Studies on Anthracnose of Beans (*Phaseolus vulgaris* L.)in Kashmir Valley

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Jan Mohd. Junaid
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Thesis

Submitted to

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in partial fulfilment of requirement for the award of the degree of

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2012

to my beloved Parents

Sher-e-Kashmir
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Certificate – I

This is to certify that the thesis entitled,**“Studies on Anthracnose of Beans (*Phaseolus vulgaris* L.) in Kashmir Valley”** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science in Agriculture (Plant Pathology),** to the **Faculty of Post-graduate Studies, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir** is a record of bonafide research work carried out by **Mr.Jan Mohd Junaid (Regd. No. 2010-A-859-M)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that information received during the course of investigation has duly been acknowledged.

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Certificate – II

We, the members of the Advisory Committee of **Mr. Jan Mohd Junaid (Regd. No. 2010-A-859-M)**, a candidate for the degree of **Master of Science in Agriculture (Plant Pathology)** have gone through the manuscript of the thesis entitled, **"Studies on Anthracnose of Beans (*Phaseolus vulgaris* L.) in Kashmir Valley"**and recommend that it may be submitted by the student in partial fulfillment of the requirements for the award of the degree.

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Certificate – III

This is to certify that the thesis entitled, **"Studies on Anthracnose of Beans (*Phaseolus vulgaris* L.) in Kashmir Valley"**submitted by **Mr. Jan Mohd Junaid (Regd. No. 2010-A-859-M)** to the **Faculty of Post graduate Studies, Sher e Kashmir University of Agricultural Sciences and Technology of Kashmir** in partial fulfilment of the requirements for the award of the degree of **Master of Science in Agriculture (Plant Pathology)** was examined and approved by the Advisory Committee and External Examiner on ………………………

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ABSTRACT

Common bean ((*Phaseolus vulgaris* L.) is one of the most important legume crops grown throughout the world, used both as green vegetable and pulse. The crop succumbs to a number of biotic factors including anthracnose of beans that poses a serious threat to its production potential. The present study was, therefore, carried out to know the status of the disease, its mode of perpetuation and screening of available germplasm against the disease. The disease was prevalent in all the potential bean growing pockets of the valley surveyed during the year 2011. The varying degree of incidence ranged from 23.51% to 82.14% with disease intensity ranging from 8.75 to 33.38%. The overall mean disease incidence and intensity on leaves and pods varied from 28.47% to 40.18% and 10.69% to 16.42%, respectively. Highest disease incidence (34.81% and 51.11%**)** and intensity (13.73 and 21.91%) on leaves and pods was recorded in district Anantnag, with least in district Shopian (24.80% and 28.99%; 9.66% and 11.93%). The pathogen associated with the disease was isolated and identified as *Colletotrichum lindemuthianum*(Sacc and Magn.) Scrib. The pathogen is mainly seed-borne but can also overwinter on the soil surface plant debris. The early symptoms of disease appear on cotyledons as dark brown to brown sunken spots with conidial mass. Moreover, fungal infection caused reduction in germination percentage of diseased seeds (53.58%) as compared to healthy (100%) and artificially inoculated seeds (89.14%). Seedlings raised from infected seeds depicted reduced vigour. Out of thirty genotypes screened, 23 were categorized as moderately susceptible (MS), 7 as moderately resistant (MR) to the pathogen. None of the genotypes screened against disease were found resistant/tolerant to anthracnose of beans. Highest disease intensity (22.08%) was recorded in genotype WB 233, while aslowest was observed by the PAS 162 (5.98%).

Key words: Anthracnose, Beans, C*olletotrichum lindemuthianum*, *Phaseolus vulgaris*(L.).

Signature of Student
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Dated: ________________

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Jan Mohd Junaid

Place **:** Shalimar, Srinagar.

Dated: ....

CONTENTS

LIST OF TABLES

LIST OF PLATES

Chapter – 1

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) belongs to family *Leguminoceae* and occupies a premier place among grain legumes in the world including India, where it is locally called as *Rajmash* (Sharma *et al*., 1994). The crop is distributed worldwide and can grow under diverse agro-ecosystems ranging from tropical, sub-tropical to temperate region (Popelka *et al*., 2004). It is also known as Common bean, Black bean, Navy bean, Pinto bean, Kidney bean, Dry bean, Snap bean and Field bean (Sardana *et al*., 2000). It is a native of Central and South America where it is being cultivated extensively and is morphologically highly variable. In India French, bean ranks second to pea in production and is extensively grown in Himachal Pradesh, Uttar Pradesh, Uttrakhand, Karnataka, Maharashtra and Jammu & Kashmir.

It is an excellent vegetable crop valued both as green vegetable as well as dry seed. It is of paramount significance for direct human consumption and a dietary supplement rich in proteins, vitamins and minerals such as calcium, phosphorus, iron, and zinc (Broughton *et al*., 2003). Beans are considered as the "meat of the poor", as they provide second most important source of protein after maize and the third most important source of calories after maize and cassava (Pachico, 1993). The importance of beans in the diet of humans can be attributed to the fact that about half the grain legumes consumed worldwide is common beans (Broughton *et al*., 2003). As vegetable it is highly nutritious being rich in vitamin C and pro-vitamin A. Quantitatively each 100g edible portion of Common bean green pod contains moisture (91.4%), carbohydrates (4.5%), proteins (1.7%), fat (0.1%), fibre (0.5%), calories (25), vitamin A (321 IU), thymine (0.08 mg), riboflavin (0.06 mg), vitamin C (16 mg), calcium (50 mg), iron (1.7 mg) and phosphorus (28 mg), on fresh weight basis. Moreover, it also contains sodium 4.30 mg, potassium 12 mg, copper 0.21 mg, sulphur 37 mg, nicotinic acid 0.30

mg (Hazra and Som, 2005). Common bean is not only a valued food or forage but also a major crop of ecological farming grown to increase the soil fertility because of its ability to fix the atmospheric nitrogen.

The ever-increasing population is concomitant with the cultivation of beans as it is easy to grow, suitable for intercropping and is a short duration crop (Wortmann *et al*., 1998). Globally common bean is cultivated over an area of 29.92 million hectares with an annual production of 23.23 million tons. In India, it is cultivated over an area of about 10.80 million hectares with an annual production of 4.87 million tons (Anonymous, 2010). In Jammu and Kashmir State common bean is grown either as sole crop or as inter-crop with maize, extending over an area of about 26.75 thousand hectares with an annual production of 14.2 thousand tons (Anonymous, 2008). As a vegetable crop, Common bean is cultivated globally over an area of 1.47 million hectares with an annual production of 17.65 million tons while in India it is cultivated over an area of about 0.20 million hectares with an annual production of 0.58 million tons (anonymous 2010). In Jammu and Kashmir it is cultivated over an area of 2000 ha with an annual production of 400 tons (Masoodi and Masoodi, 2003).

The agro-climatic conditions of the Kashmir valley offer an advantage for the cultivation of beans. However, the cool and moist climatic conditions prevalent during the growing season of beans pre-dispose the crop to a number of biotic and abiotic stresses like damage by insect-pests, diseases and nematodes that decrease its production. The attack of phyto-pathogenic microorganisms like fungi, bacteria, viruses etc. is the single significant factor that results in yield losses, either directly or indirectly.

Beans are vulnerable to a number of production constraints culminated by the fact that most of the growers lack the resources and ability to protect their crop against diseases (Wortmann and Allen, 1994). Consequently, diseases are deemed as main constraint to bean production (Opio *et al*., 2001). Most of the available cultivars are susceptible to diseases caused by fungi, bacteria and viruses. Thereby sometimes giving rise to disease complexes. Many diseases have been identified that hold back common bean production and include common bacterial blight [*Xanthomonas campestris* pv. *phaseoli* (Smith) Dye], white mould [*Sclerotinia sclerotiorum* (Lib.) de Bary], brown spot [*Pseudomonas syringae* pv. *syringae* (Van Hall)], angular leaf spot (*Phaeoisoriopsis griseola* (Sacc.) Ferraris], rust of beans [*Uromyces appendiculatus* (Pers.) Unger], anthracnose of beans and bean mosaic virus.Among these anthracnose of beans caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.)Scrib., is the most devastating disease of beans that pose a grave threat to its production worldwide.

Anthracnose of beans warrants a serious attention as it inflicts heavy economic losses to the crop, which may reach up to 100%, under conducive conditions (Pastor-Corrales *et al*., 1994).The disease affects seed quantity, quality and marketability of beans, leading to less income. It has been estimated that each 1% increase in anthracnose incidence manifest into dry bean grain yield losses of 9 kg ha^{-1} (Wortmann and Allen, 1994).

The disease may appear on any above ground plant part depending upon the phenological stage infected and source of infection but the most conspicuous symptoms appear on the immature pods. Being a short duration crop, 3-4 crops are taken in a year. In between the crops, the pathogen perpetuates in the infected seeds and plant debris. The pathogen has been found mostly in seed coat and cotyledons but rarely in embryonic axis (Ravi *et al*., 2000).

As the farming in Kashmir is of subsistence nature, the use of chemicals in the management of bean anthracnose not only makes the cultivation uneconomical but also leads to the development of resistance in the pathogen, besides being a concern of the environmental pollution. Under such circumstances it would sound better to evaluate the locally available germplasm for its resistance against the disease that could be later harvested as a seed for next year to produce disease free beans.

Although a lot of work has been done on different aspects of anthracnose of beans both at national and international levels, little work has been done to investigate the disease under the agro-climatic conditions of Kashmir. Keeping in view the significance of the disease, present study has been undertaken with the following objectives:

- To study the status of the disease in bean growing areas of Kashmir valley.
- To study the role of seed in perpetuation of the disease.
- To screen the available germplasm.

Chapter – 2

REVIEW OF LITERATURE

2.1 Status of disease

Survey and identification of plant pathogens is of utmost importance to understand its association with a specific host and to describe their geographical distribution (Agrios, 2004). Variation within a crop growing environments, crop cultivars, and farm practices determine the level of disease and their damage (Campbell and Madden, 1990)

Like other crops, Common bean is prone to myriad of diseases that are responsible for significant yield losses and anthracnose of beans is one of the most severe diseases of this crop throughout the world (Bigirimana *et al*., 2000). The disease appeared in epiphytotic form in Italy in 19th century. In New York, USA the losses varying from 30 to 100 per cent have been recorded whereas in USSR losses up to the extent of 54 percent have been estimated (Gupta and Paul, 2001).

The term anthracnose literally means 'charcoal like' and was coined first time in 1833 by Fabra and Dunal to describe the disease of grapes in which blackening of tissue was the striking feature and pathogen associated was *Colletotrichum* (Sanjaykumar and Srivastava,1983). In India the disease was first reported by Butler as early as 1918 from North India and Nilgiri hills. From Kashmir valley the disease was reported for the first time from Shopian area by Khan *et al*. (2009). The disease is wide spread in temperate to sub-temperate regions where cool and moist conditions favour the disease development with significant yield losses (Fernandez *et al*., 2000, Sharma *et al*., 2004). Frequent out breaks of the disease can severely reduce the crop yields (Schwartz, 1994). Although the susceptible cultivars get infected at all the stages of crop, yield loss depends on the critical stage at which the infection occurs. The most critical stage viz early flowering, pod filling and seed development determine yield loss (Bassanezi *et al*., 2001).

Significant disease development and yield losses occur if cool and wet climatic conditions prevail and/or prolong during the pod formation and pod filling stages of the crop (Nkalubo *et al*., 2007). This pathogen has a negative and significant impact on yield components of several *Phaseolus* beans in general and common bean in particular. The disease can cause yield losses up to 100% if contaminated seeds are sown under conditions that favor disease development (Silva *et al*., 2007; Sharma *et al*., 2008). Mahuku *et al.* (2002) reported yield losses of 95 per cent when a susceptible bean cultivar was inoculated one week after plant emergence and 38 per cent when inoculated six weeks after emergence. Sohi and Rawal (1984) observed 42.9 per cent yield losses in cowpea anthracnose caused by *C. lindemuthianum.* The highly susceptible (HS) variety of common beans in Tanzania recorded a yield loss of 86 per cent while moderately susceptible (MS) variety Mexican 142 recorded a yield loss of 27per cent (Shao and Teri, 1985). In Kenya, Makini (1995) recorded a yield loss of 38 to 95 per cent due to this disease. Investigations on yield loss in kidney bean due to anthracnose were made and it was noticed that the disease drastically affected the growth parameters and yield components of kidney beans causing significant reduction in yield with more losses in dwarf type of cultivars than pole type (Sharma *et al*., 2005). Conner *et al*. (2004) demonstrated that seed borne infection rate of 7 per cent of the viable seed of the Navigator bean resulted in yield losses ranging from 15.2 per cent to 32.1per cent. Conner *et al*. (2009) recorded a yield loss of 20 per cent in 'AC Ole' pinto bean and 27 per cent in Navigator navy bean.

Most of the locally adapted varieties in Himachal pradesh have been reported susceptible to one or the other races of pathogen and recorded disease incidence from 5 to 65 per cent with disease severity varying according to the location, cultivar and the cropping pattern (Sharma *et al*., 1994). In India disease incidence has been reported to vary between 24.59 to 51.72 per cent (Sharma and Sugha, 1995). In Ethiopia disease signature on Harricot bean was observed with an intensity of 17.2 to 76.6 per cent, and a yield loss of 67.2 per cent Bashir (1997). Qandah and Al-Momany (2003) demonstrated disease incidence ranging from 0 to 50 per cent in different parts of Jordan valley. In Jammu region of Jammu and Kashmir state disease incidence of 63.46 was recorded by Kalha *et al*. (2005).

2.2 Symptomatology

The characteristic symptoms of disease appear on any of the above ground plant parts like stems, leaves, petioles, pods and seeds. Early signs of infection appear as circular dark brown to brown spots on the germinating cotyledons due to seed infection. The disease is characterized by the production of dark brown to black lesions along the veins on under surface of leaves (Bassanezi *et al*., 2001). Eventually these spots under wet and humid conditions become covered with black fruiting bodies – acervuli and mass of salmon coloured conidia (Tu, 1988). Higher lesion density is associated with premature yellowing and defoliation of leaves resulting in lower yields (Bassanezi *et al*., 2001). Lesions are also formed on petioles and stems that develop longitudinally along the stem with sunken cankerous center. Symptoms on stem and pods appear as black to brown sunken lesions (Pastor-Corrales and Tu, 1989). Further, pod infection leads to development of light to chocolate colored sunken, cankerous spots on seed coat (Gupta and Paul, 2001).

The disease can bring about complete defoliation, stunting of plant growth and extensive yield losses if plants become diseased prior to or/and during pod filling stages (Hollingsworth *et al*., 2003). Yield loss is due to early senescence and plant death, shriveled seeds and increased amount of diseased seeds bearing lesions on seed coat (Pastor- Corrales and Tu, 1989). *C. lindemuthianum* attacks on bean leaves producing dark brown necrotic lesions with depletion of chlorophyll (Bassanezi *et al*., 2001).

2.3 Causal organism

The disease is caused by fungus *Colletotrichum lindemuthianum* (Sacc. and Magn.) Scrib. Taxonomically the fungus belong to class Coelomycetes, order Melanconiales, family Melanconiaceae, genus *Colletotrichum* and specie *lindemut hianum*. The fungus is hemi biotropphic in nature (Martinez Pacheco *et al*., 2009).

