

**Status and Management of Angular Leaf Spot of Beans
(*Phaseolus vulgaris* L.) caused by *Phaeoisariopsis griseola*
(Sacc.) Ferraris**

Uzma Rashid
(2016-A-1114-M)



Thesis

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(Plant Pathology)
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“PARENTS”

Someone who holds us in arms as we enter this world

Someone who encourages all our efforts and appreciate us wholeheartedly

Someone who listens patiently to our never ending doubts

Some who’s eyes shine with pride and happiness at our every little achievement

Someone who helps us to smile instead shedding a tear

Someone who is our life-long friend

DEDICATE MY THESIS

**“To serve whom was my dream and
Dream of serving them remain forever”**

...My Beloved Parents...

And

...My brothers...

Rohid Rashid and Enayat Rashid...

Sher-e-Kashmir
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Sopore-193201

Certificate – I

This is to certify that the thesis entitled, “**Status and Management of Angular Leaf Spot of Beans (*Phaseolus vulgaris* L.) caused by *Phaeoisariopsis griseola* (Sacc.) Ferraris**” 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 Agriculture, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir** is a record of bonafide research work carried out by **Ms. Uzma Rashid (Regd. No. 2016-A-1114-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 any help or information received during the course of investigation has duly been acknowledged.

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Spot of Beans (*Phaseolus vulgaris* L.) caused
by *Phaeoisariopsis griseola* (Sacc.) Ferraris”**

ABSTRACT

Angular leaf spot of beans (*Phaseolus vulgaris* L.) is assuming the status of major disease in Kashmir. So the present study was conducted on status and management of angular leaf spot of beans in *kharif* season during 2017-18. An extensive survey conducted in three districts of Kashmir valley *viz.*, Budgam, Baramulla and Kulgam revealed that disease was prevalent in all the three districts with overall mean disease incidence of 42.44 and 27.62 per cent on leaves and pods, respectively and overall mean disease intensity of 25.30 and 13.46 per cent on leaves and pods, respectively. The maximum disease incidence on leaves (59.20%) and pods (38.76%) and intensity on leaves (30.94%) and pods (18.14%) was observed in district Baramulla and minimum disease incidence on leaves (30.40%) and pods (18.62%) and intensity on leaves (20.07%) and pods (9.63%) was observed in district Kulgam. In the earlier stages of disease development, typical angular light brown lesions were seen on trifoliate leaves with synnemata of pathogen emerging out from the abaxial surface of leaves. The lesions then enlarged to cover larger areas of leaves resulting in premature defoliation. Maximum disease development in the field was observed during first fortnight of August. The spots on leaves attained maximum size of 2 cm and spots on pods

attained the maximum size of 2.25 cm. Based on morpho-cultural characteristics, the pathogen associated with the disease was identified as *Phaeoisariopsis griseola* (Sacc.) Ferraris. On host the pathogen showed branched, septate and smooth hyphae, measuring 3.5-4.7 μm in diameter and olivaceous brown in colour. The conidiophores were synnematosus, filliform, septate which bear obclavate conidia. The average size of conidiophores was 3.5 x 135.5 μm and that of conidia 6.1 x 34.4 μm . On culture colonies formed by the pathogen were slow growing, initially light greyish and finally become black. The hyphae were irregularly branched, sub-hyaline, septate having diameter 3.1-4.0 μm . The conidiophores were synnematosus, septate and branched which bear cylindrical to obclavate conidia. The average size of conidiophores was 3.3 x 131 μm and that of conidia 4.2 x 30.8 μm . Among the various systemic fungicides tested *in vitro* Carbendazim 50 WP proved best followed by Tebuconazole 25 WP and Myclobutanil 10 WP showing 97.41 per cent, 88.14 per cent and 81.95 per cent mean inhibition of spore germination of *P. griseola*, respectively. Among non-systemic fungicides Mancozeb 75 WP proved best with 86.62 per cent mean inhibition of spore germination of pathogen. Disease management in the field with most effective fungicides evaluated *in vitro* showed that seed treatment with Carbendazim 50 WP @ 0.1 per cent followed by three foliar sprays at 15 days interval with Carbendazim 50 WP @ 0.05 per cent effectively managed the disease. Seed treatment along with foliar sprays with Carbendazim 50 WP gave lowest disease incidence and intensity of 9.48 per cent and 3.62 per cent, respectively on leaves and showed highest pod yield of 102.08 q ha⁻¹. While as, control plot without any seed treatment and any foliar spray showed highest disease incidence and intensity of 52.13 per cent and 26.22 per cent, respectively on leaves with lowest pod yield of 72.7 q ha⁻¹.

Key words: angular leaf spot, beans, fungicides, management, status.

Signature of Student

Signature of Major Advisor

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Research is an evolving concept. Any endeavor, in this regard is challenging as well as exhilarating. It implies the testing of our nerves. It brings to light our patience, vigour and dedication. Every result arrived at is a modest beginning for a higher goal and no work can be termed as a one-man show. It needs the close cooperation and guidance of experts in the field to achieve something worthwhile and substantial.

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(Uzma Rashid)

Place: Wadura, Sopore

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Chapter – 1

INTRODUCTION

French bean (*Phaseolus vulgaris* L.) is one of the most important leguminous vegetable crops, grown throughout the world for its green pods as well as dry beans (Sharma, 2011) having its origin in South Mexico and Central America (Vavilov, 1951). French bean (*Phaseolus vulgaris* L.) is the third most important food legume crop worldwide after soybean and peanut in production and it is the most utilized legume for direct consumption in the world (Mongi, 2016). It has gained increasing popularity due to its quality proteins and nutritional balance besides certain medicinal properties (Adikshita and Sharma, 2017). Beans are also a good source of protein, folic acid, dietary fibre and complex carbohydrates providing 15 per cent of the protein and 30 per cent of the caloric requirement to the world's population and represents 50 per cent of the grain legume consumed worldwide (McConnell *et al.*, 2010). In addition to being an important source of protein and carbohydrates, common bean also supplies essential vitamins and micronutrients such as Zn and Fe (Welch *et al.*, 2000). The vitamins and minerals in the crop lower cholesterol levels and have preventive and curative properties to terminal diseases such as cancer (Hangen and Bennink, 2003). Antiviral peptides have been isolated from several *Phaseolus* spp. that inhibit activities of reverse transcriptase, an enzyme for virus replication. This may help to slow down the onset of symptoms in patients infected with HIV (Wong *et al.*, 2006). The French bean portions that are cooked to provide the nutrients are green pods, mature soft seeds and dry grain. The importance of French bean and its nutritional benefits makes it an important legume in most parts of the world.

Global production and productivity of dry beans in 2016-17 was about 26.83 million tonnes and 0.91 t ha⁻¹, respectively, on an area of 29.39 million ha, while in India the production and productivity was 3.90 million tonnes and 0.41 t ha⁻¹, respectively, on an area of 9.47 million ha. Global production and

productivity of green beans in 2016-17 was 23.60 million tonnes and 15.15 t ha⁻¹, respectively, on an area of 1.56 million ha, with India having production and productivity of 0.66 million tonnes and 2.81 t ha⁻¹, respectively, on an area of 0.23 million ha (Anonymous, 2017). In India, the crop is grown mainly in the states of Maharashtra, Jammu and Kashmir, Himachal Pradesh, Uttar Pradesh, Tamil Nadu (Nilgiri Hills, Palani Hills), Kerala (Parts of Western Ghats), Karnataka (Chickmagalur Hills) and West Bengal (Darjeeling Hills). In Jammu and Kashmir State, beans are grown either as a sole crop or inter-cropped with maize (Sultan *et al.*, 2014). It is the premier green legume crop of Jammu and Kashmir where its cultivation is mainly confined to rainfed and *Karewa* areas covering an area of about 26.75 thousand ha with an annual production of 14.2 thousand metric tonnes. As vegetable crop, common bean is grown in Kashmir over an area of 2000 ha with an annual production of 400 metric tonnes (Bhat *et al.*, 2017).

Large number of biotic and abiotic stresses like diseases, insects, physiological disorders reduce the yield of bean crop. The main limiting factors of bean production are diseases like bean rust (*Uromyces appendiculatus*), powdery mildew (*Erysiphe diffusa*), halo blight (*Pseudomonas syringae*), anthracnose (*Colletotrichum lindemuthianum*), white mold (*Sclerotinia sclerotiorum*) and angular leaf spot (*Phaeoisariopsis griseola*) (Schwartz and Harveson, 2015). Angular leaf spot (ALS) caused by *Phaeoisariopsis griseola* (Sacc.) Ferraris, is one of the major constraints in bean cultivation (Guzman *et al.*, 1995). The disease is of major importance in tropical, subtropical and temperate regions with significant economic consequences. The incidence of the disease has increased in many bean growing areas and has become a serious problem (Schwartz *et al.*, 1981; Saettler, 1991; Liebenberg and Pretorius, 1997). According to one estimate, every 10 per cent increase in ALS severity result in 7.9 per cent yield loss (Stenglein *et al.*, 2003).

The disease is favoured by intermittent dry wet and warm cool weather (Correa-Victoria *et al.*, 1989). The infected host exhibit reduced photosynthetic rate due to abnormalities in form and function of chloroplasts of the diseased tissue followed by decline in photophosphorylation, photochemical reaction and carbon dioxide assimilation reducing physiological performance of the canopy (Daly, 1976; Bergamin-Filho *et al.*, 1997; Jesus-Junior *et al.*, 2001). The infected plants initially show angular shaped grey lesions on leaves (Cardona-Alvarez and Walker, 1956). The lesions coalesce, turn brown and cause yellowing of leaves. Lesion multiplication and extension on the foliage cause premature defoliations (Correa-Victoria *et al.*, 1989) that brings the inoculum under the crop canopy with a long period of survival. On the pods and stems, dark, sunken, oval to circular spots of varying size occur. The spots may join together. Seed becomes infected beneath the spots on the pods. The resultant seeds are shrivelled and discoloured with the pathogen being embedded within the seed that facilitates its transfer from one place to another and from season to season. Furthermore, the disease also affects the quality and marketability of seed across bean-producing areas of the world (Pastor-Corrales *et al.*, 1998). Angular leaf spot disease is spread within and among fields by wind-blown particles of infested soil, windblown and rain-splashed spores. However, the primary source of infection is considered to be infested seed (Cardona-Alvarez and Walker, 1956). Other important sources of *P. griseola* inoculum include crop debris, volunteer crops and off-season bean crops (Stenglein *et al.*, 2003). In the absence of living host, the pathogen has been reported to survive upto 10 months on host plant debris. The fungus has been reported to survive on seed upto 12 months (Sindhyan and Bose, 1979). Formation of stroma after destruction of host cells permit the pathogen to remain dormant in the infected plant tissue until favourable conditions prevail (Monda *et al.*, 2001). In addition to seed, water and air currents play an important role in dissemination of *P. griseola* (Liebenberg and Pretorius, 1997). The disease is favoured by moderate temperatures (16-32°C), rain or high humidity, alternating with dry times (Pastor-Corrales *et al.* 1998). Infection and sporulation occurs in a broad

temperature range, from 10 to 33 °C (Allorent and Savary, 2005). The wet periods allow spore germination, infection and production of spores while as dry periods allow spread of the spores in the wind. *P. griseola* spores germinate on the leaf surface after 3 days of moist conditions, enter the leaf through the stomata and grow intercellularly, limited by the leaf veins resulting in an angular lesion shape (Monda *et al.*, 2001; Willocquet *et al.*, 2004).

According to the Commonwealth Mycological Institute (CMI), the disease occurs in more than 60 countries (Sartorato, 2004) and under favourable environmental conditions yield losses may reach upto 80 per cent (Ponnappa *et al.*, 1976; Gupta and Shyam, 1988; Shukla and Sharma, 2009). The disease is also quite wide spread in Kashmir and takes considerable proportion of the produce every year (Kotwal, 1994). The disease affects foliage and pods throughout the growing season and is particularly destructive in areas where warm and moist conditions are accompanied by abundant inocula from infected plant residues and contaminated seed (Saettler, 1991). Considering the economic importance of beans in Kashmir valley and the yield losses incited by *Phaeoisariopsis griseola*, the present investigation was undertaken to devise disease management strategy through chemicals with the following objectives:

- To assess the status of the disease
- To study symptomatology and etiology of the disease
- To evaluate fungicides *in vitro* and *in vivo* against the pathogen

Chapter – 2

REVIEW OF LITERATURE

2.1 Disease status

The disease is widely distributed in tropical and sub-tropical countries where it affects pod quality and causes huge yield losses (Jara *et al.*, 2001). Saccardo (1878) first reported its occurrence from Italy. The disease is sporadic in distribution in the U.S.A (Saettler and Correa, 1988), Maryland (Weaver and Zaumeyer; 1956) and Pennsylvania (Cole, 1966). *Phaeoisariopsis griseola* has been found in more than 60 countries worldwide (Guzma'n *et al.*, 1995). The pathogen has been reported in Yugoslavia (Skoric, 1930), Australia (Noble, 1929), Austria (Bubak and Kabat, 1905), Brazil (Muller, 1934), Columbia (Orejuela, 1953), England (Deighton, 1953), Eritrea (Petri, 1930), Fiji (Morwood, 1955), Guatemala (Palm, 1932), Germany (Keissler, 1923), Holland (Gremmen, 1947), Ireland (Ryan, 1965), Kenya (McDonald, 1926), Mexico (Williams and Alfonso, 1955), Mozambique (De Carvalho, 1948), Netherlands (Schoevers, 1920), New Caledonia (Bugnicourt *et al.*, 1951), New Zealand (Hill, 1982), Nicaragua (Litzenberger and Stevenson, 1957), Palestine (Ryass, 1943), Peru (Garcia and Stevenson, 1944), Rhodesia (Hopkins, 1940), Senegal (Defrancq and Collingwood, 1983), Spain (Da Camara, 1936), Siberia (Abramoff, 1931), Tanganyika (Wallace, 1932), Tanzania (Hocking, 1967), Trinidad (Thorold, 1932), Turkey (Bremer *et al.*, 1948), the United States (Gardner and Mains, 1930), USSR (Jaczewski, 1912) and Venezuela (Diaz Pollanco and Renaud, 1966).

