and Table 4.5.B.6.

4.5.B.3.1. Days to first flowering

Days to first flowering ranged from 47-52. Maximum days to first flowering were observed in BD-4097 (52 days). Minimum days to first flowering were observed in all varieties (47-50 days).

4.5.B.3.2. Days to 50% flowering

Days to 50% flowering ranged from 50-57. Maximum days to 50% flowering was observed in BD-3834 (57 days) followed by BARI musur-7, BD-3884 and BD-4097. Minimum days to 50% flowering were observed in BD-3812, BD-3872, BD-5991 and BD-3975 (50 days).

4.5.B.3.3. Days to maturity

Days to maturity ranged from 95-101. The highest days to maturity was obtained from BD-3811 and BD-3837 followed by BD-3963 and BD-3979. The lowest days to maturity were found in BD-5991, BD-3836 and BD-3974.

Table 4.5.B.4: Performances of 24 lines/varieties of lentil regarding days to 1st flowering, days to 50% flowering and days to maturity in 2012-13

Sl. No.	Name of lines/varieties	Days to 1st flowering	Days to 50% flowering	Days to maturity
1	BD-3811	49 b	54 a-d	101 a
2	BD-3812	47 b	50 d	96 b-d
3	BD-3884	48 b	55 a-d	98 a-d
4	BD-4097	52 a	55 a-c	98 a-d
5	BD-3963	48 b	53 a-d	100 ab
6	BD-3839	48 b	51 cd	98 a-d
7	BD-3837	49 b	53 a-d	101 a
8	BD-3878	47 b	51cd	96 b-d
9	BD-3962	49 b	53 a-d	96 b-d
10	BD-3877	48 b	52 b-d	97 a-d
11	BD-4105	48 b	51 cd	99a-c
12	BD-3834	49 b	57 a	99 a-d
13	BD-3872	47 b	50 d	99 ab
14	BD-6002	49 b	53 a-d	99 a-d
15	BD-3979	48 b	51 cd	100 ab
16	BD-5991	47 b	50 d	95 cd
17	BD-3975	48 b	50 d	99 a-c
18	BD-3836	49 b	52 b-d	95 d
19	BD-3974	48 b	51 cd	95 d
20	BD-4102	49 b	52 b-d	98 a-d
21	BD-4095	49 b	54 a-d	98 a-d
22	BD-3926	49 b	53 a-d	96 b-d
23	BARI musur-1	47 b	51 cd	97 a-d
24	BARI musur-7	50 b	56 ab	96 b-d
	Level of significance	*	*	*
	CV (%)	2.83	4.61	2.01

Means followed the same letter/letters do not statistically differ at 5% level tested by DMRT

* = Significant at 5% level of probability, ** = Significant at 1% level of probability, NS=Not significant

4.5.B.3.4. Plant height (cm)

The plant height differs significantly from one to another in different lines/varieties. In respect of growth and yield contributing performance under artificial inoculated condition (Table 4.5.B.5) the plant height ranged from 33.13-43.87cm. Where the tallest plant (43.87cm) was found in BD-3884 followed by BD-3926 and BD-4102, BD-4095, BD-3963, BD-6002, BARI musur-7, BD-3877, BD-3811, BD-3839 and BD-3962. The lowest plant height (33.13cm) was recorded in BARI musur-1 followed by BD-3975, BD-4105, BD-3872, BD-3979, BD-3836, BD-3812, BD-3834, BD-5991, BD-3878, BD-3837 and BD-3974.

4.5.B.3.5. Number of branch per plant

The number of branch per plant showed no significant variation among the lines/varieties (Table 4.5.B.5). The number of branch per plant ranged from 2.47 to 3.07 where the highest number of branch per plant (3.07) was produced in BD-4095 and the lowest number of branch per plant was produced in BD-3962.

4.5.B.3.6. Number of pods per plant

Wide ranges were recorded for number of pods per plant from 51.54 to 99.53. The lines BD-3926 and BD-6002 produced the highest number of pods per plant. The lowest number of pods per plant produced in BD-4097 which was identical with BD-3811 and BD-4095 (Table 4.5.B.5).

Table 4.5.B.5: Performances of 24 lines/varieties of lentil regarding plant height, no. of branch/plant and no. of pods/plant in 2012-13

Sl. No.	Name of the lines/varieties	Plant height (cm)	No. of branches /plant	No. of pods/plant
1	BD-3811	39.33 a-e	2.60	55.20 hi
2	BD-3812	36.87 b-f	2.80	74.47 b-e
3	BD-3884	43.87 a	2.60	82.07 bc
4	BD-4097	38.53 b-e	2.87	51.54 i
5	BD-3963	40.27 a-e	2.53	67.00 d-g
6	BD-3839	39.07 a-e	3.07	62.87 f-h
7	BD-3837	38.20 b-f	2.80	84.67 b
8	BD-3878	38.00 b-f	2.80	84.80 b
9	BD-3962	38.80 a-e	2.47	79.20 bc
10	BD-3877	39.40 a-e	2.87	86.47 b
11	BD-4105	35.67 def	2.67	64.47 e-h
12	BD-3834	36.87 b-f	2.80	76.27 b-d
13	BD-3872	36.00 c-f	2.80	78.47 b-d
14	BD-6002	40.07 a-e	2.87	99.53 a
15	BD-3979	36.07 c-f	2.53	81.27 bc
16	BD-5991	37.27 b-f	2.67	84.13 b
17	BD-3975	35.27ef	2.73	77.53 b-d
18	BD-3836	36.40 b-f	2.53	78.40 b-d
19	BD-3974	38.20 b-f	2.53	77.67 b-d
20	BD-4102	41.20 a-c	2.53	76.40 b-d
21	BD-4095	40.73 a-d	3.13	56.00 g-i
22	BD-3926	41.47 ab	2.67	99.53 a
23	BARI musur-1	33.13 f	2.60	70.13 c-f
24	BARI musur-7	39.50 a-e	2.57	85.00 b
	Level of significance	*	NS	*
	CV (%)	6.80	12.97	8.20

Means followed the same letter/letters do not statistically differ at 5% level tested by DMRT
* = Significant at 5% level of probability, ** = Significant at 1% level of probability, NS=Not significant

4.5.B.3.7. Number of seeds per pod

The number of seeds per pod showed no significant variation among the lines/varieties (Table 4.5.B.6). The number of seeds per pod ranged from 1.73 to 2.00 where the highest number of seeds per pod (2.00) was found in BD-3837, BD-3962, BD-6002, BD-5991, BD-3836 and BD-3926. The lowest number of seed per pod (1.73) was recorded in BD-4097.

4.5.B.3.8. 100-seed weight (g)

The different lines/varieties under investigation varied greatly in production of 100 seed weight (g). 100 seed weight (g) ranged from 1.48 to 2.47 where the highest 100 seed weight (2.47) was found in BD-5991 and BD-3836 but the rest of all lines/varieties were identical except BD-3812 and BD-3975. The lowest 100 seed weight (g) was recorded in BD-3975 (1.48), on the other hand, rest of all lines/varieties were identical except BARI musur-7, BD-3836 and BD-5991 (Table 4.5.B.6).

4.5.B.3.9. Yield (kg/ha)

The effects of stemphylium blight infection on yield of selected 24 lentil lines/varieties are presented in Table 4.5.B.6. Among the lines/varieties the yield ranged from 763 to 1738 Kg/ha. The highest yield was found in BD-6002 (1738 Kg/ha) and followed by BD-3837 (1717 Kg/ha) and BD-3926 (1705 Kg/ha) which were statistically similar to BARI Musur-7 (1631 Kg/ha) followed by BD-4102 and BD-4095. The lowest yield was found in BD-3979 (763 Kg/ha) and followed by BD-3834 (863 Kg/ha) and BD-3975 (864 Kg/ha).

Table 4.5.B.6: Performances of 24 lines/varieties of lentil regarding no. of seeds/pod, 100 seed weight and yield in 2012-13

Sl. No.	Name of the lines/varieties	No. of seed/pod	100 seed weight (g)	Yield (kg/ha)
1	BD-3811	1.87	2.20 a-c	1320 de
2	BD-3812	1.93	1.50 bc	1095 f
3	BD-3884	1.87	2.03 a-c	1456 cd
4	BD-4097	1.73	2.00 a-c	1005 fg
5	BD-3963	1.87	2.00 a-c	1543 bc
6	BD-3839	1.80	2.10 a-c	1554 bc
7	BD-3837	2.00	2.10 a-c	1717 a
8	BD-3878	1.87	1.93 a-c	1099 f
9	BD-3962	2.00	1.73 a-c	1005 fg
10	BD-3877	1.80	1.80 a-c	1268 e
11	BD-4105	1.87	1.80 a-c	1092 f
12	BD-3834	1.87	2.00 a-c	863 gh
13	BD-3872	1.87	2.27 a-c	1454 cd
14	BD-6002	2.00	2.20 a-c	1738 a
15	BD-3979	1.80	1.80 a-c	763 h
16	BD-5991	2.00	2.47 a	1270 e
17	BD-3975	1.93	1.48 c	864 gh
18	BD-3836	2.00	2.43 a	1459 cd
19	BD-3974	1.93	1.77 a-c	978 fg
20	BD-4102	1.80	1.97 c	1640 ab
21	BD-4095	1.80	2.17 a-c	1583 a-c
22	BD-3926	2.00	1.93 a-c	1705 a
23	BARI musur-1	1.87	2.18 a-c	1250 e
24	BARI musur-7	1.93	2.40 ab	1631 ab
	Level of significance	NS	*	**
	CV (%)	9.81	23.60	6.42

Means followed the same letter/letters do not statistically differ at 1% and 5% level tested by DMRT

* = Significant at 5% level of probability, ** = Significant at 1% level of probability, NS=Not significant

4.5.C. Screening of 5 lines/varieties for resistance to stemphylium blight of lentil in 2013-14 (3rd year)

Selected 3 lines and 2 lentil varieties BARI Masur-1 (susceptible check) and BARI Masur-7 (resistant check) were evaluated under natural epiphytotic condition in two locations i.e. Barisal and Rajshahi against stemphylium blight in 2013-14.

4.5.C.1. Percent disease incidence

The percent disease incidence of 5 lentil lines/varieties is presented in Table 4.5.C.1. Due to different locations variation, significant difference was found in percent disease incidence. In Barisal location, highest percent disease incidence (82.45%) was observed in BARI Masur-1 and followed by BD-3926 (67.85%). The lowest percent disease incidence was observed in BD-6002 (23.62%) and followed by BD-3837 (38.65%) and BARI Masur-7 (39.88%) which was statistically similar but differed from BD-3926 (67.85%).

In Rajshahi location, the lowest percent disease incidence was observed in BARI Masur-7 (36.43) and followed by BD-6002 (36.52%) and BD-3837 (46.33%) which was statistically similar but differed from BD-3926 (61.43%). The highest percent disease incidence (97.12%) was observed in BARI Masur1. Finally over the year, averagely the lowest percent incidence was 29.46% in BD-6002 and followed by BD-3837 and BARI Masur-7 that was statistically similar but differed from BD-3926 and the highest percent incidence was 88.95% in BARI Masur-1.