The fungus C. *lindemuthianum* was first reported on beans by Lindemuth in 1875 from Germany (Gupta and Paul, 2001). Initially the fungus was designated by various names viz, *Gleosporium lindemuthianum, Glomerella lindemuthianum, septoria legunum* but finally it was identified as fungus that presents its perfect and imperfect stages denominated as *Glomerella cingulata* and *Colletotrichum lindemuthianum,* respectively. Later, the fungus was clarified as *C. lindemuthianum* (Martinez-Pacheco *et al*., 2009). The species is easily distinguished from broad concept of conidial form of *G. cingulata* by its slow growth and dark pigmentationin culture medium. The fungus is potential cellulose producer (Martinez-Pacheco *et al*., 2009). The fungus causing anthracnose of beans is cosmopolitan in distribution with considerable pathogenic and molecular variability (Padder *et al.,* 2009). The local cultivars have been found susceptible to one or the other races of the pathogen (Pathania *et al*., 2006; Padder *et al*., 2010). More than 100 pathotypes of the pathogen have been described (Silva *et al*., 2007).

The mycelium of fungus is branched, septate, hyaline which becomes black or dark with age and maturity. Few hyphae form stromata beneath the cuticle where acervuli develop, which later rupture and become erumpent (Gupta and Paul, 2001). The acervuli are mostly in groups, coalesce together and cover lesions on infected plant parts. The acervuli bear bristle-like structures called setae. Setae are septate, few and longer than the conidial mass (Singh, 1994). The conidia of *C. lindemuthianum* are born on acervuli. The conidial masses are orange to bright orange. Fungal sporulation is favored by a pH of 5.2-6.5, and not affected by aeration or ultraviolet light (Mathur *et al*., 1950).

Conidia are hyaline, oblong to dumb-bell shaped, one-celled, straight with obtuse ends. Conidial size is about 9-15 x 3-4 μm (Mathur and Kongsdal, 2003). Kulshrestha *et al*., (1976) observed the conidial and setae dimensions as 9.0-15.0 x 3.0- 4.0 µm (with a mean of 7.0 x 3.5 µm) and 60.0-120.0 x 2.0-4.0 µm (with a mean of 90.0 x 30.0 µm), respectively. Conidia of fungus are uninucleate, uni-cellular and have a clear vacuole like body in the centre. *C. lindemuthianum* has a unicellular conidium which germinates within six to nine hours and produces 1 to 4 germ tubes (Zaumeyer and Thomas,1957). Colony of *C. lindemuthianum* is grey to deep olive grey, mycelium sparse to woolly with some aerial growth, reverse side black in colour. Further, the conidial mass are white in colour with individual conidia being hyaline, single celled, straight, cylindrical, dumb-bell shaped; oblong with round ends (Wijesekara and Agarwal, 2006)

Wijesekara and Agarwal (2006) observed the conidia with dimensions ranging from 4.88-24.39 x1.22-7.31 µm (with a mean of 14.4 x 4.27 µm). The conidia are hyaline, unicellular, cylindrical with obtuse ends, have a narrow and truncate base, dimension ranges from 9.8-11.5 x 3.8-4.5 µm with an average of 10.5 x 3.5 µm. Setae on acervuli were observed both on host as well as in culture and were 105 x 3.5 µm in size (Khan *et al*., 2009).

The perfect state of *C. lindemuthianum* with perithecia and asci has been rarely observed in nature (Tu, 1992). However, perfect state was isolated in 1913 on artificial media, and accordingly named as *Glomerella lindemuthianum* Shear. *Glomerella cingulata* is a fungus that producesperithecia with a size of 120-210 µm in diametre. The asci measure 8 x 48 to 68 µm,and contain 8 ascospores that are allantoid (6.5 x 20 µm) or ellipsoid (4 x 10 µm) in shape (Tu, 1992). The fungus has a definite plant host range comprising mainly of *Phaseolus vulgaris*, but can also colonize *P. aculatus* var. *lactifolius*, *P. cocineus*, *P. aureus*, *P. lunatus*, *P. limensis*, *Medicago sativa* and *Vicia faba* (Martinez–Pacheco *et al*., 2009).

2.4 Epidemiology

Its longevity in infected pods and seed varies considerably depending on environmental conditions; moisture and temperature being the most important factors influencing the survival of the fungus. Anthracnose conidia are spread from one plant to another mainly by rain splashes (Hirst and Stedman, 1963). The average distance of conidia spread ranges from 3 to 4.6 m per rainstorm of 10 mm or more (Tu, 1992). A 10-h wet humid period (>92%) is necessary for *C. lindemuthianum* conidia to infect, and new lesions usually appear in 3 to 7 days

(Hirst and Stedman, 1963). Although plant debris contributes greatly to pathogen perpetuation and distribution, infected seed plays a key role in the long distance dissemination of the anthracnose pathogen (Tesfaye, 2003). Where farmers continuously use infected seeds, the pathogen is distributed in all bean growing areas of the country (Leaky and Simbwa-Bunnya, 1972). Furthermore, the different tillage methods can influence anthracnose development and distribution in the dry bean fields (Ntahimpera *et al*., 1997).

In addition to seed and plant residues, human clothing, insects, machines, and animals may also disperse *C. lindemuthianum* to healthy plants (Tesfaye, 2003). The pathogen can remain viable in seed for 3-5 years and the farmers who retain seed from previously grown crop contribute to carry-over and spread of the disease (Leaky and Simbwa-Bunnya, 1972). Once on the plant, lesions on cotyledons usually serve as sources of secondary inoculum (Tesfaye, 2003). On leaves, conidia are washed down to hypocotyls and subsequently the stems.

The spread of anthracnose from primary infection in field depends on speed and direction of wind. Prevailing wind associated with rain splash is an important factor determining spread of anthracnose (Ntahimpera *et al*, 1996). Rain splashes from leaves introduce the inoculum into the soil, which act as a source of secondary infection (Tesfaye, 2003). The disease rapidly spreads by spores carried in splashing raindrops, or through human activities or implements that come in contact with diseased plants (Tu, 1983) and in one growing season, one diseased plant can spread the disease to other plants within 30 m radius. Long distance dissemination (3-5 m) may develop from raindrops being blown by gusty winds (Tu, 1983). The number of foci of initial inoculum in the field also contributes in disease dissemination, and is linearly related to the incidence of anthracnose on plant leaves (Tesfaye, 2003). Under *in vivo* conditions, anthracnose incidence is highest on leaves during rainy season and on pods during the dry season (Tesfaye, 2003).

Specifically, the conditions of high relative humidity (>92%), temperature (18^o to 22^oC) and moderate rainfall (10 mm) at frequent intervals are *sine qua* *non*for optimal survival of *C. lindemuthianum* and development of anthracnose (Tu, 1983). Moderate temperatures between 13^{o}C and 26^{o}C favour infection, with an optimum at 17^{o}C. High humidity or free moisture must be present for infection to occur successfully. Local dissemination of conidia and development of anthracnose epidemics also require moderate rainfall at frequent intervals (Pastor-Corrales *et al*., 1995).

2.5 Survival of pathogen

To manage a disease it is of great importance to unveil the modes of perpetuation of pathogen. The fungus responsible for anthracnose of beans survives in fields throughout the world wherever the crop is grown (Bigirimana *et al*., 2000). Muncie (1917) observed that anthracnose fungus overwinters in bean debris and infect bean crop the following year. The fungus can survive for five years in infected dry seeds or pods stored at 4^{0}C (Tu, 1983). However, alternating dry and wet conditions heavily affect the longevity of fungus (Siddiqui *et al*., 1983; Dillard and Cobb, 1993). Araya Fernandez *et al*. (1987) reported that anthracnose incidence in field is directly proportional to primary inoculum provided by contaminated seeds. The pathogen can survive on the infected plant debris, but the diseased seeds are the main sources of primary infection in the field (Tu, 1992). The fungus is unable to overwinter in field but is capable of surviving for two years in seed Tochinai and Sawada (1952). Tu, (1992) reported that infected seed is the common source of introducing anthracnose into a field. Dillard and Cobb (1993) demonstrated that the fungus over winters on bean debris placed at various depths for about four months in soil. The disease has been found to be externally and internally seed borne (Gupta and Paul, 2001). The infection of C. *lindemuthianum* is primarily confined to seed coat up to the extent of 74.4 per cent (Neergaard, 1979). Subsequently *C. lindemuthianum* causes embryo infection that leads to seed transmission of fungus and may be carried as hyphae in the embryo or seed coat (Neergaard, 1979). Anthracnose is often most severe when crops are exposed to inoculum originating from a combination of contaminated seed and infected stubbles from previous bean crops (Ntahimpera *et* *al*., 1997). Under favourable conditions *C. lindemuthianum* has been reported to survive in some geographical regions for at least one year on infected crop debris (Ntahimpera *et al*. 1997). There has been a direct correlation between seed discoloration and per cent seed infection with pathogen located mostly in seed coat and rarely in embryonic axis (Ravi *et al*., 2000)

Recurrence of disease in the field is mainly through the introduction of diseased seeds coupled with intermittent rainfall along with moderate temperature regimes during the vegetative stages of the bean crop (Tu, 1983). According to Melotto *et al*. (2000) the fungus survives as dormant mycelium within seed coat and cotyledons or as spore between the cotyledons in seed, but can also survive in plant debris by formation of sclerotia. Disease spreads easily in areas where the farmers depend highly on farm saved seeds (David *et al*., 2000, Opio *et al*., 2001). Opio *et al*., (2003) observed that every infected seed can give rise to infected seedling if environmental conditions are conducive for disease development. Conner *et al.,* (2009) reported that increase in rate of seed infection result in significant reduction in yield and seed mass. Moreover, increase in seed infection leads to increase in seedling infection, build-up of inoculum in the crop canopy, seedling mortality and seed discoloration. Planting infection-free seed in the field, previously infected with fungus, do not show the symptoms of disease (Yesuf and Sangchote, 2005).While stressing the importance of infected seed in transmission of the disease, planting infection-free seed was reported as primary option to manage the disease Schwartz *et al*. (1994). Yesuf and Sangchote (2005) observed a positive correlation between the seed and seedling infection. Seed infection results in reduced germination rate, poor seedling vigour and increased seedling mortality (Yesuf and Sangchote, 2007).

Seed-borne infection plays a recognizable role in initiation of disease development as no soil-borne inoculum exists in the field especially in many tropical areas where there is alternate dry-wet cycles during the off season. Healthy common bean seeds raised in a field previously infected by the disease did not show any anthracnose symptoms (Kumar *et al*., 1999).

Being mainly a seed-borne disease, it results in frequent outbreak of epiphytotic conditions in Uganda (Thomas and Sweetingham, 2003; Del Rio and Bradely, 2004). Epiphytotic of bean anthracnose resulting from primary inoculum provided by the infected seeds largely depend on the subsequent rain pattern (Araya Fernandez *et al.*, 1987). Nicholsen and Moraes (1980) demonstrated that removal of the mucilaginous matrix of conidia by water before storage could significantly reduce the conidial viability of *C. graminicola* during storage period. The crops raised from anthracnose-free seeds remain healthy throughout the cropping season; unless it is infected by the inoculum from secondary source(s) which arise from areas where local cultivars with infected seeds are grown in the vicinity of the healthy seeds (Sharma *et al*., 2003).

2.6 Screening of germplasm

Various control measures have commonly been advocated to reduce losses inflicted by anthracnose. These include: planting pathogen-free seeds, field sanitation, crop rotation, shifting planting dates, fungicides and plant resistance (Schwartz, 1989). However, farmer acceptance and utilization of many of these strategies are not always possible especially in the case of subsistence farmers in developing countries (Schwartz *et al.,* 1982). Although bean anthracnose can be controlled through use of disease-free seeds, crop rotation and seed treatments - use of host resistance have been described as most efficient and eco-friendly approach to disease management (Goncalves-Vidigal *et al*., 2007). It is envisaged that use of resistant cultivars or introgression of resistant genes in desirable susceptible cultivars is most viable option for controlling anthracnose, particularly for small-scale growers (Padder *et al.*,2010).

According to Pathania *et al.* (2006) there is wide genetic variability in most of the available germplasm, *ipso facto* it is possible to develop cultivars resistant to anthracnose with the recombination of this variability. However, the highly variable pathogenicity of the pathogen can lead to resistance breakdown in commercial bean cultivars (Mahuku and Riascos, 2004). Barros (1911) was first to report pathogenic variability in the fungus. However, different cultivars vary in their susceptibility to the same pathotype, depending on the type and number of resistant genes present in each cultivar (Ombiri *et al*., 2003).In order to characterize races of *C. lindemuthianum* and identify sources of resistance in local and exotic bean germplasm, different infection tests are required. Series of inoculation methods have been used to study infection process and to identify sources of resistance against *C. lindemuthianum* of common bean (Bigirimana and Hofte, 2001). Various inoculation techniques based on specific objective and available technical facilities have been accordingly applied. These inoculation methods include seed inoculation by immersing in a spore suspension (Bigirimana *et al*., 2000), seedling inoculation using hand sprayer and seedling stem injection using syringe (Schwartz *et al*., 1982), seedling brushing and detached leaf method (Tu, 1986).

After evaluating 72 varieties of beans for their reaction against anthracnose of beans, disease was found in all white seeded and runner varieties (Reid, 1945). Rands and Brotherton (1955) evaluated 63 bean varieties for their resistance and susceptibility against the anthracnose fungus and found 27 varieties were resistant out of which 6 were immune to all the known races of pathogen. Ganeva and Georgiena (1975) found resistance in 19 lines of beans against *C. lindemuthianum* under field conditions. Among these 5 lines viz Sperling, Dufrip, Dalgreen, Prelubel and Izomroudnaya remained disease-free when tested under the conditions of artificial inoculation in the laboratory. Minussi *et al*. (1975) studied the reaction of 60 cultivars to race BA-1of *C. lindemuthianum* and found 5 percent cultivars resistant and 88 percent as susceptible.after evaluationof over 1300 accessions for their reaction to bean anthracnose and angular leaf spot diseases, 156 accessions were resistant to all the races of *C. lindemuthianum*,30 were resistant to other races obtained from different parts of the world (Schwartz *et al*., 1982). Khare and Chacko (1983) screened 26 soybean varieties against the anthracnose and found that the disease index ranged between 0 to 58.2 per cent. Inglis *et al.* (1988) used dry inoculum to evaluate the resistance of beans against anthracnose disease and found it as effective as conidial suspension for inoculating bean foliage. Meneze *et al*. (1988) evaluated 72 cultivars and breeding lines for their resistance to 9 races of *C. lindemuthianum* and found the lines A 321, A 373, A387, and cultivar AB 136, Evolutie, Go 2338, Go3367 and Tu were resistant to all the races. Thakur and Khare (1989) tested 27 cultivars of *Vigna radiata* against the *C. dematium* and *C. lindemuthianum* and found varieties like Pusa 109 and Pusa 115 highly resistant in one season of the experiment and succumbed to disease in next season. Chakrabarty and Shyam (1990) screened 44 varieties of Common bean s and found that VL 60, VL 63, Jawala and HPR 35 were highly resistant to all the isolates of the pathogen. Wang and Li (1990) evaluated 805 varieties of *Phaseolus vulgaris* for their reaction against the anthracnose fungus, of which only 10 were resistant to the fungus. Kumar *et al*. (1997) evaluated 60 kidney bean varieties against 10 races of anthracnose in the laboratory and found two accessions AB 136 and G2333 were highly resistant to all the test races while few exhibited resistance to more than five races. Sharma *et al*. (1999) evaluated 95 kidney bean accessions including the land races and exotic germplasm against 19 races identified on the CIAT differentials and reported that exotic germplasm exhibited resistance to majority of the Indian races, while the accessions AB 136 and G 2333 showed resistant reaction to all races. After evaluation of 40 germplasm lines, 14 breeding lines viz., A 247, A 193, A 322, A 252, A 493 and AB 136, SANILAC, TO, TU, PI 207262, BLANCO INIA, G 05686, GREAT NORTHERN 31 and ICA BUNSI could be used as sources of resistance to *C. lindemuthianum* (Fernandez *et al*., 2000). Further, out of 60 genotypes of soybean screened under greenhouse conditions only three genotypes namely PK1129, DSb-2 and Cocker Staurt were resistant, 27 were moderately resistant, 19 were susceptible and 11 were highly susceptible to *C. truncatum* (Madhusudhan, 2002). Mahuku *et al*. (2002) observed that out of 75 inter specific lines screened, 49 were resistant to race 6, 15 and 3481 of anthracnose of beans.