In India, the disease was first reported from Nilgiri Hills in South India by Srinivasan (1953). The disease was also recorded from Solan (Himachal Pradesh) by Sohi *et al.* (1963) and Bangalore (Karnataka) by Ponappa *et al.* (1976) and in Chaubattia (Uttar Pradesh) by Sindhan and Bose (1979). The disease was

recorded in Tadong (Sikkim) by Srivastava and Gupta (1994). The survey and surveillance studies conducted by Bashir (2005) in three districts of Kashmir revealed that disease incidence on pods during 2002 and 2003 cropping seasons was 48.06 and 60.06 per cent with intensities of 20.45 and 32.75 per cent, respectively. Similarly on leaves, the angular leaf spot disease incidence during 2002 and 2003 was 62.79 and 77.50 per cent with intensities of 29.06 and 38.78 per cent, respectively. The survey and surveillance studies conducted by Buruchara *et al.* (2007) in Kenya revealed the moderate (33%) to high (52.9%) disease severity of angular leaf spot of bean in the bean fields located at the altitude below 1200 m and 1600 to 2000 m.a.s.l., respectively, whereas they recorded considerably less disease severity in areas above 2000m. Pamela *et al.* (2014) reported that, the incidence and severity of angular leaf spot in five bean agro-ecologies within Uganda was between 40-99 per cent and 21-80 per cent, respectively and that of rust was between 37-69 per cent and 24-44 per cent, respectively. Kijana *et al.* (2017) in Eastern Republic of Congo, assessed severity and incidence of angular leaf-spot and other occurring diseases on common bean plants in farmers' fields. Angular leaf spot in these fields had an average severity index (PSI) of 49.9 per cent. The highest severity (PSI=59.7%) was observed in Kabare district, and the lowest in Uvira district (PSI=39.5%). Van Poeteren (1922), Noble (1929) and Petri (1930) have considered the disease to be of minor importance while cases involving relatively heavier losses have also been reported (Greene, 1949; Brock, 1951; Deighton, 1953). Weevers and Zaumeyer (1956) reported that infection of *Phaeoisariopsis griseola* on beans resulted in about 20 per cent reduction of pod yield. Yield losses have been reported to be 40-60 per cent in Columbia (Barros *et al.*, 1957). Cole (1966) besides Hagedorn and Wade (1974) estimated upto 50 per cent yield losses caused by the disease. Yield losses of about 30-40 per cent in Argentina (Fortugno, 1974) and 70 per cent in Brazil (Brenes *et al.*, 1983; Rava *et al.*, 1985; Sartorato and Rava, 1992) have been reported. According to an estimate by CIAT, seed yield losses in *P. vulgaris* were 81 kg ha⁻¹ due to angular leaf spot in Columbia. Losses to the extent of 80 per cent

in Mexico (Crispin *et al.*, 1976) and Columbia (Schwartz *et al.*, 1981) have been reported due to angular leaf spot on bean. In India, Ponappa *et al.* (1976) reported 80 per cent yield loss. Singh and Saini (1980) reported the total loss of about 40-70 per cent due to the diseased and marketable pod yield. In various commercially grown French bean varieties *viz.*, Lakshmi, SVM-1, Kentucky Wonder and Contender in Himachal Pradesh Gupta and Shyam (1988) observed that the loss ranged from 53.75 to 69.33 and 52.00 to 64.45 per cent in green pod and seed yield, respectively. Rava *et al.* (1985) and Sartorato and Rava (1992) observed that every 10 per cent increase in disease severity resulted in 7.88 per cent bean yield loss. Furthermore, by increasing the number of inoculations, disease severity can be enhanced, and this may lower yield. Cultivar Rosinha G-2 was the more susceptible cultivar, losing up to 45 per cent of yield due to angular leaf spot infection. Sengooba and Mukiibi (1986) found that angular leaf spot affected seed quality. Pericarps of infected seeds can lose up to 40 per cent colour. Seed decoloration means a reduction in quality standards and this results in economic losses for farmers. Bergamin-Filho *et al.* (1997) found no relationship between yield and the area under the disease progress curve (AUDPC), calculated by trapezoidal integration in which the number of assessments, disease severity and the interval between two consecutive assessments are included. They thought that the lack of relationship between yield and AUDPC in four trials, even when the data from each trial were analyzed individually, was probably due to three main reasons: intense defoliation caused by the pathogen, the lack of an estimate of defoliation in the disease assessment method and the indeterminate growth habit of the host. Carneiro *et al.* (1997) studied disease severity in bean cultivars Rosinha G-2 and Carioca Comun, aiming to determine the damage caused by angular leaf spot. They concluded that the severity of the disease at different time intervals of evaluation and AUDPC was not correlated with yield components.

2.2 Symptomatology and etiology of disease

2.2.1 Symptomology

The pathogen affects all the green parts of the plant *viz.*, leaves, petioles, stems and pods producing varied symptoms (Skoric, 1930; Zaumeyer and Thomas, 1957; Barros *et al.* 1958a; Hocking, 1967). Skoric (1930) reported polygonal spots on the leaves and greyish black spots on pods. Miles (1951) observed that *P. griseola* was confined exclusively to the foliage and in few instances on pods. In the field, the first symptoms of angular leaf spots appear during the early stages of plant growth on primary leaves. However, the disease does not become conspicuous until late flowering or early pod set (Barros *et al.*, 1958). Zaumeyer and Thomas (1957) described the presence of circular brown lesions up to 2 cm in diameter, and not delimited by veins and veinlets. However, Barros *et al.* (1958a) described the presence of typical angular lesions on leaves and large circular elongated spots on stems and petioles. The infected pods may contain poorly developed or entirely shrivelled seeds. The lesions on diseased plants are small (0.5 cm), irregular brown to grey spots lying in the angles of dichotomized branched leaf veins (Hocking, 1967). Hagedorn and Wade (1974) reported that symptoms are more evident on leaves 9 days after infection, when lesions are greyish turning to light brown and are assuming the typical angular shape. Sharma and Sohi (1978) described the typical symptoms as angular lesions of dark brown colour on leaves. On primary leaves, the lesions are round and larger than on trifoliate leaves and develop concentric rings (Allen, 1983). Monda *et al.* (2001) reported that initial disease symptoms on leaves as small grey lesions which were angular in shape. The lesions coalesced, turned brown and eventually caused the yellowing and premature defoliation of leaves. He further recorded appearance of lesions on pods and stems and observed the sporulation of the fungus in the form of dark grey to black synnemata on the lower surface of leaves. Polanco (1970) obtained isolates from the hilum and superficial integument of

certified and registered bean seeds. Infected pods may contain poorly developed or entirely shriveled seeds that may carry the fungus internally or on the surface. Saettler and Correa (1988) found that the presence of *P. griseola* in seed is directly correlated with the physical appearance of the seed as discoloration of the seed coat, seed malformation and/or presence of dark colour around hilum area. Seed become infected only when they are attached to the pod suture directly beneath a lesion (Pastor-Corrales *et al.*, 1998). Stenglein *et al.* (2003) found that leaf lesions were brown to grey, irregular to angular to circular and 0.5 to 1 cm in diameter and lesions on pods were oval to circular with reddish brown centers surrounded by darker brown borders.

Cardona-Alvarez and Walker (1956) found that fungus grow in the substomatal cavity, chloroplasts of the cells adjacent to the stomatal cavity stained red, contrasting with normal green chloroplasts. Conidia of *P. griseola* inoculated on leaves of susceptible beans, germinated 4 h after inoculation at either one, or both ends of the spore or at its sides. Germ tubes grow following the contours of epidermal cells on the leaf surface. Penetration occurs 24 h after inoculation through stomata (Cardona-Alvarez and Walker, 1956; Wagara *et al.*, 1999; Monda *et al.*, 2001), either by formation of an apressorium-like swelling over the stoma or without it (Monda *et al.*, 2001). Three days after infection, necrosis of the guard cells and adjoining mesophyll cells occur, and the chloroplasts show signs of disintegration. The damage to the host plasma and chloroplast membranes has been attributed to the action of a toxin produced by the pathogen (Monda *et al.*, 2001). After infection, in 6-10 days, the fungus colonizes most of the intercellular spaces of the palisade cells of the leaf, resulting in cell destruction. Hyphal growth was found to be intercellular by Cardona-Alvarez and Walker (1956) and Monda *et al.* (2001). Furthermore, Monda *et al.* (2001) found that *P. griseola* did not penetrate leaf veins, probably due to the lack of easily colonizable intercellular spaces. However, Wagara *et al.* (1999), reported intercellular but also intracellular colonization, as a result of observations made with a light

microscope. Once hyphal growth occur and the intercellular space is colonized by mycelia, the conidiophores emerge through the stomata (Monda *et al.*, 2001). Synnemata are formed in association with lesions and conidia are formed at the tip of the conidiophores, generally on the leaf undersurfaces (Cardona-Alvarez and Walker, 1956; Hagedorn and Wade, 1974; Schwartz and Ga'ivez, 1980; Monda *et al.*, 2001).

2.2.2 Etiology

Angular leaf spot is one of many destructive foliar diseases of common bean. The causal organism of angular leaf spot of bean is the fungus presently known as *Phaeoisariopsis griseola* (Sacc.) Ferraris. The fungus was first reported by Saccardo (1878) from Italy, who named it *Isariopsis griseola*. Ellis (1881) described a fungus in the United States under the name *Graphium laxum* with characteristics similar to *Isariopsis griseola* Sacc. In 1886, Saccardo considered *Graphium laxa* to be synonymous to *Isariopsis griseola* and renamed the species *Isariopsis laxa*. Later, Ferraris (1909) described the same fungus and used the name *Phaeoisariopsis griseola* (Sacc.) Ferraris to accommodate the species *Isariopsis griseola* Sacc. In 1971, Ellis confirmed this name. Zaumeyer and Thomas (1957) placed *P. griseola* (Sacc.) Ferraris in the family Dematiaceae among the imperfect fungi. *P. griseola* is an imperfect fungus in the order Moniliales, family Stilbaceae (Barnett and Hunter, 1972), order Stilbellales, class Hyphomycetes (Sutton, 1980). Recently *P. griseola* has been placed in kingdom Fungi, phylum Ascomycota, subphylum Pezizomycotina, class Dothideomycetes, subclass Dothideomycetidae, order Capnodiales, family Mycosphaerellaceae, genus *Phaeoisariopsis* (Anonymous, 2018). *P. griseola* develops conidiophores that are macronematous, mononematous, and cespitose or synnematous. Conidiophores are produced in groups (synnemata) of 8 to 40 which are loosely fused along most of their length, splaying out only at the apex (Miles, 1951). Separation of synnemata is nearly at maturity (Chupp, 1925; Hocking, 1967; Ellis,

1971). Synnemata arising from globose or pyriform stromata formed within the mesophyll are brown-dark and measure upto 48 μm in diameter and 65 μm in height (Sindhan and Bose, 1972). The individual conidiophores terminate in slightly swollen integrated conidiogenous cells, which form successive conidia at the tip by sympodial growth, sometimes geniculate, cicatrized, pale grey, cylindrical to spindle shape (Lianos, 1957 and Karanja *et al.*, 1994) and tapering and rounded at one or both ends (Sindhan and Bose, 1972). They are 30-80 μm long and 3.0-8.8 μm wide. The conidia are 3-6 septate but can range from none to seven (Benloch, 1944; Hocking, 1967 and Ellis, 1971). Karanja *et al.* (1994) isolated the fungus from cultivar Rosecoco-GLP-2 in Nairobi, Kenya and showed that some conidia were Y-shaped. The number of septations varied from 1 to 11 and the size ranged from 17 to 95 μm in length to 7 to 24 μm in width. Stenglein *et al.* (2003) observed that conidia *in vivo* were curved cylindrical to obclavate with one to five septa and measured 25 to 60 \times 3.5 to 7 μm and conidiophores were 100 to 250 μm high and clustered together to form synnemata measuring 20 to 50 μm in dia. Bashir (2005) while studying the ex-situ morphology of different isolates of *P.griseola* found that a maximum average radial growth of 31.8 mm was achieved by isolate Pg-7. The stroma was Prosenchymatous, brown or olivaceous brown, the conidiophores were macronematous, mononematous and caespitose or synnematos, and the conidia were obclavate or cylindrical, subhyaline, pale olive, olivaceous brown or light brown and smooth. The maximum average conidial length of 38.5 μm was recorded for isolate Pg-9. The maximum average breadth of 5.5 μm was exhibited by isolate Pg-7. Adikshita (2012) observed that the Conidia of *Phaeoisariopsis griseola in vivo* were curved cylindrical to obclavate with one to four septa and measured 7-60 $\mu\text{m} \times$ 3.8- 30 μm . The conidiophores were 100 to 250 μm high and clustered together to form synnemata measuring 20 to 50 μm in diameter. The microscopic examination of *in vitro* culture of the test pathogen revealed that the conidia were olive to pale in colour, 2 or more celled and cylindrical to obclavate in shape. The size of conidia ranged between 5.80 -33.38 $\mu\text{m} \times$ 6.29-37.74 μm . Deighton (1990) remarked that

the arrangement of the conidiophores in synnemata is not a good character for identification, because it can also be seen in other hyphomycetus genera. In addition, the degree of darkening of the conidial scars is variable in members of other groups such as the “Cercospora-complex”. The most appropriate classification character, in combination with the synnematal conidiophores, seems to be the displacement of the old conidial scars so that they lie flat against the sides of the conidiogenous cells. Walker and White (1991) studied the ontogeny of conidia and the sympodial proliferation of the conidiogenous cell. Conidia are formed holoblastically at the apex of the conidiogenous cell. When a conidium is shed, the conidiogenous cell grows on around the conidial scar, tearing the outer wall of the cell as it grows through it. Proliferation thus is enteroblastic and sympodial. The broken area of the wall, through which the conidiogenous cell proliferates, can be seen on the conidiophores as a thin jagged tear line, encircling it just above the spore scar. This process is repeated every time proliferation occurs, so that a series of conidial scars and wall tear lines develops on the elongating conidiogenous cell. Miles (1951) and Monda *et al.* (2001) observed that young hyphae are nonseptate in the early stages of fungal growth and that there was no septum delimiting the appressorium-like structures from the germ tubes and/or young hyphae.

2.2.3 Pathogenicity test

The pathogenicity studies conducted by Srinivasan (1953) reported that typical symptom development occurred after 12 days of inoculation with conidial suspension. However, Olave (1958) observed symptoms after 8 days although characteristic symptoms developed after 12 days. Cardona – Alvarez and Walker (1956) showed that post- inoculation exposure of bean plants in a moist chamber for 8 h favoured infection and sporulation. They also observed that conidial production occurred only in moist condition within 24 h of symptom production and a period of more than 72 h was required for heavy sporulation. Stenglein and

Balatti (2006) conducted the pathogenicity test by spray inoculating the conidial suspension (2×10^4 conidia ml^{-1}) on to the upper and lower surfaces of first trifoliate leaves of bean plants, 18 days after planting.