Table 4.5.C.1: Percent disease incidence of stemphylium blight of lentil at pre-maturity stage in Barisal and Rajshahi in 2013-14

Sl. No.	Name of the lines/varieties	Disease incidence (%)		
		Barisal	Rajshahi	Average
1	BD-3926	67.85 a	61.43 b	56.21 b
2	BD-6002	23.62 b	36.52 c	29.46 c
3	BD-3837	38.65 b	46.33 c	36.78 c
4	BARI Masur-1	82.45 a	97.12 a	88.95 a
5	BARI Masur-7	39.88 b	36.43 c	41.63 bc
	Level of significance	**	**	*
	CV (%)	18.60	13.37	18.58

Means followed the same letter/letters do not statistically differ at 1% and 5% level tested by DMRT
* = Significant at 5% level of probability, ** = Significant at 1% level of probability, NS=Not significant

4.5.C.2. Disease severity

The disease severity of 5 lentil lines/varieties at pre-mature stage is presented in Table 4.5.C.2. The data were recorded 0-5 scoring scale. Out of 5 lentil lines/varieties, BD-6002 line performed better against stemphylium blight disease and graded as 1 scoring scale in both Barisal and Rajshahi locations. BARI Masur-7 and BD-3837 graded as 2 scoring scale and BD-3926 line graded as 3 scoring scale in both locations. But BARI Masur-1 severely infected and showed 4 scoring scale in Barisal and 5 scoring scale in Rajshahi.

4.5.C.3. Disease reaction

Disease reaction of 5 lentil lines/varieties in the field were recorded and found wide variation at pre-mature growth stages (Table 4.5.C.2). Over the locations, out of 5 lentil lines/varieties, the lowest infection of stemphylium blight was observed in BD-6002 line and performed resistant (R) reaction. BD-3837 and BARI Masur-7 showed moderately resistant (MR) reaction but BD-3926 showed moderately susceptible (MS) reaction. Severely infection was found in BARI Masur-1 and recorded as susceptible (S) disease reaction.

Table 4.5.C.2: Severity and diseases reaction of stemphylium blight of lentil at pre-maturity stage in Barisal and Rajshahi in 2013-14

Sl. No.	Name of the lines/varieties	Disease severity (0-5)			Disease Reaction
		Barisal	Rajshahi	Average	
1	BD-3926	3	3	2.67	MS
2	BD-6002	1	1	1.33	R
3	BD-3837	2	2	2.00	MR
4	BARI Masur-1	4	5	4.33	S
5	BARI Masur-7	2	2	2.00	MR

4.5.C.4. Growth and yield contributing characters and yield

Significant differences were found among lentil lines/varieties for days to first flowering, days to 50% flowering, days to maturity, plant height, number of branch per plant, number of pods per plant, number of seed per pod, 100-seed weight and yield were presented in Table 4.5.C.3, Table 4.5.C.4 and Table 4.5.C.5.

4.5.C.4.1. Days to first flowering

Days to first flowering ranged from 48-51. The highest days to first flowering was observed in BD-3926 and BD-6002 (51 days). The lowest days to first flowering was observed in BARI Masur-1 (48 days) and followed by BD-3837 which was statistically identical with BARI Masur-7 (Table 4.5.C.3).

4.5.C.4.2. Days to 50% flowering

Days to 50% flowering ranged from 55-59. The highest days to 50% flowering was observed in BD-6002 (59 days). The lowest days to 50% flowering were observed in BARI Masur-1 (55 days) (Table 4.5.C.3).

4.5.C.4.3. Plant height (cm)

The plant height differs significantly from one to another in different lines/varieties. The plant height ranged from 32.40-38.09 cm where the tallest plant (38.09 cm) was found in BARI Masur-1 followed by BD-3837, BD-3926 and BARI Masur-7 (Table 4.5.C.3). The dwarfs' plant (32.40 cm) was recorded in BD-6002.

Table4.5.C.3: Performances of 5 lines/varieties of lentil regarding days to 1st flowering, days to 50% flowering and plant height in 2013-14 (pooled).

Sl. No.	Name of lines/varieties	Days to 1st Flowering	Days to 50% flowering	Plant height (cm)
1	BD-3926	51 a	57 b	34.40 ab
2	BD-6002	51 a	59 a	32.40 b
3	BD-3837	49 bc	57 b	35.92 ab
4	BARI Masur-1	48 c	55 c	38.09 a
5	BARI Masur-7	50 b	57 b	34.87 ab
	Level of significance	*	*	*
	CV (%)	1.30	1.26	6.73

Means followed the same letter/letters do not statistically differ at 5% level tested by DMRT

* = Significant at 5% level of probability, ** = Significant at 1% level of probability, NS=Not significant

4.5.C.4.4. Number of branch per plant

The number of branch per plant showed significant variation among the lines/varieties. The number of branch per plant ranged from 2.13 to 3.41 where the highest number of branch per plant (3.41) was produced in BD-3837 followed by BD-6002 and BARI Masur-7 (Table 4.5.C.4). The lowest number of branch per plant was produced in BARI Masur-1 (2.13) followed by BD-3926.

4.5.C.4.5. Number of pods per plant

Wide ranges were recorded for number of pods per plant from 95.4 to 139.6 (Table 4.5.C.4). The variety BARI Masur-7 (139.6) produced the highest number of pods per plant. The lowest number of pods per plant produced in BARI Masur-1.

4.5.C.4.6. 100-seed weight (g)

The different lines/varieties under investigation varied greatly in production of 100 seed weight (g). 100 seed weight ranged from 1.68 to 2.25g where the maximum 100 seed weight was found 2.25g in BARI Masur-7 followed by BD-6002 and BD-3837. The minimum 100 seed weight (1.68g) was recorded in BARI Masur-1 followed by BD-3926 (Table 4.5.C.4).

Table 4.5.C.4: Performances of 5 lines/varieties of lentil regarding no. of branches/plant, no. of pods/plant, and 100 seed weight in 2013-14 (pooled)

Sl. No.	Name of var./lines	No. of branches/ Plant	No. of pods/ plant	100 Seed wt. (g)
1	BD-3926	2.70 b	106.2 d	1.84 b
2	BD-6002	3.40 a	129.5 b	2.23 a
3	BD-3837	3.41 a	117.3 c	2.18 a
4	BARI Masur-1	2.13 b	95.4 e	1.68 b
5	BARI Masur-7	3.40 a	139.6 a	2.25 a
	Level of significance	*	*	*
	CV (%)	12.62	4.40	9.27

Means followed the same letter/letters do not statistically differ at 5% level tested by DMRT

* = Significant at 5% level of probability, ** = Significant at 1% level of probability, NS=Not significant

4.5.C.4.7. Yield (kg/ha)

The effects of stemphylium blight infection on yield of 5 lentil lines/varieties are presented in Table 4.5.C.5. In Barisal location, the highest yield was found in BARI Masur-7 (1583 Kg/ha) which was statistically similar to BD-6002 (1561 kg ha-^{1}) and BD-3837 (1413 Kg/ha). The lowest yield was found in BARI Masur-1 (1053 Kg/ha) and followed by BD-3926 (1112 Kg/ha). In Rajshahi location, the highest yield was found in BD-6002 (1695 Kg/ha) which were statistically similar to BARI Masur-7 (1620 Kg/ha) and BD-3837 (1480 Kg/ha). The lowest yield was found in BARI Masur-1 (1046 Kg/ha) and followed by BD-3926 (1065 Kg/ha). Over the location in an average, the highest yield was found in BD-6002 (1628 Kg/ha) which was statistically identical with BARI Masur-7 (1602 Kg/ha) and BD-3837 (1447 Kg/ha). The lowest yield was found in BARI Masur-1 (1050 Kg/ha) that was no significantly differed with BD-3926 (1089 Kg/ha) (Fig. 4.5.C.1).

Table 4.5.C.5: Performances of yield of 5 lentil lines/varieties in Barisal and Rajshahi in 2013-14

Sl. No.	Name of the lines/varieties	Yield (kg/ha)		
		Barisal	Rajshahi	Average
1	BD-3926	1112 b	1065 b	1089 b
2	BD-6002	1561 a	1695 a	1628 a
3	BD-3837	1413 a	1480 a	1447 a
4	BARI Masur-1	1053 b	1046 b	1050 b
5	BARI Masur-7	1583 a	1620 a	1602 a
	Level of significance	**	**	**
	CV (%)	9.00	13.71	10.03

Means followed the same letter/letters do not statistically differ at 1% level tested by DMRT

* = Significant at 5% level of probability, ** = Significant at 1% level of probability, NS=Not significant

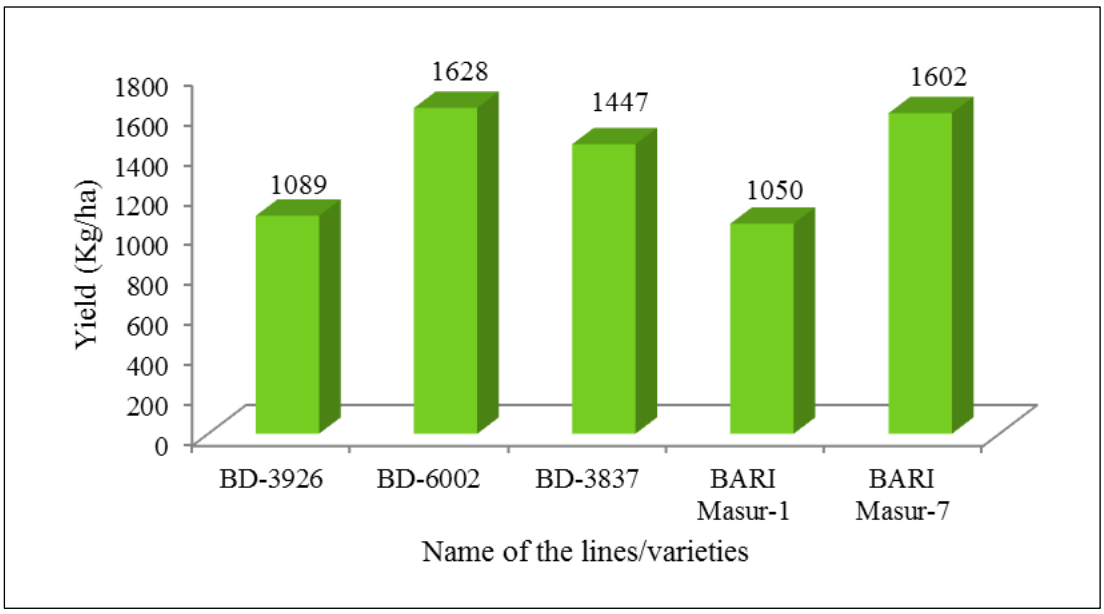


Fig. 4.5.C.1: Performances of average yield of 5 lentil lines/varieties in 2013-14

4.6. Experiment-VI: Effect of different dates of sowing and varieties on stemphylium blight of lentil (2012-2013)

Effect of 7 different sowing dates and performance of 2 varieties of BARI Masur-1 and BARI Masur-7 were evaluated against stemphylium blight in 2012-13.

4.6.1. Percent diseases incidence

The percent disease incidence against stemphylium blight of lentil is presented in Table 4.6.1. Significant difference was found in percent disease incidence at different date of sowing. Considering 7 sowing dates, the highest percent disease incidence was shown by October 25 (72.50%) followed by November 01, 8, 15, 22 and all were statistically similar with each other. The lowest percent disease incidence was observed in December 6 (30.83%) which statistically similar with November 29 but significantly differed with other sowing dates. In case of varietal performance susceptible variety BARI Masur-

1 showed 64% disease incidence. On the other hand, BARI Masur-7 showed 47% disease incidence (Table 4.6.2).