Carrijo *et al*. (2003) screened 133 common bean lines against 65, 89, and 387 races of *C. lindemuthianum* and reported that 13.82 per cent of the lines exhibited resistance to all the races while cv.Talishma exhibited a high degree of resistance to different races. Similarly, in Africa 30 widely cultivated cultivars of dry beans were tested against race 7, 73 and 89 of *C. lindemuthianum* and most of the bean cultivars were found susceptible to race 7, but resistant to race 73 and 89, respectively. However, two cultivars like Isle and Drake were resistant to all the three races (Del Rio *et al*., 2003). Gonzalez- Chavira *et al*. (2004) evaluated 21 bean genotypes for their reaction against 5 races of *C. lindemuthianum* and reported only 4 genotypes to be resistant to all the races of pathogen. Laxman (2006) observed that among the different green gram genotypes screened through artificial inoculation none were found resistant. Pathania *et al*. (2006) evaluated forty nine common bean lines comprising of exotic accessions and locally grown cultivars against *C. lindemuthianum* and found that they exhibited differential resistance to races present in Himachal Pradesh with some exotic accessions like G 2333, Cornell 49242, PI 207262, Mexique 222, TO, Perry Marrow, Kaboon and Widusa resistant to more than five Indian races, whereas two Indian accessions KRC-5 and Hans demonstrated resistance to six and four races, respectively. However, nine accessions KRC-8, KR-40, KR-43, KR-81, KR-62-2, KR-90, KR-142, KR-148, and KR-216 were resistant to three races. Vidigal-Filho *et al*. (2007) evaluated 26 landrace beans of Brazil to physiologically distinct races of *C. lindemuthianum* originating in Andean and Mesoamerican regions and reported 88 per cent landrace genotypes evaluated exhibited resistant reaction to race 31, except Carioca 3, Preto 1 and Preto 2. Moreover, about 50% of the landrace genotypes had resistance to races 9, 19, 55 and 453; and about 30% to races 7, 65, 69, 73, 81, 89 and 9, respectively.

Ferreira *et al*. (2008) evaluated 246 land races and 42 lines of beans derived from the breeding Programme against anthracnose in search of resistant lines and reported that none of the land races exhibited adequate resistance to all the five races. However, three local accessions were resistant to four races and presented intermediate or mixed reactions against the fifth one. Among the breeding lines, nine cultivars were found resistant to five races and three lines (A252, A321, and A493) were selected as resistance sources to transfer genetic resistance to Andean bean cultivar. Rajesha *et al*. (2010) evaluated 195 Dolichos bean genotypes under field conditions for their reaction against anthracnose and found nine genotypes viz., GLB 3, GLB 4, GLB 8, GLB 9, GLB 11, GLB 19, GLB 60, GLB 166 and GLB 167 were found immune, 48 genotypes were resistant, 83 genotypes were moderately resistant, 51 genotypes were moderately susceptible and 4 genotypes were susceptible to the anthracnose.

Chapter – 3

MATERIALS AND METHODS

The present research work on anthracnose of beans was carried out during 2011-2012 in the Division of Plant Pathology Sher-e- Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar Campus, Srinagar and Department of Plant Pathology, Faculty of Agriculture, Wadura Campus Sopore.

The materials used and methodology adopted in different experiments to achieve the objectives of current study is described as under:

3.1 Status of disease

For recording incidence and intensity of anthracnose of beans regular survey of potential bean growing pockets from Anantnag, Bandipora, Ganderbal and Shopian districts of Kashmir province was conducted in a systematic manner during 2011 *kharif* cropping season. The areas where beans have been intensively and extensively cultivated for years were selected in each district. The survey was conducted at seedling and pod maturity stage of the crop.

3.1.1 Assessment of disease incidence

Each district was divided into three villages and in each village three bean fields were selected randomly. The disease incidence was recorded by randomly selecting three sites of 2 x 1 msize from each selected field. At each selected site, 10 plants on an average were taken into consideration. The per cent disease incidence of anthracnose of beans at each survey plot was duly recorded on leaves and pods and accordingly computed and calculated by employing the following formula:

$$\text{Per cent disease incidence} = \frac{\text{Number of infected leaves/pods}}{\text{Total number o f leaves/pods examined}} \times 100$$

3.1.2. Assessment of disease intensity

Percent disease intensity was estimated by using the 0-9 scale proposed by Mayee and Dattar (1986) (Plate 1) with slight modifications, and was calculated by the formula:

Percent disease intensity = $\frac{\sum(\mathrm{n \ x \ v})}{\mathrm{N \ x \ G}} \times 100$

Where:

$\sum$= Summation

n = Number of diseased leaves/pods in each category

v = Category value

N = Total number of leaves/pods examined

G = Highest category value

Score/Grade	**Description**
0	No symptoms on leaf/pods
1	Small, round brown spots covering 1% or less of leaf/pod area
3	Brown, sunken spots covering 1- 10 % of leaf/pod area
5	Brown spots enlarging to form circular spots covering 11-25% of leaf/pod area
7	Circular brown, sunken spots, covering 26 – 50% of leaf/pod area
9	Circular to irregular, brown sunken spots covering 50% or more of the leaf/pod area

During the course of survey, the diseased leaves and pods bearing characteristic symptoms were recorded from field. The leaves or pods showing typical characteristic symptoms were collected in the sterilised, perforated poly ethylene bags and brought periodically to the laboratory for investigation, isolation and further study of the causal pathogen.

3.2 Isolation and Characterisation of Pathogen

3.2.1. Isolation of pathogen

Isolation of the pathogen was performed from the leaves and pods of bean plants collected from the surveyed locations and showing typical disease symptoms. The infected plant parts were first thoroughly observed under the stereoscopic microscope (25x& 50x) to ensure the existence of fruiting bodies. Further microscopy was performed to ensure the presence of conidia.

Plate-1: Characterization of beanpods infected with Anthracnose on 0-9 scale

To isolate the pathogen, diseased plant parts of leaves and/or pods along with some margin of healthy tissues around it were excised into small bits (2-3 mm) with the help of a sterilized razor. The bits were then washed with water and simultaneously surface sterilized by immersing in freshly prepared 0.1% aqueous mercuric chloride solution for 30 seconds, followed by 3-4 times washings with sterile distilled water. The bits were placed on the sterilised paper towel to remove the excess moisture. These were aseptically transferred onto sterilized petri plates containing potato dextrose agar (PDA) medium and accordingly incubated at 24 ± 1^{0}C for 7 days. The plates were regularly monitored and periodically observed for fungal growth and subsequently sub-cultured on PDA medium.

3.2.2 Purification and maintenance of pathogen

Pure culture of fungus was obtained by employing the single spore technique. Spore suspension was prepared from seven days old culture raised on potato dextrose agar medium. One ml of the suspension was poured on sterilized petri plates, filled with sterilized plain agar and spread uniformly to cover the entire surface of plates. These plates were incubated at 24 ± 1^{0}C for 24 hours. Later, the plates were accordingly observed in an inverted form under stereoscopic microscope to pinpoint. the position of single germinating spores. These were was marked with the help of a marker and lifted to transfer then aseptically into PDA slants. The pure culture so obtained was maintained by repeated sub-culturing at an interval of 30 days.

3.2.3 Identification of the pathogen

The colony characteristics such as mycelial growth, shape, septation, pigmentation: shape, size and colour of conidia, acervuli and setae were studied. The characters observed were compared with standard, authentic literature propounding detailed description of genus C*olletotrichum*. Moreover, culture was sent to the *Indian Type Culture Collection* , Division of Plant Pathology, IARI, New Delhi for further confirmation.

3.3 Phytopathogenicity test

The pathogenicity of fungus was established with the help of Koch's postulates on pods, detached leaves (Tu, 1986) and on two-week old bean seedlings. Healthy pods were obtained from the commercial bean fields during survey and placed in moist chamber to ensure the absence of latent infection. The pods were inoculated with the spore suspension of the test fungus and incubated for 4 days at 24±1 ^{0}C. An un-inoculated pod was also maintained under similar conditions as control. In detached leaf technique, the leaves of beans were used. Leaves were kept in the moist chamber to ensure the absence of latent infection. The spore suspension of test fungus was prepared from three-week old culture grown on potato dextrose agar medium. A small amount of sterilized distilled water was used to wash-off the spores from the petri plates. The suspension was filtered through the muslin cloth and its concentration adjusted, with the help of a haemocytometer, to 1.2 x 10^6 ml^{-1}. For inoculation, the leaves with their ventral side up were placed in the petri plates lined with sterilized blotter paper. The suspension was gently brushed onto the leaves. The inoculated leaves were then incubated at 24±1^{o}C for seven days. The un-inoculated leaves were also maintaine d under similar conditions as control. At the appearance of disease symptoms re-isolation of pathogen was performed from the leaf tissue exhibiting typical disease symptoms of bean anthracnose. To prove the phyto-pathogenicity, comparison was made with the inoculated pathogen.

Similarly, bean seedlings were raised in poly-bags of ½ kg capacity with sterilized soil, and sprayed with water two hours prior to inoculation for making conditions favourable for infection. The bean plants were inoculated at trifoliate stage by spraying spore suspension of pathogen on under surface of leaves with the help of an atomiser. The inoculated plants were accordingly incubated for 72 hours. An un-inoculated plant was also maintained under similar conditions as control. On the appearance of symptoms re-isolation of pathogen was performed from the tissue showing disease symptoms upon artificial inoculation. Pathogenicity was confirmed after satisfying Koch's postulates.

3.4 Morphology of fungus

The morphological characteristics of fungus were studied in laboratory on host as well as on artificial culture. Semi-permanent slides were prepared from seven days old culture stained with cotton blue in lactophenol. The slides were examined under microscope (40x) with respect to following characters:

Colony	: Colour, shape, and mycellial growth
Conidia	: Size, shape, colour, septation
Setae	: Size, shape, colour, septation
Acervuli	: Size, shape, colour

3.5 Perpetuation of pathogen

3.5.1 Perpetuation through seed

Seeds obtained from mature bean pods depicting conspicuous characteristic symptoms of anthracnose were used to determine their role in transmission of disease. The seeds were stored under conditions similar to those adopted by the farmers, to establish the relationship between seed infection and disease transmission. Blotter paper and agar plate techniques were used to determine the role of seeds in disease dissemination.

3.5.1.1 Blotter method

Twenty random bean seeds were taken at fifteen days interval after harvest and surface sterilized with 0.1 per cent mercuric chloride solution. The seeds were rinsed thrice with sterile distilled water and placed in a petri plate lined inside with moistened blotter paper. Twenty seeds were placed aseptically in the petri plate maintaining three replications. The plates were incubated at 24 ± 1^0C. The blotter paper was regularly kept moist by carefully pouring few drops of sterile distilled water as and when required. The seeds were examined from day 4^{th} up to day 15^{th} for appearance of fungal growth. The observations on the percent seed showing fungal growth were recorded by using the formula:

$$\text{Per cent seed infection} = \frac{\text{Number of seeds infected}}{\text{Total number of seeds examined}} \times 100$$

3.5.1.2 Agar plate method

Twenty seeds were randomly taken from the seed lot and surface sterilized with 0.1 percent of mercuric chloride for one minute, washed thrice with sterilized distilled water and were then aseptically placed on 2 % nutrient agar in petri plates and accordingly incubated at 24 $\pm1^0$C. The plates were examined from day 4^{th} up to day15^{th} during incubation for recording the percent infected seed.

3.5.1.3 Role of seed in transmission of disease

In order toestablish the relationship between seed infection and seed transmission of bean anthracnose, under controlled conditions, three seed lots viz., healthy, naturally infected and artificially inoculated. Healthy, naturally infected and artificially inoculated bean seeds were collected from healthy pods, severely infected pods and by overnight soaking of seeds in spore-suspension, respectively. Later, seeds were sown in pots filled with sterilized soil and sand (2:1 w/w). Seven seeds per pot were sown with four replications for each lot. To maintain the humidity pots were regularly watered with hand sprayer and then covered with polyethylene sheets. Observations were recorded for germination percentage and disease incidence on cotyledons 10 days after sowing. Disease assessment (3.1) and germination percentage were accordingly recorded:-

$$\text{Germination \%} = \frac{\text{Number of seeds germinated}}{\text{Total number of seed sown}} \times 100$$

3.5.1.4 Role of plant debris in perpetuation of pathogen

Naturally infected plant debris including the pod husk and leaves were collected from commercially grown bean fields in the last week of October 2011. Plant debris was placed in nylon pouches that permit free exchange of water, gases and microorganisms. The pouches were placed in soil at 0, 7.5 and 15 cm depth. The pouches containing the infected debris on the surface were held in place with the help of rocks. The position of pouches placed inside the soil was marked with the help of flags. A set of pouches (one each from 0, 7.5 and 15 cm depths) were retrieved at regular intervals and tested for viability of the fungus.

To check the viability of the fungus, isolations were made on potato dextrose agar medium from November 15th to March 30th, 2012.

3.6 Germplasm Screening

Seeds of 30 different bean varieties/ lines were obtained from the Department of Plant Breeding and Genetics, Faculty of Agriculture, Sheri-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Wadura Sopore (J&K) to evaluate their susceptibility/tolerance against the pathogen under artificial conditions.The seeds of each line were sown in sterilized soil filled poly bags (1/2 kg) with three replications each. Seedling inoculation method was used to evaluate the germplasm against the pathogen (Vidigal-Filho *et al*., 2007).

3.6.1. Preparation of inoculum

The test fungus was grown on potato dextrose agar medium and incubated at 24±1^{0}C for seven days. Conidia were washed off from the medium into an Erylenmeyer flasks (250 ml) containing sterilized water. The concentration was adjusted to 1.2 x 10^6 spores ml^{-1} with the help of a haemocytometre.