2.3 Evaluation of fungicides

Wallace (1952) and Oxenham (1957) used bordeaux mixture and sulphur dust and found them effective against angular leaf spot of beans. Cardenas (1958) reported persistence of protective action of Fermate, Parzate, Fuclasin, Manzate, Orthocide 50, Oxicol Ultra, Shell Copper fungicide and thiolate upto 9 days against angular leaf spot disease. Barros *et al.* (1958b) found that spraying 2.5 g l^{-1} of Zineb 5-6 times was effective in controlling angular leaf spot. Mauritius and Malawi, found that 2.5 g l^{-1} of Zineb could reduce the size of the spots considerably (Anonymous, 1959). Results based on laboratory work showed that 1 per cent Bordeaux mixture, 1 per cent lime sulphur, 0.5 per cent sukol, 0.5 per cent kumulus (micronised wettable sulphur), checked conidia of *P. griseola* from germinating and these could be useful in reducing the incidence of the disease (Milatovic, 1959). Milatovic (1959) explained that it was necessary to start spraying early and if seeds were dusted with cerasan (Ethylmercury chlorate) germisan, agrosan (phenylmercury acetate) and copper carbamate at 0.1 - 0.2 per cent, angular leaf spot disease would be reduced but this would affect germination too. The *in vivo* evaluation of fungicides against angular leaf spot conducted by Milatovic (1958) found that germisan (0.3%) limited the angular leaf spot disease effectively. Sohi and Sharma (1969a) reported that seed treatment with Cerasan dry or steeping of seeds in mercuric chloride solution (1%) for 30 minutes gave 100 per cent control against angular leaf spot. Foliar application of fungicides *viz.*, Thiovit, Dithane M-45, Zincocap, wettable sulphur, Bordeaux mixture (Sohi and Sharma, 1969b) have been reported effective to reduce disease intensity and increase yield. Foliar application of fungicides *viz.*, Derosal, SaproI (Giroto, 1976), triforine (Fortugno, 1977) have been reported effective to reduce angular

leaf spot disease intensity and increase yield. Fortugno (1978) in Columbia tried chemicals which include benzimidazole 60 per cent at 50 g 100 l⁻¹, benomyl 50 per cent at 40 g 100l⁻¹ or triforine 20 per cent at 200 ml 100l⁻¹ spraying 3 times. Gonza'lez *et al.* (1977) in Costa Rica, successfully controlled angular leaf spot on cultivar Mexico 80 and Turrialba 4 with Mancozeb, Captafol, and Metiram, 20, 30, and 40 days after sowing. Schwartz and Ga'lvez (1980) reported that several fungicides such as Benomyl, Captafol, Ferbam-Sulfur, Mancozeb, Maneb, Metiram, Copper oxychloride, Zineb, Ziram, and Bordeaux Broth, control angular leaf spot. Issa *et al.* (1982) reported that Maneb and Zn (2 kg ha⁻¹) resulted in reasonable control of angular leaf spot, which compared to the untreated control plants resulted in a yield of 75.4 per cent. Gaur and Shaikh (1983) under *in vivo* conditions evaluated six fungicides against tikka disease and rust diseases of groundnut and reported the Carbendazim (0.1%) as most effective in checking the tikka/leaf spot of groundnut. Rajagopal and Vidhyasekaran (1983) under *in vivo* conditions studied effect of fungicides on intensity of tikka disease, quality of groundnut kernel oil and oil content. They reported that Carbendazim followed by Chlorothalonil, Mancozeb and Zineb effectively controlled the disease. Combi fungicide sprays benomyl + captafol (Issa *et al.*, 1982) and chlorothalonil + copper oxychloride (Issa *et al.*, 1983) have also been found effective against angular leaf spot disease. Pastor-Corrales *et al.* (1983) obtained the best control of angular leaf spot disease and a 41 per cent yield increase over to the control plants, with Biloxazol, and a yield increment of 33 per cent with Tribasic Copper Sulphate on cultivar G 2858 when they were applied 26, 40 and 54 days after sowing. Efficacy of Benomyl, Carbendazim, Vitavax and Agrosan GN as seed dressers to reduce angular leaf spot disease intensity and increase yield have also been reported (Sindhani, 1984; Trutmann *et al.*, 1992; Vincent, 1947). Gupta (1985) under *in vivo* conditions evaluated seven fungicides against tikka of groundnut in Manipur and reported that the fungicides Bavistin followed by Tilt and Dithane M-45 significantly reduced the disease intensity, increased the number of pod/plant and gave significantly higher pod yield. Vyas *et al.* (1986)

under *in vivo* conditions evaluated five fungicides alone and in combination against leaf spots and rust diseases of groundnut. They reported that all test fungicides significantly reduced the intensity of both diseases. However, Carbendazim, alone (@ 0.15%) and Carbendazim + Mancozeb (@ 0.075+0.15%) recorded significantly reduced leaf spot intensity (1.5-2%). Shekhawat *et al.* (1987) recorded that two sprays of Mancozeb (0.2%) at 35 and 70 days after germination and 1 spray of Carbendazim (0.025%) at 50 days was the most suitable and most economic for controlling *C. arachidicola*, *C. personatum* and *P. arachidis* in the North Saurashtra Agroclimatic Zone. While evaluating the efficacy of different fungicidal formulations against *Phaeoisariopsis griseola* under glasshouse conditions Rodrigues *et al.* (1987) found that applications of Benomyl, Thiophanate methyl + Thiram and Triforine were effective when applied upto 15 days prior to inoculation with the fungus and had a curative effect when applied after inoculation under glass house conditions. Field trials conducted in Tanzania with Chlorothalonil (as Daconil or Bravo 500), Triphenylstannane (as Brestan), Carbendazim (as Bavistin 50 WP) or Mancozeb (as Dithane M 45) reduced the intensity of early and late leaf spots (*Cercospora arachidicola* [*Mycosphaerella arachidis*] and *Phaeoisariopsis personata* [*M berkeleyi*], respectively) and rust (*Puccinia arachidis*) on groundnut, and increased seed yield compared with untreated control, with the most effective control provided by chlorothalonil as Daconil (Raya, 1989). Foliar application of fungicides *viz.*, mancozeb (De-Castro *et al.*, 1991 and Vierra *et al.*, 1998), Carbendazim, Triademorph, Fernacol, Ferbam (Sindhan, 1984), Triphenyltin acetate and Triphenyltin hydroxide (De Castro *et al.*, 1991) have been reported effective to reduce disease intensity of angular leaf spot and increase yield. Oliveira *et al.* (1992) reported stannic Triphenyl (Fentin) acetate, Bitertanol and Tebuconazole as most effective as compared to Bavistin. Bhardwaj and Thakur (1992) reported that seed dressing with Carbendazim (0.25%) followed by three foliar sprays (0.1%) at 15 days interval as effective to manage the angular leaf spot disease. However, in subsequent studies Bhardwaj *et al.* (1994) reported that

fungicidal treatments coupled with careful selection of varieties and sowing dates proved highly effective to reduce disease severity and increased the yield of the crop. Labrinos and Nutter (1993) reported that Chlorothalonil (a protectant fungicide) and Tebuconazole (a systemic, sterol-inhibiting fungicide) fungicides greatly reduced spore germination of *C. personatum* when applied to groundnut leaf surfaces prior to inoculation. However, in addition to its action as a protectant, the systemic activity of tebuconazole also reduced infection frequency on nontreated leaves. Chlorothalonil had no effect on infection frequency after *M. berkeleyi* germ tubes entered the groundnut leaves. Srivastava and Gupta (1994) reported Captan, Thiophanate methyl, Carbendazim, Ziram and Captafol to be approximately equally superior to Mancozeb, Copper oxychloride and Triademorph of which applications of Captan and Zineb were the most economically viable. Gangopadhaya *et al.* (1996) under *in vivo* conditions evaluated efficacy of fungicides *Viz.* Carbendazim, Thiophenate methyle, Mancozeb and Foltaf against leaf spot of groundnut as seed treatment (@ 0.2% each) and foliar sprays (three) at recommended dosages. They reported the maximum seed germination (85.41%) in Foltaf seed treatment followed by Carbendazim (84.22%) and Thiophenate methyle (84.18%). The intensity of leaf spot was significantly reduced in all the fungicidal treatments. However, fungicide Carbendazim recorded least disease intensity (7.55-9.33%) followed by Thiophenate methyle (11.11%). Significantly highest pod yield (27.9 q ha⁻¹) was recorded with the fungicides Carbendazim and Thiophenate methyle followed by Mancozeb (23.5 q ha⁻¹). Gupta and Shyam (1996) while evaluating the *in vitro* efficacy of different fungicides against *P. griseola* reported the significantly high inhibitory effect of Bavistin and Punch at 50 ppm. Naidu and Rao (1997) reported that five sprays starting from 30 DAS with 12 days interval using the fungicides Carbendazim (0.05%) + Mancozeb (0.2%) were found to reduce the severity of rust and late leaf spot of groundnut. They also reported 57 per cent increase in dry pod yield, increased 100 kernel weight and shelling percentage. Srinivas *et al.* (1997) under *in vivo* conditions evaluated botanicals and chemicals against tikka

leaf spot disease of groundnut alone and in combination. They reported that Mancozeb (0.25%) + Carbendazim (0.1%) sprayed at 50 DAS followed by 5 per cent of leaf extract of *Calotropis procera* at 70 DAS caused significant reduction in the leaf spot severity with increased pod yield. Mathew *et al.* (1998) under *in vitro* and *in vivo* conditions evaluated 10 fungicides (Propineb, Myclobutanil, Chlorothalonil, Bitertanol, Carbendazim, Hexaconazole, Flusilazole, Penconazole, Difenconazole and Mancozeb) against angular leaf spot (*Phaeoisariopsis griseola*) of French bean. They reported that all the fungicides significantly reduced the disease severity and increased the seed yield over untreated control. However, fungicides Carbendazim (0.05%) and Hexaconazole (0.05%) were reported highly effective in reducing severity of the disease to 13.07 and 15.41% respectively, compared to untreated control (19.96%). They also studied *in vitro* effect of these fungicides on spores/ conidial germination of the test fungus and reported that fungicides Carbendazim caused maximum inhibition of spore germination (80.12%) followed by Hexaconazole (64.83%), Flusilazole (62.30%) and Mancozeb (53.39%). A single spray of Carbendazim + Mancozeb applied once in different treatments and spray timings varied from 30 DAS to 80 DAS at 10 days intervals reduced the per cent disease index in all treatments. The sprays conducted up to 50 DAS produced significantly more yield than later applications (Chandra *et al.*, 1998). Canteri *et al.* (1998a) studied the effect of fungicides in *in vitro* cultures of the fungus. They concluded that Difenoconazole (0.12 kg 100⁻¹ liters) and Tebuconazole (0.3 kg 100⁻¹ liters) controlled *P. griseola*, with an efficiency of 93 per cent and 74 per cent of control, respectively, when they were sprayed 4 days after inoculation (DAI). Canteri *et al.* (1998b) reported that spraying with Fentin hydroxide 0.1 per cent plus 0.04 per cent Tebuconazole beginning 30 days after emergence (DAE) resulted in 65.6 per cent control of angular leaf spot. Sartorato and Rava (1998) controlled angular leaf spot on dry bean cultivar Carioca in Brazil by applying the fungicide uniformly through a central pivot, a procedure that is known as fungigation (Johnson *et al.*, 1986). They concluded that the method was efficient in controlling the disease, and those

significant differences in yield were obtained only at the experimental place located at Unai', where Tebuconazole (0.25 kg ha^{-1}) was superior. Other studies, with bean cultivar Carioca, revealed that Benomyl (0.25 kg ha^{-1}), a fungicide extensively used in the control of *P. griseola*, proved to be more effective in controlling the disease in association with Mancozeb (1.6 kg ha^{-1}), except for the experiment carried out in Acreu' na (Sartorato and Rava, 1999). In the Northern region of Ri' o Grande do Sul cultivated with bean cultivar Carioca, angular leaf spot control was higher than 70 per cent for those treatments using $0.8\text{-}0.12 \text{ kg ha}^{-1}$ Azoxystrobin and Chlorothalonil 0.15 kg ha^{-1} , Thiophanate methyl and Chlorothalonil (0.7 kg ha^{-1} and 0.75 kg ha^{-1}), Propiconazol 0.125 kg ha^{-1} , Tebucanazol 0.2 kg ha^{-1} , and Triphenyltin hydroxide 0.2 and 0.3 kg ha^{-1} (Picinini and Ferna' ndez, 2000). Noriega Cantu *et al.* (2000) studied the fungicidal management of late leaf spot and rust of groundnut in North Guerrero (Maxico) during spring summer 1997-98. They reported best control of both the foliar diseases with Carbendazim, Zineb and Chlorothalonil followed by Copper-oxy chloride. Sawant (2000) recorded that all the treatments (Mancozeb and Carbendazim) were effective in controlling the tikka disease of groundnut but spraying with Mancozeb at initiation of the disease in the field, Carbendazim on 10 days after mancozeb spray and again spray of Mancozeb on 10 days after Carbendazim spray recorded lowest per cent disease index and highest pod yield. The efficacy of Carbendazim, Mancozeb and Copper oxychloride in controlling early leaf spot (*C. arachidicola*) and late leaf spot (*P. personata*) of groundnut was investigated in India. All of the treatments controlled the diseases but Carbendazim + Mancozeb gave the best control (Joshi *et al.*, 2000). Ploper *et al.* (2002) reported average yield increases between 16.8 and 28.1 per cent for single fungicide applications (35 days after sowing), and 29.0 and 35.7 per cent for double fungicide applications (35 and 50 days after sowing). The field studies conducted by Bhat (2002) for the evaluation of systemic fungicides against angular leaf spot of bean revealed the high efficacy of Benomyl (0.1%) and Carbendazim (0.1%) sprayed at 10 days interval. Jesus-Junior *et al.* (2004)

indicated that the application of fungicide in combination with Mo substantially reduced the angular leaf spot disease and increased the cumulative leaf surface area. Tiwari *et al.* (2004), under *in vivo* conditions evaluated seven fungicides alone and in combination against leaf spots and rust of groundnut and reported that all the fungicides tested significantly reduced severity of both the diseases over unsprayed control. However, Hexaconazole followed by Propiconazole and Mancozeb were recorded most effective against leaf spot with minimum disease intensity of 1.66, 6.00 and 12.50 per cent, respectively and higher pod yield and 100 kernel weight. The cost: benefit ratio (CBR) was maximum with Hexaconazole followed by Mancozeb. Sexena and Tripathi (2006) under *in vivo* conditions evaluated 10 systemic fungicides against cercospora leaf spot of mungbean and reported that all the fungicides tested significantly reduced severity of both the diseases and gave increased seed yield over untreated control and increased grain yield. However, fungicides, Carbendazim followed by Chlorothalonil, Hexaconazole and Difenoconazole significantly reduced the disease severity and increased the grain yield. Besler *et al.* (2006) found that four times spraying with Tebuconazole at 0.23 kg ai ha⁻¹ or twice with Azoxystrobin at 0.34 kg ai ha⁻¹ reduced early leaf spot severity in the single and twin-row patterns when compared to nontreated plots. Tebuconazole and Azoxystrobin increased yield compared to nontreated control. No significant differences in yield occurred between fungicide treatments. The twin-row pattern showed a yield increase compared to the single row pattern when averaged across cultivar and fungicide treatments. Nutsugah *et al.* (2007) found that Tebuconazole was the most effective fungicide in reducing early and late leaf spot severity, resulting in significantly higher biomass and pod yields compared to most of the treatments. Ali *et al.* (2008) evaluated the efficacy of fungicides as foliar spray in producing healthy seeds of mungbean. The treatments were application of Bavistin (Carbendazim) @ 2.0 g litre⁻¹, Rovral 50 WP (Iprodione) @ 2.0 g litre⁻¹, Dithane M-45 (Mancozeb) @ 2.5 g litre⁻¹, Tilt 25 EC (Propiconazole) @ 2.0 ml litre⁻¹, Ridomil (Metalaxyl + Mancozeb) @ 2.0 g litre⁻¹, Cumulax- DF (Sulphar) @ 2.0 g