4.6.2. Disease Severity

The disease severity against stemphylium blight of lentil is presented in Table 4.6.1. Wide variation was observed in disease severity at 7 different dates of sowing in both varieties of lentil. The data were recorded 0-5 scoring scale. Disease severity of stemphylium blight was decreased gradually from early sowing to late sowing of lentil. In early sowing, disease was graded as 4 and 3 scoring scale at October 25 and November 01, respectively. Disease severity was graded as 2 scoring scale at November 8, 15 and 22 sowing plots. In late sowing, November 29 and December 6, disease was graded as 1 scoring scale.

In case of varietal performance susceptible variety BARI Masur-1 was graded as 5 scoring scale. On the other hand, BARI Masur-7 was graded as 2 scoring scale (Table 4.6.2).

4.6.3. Disease reaction

The result of the Fig 4.6.1 also distinctly indicated that susceptible (BARI Masur-1) and resistant (BARI Masur-7) varieties showed different types of reactions. Considering two varieties, out of 7 sowing dates susceptible (S) and moderately susceptible (MS) diseases reaction was found at October 25 and November 1, respectively. Moderately resistant (MR) diseases reaction was found in November 8, 15, 22 and resistant (R) diseases reaction was found in November 29 and December 6.

Table 4.6.1: Effect of different dates of sowing on disease incidence, disease severity and disease reaction of lentil in 2012-13

Sl. No.	Sowing dates	Disease Incidence (%)	Disease Severity (0-5)	Disease Reaction
1	25-10-12	72.50 a	4	S
	01-11-12	63.50 a	3	MS
3	08-11-12	55.67 ab	2	MR
4	15-11-12	62.17 a	2	MR
5	22-11-12	62.17 a	2	MR
6	29-11-12	42.17 bc	1	R
7	06-12-12	30.83 c	1	R
	Level of significance	**	-	-
	CV (%)	25.26	-	-

Means followed the same letter/letters do not statistically differ at 1% level tested by DMRT

* = Significant at 5% level of probability, ** = Significant at 1% level of probability, NS=Not significant

In case of varietal performance over the all sowing dates individual susceptible variety BARI Masur-1 showed highly susceptible (HS) disease reaction. On the other hand, BARI Masur-7 showed moderately resistant (MR) reaction (Table 4.6.2).

Table 4.6.2: Varietal performances of lentil on disease incidence, disease severity and disease reaction in 2012-13

Sl. No.	Variety	Disease Incidence (%)	Disease Severity (0-5)	Disease Reaction
1	BARI Masur-1	64 a	5	HS
2	BARI Masur-7	47 b	2	MR
	Level of significance	**	-	-
	CV (%)	25.26	-	-

Means followed the same letter/letters do not statistically differ at 1% level tested by DMRT

* = Significant at 5% level of probability, ** = Significant at 1% level of probability, NS=Not significant

4.6.4. Days to first flowering

Among the seven sowing dates first flower initiation was found earlier in the December 6 sowing. First flower initiation was found late in the November 8 sowing which was significantly differed from November 15, October 25 and November 01 sowing (Table 4.6.3).

4.6.5. Days to maturity

Maturity of the plant occurred significantly among the treatments and that ranged from 87 to 118 days. Level of maturity was maximum delayed October 25 and gradually decreased the maturity November 01, 08, 15, 22 and 29. The lowest maturity level was recorded in December 06 (Table 4.6.3).

4.6.6. Plant height

The plant height differed significantly among the treatments and ranged from 36.00 cm to 47.37 cm. The tallest plant was recorded in November 01 sowing followed by November 8, 22, October 25, November 15 sowing. Dwarf plant was recorded in December 6 followed by November 29 (Table 4.6.3).

4.6.7. Number of branches per plant

The number of branches per plant differs significantly due to different date of sowing. The number of branch per plant ranged from 2.43 to 3.27. The highest number of branch per plant (3.27) was produced at November 15 sowing time which was statistically similar with November 8 sowing (Table 4.6.3). The lowest number of branch per plant was produced at December 6 sowing (2.43) followed by November 01, 22, 29 and October 25 sowing.

4.6.8. Number of pods per plant

Number of pods per plant differed significantly with each other at different date of sowing. The highest number of pods per plant was obtained from the sowing

November 8 which was statistically similar with November 15. The lowest number of pod was obtained from December 6 followed by November 29, 22 (Table 4.6.3).

4.6.9. Yield

The effects of stemphylium blight infection on yield of different sowing of lentil are presented in Table 4.6.3.The yield per plot exhibited that November 8 sowing (1200 Kg/ha) gave the highest yield which was statistically similar with November 15 (1200 Kg/ha). Yield was decreased during early or late sowing. The lowest yield was produced December 6 sowing (766 Kg/ha) followed by October 25, November 29 and November 01.

Table 4.6.3: Effects of different date of sowing regarding days to 1st flowering, days to maturity, plant height, no. of branches/plant, no. of pods/plant and yield in 2012-13

Sl. No.	Sowing dates	Days to first flowering	Days to Maturity	Plant Height (cm)	No. of Branches /plant	No. of Pods /plant	Yield (Kg/h)
1	25-10-12	53 b	118 a	42.80 ab	2.83 ab	74 bc	887 b-d
2	01-11-12	52 b	112 b	47.37 a	2.70 ab	83 b	920 bc
3	08-11-12	55 a	105 c	47.00 a	3.13 a	101 a	1200 a
4	15-11-12	53 b	103 d	42.72 ab	3.27 a	100 a	1156 a
5	22-11-12	50 c	96 e	44.85 a	2.67 ab	66 cd	968 b
6	29-11-12	48 d	93 f	40.00 bc	2.73 ab	61 cd	824 cd
7	06-12-12	46 e	87 g	36.00 c	2.43 b	53 d	766 d
	Level of significance	**	**	**	*	*	**
	CV (%)	2.16	1.24	8.56	16.81	16.03	10.16

Means followed the same letter/letters do not statistically differ at 1% and 5% level tested by DMRT

* = Significant at 5% level of probability, ** = Significant at 1% level of probability, NS=Not significant

In case of varietal performance BARI Masur-1 showed first flower initiation earlier compare to popular variety BARI Masur-7 (Table 4.6.4). In case of days to maturity both the variety matured in same time. In plant height BARI Masur-7 showed relatively dwarf compare to BARI Masur-1. The highest number of branch per plant was found in BARI Masur-7 and the lowest was found in BARI Masur-1. BARI Masur-7 produced higher number of pods compare to BARI Masur-1. BARI Masur-7 produced higher yield (1131 Kg/ha) compare to BARI Masur-1 (789 Kg/ha) (Table 4.6.4).

Table 4.6.4: Varietal performances of days to 1st flowering, days to maturity, plant height, no. of branches/plant, no. of pods/plant and yield in 2012-13

Sl. No.	Variety	Days to 1st flowering	Days to maturity	Plant height(cm)	No. of branch/plant	No. of pod/plant	Yield (Kg/h)
1	BARI Masur-1	49 b	102 a	44 a	2.65 b	74 b	789 b
2	BARI Masur-7	52 a	101 b	42 b	2.99 a	79 a	1131 a
Level of significance		**	*	*	*	*	**
	CV (%)	2.16	1.24	8.56	16.81	16.03	10.16

Means followed the same letter/letters do not statistically differ at 1% and 5% level tested by DMRT

* = Significant at 5% level of probability, ** = Significant at 1% level of probability, NS=Not significant

4.6.10. Interaction effect

The interaction effect between sowing date and variety are presented in Table 4.6.5 and Table 4.6.6. Significant difference were found among the interaction effect between sowing date and variety for days to first flowering, days to maturity, plant height, number of branches per plant, number of pods per plant and yield. It was observed that November 08 and November 15 sowing

produced better results in case of variety BARI Masur-7 but in case of variety BARI Masur-1 produced better results in November 08 sowing.

4.6.10.1. Days to first flowering

Days to first flowering ranged from 42-56 days. The highest days to first flowering was observed in BARI Masur-7 at November 08 sowing. The lowest days to first flowering was observed in BARI Masur-1 at December 6 sowing (Table4.6.5).

4.6.10.2. Days to maturity

Maturity of the plant occurred significantly among the treatments and that ranged from 86-119 days. The highest days to maturity was found in BARI Masur-7 at October 25. The lowest maturity was recorded in BARI Masur-1 at December 06 sowing that was statistically similar in BARI Masur-7 at same sowing dates (Table4.6.5).

4.6.10.3. Plant height

The plant height differed significantly among the treatments and that ranged from 35.13cm to 49.87cm. The tallest plant was recorded in BARI Masur-1 at November 8 sowing which was statistically similar with October 25, November 01, 15 & 22 sowing in BARI Masur-1 and November 01 & 08 in BARI Masur-7. The dwarves plant was recorded in BARI Masur-7 at December 6 followed by November 15, 22 & 29 in BARI Masur-7 and December 6 and November 29 sowing in BARI Masur-1 (Table 4.6.6).

Table 4.6.5: Interaction of sowing dates and varieties on days to 1st flowering and days to maturity of lentil in 2012-13

Sl. No.	Sowing dates	Variety	Days to first flowering	Days to maturity
1	25-10-12	BARI Masur-1	53 bc	116 b
2		BARI Masur-7	53 b-d	119 a
3	01-11-12	BARI Masur-1	51 c-e	111 c
4		BARI Masur-7	53 b-d	113 c
5	08-11-12	BARI Masur-1	54 b	106 d
6		BARI Masur-7	56 a	104 e
7	15-11-12	BARI Masur-1	52 b-d	103 e
8		BARI Masur-7	53 bc	102 e
9	22-11-12	BARI Masur-1	48 f	95 f
10		BARI Masur-7	51 c-e	96 f
11	29-11-12	BARI Masur-1	45 g	96 f
12		BARI Masur-7	51 de	91 g
13	06-12-12	BARI Masur-1	42 h	86 h
14		BARI Masur-7	50 ef	88 h
Level of significance			*	*
CV (%)			2.16	1.24

Means followed the same letter/letters do not statistically differ at 5% level tested by DMRT

* = Significant at 5% level of probability, ** = Significant at 1% level of probability, NS=Not significant

4.6.10.4. Number of branches per plant

The number of branches per plant differs significantly due to different date of sowing (Table 4.6.6). The number of branch per plant ranged from 2.23 to 3.57. The highest number of branches per plant was produced in BARI Masur-7 at November 15 sowing which was statistically identical with October 25, November 01, 08 & 22 in BARI Masur-7 variety and October 25, November 08, 15 sowing in BARI Masur-1. The lowest number of branch per plant was produced in BARI Masur-1 at November 29 followed by October 25, November 08, 15 sowing in BARI Masur-1 and October 25, November 01, 08, 22 & December 6 sowing in BARI Masur-7.

4.6.10.5. Number of pods per plant

Number of pods per plant differed significantly with each other at different date of sowing. The highest number of pods per plant was obtained in BARI Masur-7 in November 8 sowing (108) which was statistically similar with November 01, 08 & 15 in BARI Masur-7 and November 08 & 15 in BARI Masur-1. The lowest number of pod was obtained in BARI Masur-1 at November 29 sowing (50) that were statistically identical with October 25, November 22 & December 6 in BARI Masur-1 and November 22, 29 & December 6 in BARI Masur-7 (Table 4.6.6).