The inoculum so produced was sprayed with an atomiser on seedlings with fully expanded primary leaves on their upper and lower surfaces. The inoculated seedlings were maintained at 25±2^{0}C in a greenhouse with relative humidity of 90% for 15 days. Humidity was maintained by watering the polybags regularly.

3.6.2 Disease incidence

Disease incidence was calculated on the basis of number of diseased/infected leaves plant,$^{-1}$ out of total number of leaves plant^{-1} in each variety or line.

3.6.3 Disease intensity

Disease intensity was computed using slightly modified scale proposed by Mayee and Dattar (1986) and the genotypes were accordingly graded into various reaction types as under:

Score	Description	Reaction type
0	No disease	Immune (I)
1	Small round brown spots covering 1% or less of the area	Resistant (R)
3	Brown sunken spots covering 1- 10 % of leaf area	Moderately Resistant (MR)
5	brown spots enlarging to form circular spots covering 11 – 25% of leaf area	Moderately Susceptible (MS)
7	Circular brown sunken spots covering 26 – 50% of leaf area	Susceptible (S)
9	Circular to irregular, brown sunken spots covering 50% or more of the leaf area	Highly Susceptible (HS)

3.7 Statistical analysis

Completely randomized design and randomized block design were employed for *in vitro* and *in vivo* studies, respectively. The data was analysed by one way analysis of variance. To normalize the data, arcsine transformations were carried out, wherever applicable. The data obtained in different experiments was subjected to statistical analysis as per the methods given by Panse and Sukhatme (1985) and Mishra (2005).

Chapter – 4

EXPERIMENTAL FINDINGS

The current studies on anthracnose of beans were conducted in the Division of Plant Pathology SKUAST-K, Shalimar and department of Plant Pathology, Faculty of Agriculture, Wadura Sopore with the objectives of working out the status of disease, to screen the available germplasm and to unveil the mode of perpetuation of pathogen.

4.1 Occurrence and severity of disease

In order to determine the occurrence and severity of bean anthracnose, extensive survey of the major bean growing districts viz., Anantnag, Bandipora, Ganderbal and Shopian, of Kashmir valley were carried out during 2011). Diasease was observes on all aerial plant parts (Plate 2) The data regarding incidence and severity of the disease are presented in Table 1 and 2, respectively.

4.1.1Disease incidence

4.1.1.1Disease incidence on leaves

The data presented in Table-1 revealed that the disease was prevalent on leaves in all the districts surveyed. Highest disease incidence on leaves was recorded in district Anantnag, followed by district Ganderbal, Bandipora and Shopian. During the growing season the disease incidence ranged from 24.64 per cent to 53.79 per cent in district Anantnag, whereas it ranged from 27.02 per cent to 29.64 per cent, 23.51 to 28.15 percent and 24.11 to 25.83 per cent in district Ganerbal, Bandipora and Shopian respectively.

The data further revealed that among the different locations surveyed, the disease incidence (53.79 per cent) on leaves was highest at Larnoo region of district Anantnag while the lowest disease incidence (23.51 per cent) was recorded at Sumbal of district Bandipora. Non-significant variation in disease incidence on leaves was noted for Drad Kralipora (24.48 per cent), Herman (24.11 percent) and Sedav (25.83 per cent) of district Shopian and Dantar (24.64 percent) of district Anantnag, which was statistically at par with Sumbalof Bandipora.

Plate-2: Characteristic symptoms of Anthracnose of beans on:
(a) Cotyledons (b) Leaves (c) Stem
(d)Immature pods and (e) Seeds

Table- 1

Incidence of anthracnose of beans at various locations in Kashmir valley during 2011

District	Location	Per cent disease incidence*	
		Leaves	Pods
Anantnag	Larnoo	53.79 (47.18) **	82.14 (65.01)
	Danter	24.64 (29.72)	32.70 (34.87
	Momin	26.02 (30.04)	39.35 (38.82)
	Mean	**34.81 (35.64)**	**51.11 (46.23)**
Bandipora	Chatibandi	28.15 (32.04)	43.51 (41.27)
	Bandipora	24.65 (29.75)	29.74 (33.03)
	Sumbal	23.51 (29.00)	25.65 (30.42)
	Mean	**25.43 (30.26)**	**32.96 (34.90)**
Ganderbal	Batwani	27.05 (31.31)	43.38 (41.19)
	Yangura	29.64 (32.98)	57.79 (49.48)
	Zazun	27.02 (31.30)	40.98 (39.80))
	Mean	**27.90 (31.86)**	**47.38 (43.98)**
Shopian	Dradkralipora	24.48 (29.65)	28.19 (32.07)
	Herman	24.11 (29.40)	26.90 (31.23)
	Sedav	25.83 (30.54)	31.88 (34.37)
	Mean	**24.80 (29.86)**	**28.99 (32.56)**
Overall mean		**28.47 (32.10)**	**40.18 (39.29)**
CD$_{(P\leq 0.05)}$	District	1.59	1.73
	Location	1.32	1.44
	District x Location	3.88	4.22
CV		10.55	12.86

*Average of three sites.

**Figures in parenthesis are arcsine transformed values.

4.1.1.2 Disease incidence on pods

Perusal of Table-1revealed that the disease was prevalent on pods in all the location surveyed. The disease incidence on pods was highest in district Anantnag (51.11 per cent), followed by Ganderbal (47.38 percent), Bandipora (32.96 per cent) and Shopian (28.99 per cent). The disease incidence on Common bean pods ranged from 32.70 to 82.14 per cent in district Anantnag, whereas, it ranged from 40.98 to 57.79 per cent, 25.65 to 43.51 per cent, and 26.90 to 31.88 per cent in district Ganderbal, Bandipora and Shopian, respectively. The data further revealed that that among the different locations surveyed the disease incidence (82.14 per cent) was highest at larnoo of district Anantnag, and lowest (25.65 percent) at Sumbal of district Bandipora which was statistically at par with Herman (26.90 per cent) region of district Shopian.

4.1.1.3 Disease intensity

Perusal of the data in Table-2 revealed that the disease was prevalent all the bean growing areas of the valley with varying intensities depending upon the climatic conditions of the location and cultural practices followed. The disease severity on leaves during the crop season was highest in district Anantnag (13.73 per cent) followed by Ganderbal (9.93%), Bandipora (9.88per cent) and Shopian (9.66 %), respectively, though the districts Shopian, Ganderbal and Bandi pora were statistically at par. Disease severity on leaves varied from 9.78-21.17 per cent in Anantnag while as it ranged from 8.79 to 10.89 per cent, 8.95 to 11.82 percent and 9.03 to 10.08 per cent in districts Bandipora, Ganderbal, Shopian, respectively. Disease intensity was highest in Larnoo (21.17 per cent) of district Anantnag, followed by Yangura (11.82 percent) of Ganderbal, which was statistically at par with the Chatibandi of Bandipor.Disease on leaves intensity on pods ranged from 14.99 per cent to 33.38 per cent in district Anantnag whereas it ranged from 10.30 to 18.37, 17.37 to 22.88, and10.52 to 13.18 percent in district Bandipora, Ganderbal and Shopian, respectively.

Table – 2 Intensity of anthracnose of beans at various locations in Kashmir valley during 2011

District	Location	Per cent disease intensity*	
		Leaves	Pods
Anantnag	Larnoo	21.17 (4.60)**	33.38 (5.77)
	Danter	9.78 (3.12)	14.99 (3.876)
	Momin	10.26 (3.20)	17.44 (4.17)
	Mean	**13.73 (3.70)**	**21.91 (4.68)**
Bandipora	Chatibandi	10.89 (3.30)	18.37 (4.28)
	Bandipora	9.97(3.15)	15.07 (3.88)
	Sumbal	8.79 (2.96)	10.30 (3.20)
	Mean	**9.88 (3.14)**	**14.58 (3.81)**
Ganderbal	Batwani	9.23 (3.03)	17.37 (4.16)
	Yangura	11.82 (3.43)	22.88 (4.78)
	Zazun	8.75 (2.95)	11.51 (3.39)
	Mean	**9.93 (3.15)**	**17.27 (4.15)**
Shopian	Dradkralipora	9.87 ((3.14)	12.09 (3.47)
	Herman	9.03 (3.01)	10.52 (3.24)
	Sedav	10.08 (3.17)	13.18 (3.63)
	Mean	**9.66 (3.10)**	**11.93 (3.45)**
Overall mean		**10.69 (3.26)**	**16.42 (4.02)**
CD$_{(P \leq 0.05)}$	District	1.03	1.55
	Location	0.86	1.30
	District x Location	2.52	3.80
CV		7.96	9.02

* Average of three sites.

**Figures in parenthesis are arcsine transformed values.

Over all disease severity on pods was highest in district Anantnag (21.91percent), followed by Ganderbal (17.27 per cent), Bandipora (14.58 per cent) and Shopian (11.93 per cent) though the districts Bandipora and Shopian are statistically at par with each other. The data further revealed that the disease development increases with time reaching maximum at the pod development stage.

4.2 Isolation of the pathogen

During the present investigations, isolations made from diseased leaves and pods showing the characteristic symptoms of disease (3.2.1), yielded repeatedly a fungus which proved pathogenic and main cause of bean anthracnose under study. The fungal culture was purified by single spore technique and subsequently sub cultured on PDA medium for further studies**.**

4.3 Phytopathogenicity

Phytopathogenicity of the isolated fungus was established on pods, detached leaves and bean seedlings employing (3.3). Typical symptoms produced by the fungus on pods, detached leaves and those on seedlings started appearing four days after artificial inoculation (plate 3). The fungus proved pathogenic to the bean leaves and satisfied the Koch’s postulates.

4.4 Morphology

4.4.1 On host

Microscopic observations of the fungus revealed that the fungus produced acervuli as its fruiting structures that rupture the host epidermis and bear setae at its surface. Under humid, wet conditions the fungus produces characteristic ooze (Plate 4). Perusal of Table 3 revealed thatthe dimensions of acervuli on host range from 175-285 μm with an average of 270 μm. Inside the acervulus conidia are produced that measure about12-22 x 4-6μm with an average of 17.25 x 4.2μm. The acervuli were found to possess bristle like appendages called the setae measuring about 60-120 x 5-6μm with an average of 90 x 5.55μm.

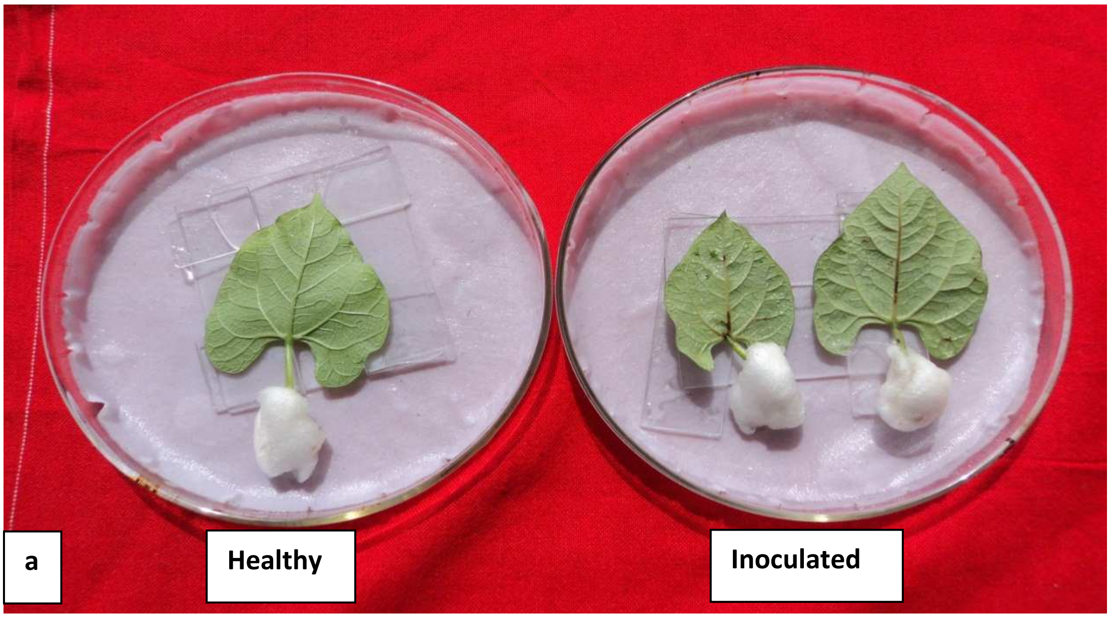
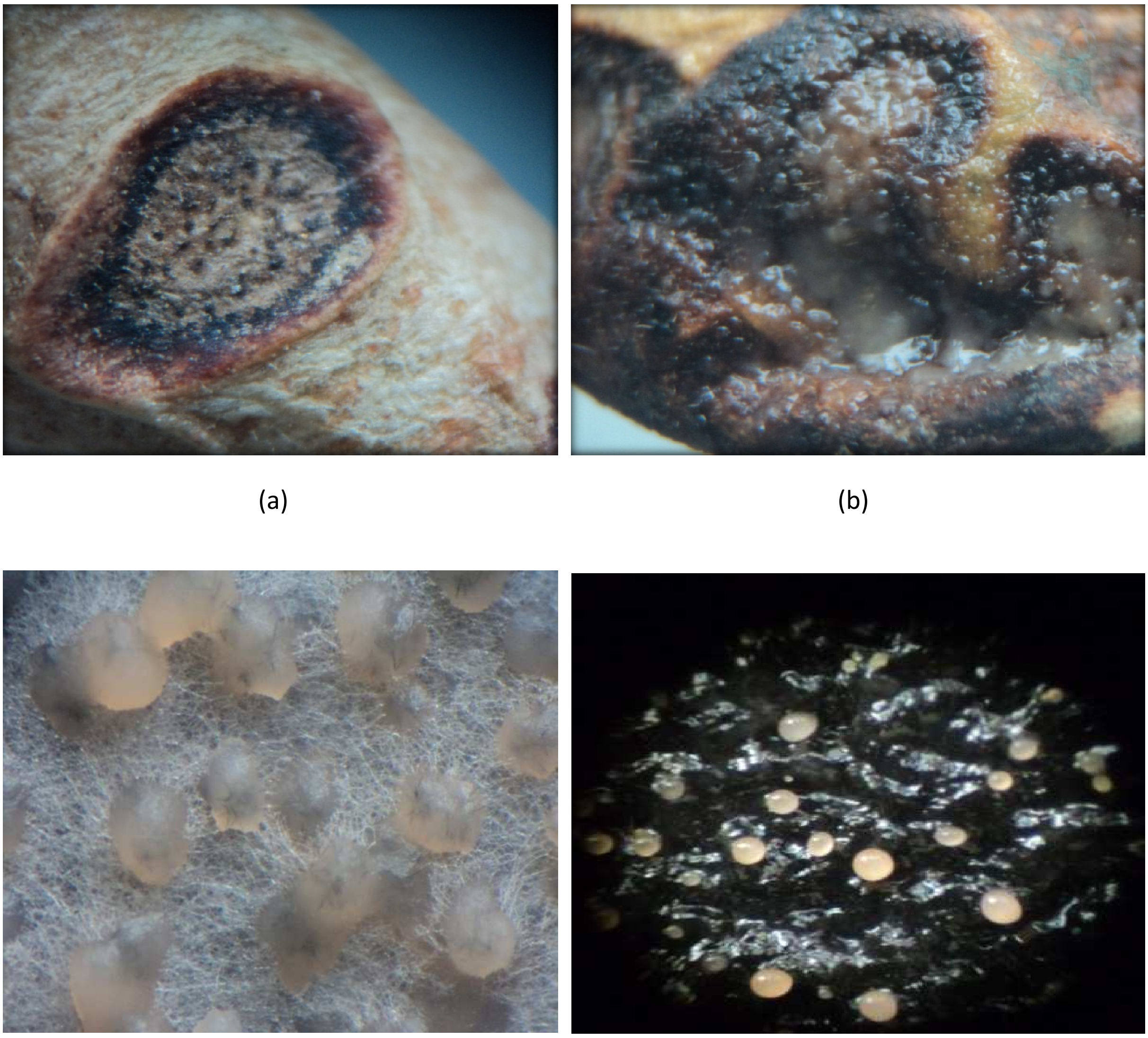