litre⁻¹, Vitavax-200 (Carboxin) @ 2.5 g litre⁻¹ plus seed treatment @ 2.5 g kg⁻¹ seed and untreated (control). Results showed that the fungicides reduced the incidence and percent disease index of *Cercospora* leaf spot. But foliar application of Bavistin 50 WP performed better than others. Highest seed yield (1324 kg ha⁻¹) was obtained in Bavistin 50 WP treated plots. Shukla and Sharma (2009) while evaluating the efficacy of seven fungicides including Carbendazim, Hexaconazole, Difenconazole, Propiconazole, Mancozeb, Propineb, and Chlorothalonil against angular leaf spot of bean under field condition found that the four sprays of Carbendazim (0.1 %) at 10 days interval restricted the disease significantly. Mesta *et al.* (2009) evaluated new molecules of fungicides and plant extracts through inhibition of spore germination test and poison food test against *Alternaria helianthi*, causal agent of Alternaria blight of sunflower. Among non-systemic fungicides, Isoprodione + Carbendazim (a combi product) was most effective which inhibited 58.39 per cent spore germination and 65.18 per cent mycelial growth whereas, systemic fungicides, Hexaconazole (63.12 % and 72.87 %, respectively) and Propiconazole (61.80 and 76.53 %) were most effective. Neem leaf extract with 38.49 per cent inhibition of spore germination and 43.90 per cent inhibition of mycelial growth was found more effective than all other plant extracts. Lemessa *et al.* (2011) also advocated the use of periodic spray of Benomyl at 7 day intervals for the successful control of angular leaf spot on beans. Taskeen-Un-Nisa *et al.* (2011) evaluated Carbendazim, Hexaconazol, Bitertanol, Myclobutanil, Mancozeb, Captan and Zineb and extracts of *Allium sativum*, *Allium cepa* and *Mentha arvensis* for their effect on the inhibition of mycelial growth and spore germination of *Fusarium oxysporum*. Maximum inhibition in mycelial growth was observed in the Hexaconazole at 1000 ppm followed by other fungicides at the same concentration. In case of botanicals, inhibition in spore germination was highest at concentration 'S'. It was followed by S/2, S/10, and S/100 concentrations of plant extracts as compared to control which showed least inhibition in spore germination. Adikshiti and Kansak (2016) evaluated the relative efficacies of different fungicides including six systemic and

nonsystemic fungicides and four combi-productss against *P. griseola* under *in vitro* and *in vivo* conditions. The *in vitro* evaluation studies indicated that Fenamidone (10%) + Mancozeb (50%) (Sectin WG), Pyraclostrobin (5%) + Metiram (55%) (Cabrio Top WG), Azoxystrobin (20%) + Difenconazole (12.5%) (Amistar Top SC), Trifloxystrobin (25%) + Tebuconazole (50%) (Nativo WG) and Azoxystrobin (25%) (Amistar SC) salone were potent growth inhibitors of *P. griseola*. Field evaluation for two consecutive years (2011 and 2012) revealed that four periodic sprays of Sectin at 15 days interval were most effective in controlling the disease and enhancing the green pod yield. Sunkad (2012) under *in vivo* conditions evaluated three formulations of Carbendazim+Mancozeb 75WP, a combi fungicide molecule, two systemic (Hexaconazole and Carbendazim) and two non-systemic (Mancozeb and Chlorothalonil) for the management of late leaf spot of groundnut and their effect on dry pod and fodder yield. Foliar application of Carbendazim with Mancozeb @ 2g l⁻¹ was found to be highly effective in the management of late leaf spot and rust of groundnut with higher pod yield (2768 kg ha⁻¹) and benefit cost ratio (2.50). Further, Carbendazim+Mancozeb @ 2 g l⁻¹ was superior not only in controlling diseases, but also resulted in higher percent increase in pod yield in farm trials and large scale demonstration trials conducted in farmers fields. Nath *et al.* (2013) observed that among the fungicides, Tebuconazole @ 0.4 per cent (87.97%) showed maximum inhibition followed by Tebuconazole @ 0.2 per cent (85.24%) and Tebuconazole @ 0.15 per cent (83.50%), among these Tebuconazole @ 0.15 per cent was found to be optimum and reduced the disease intensity to 52.42 per cent. Application of fungicidal sprays influenced the development of Cercospora leaf spot and reduced the intensity. Adikshita and Sharma (2017) integrated disease management studies conducted under field conditions revealed that combination of seed treatment with *Trichoderma harzianum* followed by pre-sowing soil application of *T. harzianum* @ 2.5 kg per 50 kg FYM ha⁻¹ and two periodic sprays of myclobutanil @ 0.2 per cent at fifteen days interval started with the first appearance of disease proved

most efficacious in limiting the angular leaf spot disease and enhanced the green pod yield.

2.4 Epidemiology

Many environmental factors are known to predispose the host to infection by *P. griseola*. Intermittent dry-wet and warm cool weather has been reported to favour the disease (Rava *et al.*, 1985; Correa, *et al.*, 1989). Infection and colonization by *P. griseola*, as well as angular leaf spot disease development, occur within temperatures between 16 and 28°C with an optimum at 24°C (Cardona-Alvarez and Walker, 1956; Hagedom and Wade, 1974; Schwartz and Ga'lvez, 1980; Inglis and Hagedom, 1986; Saettler, 1991; Cardona *et al.*, 1997) and alternate period of high and low relative humidity favour epidemic development (Sartorato *et al.*, 1988). No infection was detected with temperatures over 32°C. Therefore, in temperate regions temperature is not a limiting factor for disease development (Cardona-Alvarez and Walker, 1956). Although 24 h of high relative humidity is enough to allow the development of synnemata, a longer period of high relative humidity is necessary for the fungus to sporulate profusely. High relative humidity, rainfall or dew periods are necessary to permit successful infection and subsequent synnemata formation (Lianos, 1957; Pastor, 1985; Allen and Lenne, 1998). Similarly, Lianos (1957) reported that high humidity ranging between 95-100 percent as optimum for artificial infection, while Barros *et al.* (1958a) observed that abnormally wet season, monoculture and use of frequent irrigation resulted in severe disease development. Hocking (1967) also recorded the disease in significant proportions during exceptionally wet season. The physiological studies conducted by Sindhan and Bose (1980) revealed that spore germination of *P. griseola* occurred at temperature range of 5-33°C with an optimum temperature of 18-24°C and relative humidity more than 49 per cent. The pathogen requires an incubation period of about 5-7 days at 24°C which gets extended to 15 days under cooler conditions (Campos and Fucikovsky,

1981). They also reported that plant developed maximum infection 12 days after inoculation, when kept for 56 h at 95 per cent relative humidity and 24°C temperature. Campos and Fucikovsky (1981) and Saettler and Correa (1987) reported that *P. griseola* grow best on V-8 juice agar medium at 24°C, with sporulation occurring at 16°C and pH 6. However, among the different culture media evaluated, tomato juice agar induced maximum sporulation at 20°C (Dalla *et al.*, 1997). Gupta *et al.* (1998) also reported that epidemiological parameters such as leaf wetness for 12 h and temperature of 25°C favoured disease development. In 1998, Neitsche and co-workers reported disease development after 48 h at 20-22°C and 95 per cent relative humidity. Stenglein *et al.* (2003) also observed that angular leaf spot of bean developed rapidly at moderate temperature and at high relative humidity above 95 per cent. The epidemiological studies conducted by Pria *et al.* (2003) revealed the temperature range 20-25°C and leaf wetness period of 48 h as optimum for the angular leaf spot disease development on French bean. The epidemiological studies on angular leaf spot on French bean conducted by Gupta *et al.* (2005) indicated the progressive decline in incubation period with the increasing leaf wetness period from 3 to 9 h. They also observed that higher relative humidity (>90%) and temperature (25°C) favoured disease development and recorded the positive correlation of the disease progression with relative humidity, temperature and rainfall. Once the fungus has infected the tissue, disease development proceeded even in a relatively dry atmosphere and even under these conditions stomata in substomatal cavities are formed. The stroma remaining among leaf tissue after destruction of the host cells remains dormant. When the environmental conditions become favorable, regrowth of the fungus occurs, conidiophores emerge and form synnemata, and finally sporulation occurs (Monda *et al.*, 2001). Like other pathogens, this pathogen also has some specific nutrient requirements for growth and sporulation (Cochrane, 1958).

2.5 Survival

The *Phaeoisariopsis griseola* successfully overwinters in stem and pod tissues of infected cv. Montcalm plants that are left in the field during the winter (Correa and Saettler, 1987). The pathogen can survive up to 140 days (140-500) in soil or plant tissues (Cardona-Alvarez and Walker, 1956; Sohi and Sharma, 1967). Sindhan and Bose (1979) reported the survivability of *Phaeoisariopsis griseola* for 19 months on host plant debris and for 24 months on seed. The fungus that survived for 9 months on plant debris or those seeds left in the field after harvest are most probably more important sources of primary inoculum than stored seed (Orozco-Sarria and Cardona-Alvarez, 1959). Because the fungus is dependent upon viable conidia for primary infection, the stromata are the most important overwintering structures (Cardona-Alvarez and Walker, 1956). Under favorable environmental conditions, new synnema and conidia are produced, and they comprise the primary source of fungal inoculums (Cardona-Alvarez and Walker, 1956; Sohi and Sharma, 1967).

Sengooba and Mukiibi (1986) studied the survival of *P. griseola* in crop debris and found that longer periods of storage (laboratory, shade, outside, and soil) increased the number of conidiophores in the synnemata. Infected straw in the soil did not form synnemata, but after 31 days produced conidia on solitary conidiophores. No conidiophores were found after 63 days of storage of stroma.

2.6 Dissemination

Cardona-Alvarez and Walker (1956) concluded that wind-blown particles of recently infested soils, wind-blown spores and water-borne spores are all effective agents of angular leaf spot disease dissemination. Although infected seeds have been reported to be a source of primary infection, using various lots of seed from diseased plants, they concluded that the fungus was not carried by the seed or on its surface. Orozco-Sarria and Cardona-Alvarez (1959) concluded that

in areas where survival of the fungus in soil is limited (extremes of heat and or cold), transmission of *P. griseola* through stored seeds is relatively important. Menezes *et al.* (1978) found that among 289 seeds examined 1.1 per cent were infected with *P. griseola*, whereas Tanaka and Deslandes (1978) found infected seeds to be only 2.5 per cent out of 400 seeds examined. However, seeds, crop debris, volunteer plants, as well as offseason crops are all possible sources of *P. griseola* inoculum. Sindhan and Bose (1979) and Sengooba and Mukiibi (1986) suggested that infected seeds play an important role in transmission of disease from one season to the next and that under circumstances where aerial inoculum is absent, seed-borne inoculum is most probably the most important way of spreading the disease. The studies conducted by Liebenberg and Pretorius (1997) inferred that seed, water and air currents play an important role in dissemination of *Phaeoisariopsis griseola*.

Chapter – 3

MATERIALS AND METHODS

The present investigations on the Status and Management of Angular Leaf Spot of Beans (*Phaseolus vulgaris* L.) caused by *Phaeoisariopsis griseola* (Sacc) Ferraris were undertaken in the laboratory and experimental field of Division of Plant Pathology, at Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Wadura Sopore located at 34°-20' north latitude and 74°-24' east longitude at an elevation of 1610 meters above mean sea level (amsl) during 2017-18. However, three districts located from north to the south of Kashmir valley at 1580 meters to 2000 meters (amsl) were surveyed to assess the disease incidence and intensity. The details of materials and methodologies followed during the course of investigation are described here under:

3.1 Disease Status

Field survey of commercially important bean growing areas of three districts of Kashmir valley viz., Budgam, Baramulla and Kulgam was conducted during July-August, 2017 for ascertaining the status of angular leaf spot disease (*Phaeoisariopsis griseola*) of French bean (*Phaseolus vulgaris* L.). Each district was represented by three locations and each location by three sites. Mean of three fields represented disease incidence and intensity of respective site. Nine plants each at four corners and centre of the fields were randomly selected and their leaves and pods were examined for recording disease incidence and intensity.

S.No.	District	No. of locations	No. of sites per location	Total sites
01.	Budgam	03	03	09
02.	Baramulla	03	03	09
03.	Kulgam	03	03	09
Total	03	09	09	27

3.1.1 Disease incidence

Per cent disease incidence was calculated as per the formula:

$$\text{Per cent disease incidence} = \frac{\text{No.of diseased units}}{\text{Total No.of units examined}} \times 100$$

3.1.2 Disease intensity

The disease intensity was recorded on leaves and pods using 0-5 scale given by Inglis *et al.* (1988) with slight modifications. Six categories were made on the basis of per cent leaf/ pod area involved (Plate 1 & 2) as per the following key:

- 0 : No disease
- 1 : Lesions on leaves/pods covering 0.1-10% leaf/pod area
- 2 : Lesions on leaves/pods covering 10.1-25% leaf/pod area
- 3 : Lesions on leaves/pods covering 25.1-50% leaf/pod area
- 4 : Lesions on leaves/pods covering >50 leaf/pod area
- 5 : Lesions on leaves frequently associated with chlorosis and necrosis and pods frequently associated with necrosis

The disease intensity was calculated using formula;

$$\text{PDI} = \frac{\sum (n_i \times i)}{NG} \times 100$$

Where:

PDI = Per cent Disease Intensity

n = Number of infected leaves/pods in each category



0



1



2



3



4



5

Plate-1: Scale (0-5) for assessment of angular leaf spot intensity on bean leaves



0



1



2



3



4



5

Plate-2: Scale (0-5) for assessment of angular leaf spot intensity on bean pods

- V = Category value
- N = Total number of leaves/pods examined
- G = Maximum category value

3.2 Symptomatology and etiology of the disease

3.2.1 Symptomatology

Study on symptomatology was carried out on selected bean plants, which were kept unsprayed during the season. Symptoms of the angular leaf spot of beans were studied on leaves and pods under natural epiphytotic conditions and the selected leaves and pods were accordingly tagged. Observations were recorded on tagged leaves and pods with regard to the first appearance of disease symptoms and different stages of its development like size, shape and colour of the lesions. Infected leaves bearing typical angular leaf spot symptoms were brought to the laboratory for further examination with regard to association of causal pathogen. The diseased portion of leaves were teased and observed under microscope. Conidia were examined for their colour, shape, size and septation.