4.6.10.6. Yield

The interaction effects of stemphylium blight infection on yield of different sowing of lentil are presented in Table 4.6.6. The highest yield exhibited in BARI Masur-7 variety at November 8 sowing (1399 Kg/ha) which was statistically similar with November 15 sowing (1314 Kg/ha) in BARI Masur-7. Yield was decreased during early or late sowing. The lowest yield was found in BARI Masur-1 at December 6 sowing (583 Kg/ha) followed by November 29, October 25 and November 01 sowing in BARI Masur-1 variety.

Table 4.6.6: Interaction of sowing dates and varieties on plant height, no. of branches/plant, no. of pods/plant and yield in 2012-13

Sl. No.	Sowing dates	Variety	Plant height (cm)	No. of branch/ plant	No. of pod/ plant	Yield (Kg/h)
1	25-10-12	BARI Masur-1	43.20 a-e	2.97 a-c	70 cd	750 ef
2		BARI Masur-7	42.40 b-e	2.70 a-c	78 bc	1023 bc
3	01-11-12	BARI Masur-1	49.00 ab	2.57 bc	79 bc	734 ef
4		BARI Masur-7	45.73 a-d	2.83 a-c	87 a-c	1105 bc
5	08-11-12	BARI Masur-1	49.87 a	3.10 a-c	94 ab	1001 bc
6		BARI Masur-7	44.13 a-d	3.17 a-c	108 a	1399 a
7	15-11-12	BARI Masur-1	43.67 a-e	2.97 a-c	103 a	997 bc
8		BARI Masur-7	41.77 c-f	3.57 a	96 ab	1314 a
9	22-11-12	BARI Masur-1	48.37 a-c	2.30 bc	67 cd	792 de
10		BARI Masur-7	41.33 c-f	3.03 a-c	64 cd	1145 b
11	29-11-12	BARI Masur-1	39.33 d-f	2.23 c	50 d	667 ef
12		BARI Masur-7	40.67 d-f	3.23 ab	71 cd	981 bc
13	06-12-12	BARI Masur-1	36.87 ef	2.43 bc	54 d	583 f
14		BARI Masur-7	35.13 f	2.43 bc	51 d	948 cd
	Level of significance		*	*	*	**
	CV (%)		8.56	16.81	16.03	10.16

Means followed the same letter/letters do not statistically differ at 1% and 5% level tested by DMRT

* = Significant at 5% level of probability, ** = Significant at 1% level of probability, NS=Not significant

4.6.10.7. Disease incidence and severity

Considering percent disease incidence and severity, it was shown in graphical presentation (Fig. 4.6.1 and Fig. 4.6.2). In November 08 and November 15 sowing produced higher yield in BARI Masur-7 but in case of variety BARI Masur-1 only November 08 sowing produced higher yield.

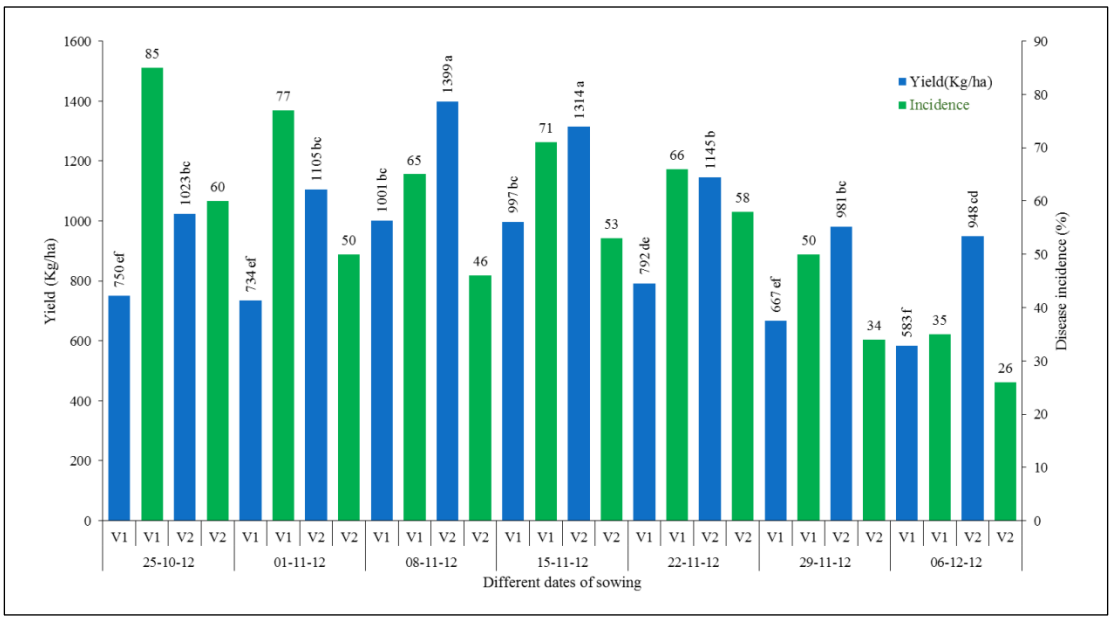


Fig. 4.6.1: Interaction of yield and percent disease incidence of two lentil varieties at different date of sowing in 2012-13

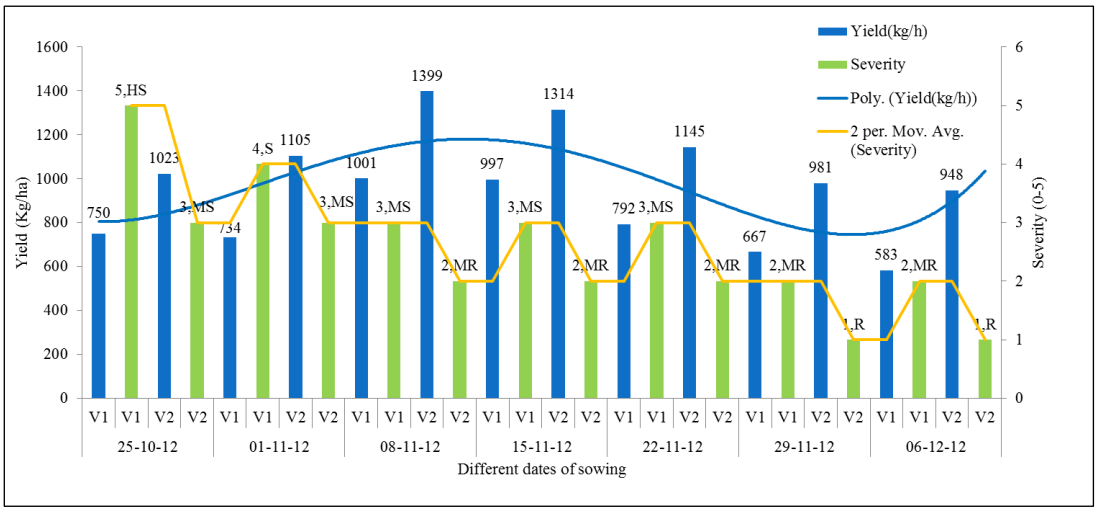


Fig. 4.6.2: Interaction of yield and severity of two lentil lines/varieties at different date of sowing in 2012-13

4.7. Experiment-VII: Efficacy of different fungicides in controlling stemphylium blight of lentil (2013-2014)

Efficacy of selected fungicides *viz*. Rovral, Compenion, Nativo, Amistar Top and Secure were assessed against *Stemphylium botryosum* in controlling stemphylium blight of lentil in 2013-14. Lentil variety BARI Masur-1 was used in this experiment and the data were presented at two locations i.e. Barisal and Rajshahi. The efficacy of the treatments was measured in respect of disease incidence, severity, yield contributing characters and yield.

4.7.1. Percent diseases incidence

Percent disease incidence of stemphylium blight of lentil was presented in Table 4.7.1. Significant difference was found in disease incidence both two locations in Barisal and Rajshahi. In Barisal, the lowest diseases incidence was observed in Rovral (30.00%) followed by Amistar Top (35.33%) and the highest incidence was observed in Control (87.33%) followed by Companion (70.00%). But in Rajshahi the lowest incidence was observed in Amistar Top (26.33%) followed by Rovral (33.67%). The highest disease incidence was observed in control treated plot (80.33%). Over the locations, averagely the lowest disease incidence was found in Amistar Top treated plot. Disease reductions over control were 63.22% and 62.02% in Amistar Top and Rovral treated plot, respectively.

4.7.2. Diseases severity

Disease severities of stemphylium blight of lentil were presented in Table 4.7.2. The data were recorded 0-5 scoring scale. In Barisal location, Rovral treated plot performed better against stemphylium blight disease and graded as 1 scoring scale. Disease severity graded as 2 in Nativo and Amistar Top treated plot, 3 in Secure and Compenion treated plot but 5 in Control treated plot. In Rajshahi location, Amistar Top treated plot performed better against

stemphylium blight disease and graded as 1 scoring scale. Disease severity graded as 2 in Rovral and Nativo treated plot. Disease severity also graded as 3, 4 and 5 in Secure, Compenion and Control treatments, respectively.

Table 4.7.1: Effect of foliar spray of fungicides on disease incidence of lentil at Barisal and Rajshahi in 2013-14

Treatments	Disease incidence (%)			Diseases reduction over control (%)
	Barisal	Rajshahi	Mean	
T_1=Rovral	30.00 d	33.67 e	31.84	62.02
T_2=Compenion	70.00 b	67.33 b	68.67	18.08
T_3=Nativo	51.67 c	44.33 d	48.00	42.74
T_4=Amistar Top	35.33 d	26.33 e	30.83	63.22
T_5=Secure	54.00 c	57.00 c	55.50	33.79
T_6=Control	87.33 a	80.33 a	83.83	-
Level of significance	**	**	-	-
CV (%)	15.06	10.44	-	-

Means followed the same letter/letters do not statistically differ at 5% level tested by DMRT

* = Significant at 5% level of probability, ** = Significant at 1% level of probability, NS=Not significant

4.7.3. Diseases reaction

Foliar spray of different fungicides resulting different disease reactions among the treatments and data were presented in Table 4.7.2. Considering both the locations, moderately resistance (MR) disease reaction was observed in Rovral, Amistar Top and Nativo treated plot. Moderately susceptible (MS), susceptible (S) and highly susceptible (HS) disease reactions were recorded in Secure, Compenion and Control treatments, respectively.

Table 4.7.2: Effect of foliar spray of fungicides on disease severity of lentil at Barisal and Rajshahi in 2013-14

Treatments	Severity (0-5)			Disease Reaction
	Barisal	Rajshahi	Mean	
T_1=Rovral	1	2	1.5	MR
T_2=Compenion	3	4	3.5	S
T_3=Nativo	2	2	2	MR
T_4=Amistar Top	2	1	1.5	MR
T_5=Secure	3	3	3	MS
T_6=Control	5	5	5	HS
CV (%)	-	-	-	-

4.7.4. Growth and yield contributing characters and yield

Significant differences were found among treatments for plant height, number of branch per plant, number of pods per plant, 100-seed weight and yield were presented in Table 4.7.3, Table 4.7.4 and Table 4.7.5 and the experimental data were presented at two locations i.e. Barisal and Rajshahi in 2013-14.