Plate-3: Phytopathogenicity test of *Colletotrichum lindemuthianum* on beans

(a) Leaves (b) Pods

Table – 3Morphological characters of fungus causing anthracnose of beans

Propagule	Size (µm)		Colour	Shape	Septation
	In culture	On host			
Conidia	9.5- 11.5 x 3.5-4.5 (10.5x 4)	12-22.0 x 4-6 (17.25 x 4.25)	Hyaline	Dumbbell, with obtuse ends	0
Setae	64-108 x 2-4 (84.22 x 3)	60- 120 x 5-6 (90 x 5.55)	Brown to Dark Brown	Cylindrical with pointed ends	0-3
Acervuli	140-320 (204.14)	175- 285 (270)	Black	Saucer Shaped	0



(a) (b)

(c) (d)

Plate-4: Morphological characters of *Colletotrichum lindemuthianum*

- **(a) Acervuli bodies on host**
- **(b) Ooze on host**
- **(c) Acervuli on culture (Under stereoscopic microscope)**
- **(d) Ooze on culture (40x)**

4.4.2 On culture

4.4.2.1 Macroscopic characters

Macroscopic observation of the fungus revealed that 5 – 7daysof incubationat 24±1^0C **,** colony of the fungus on PDA medium appeared as grey when young and became dark black having very compact mycelial growth at late stage. The acervuli were formed after 10 days on the culture first in the centre, then in rings across the colony with salmon coloured ooze extruding from them. The acervuli were found to possess dark coloured bristle like projections called setae on the surface feature characteristics of this fungus.

4.4.2.1 Microscopic characters

Morphological studies of fungus on culture are presented in Table 3 (plate 5). Persual of the data revealed that conidia were hyaline, cylindrical, unicellular with obtuse ends , narrow and truncate base, measuring about 9.5- 11.5 x 3.5-4.5µm with an average of 10.5 x 4µm. All the spores were without septa and uni-nucleate. Black acervuli were produced on 10 days old culture measuring about 140-320 µm with an average of 204.14µm. Setae were light brown to dark brown in colour measuring about 64-108 x 2- 4µm with an average of 84.22 x 3µm with 0-3 septa and few per acervuli.

4.5 Identification of fungus

On the basis of morphological characters pathogenicityand comparison with authentic description, the causal pathogen of anthracnose of beans was identified as *Colletotrichum lindemuthianum* (Sacc. and Magn.) Scrib**.**Further its identity was confirmed by Indian Type Culture Collection, Division of Mycology Plant Pathology IARI, New Delhi and culture deposited under identification No 8609.11

4.6 Perpetuation of the pathogen

Perpetuation of pathogen was studied both on seed and plant debris. Blotter paper and agar plate techniques were employed for this study.

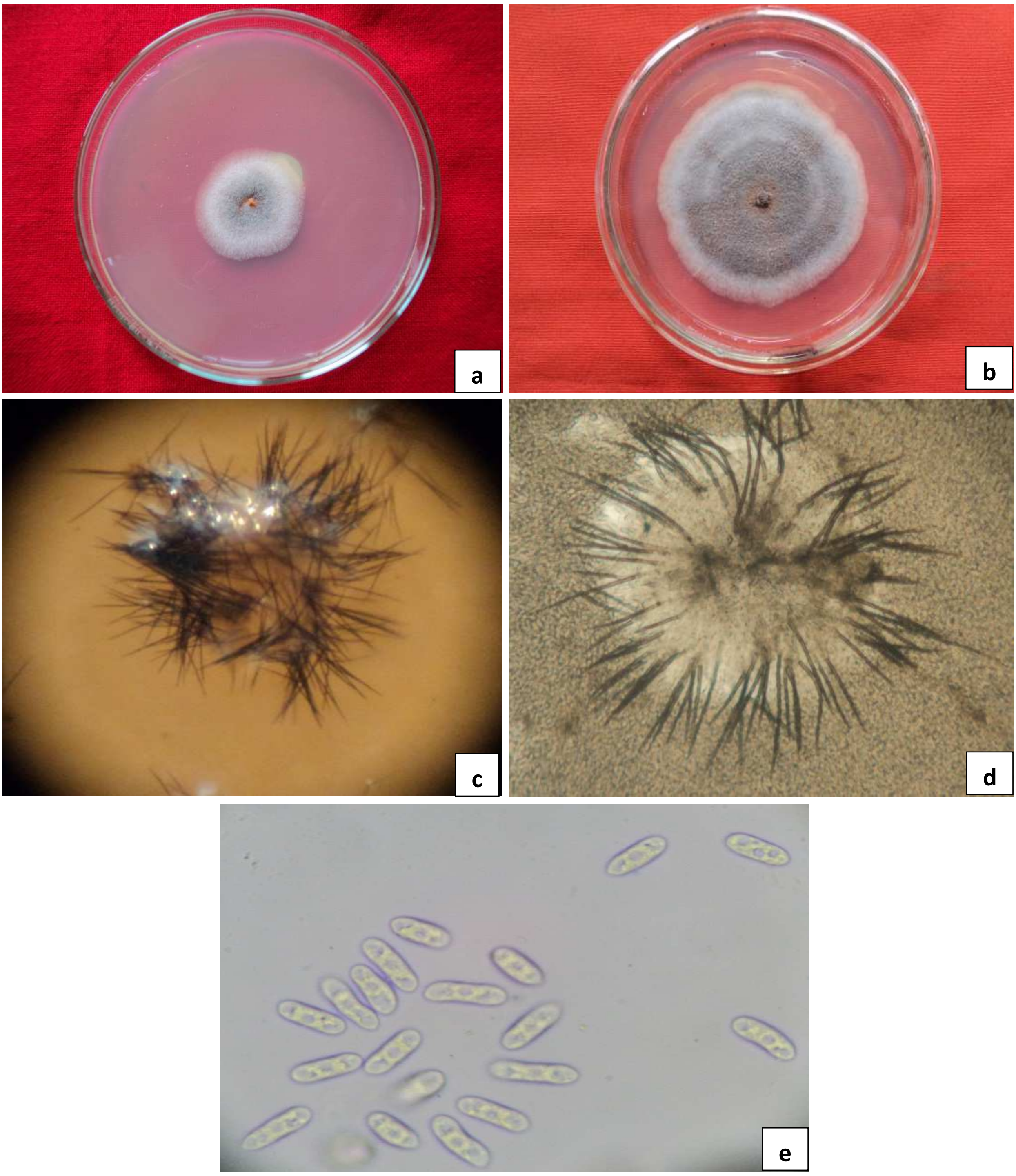


Plate-5: Colony characters of *Colletotrichum lindemuthianum*

a = 10 days old colony (PDA), b = 30 days old colony (PDA),
c = Setae in culture, d = Acervulus with setae, e = Conidia

4.6.1 Perpetuation through seed

4.6.1.1Blotter Paper and Agar Plate Method

In order to establish the relationship between the seed infection and the seed transmission of bean anthracnose blotter paper, agar plate and pot culture methods were used. The data obtained are presented in Table- 4.

Perusal of the data in Table 4 revealed that on blotter paper 72.50 per cent of seeds showed infection at the start of the experiment (15 November). The percentage of seed showing infection decreased slowly from 15 Nov till 30 March where only 62.59 percent of seeds showed the fungal growth. The table further revealed that on agar plate 79.92 percent of seed showed the fungal growth at the start of the experiment and there after decrease slowly with time to 69.11 per cent at the end of the experiment.

The Table further revealed that the percentage of seed showing the growth of *C. lindemuthianum* was higher in case of agar plate method as compared to blotter paper method. The study revealed that although, the percentage of seed exhibiting the growth of *C. lindemuthianum* decreased with the passage of time, the infection percentage recovered after135 days was sufficient to cause infection the following year and can thus act as a source of primary infection.

4.6.1.2 Pot culture

Pot culture method was used to establish the relationship between the seed infection and the transmission of disease to cotyledons and its effect on the germination percentage of seeds. The data obtained is presented in Table-5.

Perusal of the data revealed a positive correlation between the seed infection and the disease incidence on cotyledons. Highest disease incidence (100 per cent) was observed on the cotyledons of seedlings arising from the naturally infected seeds (Plate 6) followed by the artificially inoculated seeds (14.28 per cent) whereas no symptoms of bean anthracnose were recorded on the cotyledons of healthy seeds.

Plate-6: Seed transmission of bean anthracnose

a,b) Symptoms on cotyledons

c) Comparison of diseased and healthy seedlings

Table - 4 Survival of *Colletotrichum lindemuthianum* in natur-ally infected bean seeds during 2011-2012

Date	Conidial viability (%)	Seed infection* (%)	
		Blotter Paper Method	Agar Plate Method
I5 Nov	100 (10.05)	72.50 (8.57)	79. 92 (8.98)
30 Nov	97.92 (10.00)	70.53 (8.48)	78.29 (8.93)
15 Dec	90.90 (9.83)	68.08 (8.38)	75.43 (8.83)
30 Dec	87.58 (9.48)	67.82 (8.30)	75.27 (8.74)
14 Jan	84.30 (9.41)	66.18 (8.29)	74.57 (8.71)
29 Jan	81.30 (9.30)	65.90 (8.19)	72.08 (8.70)
13 Feb	79.33 (9.18)	65.11 (8.18)	71.00 (8.64)
28 Feb	**77.**03 (9.03)	63.88 (8.16)	70.65 (8.56)
14 March	76.28 (8.96)	63.26 (8.09)	69.72 (8.53)
29 M arch	76.12 (8.88)	62.59 (8.06)	69.11 (8.46)
Mean ± SE	78.04± 1.27	66.60± 0.41	76.30± 0.48
C.D(p≤0.05)	0.19	3.28	

* Average of four replication. Figures in parenthesis are arcsine transformed values.

Table – 5 Effect of bean anthracnose on seed germination percentage and disease incidence on cotyledons

Category	**Germination (%)***	**Disease incidence on cotyledons*(%)**
Healthy seeds	100 ± 0.0 **(10.05)**	0.00 ± 0.0 **(0.83)**
Naturally infected seeds	53.58 ± 0.45 **(7. 34)**	100 ± 0.01 **(88.97)**
Artificially inoculated seeds	89.14 ± 0.36 **(9.47)**	14.28 ± 0.08 **(22.194)**
C.D($p \leq 0.05$)	**1.09**	**0.16**

*Average of three replications. Figures in parenthesis are sqrt/arcsine transformed values.

The table further revealed a positive correlation between the seed infection and the germination percentage. It was observed that highest germination percentage resulted from the healthy seeds which statistically at par with germination percentage of artificially inoculated seeds.

4.6.1.3 Perpetuation in bean debris

The debris included the diseased leaves and pod husk. Prior to placement of the debris at 0, 7.5 and 15 cm depth in soil *C. lindemuthianum* was isolated on agar medium from all the pod and leaf tissue pieces. Persual of the data in Table 6 (Plate 7) revealed that the fungus was isolated throughout the experiment from pod and leaf tissue placed on the soil surface however isolation of fungus from leaves and pods placed 7.5 and 15 cm deep in soil became difficult from 75 and 90 days of their burial in soil, instead common soil inhabitants like *Fusarium, Pencillium* and *Trichoderma* were frequently isolated. The data presented in Table-7 further revealed that the fungus survives for relatively longer durations on pods as compared to leaves.

4.7 Germplasm screening

Thirty Common bean genotypes were screened for their reaction to the *C. lindemuthianum* under artificial inoculation conditions during 2011. Data on per cent disease intensity recorded 15 days after inoculation are presented in Table 8. It is evident from the data that different genotypes under evaluation have exhibited varying degrees of disease intensities and none of the genotypes was immune to *C. lindemuthianum*. The data revealed that disease intensity varied from 5.98 to 22.08 per cent. The data further revealed that among 30 genotypes evaluated PAS 162 exhibited lowest disease intensity of 5.98% followed by WB 42 (7.22%), PAS 96 (8.33%) and WB 15 (8.39%) with PAS 96 and WB15 being statistically at par with each other. Highest per cent disease intensity was recorded on WB 233 (22.08%), followed by WB 31 (18.79%). The data further revealed that genotypes PAS 186 (11.32%), PAS 204 (11.21%), PAS 95 (11.38%), WB 33

Plate-7: Perpetuation of *Colletotrichum lindemuthianum* in leaf and pod debris in soil at various depths

a,b) 0 cm

c, d)7.5 cm

e, f) 15 cm

Table – 6 Survival of *Colletotrichum lindemuthianum* on infected French bean leaves in soil

Date	Depth in Soil (cm)		
	0.0	**7.5**	**15.0**
I5 Nov 2011	+	+	+
30 Nov	+	+	+
15 Dec	+	+	+
30 Dec	+	+	+
14 Jan	+	+	+
29 Jan	+	+	+
13 Feb	+	–	–
28 Feb	+	–	–
14 March	+	–	–
29 M arch	+	–	–
13 April	+	–	–
28 April	+	–	–
13 May	+	–	–

+= *Colloetotrichum lindemuthianum* detected, –=*C. lindemuthianum* not detected

Table -7Survival of *Colletotrichum lindemuthianum* on infected French bean pods in soil

Date	Depth in soil (cm)		
	0.0	**7.5**	**15.0**
I5 Nov 2011	+	+	+
30 Nov	+	+	+
15 Dec	+	+	+
30 Dec	+	+	+
14 Jan 2012	+	+	+
29 Jan	+	+	+
13 Feb	+	+	+
28 Feb	+	_	_
14 March	+	_	_
29 M arch	+	_	_
13 April	+	_	_
28 April	+	_	_
13 May	+	_	_

+= *Colloetotrichum lindemuthianum* detected,⁻=*C. lindemuthianum* not detected

Table – 8 Screening of various *Phaseolus* genotypes against anthracnose of beans under artificial inoculation

Genotype	Per cent Disease Intensity*	Reaction type
PAS 28	11.52 (3.53)	MS
PAS 96	8.33 (3.05)	MR
WB 15	8.39 (3.06)	MR
PAS 125	17.2 (4.26)	MS
PAS 162	5.98 (2.64)	MR
PAS 50	10.26 (3.35)	MR
WB 225	11.25 (3.50)	MS
PAS 126	11.01(3.46)	MS
PAS 4	9.11(3.17)	MR
PAS 9	11.08 (3.47)	MS
WB 32	12.13 (3.62)	MS
WB 50	11.57(3.54)	MS
WB 86	11.18 (3.49)	MS
WB 233	22.08 (4.80)	MS
WB 13	10.35 (3.36)	MR
WB 80	11.01(3.46)	MS
PAS 186	11.32 (3.50)	MS
PAS 93	12.04 (3.60)	MS
PAS 204	11.21 (3.49)	MS
WB 59	11.87 (3.58)	MS
WB 42	7.22 (2.86)	MR
PAS 95	11.38 (3.51)	MS
WB 79	11.14 (3.48)	MS
WB 47	11.58 (3.54)	MS
WB 31	18.79 (4.43)	MS
WB 33	11.16 (3.48)	MS
PAS 8	11.81(3.57)	MS
PAS 49	11.16 (3.48)	MS
PAS 39	11.82 (3.57)	MS
PAS 207	11.17 (3.48)	MS
C.D ($p \leq 0.05$)	0.27	

*****Represents mean of three replications

(11.16%), WB 86 (11.18%), WB 225 (11.25%), PAS 49 (11.16%), PAS 207 (11.17%) did not differ significantly from one another,although theywere superior to WB 233 (22.08%) and WB 31 (18.79%). The test genotypes were allotted to different reaction groups on the basis of disease intensity recorded in each genotype (Table 5). Perusal of the data reveals that most of test genotypes (76.66%) were moderately susceptible (11-25% PDI) while remaining 23.33 per cent were moderately resistant (1-10% PDI) to anthracnose of beans.