3.2.2 Etiology

3.2.2.1 Isolation of pathogen

French bean leaves and pods showing typical disease symptoms, collected from susceptible plants during survey, were used for isolation of pathogen. The diseased leaves and pods showing typical symptoms of the disease were first brought to the laboratory and examined under microscope for association of conidia and synnemata by teasing the diseased portion with the help of a teasing needle. The pathogen was isolated from the host lesions by using the detached tissue technique as adopted by Loladze *et al.*, 2005 and Park *et al.*, 2008. Specimens of diseased leaves and pods were cut with the help of a sterile micro

scissors into small pieces about 1 cm long comprising of a part of diseased lesion and a portion of healthy tissue. The bits were placed in 0.1 per cent mercuric chloride solution for 30 seconds (Johnston and Booth, 1983) followed by 2-3 rinses with sterilized distilled water to remove the traces of mercuric chloride solution. The bits were then aseptically transferred to sterilized petriplates containing sterilized moist filter paper and incubated at 24 ± 2 °C. After 5 days, the spores were transferred from Coremial (Synemma) tips to V-8 juice agar plates and incubated for 10-12 days at 24 ± 2 °C temperature. Alternatively, spores were removed from sporulating lesions and transferred directly to V-8 juice agar medium. The identification of the isolated fungus was carried out in accordance with the standard keys “Illustrated Genera of Imperfect Fungi” by Barnett (1972).

3.2.2.2 Purification and maintenance of pathogen

The culture was purified by single spore technique (Johnston and Booth, 1983). The conidia from 12 days old culture on V-8 juice agar plates were harvested by flooding each plate with 5-10 ml of sterilized distilled water, serially diluted to 1:1000 and streaked on 2 per cent water agar in petridishes. After 24 hours of incubation at 24 ± 2 °C, the well separated individual germinating spores were searched under a stereomicroscope and marked with fine tip permanent marker. Fifteen such conidia were transferred separately to V-8 juice agar slants. Monosporic colonies that sporulated abundantly after 10-12 days at 24°C were maintained by periodic subculturing on fresh V-8 juice agar slants. The stock culture in V-8 juice agar slants was stored at 4°C in refrigerator.

3.2.2.3 Morphological characteristics of pathogen

The morphological characteristics of the pathogen were studied on both host as well as culture in the laboratory. Semi-permanent slides were prepared from 12 days old culture stained with cotton blue in lactophenol. The slides were

examined under microscope with respect to following characteristics of the causal organism.

Colony	:	Colour, shape and size
Mycelium	:	Colour, breadth, septation and branching
Conidiophore	:	Colour, shape and size
Conidia	:	Colour, shape, size and septation

Measurements regarding the above mentioned characteristics were taken with the help of two micrometers *viz.*, ocular micrometer and stage micrometer. The ocular micrometer was fitted into the circular shelf inside the eye piece and the stage micrometer was fitted to the stage of microscope. Using the objective lense, the graduations on the ocular micrometer were calibrated against the standard graduations on the stage micrometer. For calibration, both the micrometer etchings were superimposed by rotating the eyepiece. The number of ocular divisions coinciding with the number of stage divisions were find out and the calibration factor was calculated. After calibration, the stage micrometer was removed and the pathogen was placed on the stage on a slide and focused. The size was measured by counting the number of ocular divisions occupied by mycelium, conidiophore and conidia and multiplying with the calibration factor (Yang and Heinsohn, 2007).

3.2.3 Pathogenicity test

The pathogenicity test of the isolated fungal pathogen (one isolate) was conducted on French bean cv. Shalimar French Bean-1, susceptible to angular leaf spot disease in glass house. The pathogenicity of *Phaeoisariopsis griseola* was established by following the Koch's postulates (Nasehi *et al.*, 2012). The plants

were raised in earthen pots using sterilized soil and apparently healthy seeds. The soil was sterilized by autoclaving at 1.05 kg cm^{-2} for 20 minutes for three consecutive days and 2 kg of the sterilized soil was put in each pot (with 18 cm dia). The seeds of French bean, sterilized by immersing in mercuric chloride solution (0.1%) for 1 minute and subsequently rinsed with sterilized distilled water and sown in pots (Johnston and Booth, 1983). Twelve days old culture of *P. griseola* grown on V-8 juice agar medium was flooded with 5 ml distilled water, conidia were loosened and the resulting suspension was filtered through four thickness of cheese cloth. Conidial concentrations were adjusted to about 2×10^4 conidia ml^{-1} with the help of haemocytometer. The bean plants were inoculated at primary trifoliate leaf stage with a suspension of *P. griseola* at a concentration of 2×10^4 conidia ml^{-1} by spraying thoroughly with glass atomiser and incubated under moist chamber for 72 hours. An uninoculated bean plant was also maintained under similar conditions to serve as control. The observations on symptom development was recorded at regular intervals. The organism was re-isolated from artificially inoculated leaves showing characteristic symptoms. The culture obtained was compared with original culture to satisfy Koch's postulates and to confirm identity of the pathogen.

3.3 Evaluation of fungicides

3.3.1 *In vitro* evaluation of fungicides

The following six systemic and four non-systemic fungitoxicants were evaluated *in vitro* for their efficacy against the pathogen causing angular leaf spot by using spore germination inhibition technique (Begum *et al.*, 2010). Each test fungitoxicant was evaluated at three different concentrations (C_1 , C_2 , C_3) including control as under:

3.3.1.1 *In vitro* evaluation of systemic fungicides

S.No	Fungicide	C ₃ (ppm)	C ₂ (ppm)	C ₃ (ppm)
01.	Hexaconazole 5 EC	100	300	500
02.	Carbendazim 50 WP	100	300	500
03.	Tebuconazole 25 WP	100	300	500
04.	Myclobutanil 10 WP	100	300	500
05.	Trifloxystrobin 25 WG + Tebuconazole 50 WG	100	300	500
06.	Metiram 55 WG + Pyraclostrobin 5 WG	100	300	500
07.	Control	DW	DW	DW

DW: Distilled water

3.3.1.2 *In vitro* evaluation of non-systemic fungicides

S. No.	Fungicide	C ₁ (ppm)	C ₂ (ppm)	C ₃ (ppm)
01.	Mancozeb 75 WP	500	1000	1500
02.	Copper oxychloride 50 WP	500	1000	1500
03.	Captan 50 WP	500	1000	1500
04.	Chlorothalonil 75 WP	500	1000	1500
05.	Control	DW	DW	DW

DW: Distilled water

Spore suspension of 2×10^4 spores ml⁻¹ concentration in sterile water was prepared aseptically from twelve days old culture of *Phaeoisariopsis griseola* on V-8 agar media against three different concentrations of the chemical fungicides. Fungitoxicant suspension of double the concentration than the desired one was prepared separately in 150 ml conical flasks. Each fungitoxicant treatment was replicated thrice in a completely randomized design (CRD). One drop (50 µl) each of fungitoxicant was mixed with one drop of spore suspension on a clean two concavity glass slide. The slides kept in petriplates lined inside with moist filter paper were incubated at 24°C for 48 hours. The slides were finally examined under microscope (400x) for recording the percentage of conidial germination and were compared with the check. Average per cent inhibition of conidial germination for each fungitoxicant was calculated by employing the following formula of Vincent (1947).

$$\text{Per cent germination inhibition} = \frac{\text{Germination in Check} - \text{Germination in Treatment}}{\text{Germination in Check}} \times 100$$

3.3.2 *In vivo* evaluation of fungicides

The most effective fungicides (Carbendazim, Tebuconazole, Myclobutanil and Mancozeb) evaluated *in vitro* were further evaluated under field conditions against angular leaf spot of bean. Field trial on management of the disease was conducted during *kharif* 2017-18 at Faculty of Agriculture, SKUAST-K Wadura. The experiment was laid in Randomized Complete Block Design with 2m x 1m plot size, with 60 plants in each plot and replicated thrice. The crop husbandry was followed as per SKUAST-K package of practices (Anonymous, 2011). The bean seeds of susceptible cv. Shalimar French Bean-1 were sown after treating with two fungicides (Carbendazim 50 WP and Mancozeb 75 WP). The first four fungicides that proved best under *in vitro* conditions, were sprayed thrice at 15 days interval starting from first appearance of the disease. Control plots were sprayed with sterile distilled water. The details of treatments imposed in trial are as under:

Details of treatment combinations

Treatment No.	Treatment Details
T1	Control
T2	Foliar spray with Carbendazim 50 WP @ 0.05%
T3	Foliar spray with Tebuconazole 25 WP @ 0.025%
T4	Foliar spray with Myclobutanil 10 WP @ 0.05%
T5	Foliar spray with Mancozeb 75 WP @ 0.25%
T6	Seed treatment with Carbendazim 50 WP @ 0.1%
T7	Seed treatment with Carbendazim 50 WP @ 0.1% + Foliar spray with Carbendazim 50 WP @ 0.05%
T8	Seed treatment with Carbendazim 50 WP @ 0.1% + Foliar spray with Tebuconazole 25 WP @ 0.025%
T9	Seed treatment with Carbendazim 50 WP @ 0.1% + Foliar spray with Myclobutanil 10 WP @ 0.05%
T10	Seed treatment with Carbendazim 50 WP @ 0.1% + Foliar spray with Mancozeb 75 WP @ 0.25%
T11	Seed treatment with Mancozeb 75 WP @ 0.3%
T12	Seed treatment with Mancozeb 75 WP @ 0.3% + Foliar spray with Carbendazim 50 WP @ 0.05%
T13	Seed treatment with Mancozeb 75 WP @ 0.3% + Foliar spray with Tebuconazole 25 WP @ 0.025%
T14	Seed treatment with Mancozeb 75 WP @ 0.3% + Foliar spray with Myclobutanil 10 WP @ 0.05%
T15	Seed treatment with Mancozeb 75 WP @ 0.3% + Foliar spray with Mancozeb 75 WP @ 0.25%

Observations on disease intensity were recorded on leaves using modified 0-5 disease rating scale (Inglis *et al.*, 1988) at pod formation stage. The pod yield data was also recorded on maturity and data was analyzed using standard statistical procedure (Gomez and Gomez, 1984).

Chapter-4

EXPERIMENTAL FINDINGS

The experimental findings of research work on “Status and Management of Angular Leaf Spot of Bean (*Phaseolus vulgaris* L.) caused by *Phaeoisariopsis griseola* (Sacc.) Ferraris” are presented under different heads as given below:

4.1 Disease status

With a view to find out the status of angular leaf spot of beans in Kashmir valley, the survey was carried out in three districts *viz.*, Baramulla, Budgam and Kulgam of Kashmir valley for the prevalence of angular leaf spot of beans during *kharif* 2017-18. The data on disease incidence on leaves and pods recorded are presented in Table-1 and 2 and data on disease intensity on leaves and pods recorded are present in Table-3 and 4, respectively.

4.1.1 Disease incidence

(a) Disease incidence on leaves

The data presented in Table-1 revealed that angular leaf spot of beans was prevalent in all the sites of districts surveyed with varying levels of incidence ranging from 22.50 per cent to 68.91 per cent on leaves. The highest disease incidence on leaves was recorded in district Baramulla (59.20%) followed by district Budgam (37.70%) while as the district Kulgam (30.40%) exhibited the least disease incidence (Fig.1). The disease incidence on leaves ranged from 49.00 to 68.91 per cent in district Baramulla, whereas in Budgam from 25.50 to 46.50 per cent and in Kulgam it ranged from 22.50 to 36.40 per cent. Among the locations surveyed highest disease incidence of 65.61 per cent was recorded in Sopore followed by Pattan (60.10%), lowest disease incidence was recorded in Kulgam (25.90%). On an overall comparison amongst different sites surveyed, the

highest disease incidence of 68.91 per cent was recorded in Arampora followed by Chinkipora (66.40%) and Wusan (63.10%). The least disease incidence of 22.50 per cent was recorded in Banimullah.

Statistical analysis of the surveyed data revealed that the limits for average disease incidence fluctuated between 54.15 to 64.24 per cent for Baramulla district and 32.22 to 43.20 per cent for Budgam district. Similarly, for Kulgam district the limits fluctuated between 27.16 to 33.75 per cent respectively. However, the average statistical limits in all the three districts at all the locations in incidence fluctuated between 37.01 to 47.89 per cent.

(b) Disease incidence on pods

The data presented in Table-2 revealed that angular leaf spot of beans was prevalent in all the sites of districts surveyed with varying levels of incidence ranging from 12.50 per cent to 48.50 per cent on pods. The highest disease incidence on pods was recorded in district Baramulla (38.76%) followed by district Budgam (25.49%) while as the district Kulgam (18.62%) exhibited the least disease incidence (Fig.1). The disease incidence on pods ranged from 30.60 to 48.50 per cent in district Baramulla, whereas in Budgam from 14.76 to 32.83 per cent and in Kulgam it ranged from 12.50 to 26.13 per cent. Among the locations surveyed highest disease incidence of 45.27 per cent was recorded in Sopore followed by Pattan (39.36%), lowest disease incidence was recorded in Kulgam (14.86%). On an overall comparison amongst different sites surveyed, the highest disease incidence of 48.50 per cent was recorded in Arampora followed by Palhalan (45.38%) and Wadura (44.91%). The least disease incidence of 12.50 per cent was recorded in Banimullah.

Statistical analysis of the surveyed data revealed that the limits for average disease incidence fluctuated between 33.65 to 44.03 per cent for Baramulla district and 20.68 to 30.30 per cent for Budgam district. Similarly, for Kulgam

district the limits fluctuated between 15.35 to 21.89 per cent. However, the average statistical limits in all the three districts at all the locations in incidence fluctuated between 23.59 to 31.66 per cent.

4.1.2 Disease Intensity

(a) Disease intensity on leaves

The data presented in Table-3 revealed that angular leaf spot of beans was prevalent in all the sites of districts surveyed with varying levels of intensity ranging from 15.14 per cent to 38.33 per cent on leaves. The highest disease intensity on leaves was recorded in district Baramulla (30.94%) followed by district Budgam (24.89%) while as the district Kulgam (20.07%) exhibited the least disease intensity (Fig.2). The disease intensity ranged from 24.81 to 38.33 per cent in district Baramulla, whereas in Budgam from 17.51 to 30.52 per cent and in Kulgam it ranged from 15.14 to 23.41 per cent. Among the locations surveyed highest disease intensity of 34.94 per cent was recorded in sopore followed by Pattan (30.93%), lowest disease intensity of 17.16 per cent was recorded in Kulgam. On an overall comparison amongst different sites surveyed, the highest disease intensity of 38.33 per cent was recorded in Arampora followed by Chinkipora (36.13%) and Wusan (32.00%). The least disease intensity of 15.14 per cent was recorded in Banimullah.