4.7.4.1. Plant height

The plant height differed significantly among the treatments in Rajshahi location and ranged from 40.33 to 45.03cm. But in Barisal significant difference was not found and ranged from 38.40 to 40.87cm due to the application of different fungicides (Table 4.7.3). The tallest plant was found spray with Companion (40.87cm) and Amistar Top (45.03cm) in Barisal and Rajshahi, respectively. Dwarf plant was found spray with Rovral (38.40 cm) and Companion (40.33 cm) in Barisal and Rajshahi, respectively but dwarf plant height 40.33 cm was identical with other treatments except Amistar Top. Vigorous growth was found in Amistar Top treated plot compare to others.

4.7.4.2. Number of branches per plant

Number of branches per plant differed significantly among the treatments in Rajshahi location and ranged from 2.22 to 2.95. In Barisal location significant difference was not found and ranged from 2.04 to 2.45 due to the application of different fungicides (Table 4.7.3). In Rajshahi the maximum number of branches was recorded in Amistar Top sprayed plot (2.95) which was statistically identical with Rovral (2.88). Non-treated Control plot was produced the lowest (2.22) number of branches per plant which was statistically identical with Companion treated plot.

Table 4.7.3: Effect of foliar spray of fungicides on plant height and number of branches/plant of lentil at Barisal and Rajshahi in 2013-14

Treatments	Plant height (cm)		No. of branches/plant	
	Barisal	Rajshahi	Barisal	Rajshahi
T_1=Rovral	38.40	42.40 ab	2.35	2.88 a
T_2=Compenion	40.87	40.33 b	2.04	2.34 c
T_3=Nativo	39.20	41.47 ab	2.19	2.57 b
T_4=Amistar Top	39.93	45.03 a	2.45	2.95 a
T_5=Secure	39.40	41.47 ab	2.15	2.64 b
T_6=Control	40.20	41.33 ab	2.04	2.22 c
Level of significance	NS	*	NS	**
CV (%)	4.43	4.83	9.64	3.84

Means followed the same letter/letters do not statistically differ at 1% and 5% level tested by DMRT

* = Significant at 5% level of probability, ** = Significant at 1% level of probability, NS=Not significant

4.7.4.3. Number of pods per plant

Different fungicides had significant influence on number of pods per plant of lentil in both locations (Table 4.7.4). The maximum number of pods per plant was obtained from the plot sprayed with Rovral (95.57) which was statistically identical with Amistar Top (91.64) in Barisal. In Rajshahi the maximum number of pods per plant was obtained from the plot sprayed with Amistar Top (94.00) that was statistically similar with Rovral. The lowest value was found in controlled treatment in both locations.

4.7.4.4. 100-Seed weight

Different fungicides had also significant influence in hundred seed weight of lentil in both the locations and weight was increased over control (Table 4.7.4). In Barisal location maximum 100 seed weight was recorded from Amister Top treated plot (1.70g) which was statistically identical with Rovral treated plot. Minimum 100 seed weight was recorded from Control treated plot (1.21g) and it was statistically similar with all treatments except Amister Top and Rovral treated plot. In Rajshahi location maximum 100 seed weight was recorded from Amister Top treated plot (1.44g) followed by Rovral and Secure. Minimum 100 seed weight was recorded from Control treated plot (1.18g) which was statistically identical with all treatments except Amister Top and Rovral treated plot.

Table 4.7.4: Effect of foliar spray of fungicides on number of pods/plant and 100 seed weight of lentil at Barisal and Rajshahi in 2013-14

Treatments	No. of pods/plant		100 seed wt. (g)	
	Barisal	Rajshahi	Barisal	Rajshahi
T_1=Rovral	95.57 a	89.00 ab	1.65 a	1.35 ab
T_2=Compenion	75.51 c	78.00 c	1.24 b	1.23 bc
T_3=Nativo	77.12 c	82.00 bc	1.30 b	1.21 bc
T_4=Amistar Top	91.64 ab	94.00 a	1.70 a	1.44 a
T_5=Secure	87.84 b	79.00 c	1.40 b	1.31 a-c
T_6=Control	72.37 c	74.40 c	1.21 b	1.18 c
Level of significance	**	**	**	*
CV (%)	3.99	5.01	7.69	6.10

Means followed the same letter/letters do not statistically differ at 1% and 5% level tested by DMRT
* = Significant at 5% level of probability, ** = Significant at 1% level of probability, NS=Not significant

4.7.4.5. Yield

Remarkable effect of fungicides was noticed on the grain yield of lentil and yield was increased considerably compared to control (Table 4.7.3). In Barisal location the fungicide Rovral (1237 Kg/ha) sprayed plot produced the highest grain yield. The lowest yield was produced in the Control (815 Kg/ha) treated plot. In Rajshahi location the highest grain yield was produced in the Amistar Top (1216 Kg/ha) sprayed plot which was statistically identical with Rovral (1194 Kg/ha) but significantly differed from Nativo (1078 Kg/ha). The lowest yield was produced in the non-treated Control (748 Kg/ha) plot. Considering over locations, grain yield increased over control were 55.50% and 53.58% in Rovral and Amistar Top treated plot, respectively.

Table 4.7.5: Effect of foliar spray of fungicides on yield of lentil at Barisal and Rajshahi in 2013-14

Treatments	Yield (Kg/h)			Yield increased over control (%)
	Barisal	Rajshahi	Mean	
T_1=Rovral	1237 a	1194 a	1216	55.50
T_2=Compenion	912 d	850 d	881	12.66
T_3=Nativo	1051 c	1078 b	1065	36.19
T_4=Amistar Top	1185 b	1216 a	1201	53.58
T_5=Secure	1082 c	1023 c	1053	34.65
T_6=Control	815 e	748 e	782	-
Level of significance	**	**	-	-
CV (%)	2.67	2.54	-	-

Means followed the same letter/letters do not statistically differ at 5% level tested by DMRT

* = Significant at 5% level of probability, ** = Significant at 1% level of probability, NS=Not significant

4.7.5. Relation of yield and diseases

Relation of yield with disease incidence or disease severity was presented in Fig. 4.7.1 and Fig. 4.7.2. Lower percent disease incidence of stemphylium blight of lentil produced higher yield and higher percent disease incidence produced lower yield. Same trend was found in case of disease severity where lower disease severity of stemphylium blight of lentil produced higher yield and higher disease severity produced lower yield.

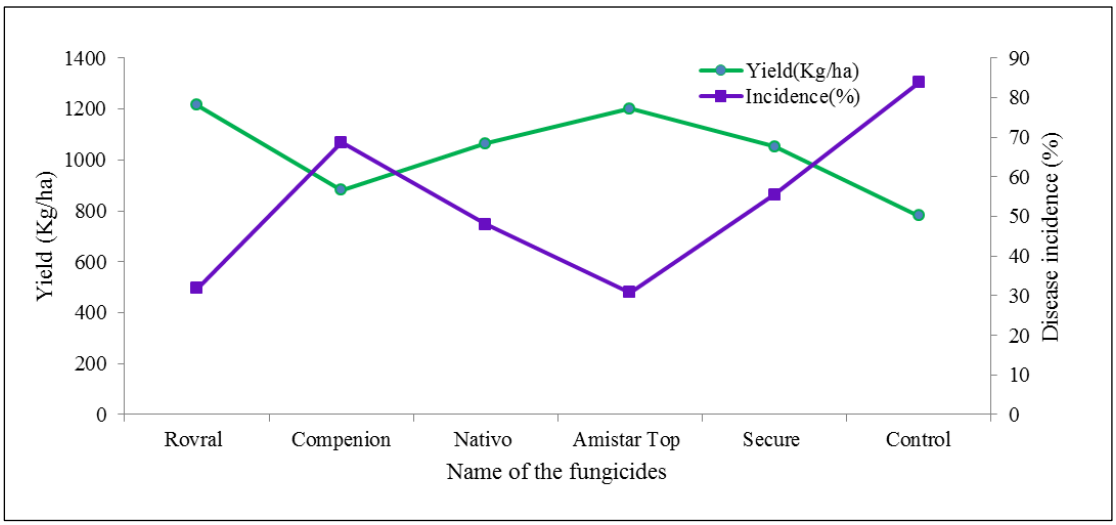


Fig. 4.7.1: Relation of yield and diseases incidence of stemphylium blight of lentil due to application of different fungicides in 2013-14

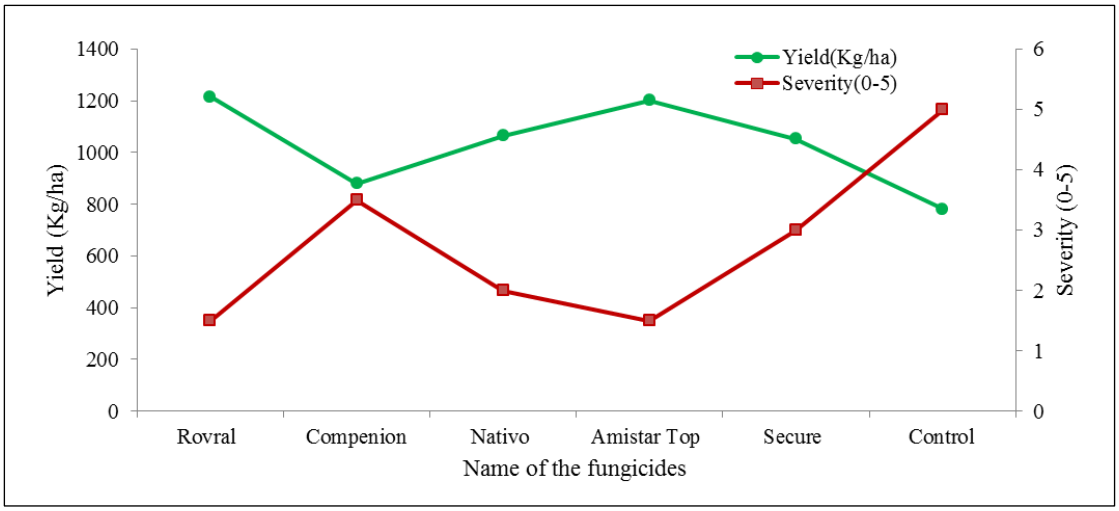


Fig. 4.7.2: Relation of yield and diseases severity of stemphylium blight of lentil due to application of different fungicides in 2013-14

4.8. Experiment-VIII: Efficacy of different fungicides with foliar spray and seed treatment in controlling stemphylium blight of lentil (2014-2015)

Selected fungicide Rovral was sprayed as a foliar spray and Bavistin or Provax as a seed treating chemical were effectively controlled the stemphylium blight of lentil variety BARI Masur-1 under field condition. The efficacy of the treatments was measured in respect of incidence, severity and yield at Rajshahi in 2014-15.

4.8.1. Percent diseases incidence

The percentage disease incidence of stemphylium blight of lentil is presented in Table 4.8.1. Significant difference was found in percent disease incidence among the treatments. The lowest diseases incidence was recorded in the treatment T_1 (Seed treatment with Provax + Foliar spray with Rovral) (25.81%). The highest diseases incidence was recorded in the treatment T_4 (No seed treatment + No foliar spray with Rovral) (81.43%). Disease reductions over control were the highest in T_1 (68.30%) followed by T_2 (62.26%) and then T_3.