Chapter – 5

DISCUSSION

Common bean (*Phaseolus vulgaris* L.) is one of the most important*Leguminous* crops cultivated throughout the world (Broughton *et al*., 2003). The cultivation of the crop has increased manifold on account of its high palatability and economic value. However, it succumbs to a number of biotic and abiotic stresses that circumvent its production and grain quality. Among the biotic factors, the most destructive disease encountered is anthracnose of beans which, *inter alia,* is the main constraint to its production especially in regions where agro-climatic conditions are conducive for disease development (Pastor-Corrales and Tu, 1989). The disease infect the crop at various phenological stages and affect leaves, petioles, stems, pods and seeds (Lobato *et al*., 2009). The disease is prevalent in different parts of the world and almost in all local and commercial cultivars of the beans (Pastor-Corrales and Tu, 1989). Its occurrence with varying degree of incidence and intensity has also been reported from India (Sharma *et al*. 1994).

The anthracnose of beans occurs in mild to severe form in every bean growing area of Kashmir valley, causing considerable losses (Khan *et al*., 2009). Establishing the status is pre-requisite to decide the adoption of disease management strategies. The objective was achieved in the present study by undertaking surveys in four commercially important bean growing districts of Anantnag, Bandipora, Ganderbal and Shopian during *kharif* 2011. It was observed that bean anthracnose was present in all the locations in varying proportions. The disease, however, varied from cultivar to cultivar and location to location due to difference in micro- and macro-climatic conditions.

During the course of survey incidence and intensity of bean anthracnose in the valley ranged from 23.51 to 82.14% and 8.75 to 33.38%, respectively. Highest mean disease incidence and intensity, ranged from 34.81% and 13.73% on leaves was recored in district Anantnag followed bydistrct Ganderbal (27.90 & 9.95 %), Bandipora (25.43 & 9.88 %) and Shopian (24.80 & 9.66%), respectively. Though district Bandipora and Shopian were statistically at par. Least disease incidence and intensity of 24.80 & 9.66% was recorded in Shopian. Similarly, maximum disease incidence and intensity on pods was observed in district Anantnag (82.14 & 33.38%) followed by Ganderbal (47.38% &17.27%), Bandipora (32.96 & 14.58%) and Shopian (28.99 & 11.93%), respectively. The findings are in confirmation with the well-established phenomenon of disease pathogenesis i.e., more the disease incidence, more will be the disease intensity (Fry, 1982). The disease incidence and intensity of beans was found to increase with the advance in phenological stages of crop, with a maximum increase at pod formation. The findings are also corroborated by Rajappan *et al*. (2001), Ambardar and Sidha (2009) and Mudawi *et al*. (2008). It has also been ratified that when no heavy seedling infection is observed, another phase of marked susceptibility is at early stage of pod formation (Wheeler, 1975. Gupta and Mathew, 2005).Increase in the disease severity with age may also be attributed to increase in susceptibility at pod development stage (Mudawi *et al*., 2008).

Maximum disease incidence and intensity on leaves and pods was recorded at Larnoo (53.79 & 82.14%, 21.17 & 33.38%) of district Anantnag followed by Yangura (29.64 & 57.79%, 11.82 & 22.88%) of district Ganderbal, whereas lowest incidence and intensity was recorded at Sumbal of Bandipora (23.51% ; 25.65% and 8.79% ; 10.30%). The variation in disease incidence and intensity observed may be attributed to differences in the environmental factors, high dependence on farm saved seeds, exchange of diseased seeds among farmers, crop care practices, type of cultivar and the cropping pattern (David *et al*. 2000; Opio *et al*., 2001, Nkalubo *et al*., 2007). Adoption of different in crop cultivation practices under diverse topographic locations with moist, cool climate, low night temperatures coupled with the race variability of the pathogen may also be deemed as feasible factors for disease variation (Pastor-Corrales, 1995; Bassenezi *et al*., 2001; Opio *et al*., 2006). While studying the incidence of bean anthracnose, Padder *et al.* (2010) also recorded an incidence of 0 to 88% from Himachal Pradesh.

The initial symptoms of the disease appeared 15 DAS though plants get infected at different growth stages but conspicuous necrotic spots appeared on cotyledons followed by leaves and pods. Characteristic symptoms like dark brown to brown spots with cankerous sunken centres also appeared on pods. The spots become dark charcoal like in colour on the basis of which the disease is designated as anthracnose. The older leaves were susceptible to the disease than the young ones. Moreover, the immature pods were more vulnerable to the attack of disease than the leaves. Under severe infection pre mature yellowing of leaves followed by defoliation was observed. These observations are in conformity with those of Singh (1999), Bassenezi *et al*. (2001), Gupta and Paul (2001) and Gupta and Mathew (2005).

The causal organism was isolated from diseased leaves and pods by tissue bit method and simultaneously purified by single spore technique. Koch's postulates were satisfied through protocols of detached leaf and seedling techniques on common beans.

Morphological studies and colony characters were studied on potato dextrose agar medium. Colony colour of fungus was grey when young and later on became dark black having very compact mycelial growth. Conidia were hyaline, cylindrical, unicellular with obtuse ends and narrow and truncate base measuring about 12-22 x 4-6 μm with an average of 17.25 x 4.2 μm on host and 9.5- 11.5 x 3.5-4.5 μm with an average of 10.5 x 4 μm. All the spores were without septa and uninucleate. The findings about spore-dimensions on culture are in consonance with those of Wijesekara and Agarwal (2006) and Khan *et al*. (2009).

Acervuli were produced on 10 days old PDA culture, first in centre then in rings across the colony with salmon coloured ooze exuding from them. These findings are scientifically substantiated by Khan e*t al*. (2009). The dimensions of acervuli ranged from 175-285 µm with an average of 270 µm on host. In artificial culture the dimensions ranged from 140-320 µm with an average of 204.14 µm. The acervuli possessed on the surface dark coloured bristle-like projections called setae, a conspicuous characteristic of this fungus. Setae were light brown to dark brown in colour measuring 60-120 x 5-6 µm with an average of 90 x 5.55 µm on host. In culture the corresponding dimensions were 64-108 x 2-3 µm with an average of 84.22 x 3.0 µm, with 0-3 septa and few per acervuli. Similar findings have also been put forth by Kulshrestha *et al*. (1976).

On the basis of symptomatology, morphological characters and phytopathogenicity test and their comparison with different identifying keys available in authentic taxonomic literature, the fungal pathogen was identified as *Colletotrichum lindemuthianum* (Sacc. and Magn.) Scrib.(Tu, 1992; Alexopouls *et al.*, 2004). These characteristics are also in conformity with those reported by Singh (1994), Prusky *et al*. (2000) and Gupta and Paul (2001).

The mode of fungal perpetuation through seed and plant debris was also studied during the current course of research. It was observed that seed acted as a source of primary infection. The percentage of seed showing the fungal growth during the experimental period of 135 days through blotter paper and agar plate methods was 62.59 & 69.11%, respectively. This was enough to infect the crop in next year. According to Yesuf and Sangchote(2005) also seed is the primary source of inoculum for bean anthracnose. Our studies are substantiated by the findings of Tu (1983), who reported that the fungus can survive for five years in infected dry seeds or pods stored at 4^0C. He also observed that the beans planted in the field, heavily infested with disease the previous year, did not show any anthracnose disease. The infected seed has been found as common source of introducing anthracnose in the field (Tu, 1992; Allen *et al.*, 1998; Bhale *et al.*, 2001; Gupta and Paul, 2001; Qandah and Al-Momany, 2003; Gupta and Mathew, 2005 and Vishunavat, 2009).

The role of seed in the perpetuation of the fungus is further explained by the findings of the current study when pots planted with disease-free seeds failed to show any anthracnose symptoms, whereas those planted with naturally infected seeds developed typical symptoms, mainly on cotyledons. Appearance of such typical symptoms within few days of plant emergence was also observed by Yesuf and Sangchote (2005). There is a positive and significant correlation between the seed infection and seedling infection (Yesuf and Sangchote, 2005). These findings are in accordance with that of Tu (1983), who observed a positive and linear relationship between the seed infection and per cent seedling infection in greenhouse studies on anthracnose of beans.

Furthermore, the results reveal that per cent disease on cotyledons was more in case of naturally infected seeds (100%) as compared to artificially inoculated seeds (14.28%), thereby depicting that disease is internally seed-borne. The fungus *C. lindemuthianum,* cause embryo infection that might lead to non-systemic seed transmission and may be carried as hyphae in the embryo or seed coat Neergaard (1979). In an analogous study similar observations have been made by David *et al*. (2000), Melotto *et al*. (2000), Ravi *et al*. (2000), and Opio *et al*. (2001).

During the current study it was observed that with increase in level of seed infection germination percentage of seed got decreased. Highest germination per centage (100%) was observed in healthy seeds, which was statistically at par with that of artificially inoculated seeds whereas lowest germination percentage (53.58 %) was recorded from naturally infected seeds. Observations of present study are in confitmity with those of Qandah and Al-Momany (2003) who found that bean anthracnose results in reduced seed germination, seed rot and seedling mortality. The pre- and post- emergence seedling mortality has also been noticed by Yesuf and Sangchote (2005).

In the present investigations plant debris on the surface of field had a significant role in perpetuation of bean anthracnose for next crop. The results are almost in agreement with those of Dillard and Cobb (1993) who reported that disease inoculum can survive on crop debris at different soil depths during winter seasons and cause disease in next bean crop. The *C. lindemuthianum* can alsosurvive for more than one season in infected vines and pods, but spores in soil were not viable after 7 weeks (Barrus, 1921).

The burial of plant debris in soil (7.5 and 15 cm depth) decreased the viabi lity of fungus, for it could not be isolated from plant debris after 75 and 90 days of incorporation in soil. It has been also found by other workers that development of anthracnose was significantly influenced by spring tillage while as highest disease was recorded in plots with bean debris left on soil surface (Ntahimpera *et al.,* 1997). The loss of viability due to incorporation of plant debris in soil may be due to microbial decomposition of plant debris (Ntahimpera *et al.,* 1997). The results are, however, in contrast with those of Tu (1983) who reported that the fungus failed to overwinter in infected bean debris unless protected from water. The differences in findings may be attributed to the fact that in Kashmir valley beans are harvested in the last week of October and because of wet conditions that prevail in autumn, bean producers usually delay incorporation of bean residues into the soil until next growing season. Ergo, crop debris remains on soil surface and may act as a reservoir of inoculum for the next growing season.

Inherent resistance or tolerance of plants to infection by the pathogen can likely be a safe, economical and eco-friendly disease management venture. Attempts were, therefore, made in identifying the bean genotypes/lines/cultivars showing tolerance to *C. lindemuthianum* under in-house conditions. The observations vis-a-vis reaction of thirty common bean genotypes revealed that one was immune/resistant or tolerant against anthracnose. Most of th e cultivars (76.66%) were moderately susc-eptible to the disease while as remaining (23.34 %) were moderately susceptible to disease. A similar observation was realized by Pathania *et al.* (2006) wherein 35 out of 49 bean varieties were susceptible to race 3 of *C. lindemuthianum*. Likewise a study on 26 Brazilian bean lines to nine races of *C. lindemuthianum* showed that the Andean genotypes were generally more susceptible to anthracnose than their Mesoamerican counterparts (Vidgal-Filho *et al*., 2007). Similar results about susceptibility of local germplasm to different races of pathogen were inferred even by Kumar *et al*. (1997), Sharma *et al*. (1999), and Padder *et al*. (2010).

The present study revealed that bean anthracnose pathogen *C. lindemuthianum* causes an appreciable reduction in bean productionwhich is considered as meat of poor because of its nutritive value.There is enough scope in futu re to contemplate the role of disease management by developing the resistant/toler ant cultivars with acceptable varietal characters.

Chapter – 6

SUMMARY AND CONCLUSION

Due to dynamic nature of micro-organisms, routine disease surveys and identification work is important for better understanding of fungal mycoflora associated with bean seeds. Such of information enables to prioritize research activities to address production constraints in different bean growing agro-ecologies. The disease survey data indicate that anthracnose of beans is prevalent in all the bean growing areas of the Kashmir valley with varying degree of incidence and intensity. The incidence and intensity of the disease, irrespective of phonological stage ranged from 23.51 to 82.14% and 8.29 to 33.38%, respectively. Highest incidence and intensity of the disease both on leaves and pods was recorded in district Anantnag (35.71%; 51.11% and 13.73%; 21.19%) while as it was lowest in district Shopian (24.80% and 9.66%), respectively. Further among the various locations surveyed highest disease incidence and intensity on leaves and pods was recorded at larnoo of district Anantnag (53.79% ; 82.14% and 21.17% ; 33.38%), while it was lowest in Sumbal of district Bandipora (23.51% ; 25.65% and 8.79% ; 10.30%). In the current studies it was also observed that disease incidence was more at higher altitudes where cool and moist climatic conditions prevail during cropping season which predisposes the crop to the disease.

The disease appears at various phonological stages of the crop but is more conspicuous on cotyledons, leaves and pods. The dark brown spots with sunken centers on pods are characteristic symptoms of the disease. The older leaves and pods are more succeptible. The causal organisms isolated and purified by single spore technique have been declared as *Colletotrichumm lindemuthianum* (Sacc. and Magn.) Scrib. Colony colour of fungus was grey when young and later on became dark black having very compact mycelial growth. Conidia were hyaline, cylindrical, unicellular with obtuse ends and narrow and truncate base measuring about 12-22 x 4-6 μm with an average of 17.25 x 4.2 μm on host and 9.5- 11.5 x 3.5-4.5 μm with an average of 10.5 x 4 μm. Spores were aseptate and uninucleate.