Statistical analysis of the surveyed data revealed that the limits for average disease intensity fluctuated between 27.75 to 34.14 per cent for Baramulla district and 21.81 to 27.95 per cent for Budgam district. Similarly, for Kulgam district the limits fluctuated between 18.06 to 22.12 per cent respectively. However, the average statistical limits in all the three districts at all the locations in intensity fluctuated between 23.04 to 27.57 per cent.

(b) Disease intensity on pods

The data presented in Table 4 revealed that angular leaf spot of beans was prevalent in all the sites of districts surveyed with varying levels of intensity ranging from 6.31 per cent to 21.86 per cent on pods. The highest disease intensity on pods was recorded in district Baramulla (18.14%) followed by district Budgam (12.62%) while as the district Kulgam (9.63%) exhibited the least disease intensity (Fig.2). The disease intensity on pods ranged from 15.33 to 21.86 per cent in district Baramulla, whereas in Budgam from 8.83 to 16.30 per cent and in Kulgam it ranged from 6.31 to 12.37 per cent. Among the locations surveyed highest disease intensity of 20.42 per cent was recorded in Sopore followed by Pattan (18.22%), lowest disease intensity of 7.00 per cent was recorded in Kulgam. On an overall comparison amongst different sites surveyed, the highest disease intensity of 21.86 per cent was recorded in Arampora followed by Palhalan (20.06%) and Wadura (19.96%). The least disease intensity of 6.31 per cent was recorded in Banimullah.

Statistical analysis of the surveyed data revealed that the limits for average disease intensity fluctuated between 16.40 to 19.88 per cent for Baramulla district and 10.95 to 15.93 per cent for Budgam district. Similarly, for Kulgam district the limits fluctuated between 7.80 to 11.45 per cent respectively. However, the average statistical limits in all the three districts at all the locations in intensity fluctuated between 11.75 to 15.18 per cent.

Table -1: Disease incidence of angular leaf spot of beans on leaves at various locations of Kashmir valley during the year 2017-18

District	Location	Site	Disease Incidence on leaves (%)	95% C.I.**	
				Lower limit	Upper limit
Baramulla	Sopore	Arampora	68.91*	56.52	74.95
		Chinkipora	66.40		
		Wadura	61.52		
		Sub mean	65.61		
	Pattan	Palhalan	59.10	53.87	66.50
		Wusan	63.10		
		Nehalpora	58.40		
		Sub mean	60.10		
	Baramulla	Stadium colony	49.00	45.37	58.22
		Johama	54.10		
Chakla		52.30			
Sub mean		51.80			
Over all mean			59.20	54.15	64.24
Budgam	Khansahib	Kremshore	41.50	38.58	47.47
		Yarikhah	45.00		
		Wager	42.60		
		Sub mean	43.00		
	Chadoora	Nowbug	38.60	20.22	57.50
		Ranger	31.50		
		Charisharief	46.50		
		Sub mean	38.90		
	Mazhama	Kawoosa	30.70	16.38	46.01
		Kawlatpora	37.40		
Kuthipora		25.50			
Sub mean		31.20			
Over all mean			37.70	32.22	43.20
Kulgam	Devsar	Bonigam	36.40	25.43	42.23
		Kilan Bozgam	35.10		
		Nowgam Devsar	30.00		
		Sub mean	33.80		
	Kulgam	Banimullah	22.50	18.44	33.49
		Mirhama	27.30		
		Laroo	28.10		
		Sub mean	25.90		
	Qaimoh	Bachroo	29.50	26.93	35.99
		Khudwani	31.80		
Baloo		33.10			
Sub mean		31.50			
Over all mean			30.40	27.16	33.75
Over all disease incidence			42.44	37.01	47.89

* Average of three fields at each site.

**Confidence Interval

Table -2: Disease incidence of angular leaf spot of beans on pods at various locations of Kashmir valley during the year 2017-18

District	Location	Site	Disease Incidence on pods (%)	95% C.I. **	
				Lower limit	Upper limit
Baramulla	Sopore	Arampora	48.50*	37.64	52.88
		Chinkipora	42.42		
		Wadura	44.91		
		Sub mean	45.27		
	Pattan	Palhalan	45.38	26.05	52.65
		Wusan	37.55		
		Nehalpora	35.14		
		Sub mean	39.36		
	Baramulla	Stadium colony	30.60	28.01	35.29
		Johama	31.04		
		Chakla	33.33		
		Sub mean	31.66		
	Over all mean			38.76	33.65
Budgam	Khansahib	Kremshore	29.88	28.80	32.17
		Yarikhah	31.22		
		Wager	30.36		
		Sub mean	30.48		
	Chadoora	Nowbug	26.45	15.66	39.48
		Ranger	23.44		
		Charisharief	32.83		
		Sub mean	27.57		
	Mazhama	Kawoosa	18.19	9.00	27.85
		Kawlatpora	22.34		
		Kuthipora	14.76		
		Sub mean	18.43		
	Over all mean			25.49	20.68
Kulgam	Devsar	Bonigam	26.13	14.16	32.22
		Kilan Bozgam	24.33		
		Nowgam Devsar	19.13		
		Sub mean	23.19		
	Kulgam	Banimullah	12.50	9.74	19.97
		Mirhama	15.76		
		Laroo	16.31		
		Sub mean	14.86		
	Qaimoh	Bachroo	16.88	14.96	20.65
		Khudwani	17.46		
		Balsoo	19.09		
		Sub mean	17.81		
	Over all mean			18.62	15.35
Over all disease incidence			27.62	23.59	31.66

* Average of three fields at each site.

**Confidence Interval

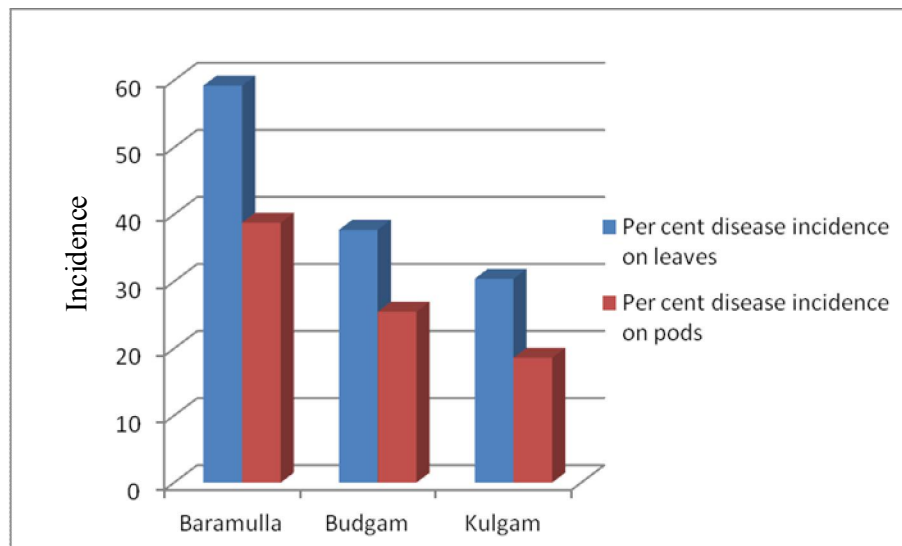


Figure 1: Disease incidence of Angular Leaf Spot of bean in three districts of Kashmir valley during the year 2017-18

Table -3: Disease intensity of angular leaf spot of beans on leaves at various locations of Kashmir valley during the year 2017-18

District	Location	Site	Disease Intensity (%) on leaves	95 % C.I. **	
				Lower limit	Upper limit
Baramulla	Sopore	Arampora	38.33*	24.78	45.10
		Chinkipora	36.13		
		Wadura	30.42		
		Sub mean	34.94		
	Pattan	Palhalan	30.89	28.32	33.54
		Wusan	32.00		
		Nehalpora	29.93		
		Sub mean	30.93		
	Baramulla	Stadium colony	24.81	22.26	31.67
		Johama	28.33		
		Chakla	27.82		
		Sub mean	26.97		
	Over all mean			30.94	27.75
Budgam	Khansahib	Kremshore	25.81	21.15	33.96
		Yarikhah	30.52		
		Wager	26.34		
		Sub mean	27.56		
	Chadoora	Nowbug	24.61	16.80	35.06
		Ranger	23.11		
		Charisharief	30.09		
		Sub mean	25.94		
	Mazhama	Kawoosa	22.53	13.14	29.26
		Kawatpota	23.58		
		Kuthipora	17.51		
		Sub mean	21.21		
	Over all mean			24.89	21.81
Kulgam	Devsar	Bonigam	23.41	19.09	25.76
		Kilan Bozgam	23.00		
		Nowgam Devsar	20.9		
		Sub mean	22.43		
	Kulgam	Banimullah	15.14	12.35	21.96
		Mirhama	17.33		
		Laroo	19.00		
		Sub mean	17.16		
	Qaimoh	Bachroo	19.86	18.94	22.38
		Khudwani	21.00		
		Baloo	21.11		
		Sub mean	20.65		
	Over all mean			20.07	18.06
Over all disease intensity			25.30	23.04	27.57

* Average of three fields at each site.

**Confidence

Interval

Table -4: Disease intensity of angular leaf spot of beans on pods at various locations of Kashmir valley during the year 2017-18

District	Location	Site	Disease Intensity (%) on pods	95% C.I. **	
				Lower limit	Upper limit
Baramulla	Sopore	Arapora	21.86	17.18	23.62
		Chinkipora	19.38		
		Wadura	19.96		
		Sub mean	20.42		
	Pattan	Palhalan	20.06	14.03	22.41
		Wusan	17.88		
		Nehalpora	16.74		
		Sub mean	18.22		
	Baramulla	Stadium colony	15.33	14.73	16.88
		Johama	15.91		
Chakla		16.18			
Sub mean		15.81			
Over all mean			18.14	16.40	19.88
Budgam	Khansahib	Kremshore	15.77	13.20	17.83
		Yarikhah	16.30		
		Wager	14.49		
		Sub mean	15.52		
	Chadoora	Nowbug	12.78	8.91	17.22
		Ranger	11.56		
		Charisharief	14.87		
		Sub mean	13.07		
	Mazhama	Kawoosa	9.06	7.79	10.78
		Kawlatpora	9.97		
		Kuthipora	8.83		
		Sub mean	9.28		
	Over all mean			12.62	10.95
Kulgam	Devsar	Bonigam	12.37	11.65	12.70
		Kilan Bozgam	12.22		
		Nowgam Devsar	11.95		
		Sub mean	12.18		
	Kulgam	Banimullah	6.31	5.43	8.56
		Mirhama	7.14		
		Laroo	7.55		
		Sub mean	7.00		
	Qaimoh	Bachroo	8.32	6.13	13.22
		Khudwani	9.66		
		Bal soo	11.15		
		Sub mean	9.71		
	Over all mean			9.63	7.80
Over all disease intensity			13.46	11.75	15.18

* Average of three fields at each site.

** C.I. = Confidence Interval

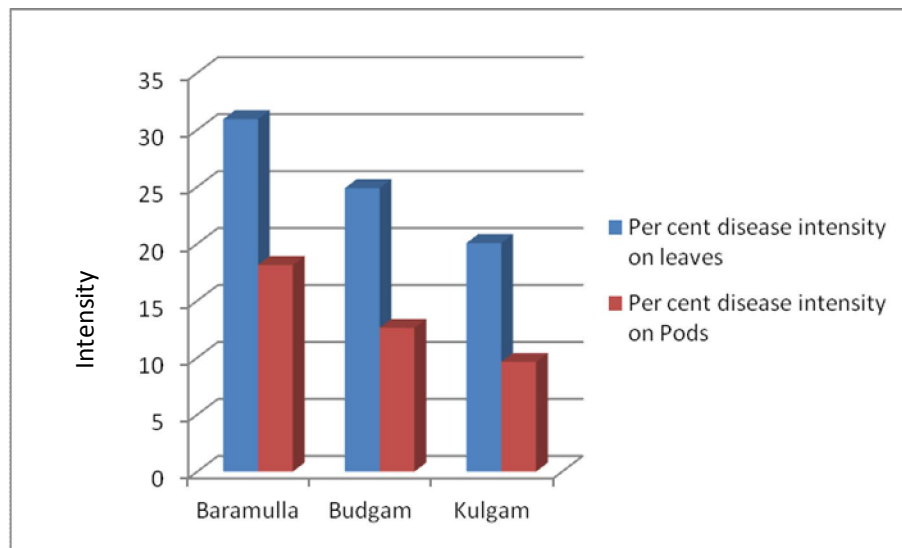


Figure 2: Disease intensity of Angular Leaf Spot of bean in three districts of Kashmir valley during the year 2017-18

4.2 Symptomatology and etiology of the disease

4.2.1 Symptomatology

Periodical observations on symptomatological development were recorded at an interval of seven days on leaves. The first symptoms of angular leaf spot appeared during the early stages (2nd week of June) of plant growth on primary leaves. However, the disease did not become conspicuous until late flowering or early pod set. The first symptoms appeared on the primary leaves in the form of circular greyish lesions with concentric rings, greater in size than first trifoliolate, about 0.7-1.3 cm in diameter. On the first trifoliolate, the lesions appeared as angular greyish-brown colouration of approximately 0.3-0.7 cm in diameter, surrounded by a yellow halo delimited by the ribs. Symptoms became more evident on leaves after a period of two weeks when lesions were light brown and were assuming the typical angular shape. Periodic changes in size, shape, and colour of the lesions were observed. The initial development of the lesions was slow up to second week of July after which it showed a curvilinear behaviour upto the second week of August beyond which the lesion enlargement declined sharply. No symptoms were seen on other trifoliolate leaves before first week of July (Plate-3 a). From first week of July symptoms were observed on other trifoliolate leaves in the form of angular light brown lesions of size 0.1-0.3cm (Plate-3 b). In the second week of July the lesion size increased from 0.3-0.5cm with synnemata emerging from abaxial surface of leaves. In third week of July lesion size increase from 0.5-0.7cm (Plate-3 c). Numerous lesions with profuse sporulation were seen in the fourth week of July with size of lesions ranging from 0.7-1.0 cm (Plate-3 d). In first week of August irregular lesions are formed due to coalescing of 2-5 spots with lesion size ranging from 1.0-2.0 cm (Plate-3 e). In second week of August larger irregular patches on leaves were formed, giving blighted appearance to leaves (Plate-3 f). In third and fourth week of August lesions covering larger areas on leaves were seen showing excessive chlorosis and



(a)

(b)

(c)



(d)

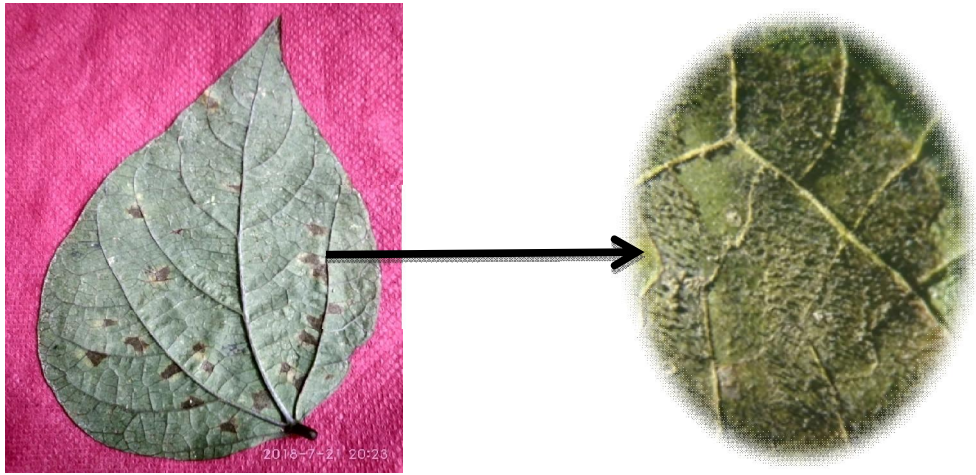
(e)

(f)

(g)

Plate-3: Symptomatological development of angular leaf spot of beans on leaves

- (a) No symptom
- (b, c) Angular light brown lesions of small size
- (d) Numerous spots with profuse sporulation
- (e) Irregular lesions due to coalescing of spots
- (f) Larger irregular patches on leaves
- (g) Lesions showing excessive chlorosis



(h)

(i)

Plate-3: Symptomatology development of angular leaf spot of beans on leaves

(h) Spots on abaxial surface of leaf

(i) Synnemata distribution on leaves



(a)



(b)



(c)



(d)

Plate-4: Symptomatological development of angular leaf spot of beans on pods

- (a) Small oval to circular brown spots
- (b) Circular reddish brown spots with dark edges
- (c) Numerous spots of larger size
- (d) Spots coalescing to cover larger areas

premature leaf fall (Plate-3 g) (Table-5). In the later stages the spots were also seen on pods and stem. On pods symptoms appeared in first week of August in the form of small oval to circular brown spots of size 0.1-0.7 cm (Plate-4 a), which turned reddish brown with dark edges approximately 1.25cm in diameter in the second week of August (Plate-4 b). Numerous spots of larger size 1.25-1.75 were observed in third week of August (Plate-4 c), spots coalesced to attain a maximum of 2.25 cm diameter in the last week of August (Plate-4 d) (Table-6). Lesions on the stem were dark brown and elongated.