4.8.2. Diseases severity

The disease severity of stemphylium blight of lentil is presented in Table 4.8.1.The data were recorded 0-5 scoring scale. The lowest disease infection was recorded in T_1 and T_2 were graded as 1 scoring scale. T_3 graded as 2 scoring scale and T_4 graded as 5 scoring scale.

4.8.3. Diseases reaction

The disease reaction of stemphylium blight of lentil is presented in Table 4.8.1. Disease reaction of lentil in the field were recorded and found wide variation among the treatments. Resistant (R) disease reaction was showed both the

treatments T_1 and T_2. Moderately resistant (MR) disease reaction was recorded in the treatment T_3 (No seed treatment + Foliar spray with Rovral). Sever infection was found in the non-treated Control plot i.e. treatment T_4 (No seed treatment + No foliar spray with Rovral) which showed highly susceptible (HS) disease reaction.

Table 4.8.1: Effect of foliar spray with seed treatment of fungicides on incidence, severity and disease reaction of lentil at Rajshahi in 2014-15

Treatments	Disease Incidence (%)	Disease reduction over control (%)	Disease Severity (0-5)	Disease Reaction
T_1=Seed treatment with Provax + Foliar spray with Rovral	25.81 d	68.30	1	R
T_2=Seed treatment with Bavistin + Foliar spray with Rovral	30.73 c	62.26	1	R
T_3=No seed treatment + Foliar spray with Rovral	39.19 b	51.87	2	MR
T_4=No seed treatment +No foliar spray with Rovral	81.43 a	-	5	HS
Level of significance	**	-	-	-
CV (%)	4.55	-	-	-

Means followed the same letter/letters do not statistically differ at 1% level tested by DMRT

* = Significant at 5% level of probability, ** = Significant at 1% level of probability, NS=Not significant

4.8.3. Yield

Remarkable effect of fungicides was noticed on the grain yield of lentil variety BARI Masur-1 and yield was increased considerably compared to control (Table 4.8.2). Significant difference was recorded among the treatments and the yield ranged from 847 to 1314 Kg/ha. The highest yield was recorded in the treatment T_1 (Seed treatment with Provax + Foliar spray with Rovral) (1314

Kg/ha). The lowest yield was recorded in the treatment T_4 (No seed treatment+No foliar spray with Rovral) (847 Kg/ha). Yield increased over control were 55.14%, 51.71% and 31.64% in the treatments T_1, T_2 and T_3, respectively.

Table 4.8.2: Effect of foliar spray with seed treatment of fungicides on incidence, severity and yield of lentil during 2014-15 in Rajshahi

Treatments	Yield (Kg/ha)	Yield increased over control (%)
T_1=Seed treatment with Provax + Foliar spray with Rovral	1314 a	55.14
T_2=Seed treatment with Bavistin + Foliar spray with Rovral	1285 b	51.71
T_3=No seed treatment +Foliar spray with Rovral	1115 c	31.64
T_4=No seed treatment +No foliar spray with Rovral	847 d	-
Level of significance	**	-
CV (%)	6.4	-

Means followed the same letter/letters do not statistically differ at 1% level tested by DMRT

* = Significant at 5% level of probability, ** = Significant at 1% level of probability, NS=Not significant

CHAPTER 5

DISCUSSION

During last three decades stemphylium blight of lentil caused by S*temphylium botryosum* have emerged as devastating pathogen in Bangladesh. It is considered prime limiting factor in lentil production in our country causing huge economic losses and threatening lentil production. The present research was carried out to investigate the performance of different lentil lines/varieties in the southern region of Bangladesh and different management practices in controlling this disease. This study was done at Regional Agricultural Research Station (RARS), Bangladesh Agriculture Research Institute (BARI), Rahmatpur, Barisal during rabi season of 2011-2014 and Fruit Research Station, BARI, Binodpur, Rajshahi, Bangladesh during 2013-2015.

5.1. Experiment-I: Survey of stemphylium blight of lentil at farmer's field indifferent lentil growing areas of Bangladesh (2012-13)

A stemphylium blight disease survey was conducted in 11 districts and found that the highest disease incidence was found in southern district and the lowest disease incidence was found a north-western district. Out of 11 districts disease severity graded as 2, 3 and 4 scoring scale in the number of 3, 5 and 3 districts, respectively. The cause of variation of infection might be due to influence of prevailing of different weather conditions of the locations and variation of isolate of the pathogen. These findings corroborate with the findings of other researchers. Bakr (1991), Bakr (1993), Sinha and Singh (1993) and Bakr and Ahmed (1993) reported that stemphylium blight caused by *Stemphylium* sp. was becoming a serious threat to lentil cultivation in Bangladesh. It was a major disease estimated yield losses of 62% and total crop failure have been reported in some cases. This disease was epidemic in Bangladesh and also in India that was revealed by Sinha and Singh (1993) who reported that disease intensity as high as 83% was observed on an unsprayed local cultivar in Bihar of India causing above 90% yield loss. Moreover, stemphylium blight of lentil

also major disease in Nepal reported by Subedi *et al*. (2015). Stemphylium blight disease caused by the fungus *S. botryosum* is occurring in Australia as a saprophyte but could be a potential threat for the Australian lentil industry. But in Canada it was sporadic incidences (PBA, 2013). Stemphylium blight of lentil was occurred as a minor disease in many countries like Ethiopia (Abraham, 2015).

Stemphylium blight of lentil showed huge variation of disease incidence and severity in surveyed area. The highest disease incidence was found in local variety (74.33%) and the lowest disease incidence was BARI Masur-7. Highly susceptible (HS) disease reaction was found in local variety, moderately susceptible (MS) were showed in 2 varieties (BARI Masur-3 and BARI Masur-4) but moderately resistant (MR) disease reactions were found in 3 varieties (BARI Masur-5, BARI Masur-6 and BARI Masur-7). This variation of disease incidence might be due to the variation in their genetic makeup of the specific variety and the findings of this investigation supported by Polfliet (2002) who stated that *Stemphylium* spp. was pathogenic on many crops throughout the world and cause varying degrees of losses on different crops.

During field survey disease incidence and severity was observed in different growth stages of lentil and found highly significant difference within the growth stages. Similar result was obtained and agreed by Islam, 2014.

Out of total diseased samples, collected during survey work, 100% samples were infected by stemphylium blight and found alternaria, rust, leaf rot and botrytis gray mold (BGM) as an associated fungi. Rashid *et al*. (2007) reported that fifteen pathogens causing 17 diseases have so far been recorded in Bangladesh but only few are severe causing severe losses in yield which was an agreement with the results obtained in the present study. Similar result were described by Uddin *et al*. (2008) that in lentil stemphylium blight (*Stemphylium botryosum*) causes 88%, collar rot (*Sclerotium rolfsii*) 44.4 %, rust (*Uromyces fabae*) 34.4 % yield loss in Bangladesh.

5.2. Experiment-II: Isolation, identification and morphology of *Stemphylium botryosum* in lentil (2013)

The symptoms of stemphylium blight disease of lentil were well characterized with chronological photography of the disease in this investigation. Because of chronological photography of the disease symptoms might be helpful to the farmers or researchers. This study also helpful for identification of the disease correctly. Stemphylium blight disease of lentil could not occur at seedling stages and should not confuse with root rot occurring at seedling stages. Root rot shown brown or light yellow colored leaf looking stemphylium blight.

The results i.e. characterization of the stemphylium blight disease symptoms were in agreement with the findings of Bakr (1991) who reported that the symptoms of the disease in Bangladesh included the appearance of small pin-headed light brown to tan colored spots on the leaflets which later enlarged. A blighted dull yellow appearance was observed with infected foliage and branches. Defoliation occurred rapidly, leaving the branches with terminal leaves. The stems and branches also bend down and dry up but the pods remain green. White mycelia growth could also be observed on the infected stems. Barker, 2009 again confirmed the symptoms. There were many researcher agreed with the present findings (Erskine and Sarker, 1997; Bayaa and Erskine, 1998; Chen *et al*., 2009 and McGreevy, 2013). But Morrall *et al*. (2004) reported that in Saskatchewan, it was suspected that stemphylium blight has not been correctly identified in the field, as the lesions closely resembled those of ascochyta blight. Isaacs, 2014 also similar commented that stemphylium blight caused by the fungal pathogen *S. botryosum*, is a lentil disease that has only been identified as a problem in recent years and according to Sabine Banniza, a researcher at the University of Saskatchewan, this might be due to misdiagnosis in the past.

5.3. Experiment-III: Searching of the time of first appearance of stemphylium blight in lentil (2013-2014)

For successful management, appropriate time of the first appearance of the disease in the field might be important. During 2013-14, out of 7 different date of sowing stemphylium blight of lentil was searched minutely and recorded first appearance of the disease in the field at pre-flowering or flowering stages. Two lentil varieties were under investigation and found similar result in both varieties.

The results were in agreement with the findings of Polfliet (2002) who reported that stemphylium blight usually affects different crops from the flowering stage onwards. Shahiduzzaman *et al*. (2015) stated similar things that stemphylium blight generally appears at flowering stage of the crop. Islam (2014) also agreed with this type of findings. Findings in the present investigation were under natural epiphytotic conditions. But it might be contradictory in artificial condition where Kumar (2007) found during indoor screening, two lentil cultivars Eston and CDC Milestone inoculated by *S. botryosum* at 14, 28, 42 and 56 days after planting under greenhouse conditions and disease symptoms were developed in all plants. This was also supported by Banniza *et al*. (2005) and Saha (2009).

So, it might be concluded that first appearance of stemphylium blight in lentil in the field at pre-flowering or flowering stages but in artificial condition, in presence of available inoculum with favorable environment, might be possible to develop disease symptoms in any growth stage of lentil.

5.4. Experiment-IV: Searching of the alternate host of *Stemphylium* spp. in different weed species in lentil field (2012-2013)

During survey (2012-2013) of stemphylium blight of lentil in 11 districts, it also surveyed different weed species grown in lentil fields and found 8 different weed species. From the collected weed samples and after investigation under compound microscope *Stemphylium botryosum* were found only from bathua weed (*Chenopodium album*). Pathogenicity test were successfully carried out in case of stemphylium blight of bathua by following Koch's postulates procedure. It was first report in Bangladesh. The results were in agreement with the findings of Aly, 2010 who reported that *S. botryosum* was isolated from leaves of bathua weed (*C. album*) collected in Egypt. Hanse *et al*. (2015) had also similar observation that in the Netherlands, during the summer of 2007, *C. album* was identified as hosts in an assay of plants grown and inoculated in climate rooms.

5.5. Experiment-V: Screening of lines for resistant to stemphylium blight of lentil (2011-2014)

The tested 214 lentil lines/varieties showed wide variation in reaction to stemphylium blight under field condition at different growth stage. The sensitivity of the tested lentil lines/varieties increased with the increase in age of the plants. The tendency of prevalence of stemphylium was as follows: flowering stage>pod setting stage>maturity stage. But this tendency was not always a regular pattern to, susceptible all the lines/varieties. Some materials were sensible at flowering stage. Moreover, the tested lines/verities showed variation in tolerance/resistance in the experimental period. These findings corroborate with the findings of other researchers. 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 resistance and susceptible. The finding of the present study revealed that the tested lentil lines/verities showed different types of reaction to stemphylium blight under field condition. The variation in respect of disease reaction among the lentil lines/verities from flowering, pod setting and maturity stage may be due to genetic variation among the lines/varieties that govern the resistance mechanism of plant against stemphylium blight or variation of the pathogenic strain/races of *S. botryosum* since some materials showed different resistant reaction, these may be due to exposure of different resistant mechanism of plant. From this study it is clear that none of the tested materials were immune to *S. botryosum*. Ahmed *et al.* (1981) also did not find any material immune lines/verities to *S. botryosum*.