The source of primary bean anthracnose inoculum in field is mainly through infected seeds. Among the two techniques employed to establish the role of seed in disease transmission agar plate proved more efficient (76.30 ± 0.48%) gave better results as compared to blotter paper technique (66.60 ± 0.41%). Further, in pot-culture studies, a positive and significant correlation was observed between the seed infection and the disease transmission. Disease development in the field and subsequent pod and seed infection is favoured by high and frequent rainfall with moderate temperature during the crop season. Seed infection was positively correlated with severity of pod infection. The disease results in reduction of germination percentage of infected seeds, due to pre and post emergence mortality. Since there is no government or private institution dealing with the production of disease free or improved seeds, the majority of common bean growers in the valley depend on their own seeds saved from the previous harvest. Moreover, most of these farmers are not aware of seed-borne infection. Therefore, production of disease-free seeds in areas, where conditions are not conducive for bean anthracnose infection, could play a vital role in integrated management of the disease.

Present investigations propounded that bean debris on soil surface act as a potential source of disease inoculum for the next crop and have a positive (+) correlation with the disease development. However, bean debris incorporated in soil has no role in perpetuation of pathogen. Since most of the bean growers in the valley don't plough their fields after the harvest of crop due to wet conditions that prevail at the fag end of the season. Consequently, plant debris remain on soil surface allowing pathogen to perpetuate during winter season. Moreover, shattering of pods also results in the dispersal of bean seeds, which if infected, can act as a primary source of disease inoculum for the next crop. Among the thirty genotypes evaluated for their reaction to bean anthracnose none was found immune/resistant or tolerant to the disease. However, there are germplasm lines which exhibit moderate resistance (MR) to the pathogen. The genotypes PAS 162 exhibited lowest disease intensity (5.98%) and was accordingly graded as moderately resistant cultivar against bean anthracnose fallowed by PAS 96 (8.33 %), WB 15 (8.39 %) PAS 4 (9.11 %), while as the highest disease intensity of 22.08 %was recorded in WB 233. These findings suggest that there are sources which can be explored and exploited to develop varieties resistant to anthracnose of beans by applying suitable breeding techniques that suit to the floral morphology of bean plant. The non-existence of any resistant cultivar against the disease is mainly because of the considerable pathogenic variability.

- In light of the present investigations it is concluded that anthracnose of beans is an economically important disease, present in almost all bean growing areas of Kashmir valley especially in high altitudes where cool and moist conditions prevail during the most part of the cropping season.
- Disease is caused by *Colletotrichum lindemuthianum.*
- The disease is mainly seed borne in nature. However, can survive on plant debris left on the surface of soil.
- The disease significantly reduces the germination percentage of infected seeds.
- Seedlings raised from the infected seeds show reduced vigour.
- Initial symptoms of the disease appear on the cotyledons as dark brown to brown spots with cankers centers that contain salmon coloured ooze representing conidial mass which are the sources of secondary infection in the field.
- There is an ample scope for identifying the sources of resistance in the locally available germplasm.

Based on the findings of the current studies it is as such suggested that anthracnose of beans can be managed by:

- Multiplying seeds in low altitude belts.
- Using disease free seeds.
- Destruction of crop debris.
- Use of resistant varieties.

The current investigations, in fact unveil the preliminary aspects of the disease. There is an ample scope for further in-depth research and investigation so far as the pathogenic and/or molecular diversity of the *C. lindemuthianum* is concerned.

LITERATURE CITED

Agrios, G.N.2004.*Plant Pathology***.**Academic Press, San Diego, USA, pp. 948.

Alexopoulos, C.G., Mims, C.W. and Blackwell, M. 2004. *Introductory Mycology*.John Wiley & Sons, New York, USA, pp. 869.

Allen, D.J., Buruchara, R.A. and Smithson, J.B. 1998. Diseases of common bean.pp.179-265. **In**:*The Pathology of Food and Pasture Legumes* (Eds. D.J. Allen and J.M. Lenne), CAB International, Wallingford Oxon, UK.

Ambardar, V.K. and Sidha, K. 2009. Distribution and management of anthracnose of beans (*Phaseolus vulgaris* L.) caused by *Colletotrichum lindemuthian-um*. *Plant Disease Research* **24**: 184-186.

Anonymous, 2008.*Digest of Statistics*. Directorate of Economics and Statistics, Planning and Development Department, Government of Jammu and Kashmir, pp. 96-98.

Anonymous, 2010.Food and Agriculture Organisation of the United Nations.FAOSTAT database.http://www.fao.org.

Araya-Fernandez, C.M., Dhingra, O.D. and Kushalappa, A.C. 1987. Influence of primary inoculum on bean anthracnose prevalence. *Seed Science and Tec hnology***15**: 45- 54.

Barrus, M.F. 1911.Variation of varieties of beans in their susceptibility to anthrac-cnose. *Phytopathology***1**: 190-199.

Barrus, M.F. 1921. Bean anthracnose. *Cornell University of Agricultural Experim ent Station Bulletin* **42**: 101-125.

Bashir, T. 1997. Loss assessment study on haricot beans due to anthracnose. *Pest Management Journal of Ethiopia***1**: 69-72.

Bassanezi, R.B., Amorin, L., Bergamin, F.A., Hau, B. and Berger, R. D. 2001. Ac counting for photosynthetic efficiency of bean leaves with rust, angular leaf spot and anthracnose to assess the crop damage.*Plant Pathology***50**: 443-452.

Bhale, M.S., Khare, D., Raut, N.D. and Singh, D. 2001. *Seed-borne Diseases:Objectionable in Seed Production and their Management*. Scientific Publishers, Jodhpur, India, pp. 185

Bigirimana, J. and Hofte, M. 2001. Bean anthracnose: inoculation methods and influence of plant stage on resistance of *Phaseolus vulgaris* cultivars. *Journal of Phytopathology***149**: 403-408.

Bigirimana, J., Fontaine, R. and Hofte, M. 2000. Bean anthracnose: virulence of *Colletotrichum lindemuthianum* isolates from Burundi, Central Africa**.** *Plant Disease***84**: 491.

Broughton, W.J., Hernandez, G., Blair, M. and Beebe, S. 2003. Bean (*Phaseolus vulgaris*) - model food legume. *Plant and Soil***252**: 55-128.

Butler, E.J. 1918. *Fungal Diseases in Plants*.Thacker Spring and Co. Calcutta, India, pp. 547.

Campbell, C.L. and Madden, L.V.1990. *Introduction to Plant Disease Epidemiology*.John Wiley and Sons, New York, USA, pp.532.

Carrijo, F.R.F., Souza, E.A. and Ramlho, M.A.P. 2003. Common bean lines reaction to anthracnose pathogen. *Crop Breeding and Applied Biotechnology***3:** 279-289.

Chakrabarty, P.K. and Shyam, K.R. 1990. Reaction of some promising lines of French bean to different isolates of *Colletotrichum lindemuthianum* in Himachal Pradesh. *Indian Journal of Plant Pathology***8**: 86-88.

Conner, R.L., McAndrew, D.W., Kiehn, F.A., Chapman, S.R. and Froese, N.T. 2004.Effect of foliar fungicide application timing on the control of bean anthrac-nose in the navy bean ‘Navigator’. *Canadian Journal of Plant Pathology* **26**: 299-303.

Conner, R.L., Chen,Y., Hou, A., Balasubramanian, P. M., McLaren, D.L. and Mc Ra, K. B. 2009. Seed-borne infection affects anthracnose development in two dry bean cultivars. *Canadian Journal of Plant Pathology***31**: 449-455.

David,S., Kirkby,R. and Kasozi, S. 2000. Assessing the impact of bush bean varieties on poverty reduction in Sub-Saharan Africa: Evidence from

Uganda.**In:***Assessing the Impact of Agriculture Research on Poverty Alleviation*. San Jose, Costica Rica, 14-16 September.

Del Rio, L. and Bradely, C. 2004. *Anthracnose of Dry Beans*.North Dakota State University Extension Service, Frago, North Dakota, USA.pp.23.

Dillard, H.R. and Cobb, A.C. 1993. Survival of *Colletotrichum lindemuthianum* in bean debris in New York State.*Plant Disease***77**: 1233-1238.

Fernandez, M.T., Fernandez, M., Casares, A., Rodriguez, R and Fueyo, M. 2000. Bean germplasm evaluation for anthracnose resistance and characterization of agronomic traits: A new physiological strain of *C. lindemuthianum* infecting *Phaseolus vulgaris* L. in Spain. *Euphytica***114**: 143-149.

Ferreira, J.J., Campa, A., Pérez-Vega, E., and Giraldez, R. 2008.Reaction of a bean germplasm collection against five races of *Colletotrichum lindemuth ianum* identified in northern Spain and implications for breeding.*Plant Disease***92**: 705-708.

Fry, W.E. 1982.*Principles of Plant Disease Management*. Academic Press, New York, USA, pp. 378.

Ganeva , D. and Georgiena, M. 1975. Resistance to introduced and local garden bean samples to anthracnose. *Gradinarska Lozarska Nauka***12**: 53-60.

Goncalves-Vidigal, M.C., da Silva, C.R., Filho, P.S.V., Gonela, A. and Kvitschal, M. V. 2007. Allelic relationships of anthracnose (*Colletrotrichum lindem uthian-um*) resistance in the common bean (*Phaseolus vulgaris* L.) cultivar Michelite and the proposal of new anthracnose resistance gene, Co-11. *Genetics and Molecular Biology***30**: 589-593.

González-Chavira, M., Guerra, R.R., Hernández-Godínez, F., Acosta-Gallegos, J.A., Martínez de la Vega, O., and Simpson, J. 2004. Analysis of pathotypes of *Colletotrichumlindemuthianum* found in the central region of Mexico and resistance in elite germplasm of *Phaseolus vulgaris. Plant Disease***88**: 152-156.

Gupta, S.K. and Mathew, K.A. 2005. Diseases of french bean and pea and their management. pp. 325-352. **In**: *Diseases of Fruits and Vegetables and their Management* (Ed. T. S. Thind). Kalyani Publishers, New Delhi, India.

Gupta, V.K. and Paul, Y.S. 2001.*Diseases of Vegetable Crops*. Kalyani Publishers, New Delhi, India, pp. 277.

Hazra, P. and Som, M.G. 2005.*Vegetable Science*. Kalyani Publishers, Ludhiana, India, pp. 491.

Hirst, J.M. and Stedman, O.J. 1963. Dry liberation of fungus spores by raindrops. *Journal of General Microbiology***33**: 335-344.

Hollingsworth, C.R., Rio, L. and Bradley, C. 2003. Dry bean anthracnose in Minn es-ota. http//www.umes.org.

Inglis, D.A., Hagedorn, D.J. and Rand, R.E. 1988. Use of dry inoculum to evaluate beans for resistance to anthracnose and angular leaf spot. *Plant Disease***72:** 771-774**.**

Kalha, C. S., Gupta, S. and Vaid, A. 2005. Status of bean anthracnose in Jammu division of Jammu and Kashmir.*Indian Journal of Mycology and Plant Pathology* **19:** 151-156.

Khan, N.A., Sagar, V., A. Khursheed., Ahmad, M. and Beigh, M. A. 2009.Occurr ence of bean anthracnose in Kashmir valley –A Preliminary Study.*SKUAST Journal of Research***1**: 164-166.

Khare, M. N. and Chacko, S. 1983. Factors affecting seed infection and transmissi on of *Colletotrichum truncatum* in soybean. *Seed Science and Technology* **11**: 853-858.

Kulshrestha, D.D., Mathur, S.B. and Neergard, P. 1976. Identification of seed-born e species of *Colletotrichum*. *Friesia Bind* **2**:116-124.

Kumar, A., Sharma, P.N. and Sharma, O.P. 1997. Resistance to *Colletotrichum lin de-muthianum* (bean anthracnose) of kidney bean accessions of diverse origin in Himachal Pradesh. *Indian Phytopathology***50**: 59-64.

Kumar, A., Sharma, P.N., Sharma, O.P. and Tyagi, P.D. 1999. Epidemiology of b ean anthracnose (*Colletotrichum lindemuthianum*) under sub-humid mid-hill zone of Himachal Pradesh.*Indian Phytopathology* **52**: 393-397.

Laxman, R. 2006. *Studies on leaf spot of green gram caused by Colletotrichum truncatum (Schw.) Andrus and Moore*. M. Sc. (Agri.) thesis submitted to the University of Agricultural Sciences, Dharwad,Karnataka, India, pp. 128.

Leaky, C.L.A. and Simbwa-Bunnya, M. 1972. Races of *Colletotrichum lindemut-hianum* and implications for bean breeding in Uganda. *Annals of Applied Biology* **70**: 25-34.

Lobato, A.K.S., Gonclaves-Vidgal, M. C., Vidgal-Filho, P.S., Costa, R.C.L., Lopes, M.J.S., Cruz, A.S., Meirelles A.C.S. and Gonclaves, A.M.O. 2009. Nitrogen compound responses in two cultivars of common bean inoculated with *Colletotrichum lindemuthianum. Research Journal of Biological Science***4**: 293-297.

Madhusudhan, B. S. 2002. *Studies on soybean anthracnose caused by Colletotrich um truncatum (Schw.) Andrus and Moore*. M. Sc. (Agri.) thesis submitted to the University of Agricultural Sciences,Bangalore, Karnataka, India, pp.123.

Mahuku, S.G., Jara, C.E., Cajiao, C. and Beebe, S. 2002. Sources of resistance to *Colletotrichum lindemuthianum* in the secondary gene pool of *Phaseolus vulgaris* and in crosses of primary and secondary gene pools**.***Plant Disease* **86:**1383–1387.

Mahuku, S.G. and Riascos, J. J.2004. Virulence and molecular diversity within *Co lletotrichum lindemuthianum* isolates from Andean and Mesoamerican be an varieties and regions. *European Journal of Plant Pathology***110**: 253–263.

Makini, F.W. 1995. Bean production problems and constraints in Kenya with emp ha-sis on disease.pp.104-109. **In*:*** *Proceedings of a Regional Workshop for Eastern, Central and Southern Africa,* Kenya, October 2-6, 1994.

Martinez-Pacheco, M.M., Saucedo-L.J., Flores-G. A. Martinez-M., Campos-G.J. 2009.*Colletotrichum lindemuthianum* is a potential cellulases producer mi croorganism. *Microbiologica* **51**: 23-31.

Masooodi, M.A. and Massoodi, S.D, 2003. *Agriculture in J&K, A Perspective in Production of Vegetables.*Mohisarw Book Series, Srinagar, Kashmir, India, pp.111-126.

Mathur, S.B. and Olga Kongsdal. 2003. *Common Laboratory Seed Health Testing Methods for Detecting Fungi*. International Seed Testing Association, Bas ser-sdorf, Switzerland, pp. 447.

Mathur, S.R., Barnett, H. L. and Lilly, V.G. 1950. Sporulation of *C. lindemuthian um* in culture.*Phytopathology* **40:** 104-114**.**

Mayee, C.D. and Dattar, V.V. 1986. *Phytopathometry*. Marathwada Agricultural University, Parbhani, Maharashtra, India, pp. 85.