4.2.2 Etiology

4.2.2.1 Isolation of pathogen

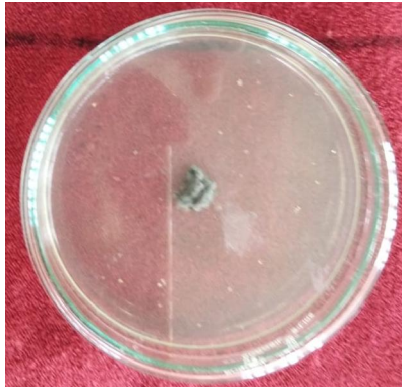
The isolation of the pathogen associated with angular leaf spot of french bean were made (3.2.2.1) and the fungus emerging from leaf bits was observed to have olivaceous grey mycelial mat (Plate-5 a).

4.2.2.2 Purification and maintenance of the pathogen

The pure culture was further sub cultured at monthly intervals and stored in a refrigerator for further studies (Plate-5 b, c, d).

4.2.2.3 Morphological characteristics of pathogen

The morphological and cultural characteristics of the isolated pathogen are presented in Table-7 and depicted in Plate-6 &7.



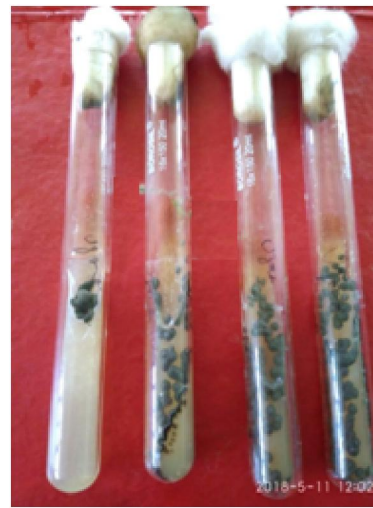
(a)



(b)



(c)



(d)

Plate-5: Isolation, Purification and Maintenance of *Phaeoisariopsis griseola* causing angular leaf spot of beans

- (a) Isolation
- (b) Subculture
- (c) Purification
- (d) Maintenance

Table -5: Symptomatological development of angular leaf spot of beans on leaves during *kharif* 2017-18.

Time of observation		Symptoms on leaves	Lesion size(cm)
Month	Week		
June	I	No symptoms appeared	0
	II	On primary leaves - circular greyish lesions with concentric rings	0.7-1.3
	III	On first trifoliolate leaves - angular greyish brown lesions with a yellow halo	0.2-0.3
	IV	-do-	0.3-0.7
July	I	Angular light brown lesions of small size on other trifoliolate leaves	0.1-0.3
	II	Angular brown lesions with synnemata emerging from abaxial surface of leaves	0.3-0.5
	II	-do-	0.5-0.7
	IV	Numerous spots with profuse sporulation	0.7-1.0
August	I	Irregular lesions due to coalescing of 2-5 spots	1.0-2.0
	II	Larger irregular patches on leaves giving blighted appearance	—
	III	Lesions covering larger areas on leaves showing excessive chlorosis and premature leaf fall (defoliation)	—
	IV	-do-	

Table - 6: Symptomatological development of angular leaf spot of beans on pods during *kharif* 2017-18.

Time of observation		Symptoms on pods	Lesion size (cm)
Month	Week		
August	I	Small oval to circular brown spots on pods	0.1 - 0.7
	II	Circular reddish brown spots with dark edges on pods	0.7 - 1.25
	III	Numerous spots of larger size on pods	1.25 - 1.75
	IV	Spots coalescing to cover larger areas on pods	1.75 - 2.25

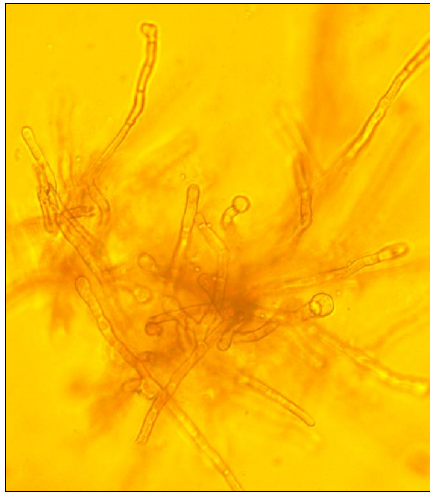
4.2.2.3.1 Morphological characteristics on host

The diseased samples collected from the field observer under microscope revealed that the hyphae were branched, septate, smooth, hyaline to olivaceous brown in colour and measuring 3.5-4.7 μm in width with an average of 4.1 μm (Table-7). Conidiophores numerous, ranging from 26 - 35, in dense fascicles, often forming synnemata, erumpent and measured 2-4.9 \times 102.5-171 μm with an average size of 3.5 \times 135.5 μm , olivaceous-brown, conidiophores splaying out at the end of the synnemata. Individual conidiophores filiform 2-5 μm wide, septate, subhyaline to olivaceous-brown. Conidia obclavate-cylindrical, broadly subfusiform, short conidia ellipsoid to cylindrical, straight to curved, 5-7.9 \times 32.6-55.4 μm with an average size of 6.1 \times 34.4 μm , 1-6 septate, subhyaline to olivaceous-brown, smooth, truncate to rounded base, 1.5-2.5 μm wide (Plate-6).

4.2.2.3.2 Morphological characteristics in culture

The morphological characters of the isolated pathogen infecting beans were studied from the culture growth on V-8 agar juice media for 5-15 days at 24 \pm 1 $^{\circ}\text{C}$. Observations regarding morphological characters of different structures *viz.*, mycelium, conidiophores, conidia were noted (Plate-7) and are presented in Table-7.

The slants inoculated with fungal bits, obtained from pure culture of the fungus, were critically observed for colony characters and growth behaviour. The fungus is slow growing so visual growth of the fungus was first observed after 5 days of inoculation as light greyish growth of fungal hyphae up to a diameter of 1mm attained 3-4 mm in 15 days. The colonies formed were erumpent with moderate aerial mycelium initially light grey which turned dark olivaceous gray and finally black after 15 days of incubation.



(a) Mycelium



(b) Conidia



(c) Conidia with septation



(d) Synnemata



(e) Conidiophore

Plate-6: Morphological characters of *Phaeoisariopsis griseola* on host

a) Mycelium

Microscopic observations of the isolated fungus revealed that the mycelium was septate, with subhyaline hyphae when observed singly and irregularly branched, measuring 3.1-4.0 μm in width. The conidiophores were pale olive to olivaceous brown in color, septate, branched and measured 95-159 μm in length and 1.8-4.6 μm in width. The mycelium was light grey when young and turned darker with age.

b) Conidia

Conidia were olivaceous grey to olivaceous brown in colour slightly curved with cylindrical to obclavate shape measuring 24.0-37.6 μm in length and 2.7-5.3 μm in width having 1-3 longitudinal septa (Plate-7).

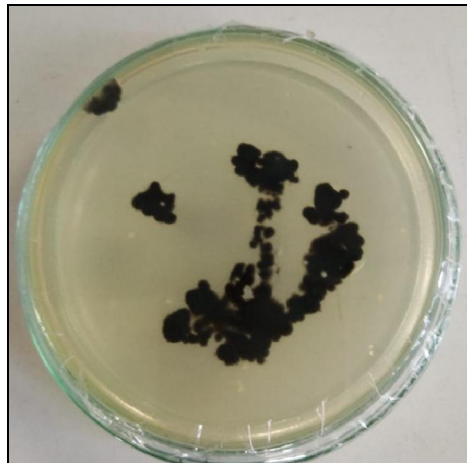
Microscopic examination also revealed that the colony was flat to slightly erumpent, spreading with moderate aerial mycelium, margins smooth, regular, surface with olivaceous-grey and smoke-grey patches, mycelium was branched and septate. The stroma was Prosenchymatous, brown or olivaceous brown, the conidiophores macronematous, mononematous and synnematous, and the conidia solitary, dry acropleurogenous, simple, obclavate or cylindrical, sub-hyaline, pale olive, olivaceous brown or light brown and smooth.

4.2.3 Pathogenicity test

The *Phaeoisariopsis griseola* (Sacc.) Ferraris isolated from infected leaf tissue of beans showing angular leaf spots was found pathogenic when inoculated with 12 days old culture of the fungus on healthy bean leaves. The mycelial cum conidial suspension (2×10^4 conidia ml^{-1}) of isolated fungus was inoculated on susceptible cultivar 'Shalimar French Bean-1'. On primary trifoliolate leaves, the symptoms were observed after 12 days of inoculation as typical angular shaped brown or grey lesions delimited by veins and veinlets, later becoming necrotic



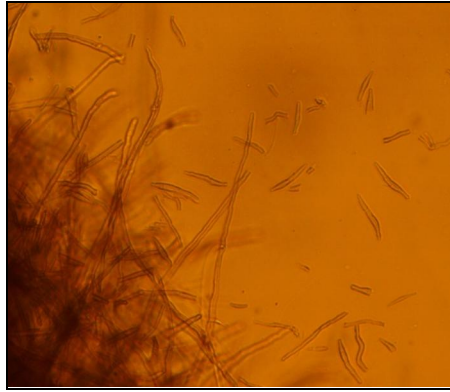
(a)



(b)

Plate-7: Morphological characters of *Phaeoisariopsis griseola* in culture

- (a) Upper side of culture
- (b) Lower side of culture



(c)



(d)



(e)



(f)



(g)

Plate-7: Morphological characters of *Phaeoisariopsis griseola* in culture

- (c) Mycelium
- (d) Conidia
- (e) Conidia showing septation
- (f) Synnemata
- (g) Conidiophore

(Plate-8). In some cases the lesions coalesced to produce lesions of greater dimensions. On trifoliolate leaves, the symptoms appeared first on the lower leaf surface as light grey lesions. In the advanced stages of the disease, leaf spots coalesced causing extensive necrosis, chlorosis, leaf curling, drying and eventually defoliation. The results of the pathogenicity test on the leaves of French bean plant indicated that inoculated plant showed symptoms similar to those observed in the field while the control plants remained healthy. The fungus that was consistently reisolated from lesions in the inoculated plants was identical to that of isolated from leaf tissues of bean collected from the field and were identified as *Phaeoisariopsis griseola* on the basis of fungal morphology (Bartnett, 1972), symptoms produced on the leaves, hence proving the pathogenicity of the pathogen by Koch's postulates.

4.3 Evaluation of fungicides

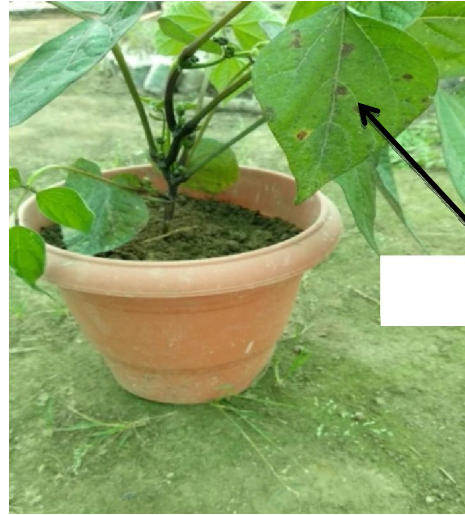
4.3.1 *In vitro* evaluation of fungicides

The efficacy of various systemic and non-systemic fungitoxicants against *Phaeoisariopsis griseola* was evaluated *in vitro* by spore germination inhibition technique.

Six systemic and four non-systemic fungitoxicants at three different concentrations (100, 300, 500 ppm) were evaluated for their comparative efficacy against spore germination of *Phaeoisariopsis griseola* by spore germination inhibition technique.



(a)



(b)

symptom

Plate-8: Pathogenicity test of *Phaeoisariopsis griseola* on beans

(a) Uninoculated host

(b) Inoculated host

Table-7: Morphological characteristics of isolated pathogen

Thallus part	Shape and physical appearance	Colour	Size (μm)	Septation
On host				
Hyphae	Branched, septate, smooth	Hyaline to olivaceous brown	3.5-4.7 (4.1)*	Septate
Conidiophore	Synnematosus, filiform, septate	Subhyaline to olivaceous-brown	2-4.9 \times 102.5-171 (3.5 \times 135.5)*	Septate
Conidia	bclavate-cylindrical	olivaceous-brown	5-7.9 \times 32.6-55.4 (6.1 \times 34.4)*	1-6 septate
In culture				
Colony	Slow growing, irregular	Initially light greyish finally becomes black	1-2 mm after 5 days on V-8 agar juice media at 25 \pm 1 $^{\circ}$ C	–
Mycelium	Irregularly branched septate	Subhyaline	3.1-4.0 μm (3.4)*	Septate
Conidiophore	Septate branched	Olive to olivaceous brown	1.8-4.6 \times 95-159 μm (3.3 \times 131)*	Septate
Conidia	Slightly curved cylindrical to obclavate	Olivaceous grey to olive brown	2.7-5.3 \times 24.0-37.6 μm (4.2 \times 30.8)*	1-3 septate

*Figures in the parenthesis are average values

4.3.1.1 *In vitro* evaluation of systemic fungicides

Perusal of data (Table-8) revealed that all the six systemic fungitoxicants at all the test concentrations inhibited spore germination. Among the systemic fungitoxicants tested, Carbendazim 50 WP proved to be most effective as it provided 97.41 per cent spore germination inhibition over control (Plate-9) followed by Tebuconazole 25 WP and Myclobutanil 10 WP, exhibiting 88.14 and 81.95 per cent spore germination inhibition, respectively. However, Trifloxystrobin 25 WG +Tebuconazole 50 WG proved to be least effective with only 69.05 per cent germination inhibition.