Temperature, leaf wetness and relative humidity (RH) are the most important environmental factors affecting the development of the disease (Basallote-Urbea *et al.*, 1999). The surveys and field experiment carried out in India and Bangladesh on *S. botryosum*, have confirmed the importance of temperature and RH for successful development of stemphylium blight in lentil (Bakr, 1993; Sinha and Sing, 1993).

It was observed that the tested lentil lines/verities differed significantly in respect of plant height, number of pod per plant, number of branch per plant, 100 seed weight and yield. The variation of the materials in respect of above parameters may be due to i) stemphylium blight diseases reaction to the respective materials. ii) genetic constitution and variation of materials and iii) environmental effect of the growing period in the field. The findings of the study is closely related with the study of Sarwar *et al.* (1984), Saraf *et al.* (1985) and Zaman *et al.* (1989). They reported that the lentil line differed significantly in respect of agronomic traits and yield parameters. Regarding the variation in yield of lentil due to stemphylium blight was observed that the lines/verities differed significantly from one to another. This variation may be due to i) the effect of S*temphylium botryosum* on formation of pod. ii)

variations of genetic makeup of lentil lines/verities and iii) growing conditions of plants. Bakr (1993), Mwakutuya (2002) and Neubauer (1998) 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 materials of lentil that showed resistant reaction in November-2011 to April-2014 to *S. botryosum* need to be tested for furthers conformation of the result of this study. In the experimental period it was concluded that after harvesting of all lines/varieties it can be seen that two lines/varieties were gave the highest yield BD-6002 (1628 Kg/ha) followed by BARI Masur-7 (1602 Kg/ha) and BD-3837 (1447 Kg/ha).

5.6. Experiment-VI: Effect of different dates of sowing and varieties on stemphylium blight of lentil (2012-2013)

Time of sowing had marked effect upon level of disease incidence and thus manipulating the sowing time infection may be avoided. Many field crops can escape various diseases with the shifting of sowing time. Optimum time of sowing is the important factor for profitable lentil cultivation. During the investigation, in early sowing (October 25 and November 1) plot yield were produced lower due to higher disease severity and in this time vegetative growth was resulting delayed maturity. Optimum sowing (November 08 and November 15) produced higher yield. During late sowing (November 29 and December 06) disease incidence and severity were recorded lower as well as lower yield. Due to late sowing maturity might be forced. In that aspect late sowing having forced maturity might be a cause of yield loss.

These finding was closely favored with Hawthorne *et al*. (2016) who obtained that early sowing of lentil resulted in more vegetative growth and crops prone to lodging, increasing the risk of disease infection and subsequent poor grain

quality. Later sowings reduce disease risk but can result in lower yields due to the risk of dry conditions, high temperatures at flowering and pod filling stage. Many previous researchers supported that incidence and severity of disease were varied by changing the sowing time resulting the effect on yield. Ahmed *et al*. (2002) reported that sowing date of lentil is considered as one limiting factor for disease incidence in field. Jain *et al*. (1987) confirmed that effect of sowing date on yield of lentil on November 01 showed significantly the lowest PDI and the highest yield followed by November 10 and November 20. Sowing on early November could avoid disease significantly and increase yield. Before few years, in the same location (Barisal) of the present investigation, Huq and Khan (2008) conducted an experiment and found similarity of the result with some extents that was effect of sowing date on yield of lentil on November 01 showed significantly the lowest PDI and the highest yield followed by November 10 and November 20. Sowing on early November in Barisal region of Bangladesh could avoid disease significantly and increase yield. After above discussion it might be concluded that optimum sowing time of lentil was November 8 to 15 in Bangladesh condition for minimized the disease and increased the yield. So it might be recommended that lentil may be sown before November 20 for maximum yield by the reduction of disease severity significantly.

5.7. Experiment-VII: Efficacy of different fungicides in controlling stemphylium blight of lentil (2013-2014)

Successful management of the disease was achieved through application of chemical fungicides. All the tested fungicides reduced the disease score and affect the plant growth parameters and increased yield of lentil as compare to control plot. Considering both locations, the lowest disease incidence with moderately resistance (MR) disease reaction were recorded in case of Rovral 50WP (Iprodione) and Amistar Top (Azoxystrobin 20% + Difenoconazole 12.5%) treated plot and there were no significant difference among them as

well as increased yield compare to Control. The maximum number of pods per plant and increased 100-seed weight was obtained from the plot sprayed with Rovral and Amistar Top treated plot. On the other hand the highest disease incidence with highly susceptible (HS) disease reaction was recorded in case of Control as well as yield was the lowest.

Similar findings had been recorded by many researchers. Bakr and Ahmed (1992) reported that disease score was the lowest whereas yield was the highest in plots treated with Rovral 50WP @ 0.2%. Haque *et al*. (2013) also reported that the lowest disease was obtained from the Rovral 50WP treated plot. Foliar spray (4 sequences) with Rovral 50WP and Secure 600WG (Fenamidione + Mancozeb) at an interval of 7 days effectively controlled the disease and increased yield of lentil by 31.99% and 28.20%, respectively (Shahiduzzaman *et al*., 2015). In the present investigation stemphylium blight of lentil caused by *S. botryosum* effectively controlled by Amistar Top that was supported by many other researchers in other host plant. Azoxystrobin is registered in Florida and Washington to manage stemphylium leaf spot of spinach (Raid and Kucharek, 2003; PICOL, 2004). du Toit and Derie (2003a) and du Toit *et al*. (2004 and 2005) documented that fungicides in the strobilurin family as well as iprodione were highly efficacious against stemphylium leaf spot.

In Bangladesh condition, farmer's practice was observed sprayed of foliar spray by Rovral in controlling stemphylium blight of lentil. But to avoid development of resistance to fungicides by *S. botryosum*, fungicides with different modes of action should be alternated or mixed to manage this disease for the long term. Already research finding was found in the present investigation that *S. botryosum* effectively controlled in lentil by spray with Amistar Top or Rovral 50WP. So, Amistar Top might be suggesting for alternating spray with Rovral 50WP in controlling stemphylium blight of lentil.

5.8. Experiment-VIII: Efficacy of different fungicides with foliar spray and seed treatment in controlling stemphylium blight of lentil (2014-2015)

Infected seed is an important means of transmission of the disease from region to region and also serve as a source of initial inoculum early in the season. It overwinters on seed and as mycelium on dead stems and leaves in many cropping systems. Limited information is available on whether the pathogen is seed-borne in nature on lentil. Selected fungicide Rovral was sprayed as a foliar spray and Bavistin or Provax as a seed treating chemical were effectively controlled the stemphylium blight of lentil variety BARI Masur-1. Remarkable effect of fungicides was reported on the grain yield of lentil and yield was increased considerably compared to control.

Though seed infection by *S. botryosum* has been reported in previous studies, there is no clear understanding of the significance of seed borne *S. botryosum* inoculum on disease initiation of lentil (Mwakutuya, 2006). McGreevy, 2013 reported that *S. botryosum* infected the lentil seed, but seed-to-seedling transmission of the disease has not been demonstrated. In case of onions fungicide use was found to be effective against seed borne *Stemphylium* sp. (Aveling *et al*., 1993). However many researchers supported *S. botryosum* infected the seeds. Seed infection of lentil with *S. botryosum* has been observed yearly since 2000, as reported in the Canadian Plant Disease Survey (Dykstra *et al*., 2004).

From above discussion it might be understood that *S. botryosum* infected the seeds and reduced the incidence of the disease by seed treatment. Due to lack of availability of research paper on seed treatment for controlling of stemphylium blight of lentil, seed treating agent were chosen as Bavistin and Provax in the present investigation which also might be controlled the root rot or wilt in lentil.

CHAPTER 6

SUMMARY AND CONCLUSION

Stemphylium blight caused by *Stemphylium botryosum* Wallroth is considered as the most devastating foliar diseases of lentil (*Lens culinaris* Medikus) in Bangladesh. Eight experiments were conducted during October 2011 to March 2015 to know the prevalence of the diseases with identification the causal agent, finding alternate host and determine different type of management practices.

A survey was conducted at 11 lentil growing districts of Bangladesh during cropping season of 2012-13 to assess the percent disease incidence and severity of stemphylium blight of lentil. The incidence and severity were varied from location to locations. The highest disease incidence was found in Jhalokathi (Sadar) (77.90%) and the lowest disease incidence was found in Pabna (Ishardi) (45.50%). The highest disease severity was found in Faridpur (Sadar), Jhalokathi (Sadar) and Khulna (Paikgasa) and the lowest was found Kushtia (Kumarkhali), Pabna (Ishardi) and Barisal (Babuganj). The highest disease incidence was found in local variety (74.33 %) followed by BARI Masur-3 (68.67%) and BARI Masur-4 (68.33%) which was not significantly difference with each other but significantly difference with BARI Masur-6 (41.00 %) and BARI Masur-7 (31.00 %). The lowest disease incidence was recorded in BARI Masur-7. In case of disease reaction, local variety performed highly susceptible (HS), BARI Masur-3 and BARI Masur-4 showed moderately susceptible (MS) and BARI Masur-5, BARI Masur-6 and BARI Masur-7 showed moderately resistant (MR) disease reaction under field condition. Lower disease incidence (17%) was observed at flowering stage. Moderately disease incidence was recorded at pod setting stage (54%) and severely incidence was recorded (76%) at pre-maturity stage. Same trend occurred in case of disease severity. A total of 330 diseased samples were collected from 110 fields and found that 100%

samples were infected by stemphylium blight. In the same samples 30%, 7%, 2% and 1% samples were associated by alternaria blight, rust, leaf rot and botrytis gray mold, respectively. Out of 11 districts only 3 districts named Faridpur (sadar), Madaripur (sadar) and Khulna (Paikgasa) were infected by rust and rest of the districts were not found rust disease in any field.

Spore of *Stemphylium botryosum* landing and infect the leaflet and formed of small pinhead gray spots or light brown on the leaflets which smaller lesions later irregularly enlarged, covering the surface of the leaflet within 2 to 3 days and killed single leaflet. In presence of more inoculum more than one leaflet infected and twigs showed special type symptoms like fishing hook. Later infection was rapidly spread over the leaf, shoots or twigs but pod remained green. The above symptoms clearly differ from other foliar lesions of lentil. These findings were recorded by frequently field visit with closed observation and collected samples were examined under microscope. During susceptible condition of the disease within 7 to 10 days farmers' field might be possible to reach brownish color and looking just burn by fire. Stemphylium blight could not occur at seedling stages and should not confuse with foot rot occurring at seedling stages. *S. botryosum* were isolated by using V-6 media (modified of V-8 media) and found pure culture. Conidia were oblong rounded at the ends, broadly ellipsoidal or sub-spherical, usually 3 transverse and 1-3 longitudinal septa, minutely verrucos or echinulate and muriform with length to breadth ratio (L/B) about 1.5. Conidiophores were brown with terminal swellings.