Melotto, M., Balardin, R.S. and Kelly, J.D. 2000.Host-pathogen interactions and variability of *Colletotrichum lindemuthianum*. pp. 346-36.**In:** *Colletotric hum: Host Specificity, Pathology and Host-Pathogen Interaction* (Eds. D. Prusky, S. Freeman and M.B. Dickman). American Phytopathological So cietySt. Paul, Minnesota, USA.

Menezes, J.R. and Dianese, J.C. 1988. Race characterization of Brazilian isolate o f *Colletotrichum lindemuthianum* and detection of resistance to anthracno se in *Phaseolus vulgaris. Phytopathology* **78:** 650-655.

Minussi, E., Neto, T.A. and Kimati, H. 1975. Reaction of 60 bean (*Phaseolus vulgaris* L.) varieties to race BA 1 (alpha) of *Colletotrichum lindemuthianum* (Sacc. and Magn). Scrib.*Revista do Centro de Ciencias Rurais* **5**: 275-280.

Mishra, B.L. 2005. *Design and Analysis of Experiments for Agriculture Workers.*Kalyani Publishers New Delhi, India, pp. 219.

Muncie, J.H. 1917. Experiments on the control of bean anthracnose and bean blight. *Michigan Agriculture Experiment Station Technical Bulletin***38**: 50.

Mudawi, H.I., Idris, M.O. and El Balla, M.M.A.2008Anthracnose disease in common bean (*Phaseolus vulgaris* L.) in Shambat, Sudan. II. Disease Incidence, Severity and effect on yield University of Khartoum. *Journal of Agricultural Sciences* **16**: 477-489.

Neergaard, P. 1979. *Seed Pathology.*The Macmillan Press Ltd. London, UK , pp. 1191.

Nicholsen, R. I. and Moraes, W. B.C. 1980. Survival of *Colletotrichum graminicola*: importance of the spore matrix. *Phytopatholog***y** **70**: 255-261.

Nkalubo, S., Melis, R., Laing,M.D.and Opio, F. 2007. Yield losses associated with anthracnose disease on Ugandan market-class dry bean cultivars. *African Crop Science Society* **8***:* 869-874.

Ntahimpera, N., Dillard, H.R., Cobb, A.C. and Seem, R.C. 1997. Influence of tillage practices on anthracnose development and distribution in dry bean fields. *Plant Disease***81**: 71-76.

Ntahimpera, N., Dillard, H.R., Cobb, A.C., and Seem, R.C. 1996. Anthracnose development in mixtures of resistant and susceptible dry bean cultivars.*Phytopathology***86**: 668-673.

Ombiri, J., Zinkernagel, V., Gathuru, E.M., Achwanya, O. and Lebeda, A. 2003. Induction of post-infection ethylene and its role in resistance of bea n (*Phaseolus vulgaris*) against *Colletotrichum lindemuthianum.* *Plant Protection Science* **39**: 79-87.

*Opio, F., Mugagga., Mawejje, D. and Nkalubo, S. 2006. *Progress Report on Bean Anthracnose Research in Uganda*.MAURIK Bullitin. (cf: *African Crop Science Society***8**: 869-874).

Opio, F., Ugen, M.A., Kayamanywa, S., David, S. and Mugisha-Mutetikka, M. 2001. Beans. pp. 112-120. **In**: *Agriculture in Uganda.Vol. II Crops*. (Eds. J. K. Mukiibi). NARO. Kampala, Uganda.

Opio, F., Mugagga, I. and Mawejje, D. 2003. Studies on transmission of *Colletotrichum lindemuthianum* on common beans in Uganda. *African Crop Science Conference Proceedings* **6**: 385-389.

Pachico, D. 1993. The demand for bean technology. pp. 60-73 **In:** *Trends in CIAT Commodities* (Ed. G. Henry). Working document No. 128. CIAT California, Colombia.

Padder, B.A., Sharma, P.N. and Sharma, O.P. 2009.Virulence and RAPD data-A tool to study the evolutionary trends of *Colletotrichum lindemuthianum* virulences in north western Himalayan region.*Archives ofPhytopathology and Plant Protection* **42**: 610-617.

Padder, B.A., Sharma, P.N. and Sharma, O.P. 2010.Distribution of *Colletotrichum lindemuthianum* race flora and its implication in deploym ent of resistant sour-ces in Himachal Pradesh.*Research Journal of Agricultural Sciences***1**: 1-6.

Panse, V.G. and Sukhatme, P.V. 1985. *Statistical Methods for Agricultural Workers.* Indian Council of Agricultural Research, New Delhi, India, pp. 347.

Pastor-Corrales, M.A., Otoya, M.M., Molina, A. and Singh, S.P. 1995. Resistance to *Colletotrichum lindemuthianum* isolates from Middle America and Andean South America in different common bean races. *Plant Disease***79**: 63-67.

Pastor-Corrales, M. A. and Tu, J. C. 1989. *Anthracnose* PP. 77-104 **In**: *Bean Production Problems in Tropics* (Eds. H.F. Schwatz and M.A. Pastor Corrales. Cent-ro internacional de Agricultura Tropical (CIAT), California, Colombia.

Pathania, A., Sharma, P.N., Sharma, O.P., Chahota, R.K., Bilal, A. and Sharma, P. 2006. Evaluation of resistance sources and inheritance of resistance in ki dney bean to Indian virulences of *Colletotrichum lindemuthianum***.** *Euphy tica* **149**: 97-103.

Popelka, J., Carlos, N. T. and Higgins, T. J.V. 2004. Gene technology for grain legumes: can it contribute to the food challenge in developing countries. *Plant Science* **167**: 195-206.

Prusky, D. freeman, S. and Dickman, M. 2000. *Colletotrichum: Host Specificity, Pathology and Host-Pathogen Interaction.* American Phytopathological Society, St. Paul. Minnesota, USA, pp. 400.

Qandah, I. S. and Al-Momany, A. 2003. The Occurrence and seed transmission on bean anthracnose caused by *Colletotrichum lindemuthianum* in Jordan. , *International Refereed Research Journal-Dirasat* **30**: 205-210.

Rajappan, K., Ramaraj, B. and Nirmal, J.S. B. 2001. Anthracnose of Beans. The Hindu Indiaserver.com http// www.hinduonnet.com/ the hindu/2001/01/0 4/stor- ies/08040028.htm.

Rajesha, G., Mantur, S.G., Ravishankar, M., Shadakshari, T.V. and Boranayaka, M.B. 2010. Screening of Dolichos bean (*Dolichos lablab* L.) genotypes for resistance to anthracnose disease caused by *Colletotrichum lindemuthi an-um.* *International Journal of Plant Protection* **3**: 135-136.

Rands, R.D. and Brotherton, W. 1995. Bean varietal tests for disease resistance. *Journal of Agricultural Research* **2**: 101-104.

Ravi, S., Doraiswamy, Sabitha., Valluvaparadisan, V. and Jeyalakshmi, C. 2000. Impact of seed born *Colletotrichum lindemuthianum* on French bean seed quality. *Acta Phytopathologica et Entomologica Hungarica* **34**:283-291.

Reid, W. D.1945. Resistance of beans to halo blight and anthracnose and occurrence of bean mosaic and bean weevil. *N.Z. J. Sci. Tech.* pp. 331-335.

Sanjaykumar and Srivastava, S. N. 1983, Taxonomy of *Colletotrichum corde* causing anthracnose disease on tropical fruits in India. *Indian Journal of Mycology and Plant Pathology* **13** : 7.

Sardana, V., Dhingrae, K K., Gill, M. S. and Singh, I.J. 2000.Production technology of French beans (*Phaseolus vulgaris* L.). *Agriculture Review***21**:141-154.

Schwartz, H. F. 1994. Anthracnose.pp.16-17. **In**:*Compendium of Bean Diseases.*American Phytopathological Society, St. Paul, Minnesota, USA.

Schwartz, H.F. 1989.Additional fungal pathogens. pp. 231-259. **In**: *Bean Production Problems in the Tropics*(Eds. M.A. Pastor-Corrales and H.F. Schwartz). Centro International de Agricultura Tropical (CIAT). California, Colombia.

Schwartz, H.F., Pastor-Corrales, M.A. and Singh, S.P. 1982. New sources of resistance to anthracnose and angular leaf spot of beans *(Phaseolus vulgaris*). *Euphytica* **31**: 741-754.

Shao, F.M. and Teri, J.M. 1985. Yield losses in *Phaseolus* beans induced by anthracnose in Tanzania. *Tropical Pest Management***31**: 60-62.

Sharma, P.N. and Sugha, S.K. 1995. Management of bean anthracnose through chemicals.*Indian Phytopathology***48**: 304-307.

Sharma, P.N., Patial, S., Sharma, O.P., and Kapil, R. 2005. Yield loss assessment in kidney beans due to bean anthracnose (*Colletotrichum lundemuthianu m*) under sub-temperate conditions of Himachal Pradesh. *Indian Journal of Mycology and Plant Pathology* **58**: 373.

Sharma, P.N., Sharma, O.P. Padder, B.A. and Kapil, R. 2008. Yield loss assessment in kindney bean due to bean anthracnose (*Colletotrichum lundemuthianum*) under sub temperate conditions of nort h western Himalayas. *Indian Phytopa-thology***61**: 323-330.

Sharma, P.N., Sharma, O.P. and Tyagi, P.D. 1994. Status and distribution of bean anthracnose in Himachal Pradesh. *Himachal Pradesh Journal of Agriculture Research***20**: 91-96.

Sharma, P.N., Sharma, O.P., Sharma, P. and Pathania, A. 2003.RAPD analysis of diverse isolates of *Colletotrichum lindemuthianum* infecting kidney bean in Himachal Pradesh.pp.1-26 **In**: *Symposium onChallenging Problems in Horticultural and Forest Pathology,*Solan, 14-15 November.

Sharma, P.N., Kumar, A., Sharma, O.P., Sud, D. and Tyagi, P.D. 1999. Pathogeni c variability in *Colletotrichum lindemuthianum* and evaluation of resistan ce in *Phaseolus vulgaris* in the North-Western Himalayan region ofIndia. *Journal of Phytopathology***147**: 41-45.

Siddiqui, M.R., Mathur, S.B. and Neergaard, P. 1983. Longevity and pathogenicit y of *Colletotrichum* species in seed stored at 5^0C. *Seed Science and Techn ology* **11**: 353-361.

Silva, K. J. D., Souza, E. A. and Ishikawa, F. H. 2007. Characterization of *Colleto tri-chum lindemuthianum* isolates from the state of Minas Gerais, Brazil. *Journal of Phytopathology* **155**: 241-247.

Singh, R. S. 1994. *Plant Pathogens—The Fungi*. Oxford & IBH Publising Co., New Delhi, India, pp. 443.

Singh, R. S. 1999. *Diseases of Vegetable Crops.*Oxford & IBH Publishing Co., New Delhi, India, 406.

Sohi, H. S. and Rawal, R. D. 1984. Studies on the efficacy of various fungicides for the control of anthracnose of cowpea caused by *Colletotrichum lindemuthianum. Pesticides***18**: 33-34.

Tesfaye, B. M. 2003. *Biology and Control of Bean Anthracnose i*nEthiopia.Ph.D. Thesis submitted to the Faculty of Natural and Agricultu ral Sciences University of Free State, pp. 143.

Thakur, M. P. and Khare, M. N. 1989. Evaluation of mung bean varieties for resi-stance to anthracnose.*Indian Journal of Plant Protection***17**: 107-108. .

Thomas, G.J. and Sweetingham, M.W. 2003. Fungicide treatments reduce seed transmission and severity of lupin anthracnose caused by *Colletotrichum gloeosporioides.* *Australian Plant Pathology* **32**: 39-46.

Tochinai, Y. and Sawada, K.1952. Observation on the overwintering of thebean anthracnose fungus, *Colletotrichum lindemuthianum* Briosi *et* Cavara. Ho kkaido University, Faculty, Agriculture.Mem.**1**: 103-112.

Tu, J.C. 1986. A detached leaf technique for screening bean (*Phaseolus vulgaris* L.) *in vitro* against anthracnose (*Colletotrichum lindemuthianum*). *Canadian Journal of Plant Science* **66**: 805-809.

Tu, J.C. 1988. Control of bean anthracnose caused by the delta and lambda races of *Colletotrichum lindemuthianum* in Canada. *Plant Disease* **72**: 5-8.

Tu, J.C. 1992. *Colletotrichum lindemuthianum* on bean: population dynamics of the pathogen and breeding for resistance. pp.203-224. **In:** *Colletotrichum: Biology, Pathology and Control.* (Eds. J.A. Bailey and M. J. Jeger). CAB International,Wallingford, U.K.

Tu, J.C. 1983. Epidemiology of anthracnose caused by *Colletotrichum lindemuthian-um* on white bean *(Phaseolus vulgaris*) in southern Ontario: survival of the pathogen. *Plant Disease* **67**: 402-404.

Vashunavat, K. 2009. *Fundamentals of Seed Pathology*. Kalyani Publishers, New Delhi, India, pp. 260.

Vidigal-Filho, P.S., Goncalves-Vidigal, M.C., Kelly, J.D. and Kirk, W.W.2007. Sources of resistance to anthracnose in traditional common bean cultivars from Parana, Brazil. *Journal of Phytopathology* **155**: 108-113.

Wang, X. M. and Li, Y. L. 1990.Evaluation of resistance to anthracnose in French bean varieties grown for seed.*Crop Genetic Resource* **3**: 21-23.

Wheeler, B. E. J. 1975. *An Introduction to Plant Diseases*. John Wiley and Sons, London, UK,pp. 374.

Wijesekara, H. T. R. and Agarwal, D. K. 2006. Taxonomic studies of five species of genus *Colletotrichum*. *Indian Phytopathology* **59**:203-209.

Wortmann, C. S. and Allen, D.J. 1994. *African Bean Production Environments, their Characteristics and Constraints*.Network on BeanResearch in Africa.Occasional paper series No. 11, Dar-es-Salaam, Tanzania.

Wortmann, C. S. Krikby, R.A., Eluedu, C.A. and Allen, D.J. 1998.*Atlas of Common Beans* (*Phaseolus vulgaris* L.).Pan African Bean Research Alliance.CIAT.pp. 133.

Yesuf, M. and Sangchote, S. 2005. Seed transmission and epidemics of *Colletotrich-um lindemuthianum* in the major bean growing areas of Ethiopia.*Kasetsart Journal of National Science* **39**: 34-45.

Yesuf, M. and Sangchote, S. 2007. Survival and transmission of *Colletotrichum lindemuthianum* from naturally infected bean seeds to seedlings. *Tropical Science***47**: 96-103.

Zaumeyer, W.J. and Thomas, H.R.1957. *A Monographic Study of Beans Disease and Methods for their Control.* United States Department of Agriculture, Technical Bulletin 868.Washington, DC, USA, pp. 255.

*Original not seen.

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Certificate

Certified that all the corrections/amendments as suggested by External Examiner Dr. R. K. Verma, Principal Scientist (Plant Pathology), Central Institute of Temperate Horticulture, Rangreth Srinagar during viva-voce examination held on ………………… have been incorporated in the manuscript entitled **"Studies on Anthracnose of Beans (*Phaseolus vulgaris* L.) in Kashmir Valley"** submitted by **Mr. Jan Mohd Junaid (Regd. No. 2010-A-859-M).**

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