Out of 7 different dates of sowing stemphylium blight of lentil was searched minutely and recorded first appearance of the disease in the field at pre-flowering or flowering stages. Two lentil varieties named BARI Masur-1 (susceptible variety) and BARI Masur-7 (resistant variety) were investigated for searching the appropriate time of disease at Barisal, Bangladesh during 2013-14.

Eight weed species were recorded grown in lentil fields during survey period (2012-2013) at 11 districts. Conidia of *Stemphylium botryosum* were isolated only from the collected leaf samples of Bathua weed (*Chenopodium album*). Pathogenicity test were confirmed following standard procedure and it was first report in Bangladesh.

A total of 214 lentil lines/varieties were screened out against stemphylium blight in the field under artificial inoculated condition in 3 subsequent years of 2011-12, 2012-13 and 2013-14 in Barisal. In the first year 214 lines/varieties were screened including 4 check varieties BARI Masur-1, BARI Masur-4, BARI Masur-6 and BARI Masur-7 and 22 lines were selected. At pre-maturity stage out of 214 lines/varieties 24, 41, 82 and 67 lines were recorded as MR, MS, S and HS, respectively. Result reveled that four lines performed higher amount of grain yield and ranged from 1401 to 1700 Kg/ha. Eighteen lines produced medium ranged of grain yield and ranges from 1001 to 1400 Kg/ha. In the second year out of 24 lines/varieties including 2 check varieties BARI Masur-1 (susceptible) and BARI Masur-7 (resistant), 3 lines named BD-6002, BD-3926 and BD-3837 were selected. Disease incidence increased gradually flowering to pod setting stage and finally severely infected at pre-maturity stage. Percent disease incidence ranged from 0.71-3.16, 10.80-32.67 and 26.67-86.96 at flowering stage, pod setting stage and pre-mature stage, respectively. At pre-maturity stage 13, 10 and 01 lines/varieties were recorded as MR, MS and S, respectably. Among the lines/varieties the yield ranged from 763 to 1738 Kg/ha. In the third year out of 5 lines/varieties including 2 check varieties BARI Masur-1 and BARI Masur-7 were screened at Barisal and Rajshahi and finally 2 lines *viz*. BD-6002 and BD-3837 found better performance considering different parameters. Resistant (R) and moderately resistant (MR) disease reaction were showed in BD-6002 and BD-3837, respectively with lower disease incidence. The highest yield were found over the locations in

BD-6002 line (1628 Kg/ha) followed by BARI Masur-7 (1602 Kg/ha) and BD-3837 (1447 Kg/ha) that were statistically similar.

Effect of different dates of sowing and varieties on stemphylium blight of lentil was carried out in Barisal in 2012-13. The higher number of branches per plant and number of pods per plant in November 08 and November 15 sowing plots were contributed the highest seed yield in case of variety BARI Masur-7 but in case of variety BARI Masur-1 produced better results November 08 sowing plot. In early sowing (October 25 and November 1), yield were produced lower due to higher disease severity and in this time vegetative growth was resulting delayed maturity. Optimum sowing (November 08 and November 15) produced higher yield. During late sowing (November 29 and December 06) disease incidence and severity were recorded lower as well as lower yield. Due to late sowing maturity might be forced resulting yield loss. In case of varietal performance, disease incidence was 64% and 47% in BARI Masur-1 and BARI Masur-7, respectively. BARI Masur-1 showed highly susceptible (HS) disease reaction on the other hand BARI Masur-7 showed moderately resistant (MR) reaction. And yield was 789 Kg/ha and 1131 Kg/ha in BARI Masur-1 and BARI Masur-7, respectively.

Five selected fungicides *viz*. Rovral, Compenion, Nativo, Amistar Top and Secure were assessed against stemphylium blight of lentil in Barisal and Rajshahi in 2013-14. Results revealed that 3 sequences foliar spray with Rovral 50WP (Iprodione) @ (0.2%) and Amistar Top 325 SC (Azoxystrobin 20% + Difenoconazole12.5%) @ (0.1%) at an interval of 12 days effectively controlled the disease. Considering over locations, moderately resistance (MR) disease reaction was observed in Rovral, Amistar Top and Nativo treated plot. Moderately susceptible (MS), susceptible (S) and highly susceptible (HS) disease reactions were recorded in Secure, Compenion and Control treatments, respectively. Grain yield increased over control were 55.50% and 53.58% in Rovral and Amistar Top treated plot, respectively.

In another field experiment, efficacy of different fungicides with foliar spray and seed treatment were evaluated against stemphylium blight of lentil in Rajshahi in 2014-15. Seed treatment with Bavistin or Provax and selected fungicide Rovral was sprayed as a foliar spray were effectively controlled the stemphylium blight of lentil variety BARI Masur-1. Significant difference was recorded among the treatments and the yield ranged from 847 to 1314 Kg/ha.

From the study it might be concluded

- The present investigations have clearly pointed out the importance of stemphylium blight disease of lentil in Bangladesh. Most of the farmers' fields were infected by the disease, so it is a threat to lentil cultivation in Bangladesh.
- Symptoms of stemphylium blight of lentil were distinctly characterized and reported.
- The first appearance of the disease in the field was observed at pre-flowering or flowering stages of lentil. So control measures should be started at this stage.
- Bathua weed (*Chenopodium album*) is an alternate host of stemphylium blight that was confirmed by pathogenicity test. It should be removed from the lentil field. It was the first report in Bangladesh.
- A total of 214 lentil lines/varieties were screened out against stemphylium blight in the field under artificial inoculated condition in three subsequent years and finally 2 lines *viz.* BD-6002 and BD-3837 found better performance considering different parameters.
- The optimum sowing time (November 8 to 15) was important for maximum yield by the reduction of disease severity significantly.
- Rovral 50WP (Iprodione) @ (0.2%) and Amistar Top 325 SC (Azoxystrobin 20% + Difenoconazole 12.5%) @ (0.1%) effectively controlled the stemphylium blight disease of lentil with 3 sequences foliar spray at an interval of 12 days. Seed treatment with Bavistin or Provax provided better performance.

CHAPTER 7

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APPENDICES

Appendix 1: Different Meteorological Data

Table 1: Monthly mean temperature, relative humidity and total rainfall during crop period at the experimental site, Regional Agricultural Research Station (RARS), Rahmatpur, Barisal

Month/Season	Temperature (^{0}C)			Average relative humidity (%)	Total rainfall (mm)
	Maximum	Minimum	Average		
2011-12 Season					
October-2011	35.00	20.00	27.50	84.00	56.00
November-2011	32.50	15.00	23.75	82.00	1.00
December-2011	30.60	9.80	20.20	84.00	0
January-2012	28.80	9.00	18.90	82.00	31.00
February-2012	34.20	9.50	21.85	72.00	2.00
March-2012	35.80	16.80	26.30	74.00	11.00
2012-13 Season					
October-2012	34.60	18.80	26.70	86.00	70.00
November-2012	32.70	13.00	22.85	82.00	44.00
December-2012	28.80	8.50	18.65	85.00	0
January-2013	28.70	6.50	17.60	79.00	0
February-2013	32.30	12.00	22.15	76.00	6.00
March-2013	36.20	14.00	25.10	75.00	5.00
2013-14 Season					
October-2013	34.20	20.20	27.20	87.00	181.00
November-2013	32.20	15.10	23.65	80.00	0
December-2013	29.50	10.40	19.95	82.00	0
January-2014	28.80	8.50	18.65	83.00	0
February-2014	29.70	10.20	19.95	76.00	15.00
March-2014	37.40	15.00	26.20	71.00	13.00

Source: Bangladesh Meteorological Department, Climate Division, Agargaon, Dhaka-1207

Table 2: Monthly mean temperature, relative humidity and total rainfall during crop period at the experimental site, Fruit Research Station (FRS), Binodpur, Rajshahi

Month/Season	Temperature (^{0}C)			Average relative humidity (%)	Total rainfall (mm)
	Maximum	Minimum	Average		
2013-14 Season					
October-2013	34.80	18.20	26.50	87.19	204.00
November-2013	32.00	12.00	22.00	75.90	0
December-2013	29.60	9.20	19.40	81.32	0
January-2014	27.50	7.00	17.25	82.68	0
February-2014	30.20	9.20	19.70	77.36	26.40
March-2014	39.60	13.40	26.50	66.48	8.80
2014-15 Season					
October-2014	35.70	18.0	26.85	83.16	5.00
November-2014	33.80	11.30	22.55	78.13	0
December-2014	28.10	7.40	17.75	83.94	0
January-2015	28.70	6.70	17.70	83.58	13.80
February-2015	34.60	7.60	21.10	78.18	14.20
March-2015	37.20	11.60	24.40	66.39	0.40

Source: Bangladesh Meteorological Department, Rajshahi Center, Rajshahi

Appendix 2: Fertility status of initial soil of the experimental field

Sl. No.	Properties of soil	Contents	
		Barisal Field	Rajshahi Field
1	P^H	5.50	8.00
2	Organic Matter (%)	1.10	1.61
3	Nitrogen (%)	0.11	0.09
4	Potassium (meq/100gm)	0.18	0.59
5	Phosphorus (µg/g)	15.00	145.00
6	Sulfur (µg/g)	17.00	17.20
7	Boron (µg/g)	0.20	0.80
8	Zinc (µg/g)	2.20	1.73

Sources: Regional Laboratory, Soil Resource Development Institute, Barisal and Rajshahi.

Appendix 3: Global lentil production calendar

Name of the country	Name of the months											
	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
Australia					↔					↔		
Canada				↔				↔				
Turkey/Syria					↔						↔	
India (Rabi)				↔						↔		
Pakistan			↔							↔		
Egypt				↔				↔				
EU (Spring)		↔						↔				
China (North)		↔							↔			
China (South)				↔					↔			

*Direction: Sowing ↔ Harvest ↔

Appendix 4: Different plates showed experimental fields

Plate 1: Data and sample collection in field and investigated in laboratory

Plate 2: Field view of non-infected farmers field

Plate 3: Field view of screening from 214 lines/varieties

Plate 4: Field view of screening from 24 lines/varieties

Plate 5: Field view of screening from 5 lines/varieties

Plate 6: Screening from 5 lines/varieties and view of different plots

Plate 7: PhD evaluation committee visited the field

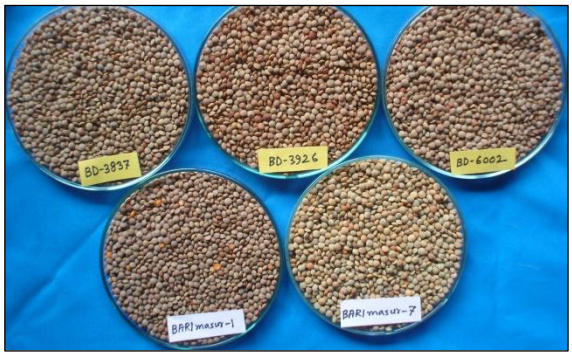

Plate 8: Picture showed seed of 5 lines/varieties

Plate 9: Field view of efficacy of different fungicides

Plate 10: Efficacy of different fungicides with different plots

Plate 11: Field view of efficacy of different fungicides with foliar spray and seed treatment

Plate 12: Field view of different time of sowing

Plate 13: Field view of searching the time of first appearance of the disease

Plate 14: The researchers diagnosis the disease with the help of co-supervisor

Plate 15: Performance of BARI Masur-1 and BARI Masur-7 in the research field