On an overall basis the inhibitory effect of test fungitoxicants increased with increase in concentration. A minimum inhibition of 58.26 per cent was achieved at 100 ppm which increased gradually to 81.06 per cent at 500 ppm concentration. A non-significant interaction existed between Carbendazim concentrations with cent per cent inhibition beyond 100 ppm.

4.3.1.2 *In vitro* evaluation of non-systemic fungicides

Perusal of the data (Table-9) revealed that all the non systemic fungitoxicants differ significantly among each other. Among non systemic fungitoxicants tested, Mancozeb 75 WP proved to be most effective as it provided 86.62 per cent spore germination inhibition over control followed by Chlorothalonil 75 WP exhibiting 64.73 per cent spore germination inhibition. However, Captan 50 WP proved to be least effective with only 51.26 per cent germination inhibition. Mancozeb 75 WP at each respective concentration was found to be statistically superior to all test fungitoxicants. The data further reveals that the mancozeb 75 WP proved significantly most effective by providing cent per cent germination inhibition at 1500 ppm concentration.

Table - 8: Effect of different systemic fungicides on spore germination of *Phaeoisariopsis griseola* (Sacc.) Ferraris

Fungitoxicant	Trade name	Inhibition of spore germination (%)			
		Concentration (ppm)			
		100	300	500	Mean
Carbendazim 50 WP	Bavistin	92.27 (73.83)*	100 (89.39)	100 (89.39)	97.41 (84.20)
Myclobutanil 10 WP	Grapple	64.15 (53.21)	81.71 (64.67)	100 (89.39)	81.95 (69.09)
Tebuconazole 25 WP	Caviet	78.49 (62.36)	85.94 (67.96)	100 (89.39)	88.14 (73.24)
Metiram 55 WG + Pyraclostrobin 5 WG	Cabrio Top	59.88 (50.68)	73.74 (59.17)	93.41 (75.11)	75.67 (61.65)
Trifloxystrobin 25 WG + Tebuconazole 50 WG	Nativo	54.30 (47.45)	66.23 (54.45)	86.62 (68.52)	69.05 (56.81)
Hexaconazole 5 EC	Anvil	58.72 (50.00)	69.21 (56.28)	87.46 (69.24)	71.80 (58.51)
Control		0.00 (0.57)	0.00 (0.57)	0.00 (0.57)	0.00 (0.57)
Mean		58.26 (48.30)	68.11 (56.07)	81.06 (68.80)	

C.D (p≤0.05)

Fungicide 0.95

Concentration 0.62

Fungicide×Concentration 1.64

*Figures in the parenthesis are arcsine transformed values

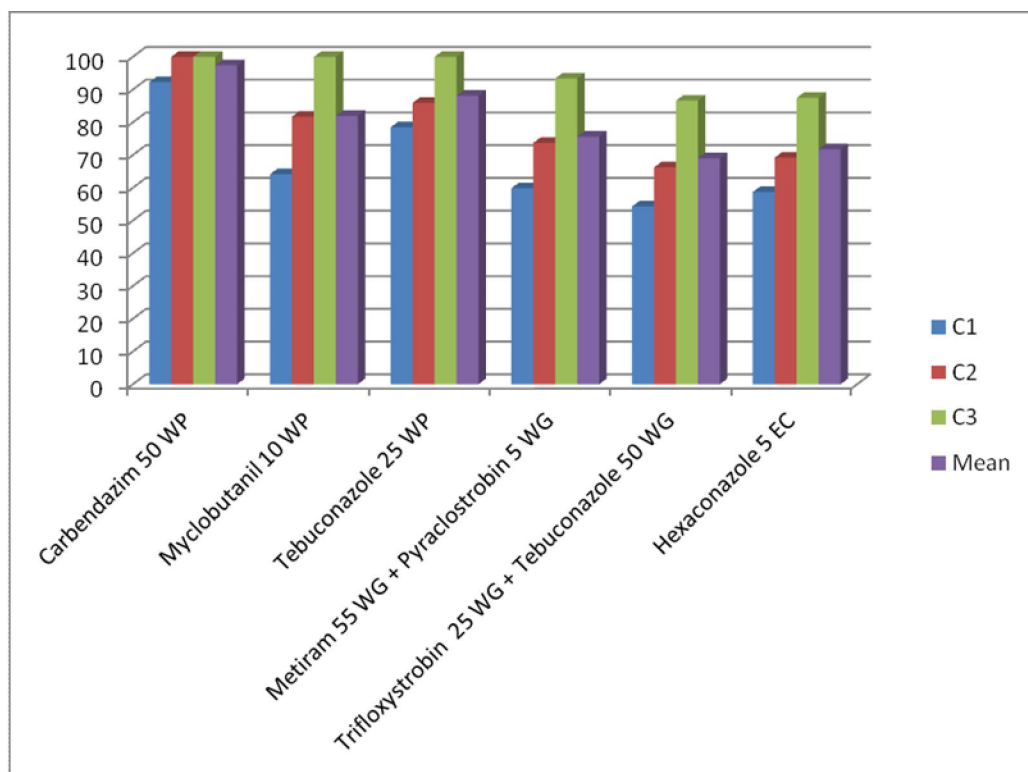


Figure 3: Effect of different systemic fungicides on spore germination of *Phaeoisariopsis griseola* (Sacc.) Ferraris

On an overall basis, the inhibitory effect of fungitoxicants increased with increase in concentration of fungitoxicants. A minimum inhibition of 42.61 per cent was achieved at 500ppm which increased gradually to 62.74 per cent at 1500 ppm concentration.

4.3.2 *In vivo* evaluation of fungicides

Factorial experiment in RCBD was conducted during 2017-18 to study the combined effect of seed treatment and foliar sprays of those fungitoxicants that proved most effective in inhibiting spore germination of the test fungus under *in vitro* evaluation (Plate-10). Among the systemic and non systemic, the most effective four fungitoxicants were selected for foliar sprays (Carbendazim 50 WP, Tebuconazole 25 WP, Myclobutanil 10 WP and Mancozeb 75 WP) and one from systemic (Carbendazim 50 WP) and one from non-systemic (Mancozeb 75 WP), that proved most effective were selected for seed treatment. The results on disease incidence, disease intensity and pod yield of Shalimar French Bean-1 are presented in Table 10.

Data on effect of various seed treatments and foliar sprays on incidence of angular leaf spot of beans under field conditions during 2017-18 (Table-10) reveal that all the seed treatments and foliar sprays and their combinations were superior over the control in controlling the disease incidence. In the experiment the disease incidence ranges from 9.48 to 52.13 per cent. Lowest disease incidence of 9.48 per cent was recorded in treatment combination of seed treatment with Carbendazim 50 WP @ 0.1 per cent + foliar spray of Carbendazim 50 WP @ 0.05 per cent and proved superior over all other treatments. Highest disease incidence of 52.13 per cent was observed in treatment combination of seed treatment with sterile distilled water + foliar spray with SDW (Control). Other treatment combinations which followed in decreasing order of efficacy were seed treatment with Mancozeb 75 WP @ 0.3 per cent + foliar spray with

Table -9: Effect of different non-systemic fungicides on spore germination of *Phaeoisariopsis griseola* (Sacc.) Ferraris

Fungitoxicant	Trade name	Inhibition of spore germination (%)			
		Concentration (ppm)			
		500	1000	1500	Mean
Mancozeb 75 WP	Dithane M-45	77.63 (61.77)*	82.24 (65.06)	100 (89.39)	86.62 (72.07)
Chlorothalonil 75 WP	Kavach	51.11 (45.62)	64.16 (53.21)	78.91 (62.66)	64.73 (53.83)
Copper oxychloride 50 WP	Blue copper	43.60 (41.30)	56.13 (48.50)	74.93 (59.94)	58.22 (49.91)
Captan 50 WP	Kohicap	40.70 (39.62)	53.19 (46.81)	59.88 (50.68)	51.26 (45.70)
Control		0.00 (0.57)	0.00 (0.57)	0.00 (0.57)	0.00 (0.57)
Mean		42.61 (37.77)	51.15 (42.83)	62.74 (52.65)	

C.D (p≤0.05)

Fungicide 1.28

Concentration 0.99

Fungicide×Concentration 2.23

*Figures in the parenthesis are the arcsine transformed values

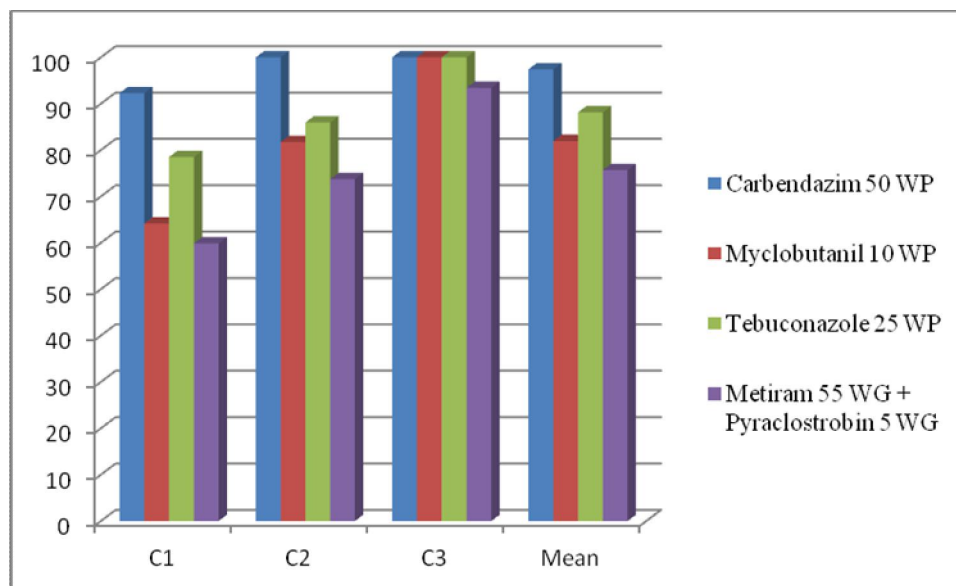
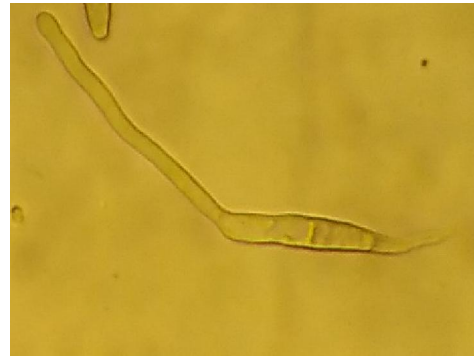


Figure 4: Effect of different non systemic fungicides on spore germination of *Phaeoisariopsis griseola* (Sacc.) Ferraris



(a)



(b)

Plate-9: Spore germination inhibition of *Phaeoisariopsis griseola* (Sacc.) Ferraris

(a) Under carbendazim

(b) Under control

Carbendazim 50 WP @ 0.05 per cent giving an incidence of 10.02 per cent, seed treatment with Carbendazim 50 WP @ 0.1 per cent + foliar spray with Tebuconazole 25 WP @ 0.025 per cent (11.15%), seed treatment with Carbendazim 50 WP @ 0.1 per cent + foliar spray with Myclobutanil 10 WP @ 0.05 per cent (12.35%), seed treatment with Mancozeb 75 WP @ 0.3 per cent + foliar spray with Tebuconazole 25 WP @ 0.025 per cent (12.56%), seed treatment with Mancozeb 75 WP @ 0.3 per cent + foliar spray with Myclobutanil 10 WP @ 0.05 per cent (13.38%), seed treatment with Carbendazim 50 WP @ 0.1 per cent + foliar spray with Mancozeb 75 WP @ 0.25 per cent (18.52%) and seed treatment with Mancozeb 75 WP @ 0.3 per cent + foliar spray with Mancozeb 75 WP @ 0.25 per cent (19.02%), respectively. Amongst the foliar sprays alone, foliar spray with Carbendazim 50 WP @ 0.05 per cent proved best followed by Tebuconazole 25 WP @ 0.025 per cent, Myclobutanil 10 WP @ 0.05 per cent and Mancozeb 75 WP @ 0.25 per cent in management of angular leaf spot disease of beans compared to other foliar sprays. In the experiment, disease incidence of foliar sprays was observed lowest in case of Carbendazim 50 WP @ 0.05 per cent (15.69%) followed by Tebuconazole @ 0.025 per cent (19.29%), Myclobutanil 10 WP @ 0.05 per cent (20.22%) and Mancozeb 75 WP @ 0.25 per cent (27.45%).

Further it was observed that amongst the seed treatments, treatment with Carbendazim 50 WP @ 0.1 per cent proved superior than other seed treatments. Disease incidence in case of seed treatments, was observed least in Carbendazim 50 WP @ 0.1 per cent (30.37%) followed by Mancozeb 75 WP @ 0.3 per cent (31.14%).

Data on effect of various seed treatments and foliar sprays on intensity of angular leaf spot of beans under field conditions during 2017 revealed that, all the seed treatments and foliar sprays and their combinations were superior over the control in controlling the disease. In the experiment the disease intensity ranges from 3.26 to 26.22 per cent. Lowest disease intensity of 3.26 per cent was

recorded in treatment combination of seed treatment with Carbendazim 50 WP @ 0.1 per cent + foliar spray of Carbendazim 50 WP @ 0.05 per cent and proved superior over all other treatments. Highest disease intensity of 26.22 per cent was observed in treatment combination of seed treatment with sterile distilled water + foliar spray with SDW (Control). Other treatment combinations which followed in decreasing order of efficacy were seed treatment with Mancozeb 75 WP @ 3g kg⁻¹ of seed + foliar spray with Carbendazim 50 WP @ 0.05 per cent exhibiting an intensity of 3.73 per cent, seed treatment with Carbendazim 50 WP @ 0.1 per cent + foliar spray with Tebuconazole 25 WP @ 0.025 per cent (4.39%), seed treatment with Carbendazim 50 WP @ 0.1 per cent + foliar spray with Myclobutanil 10 WP @ 0.05 per cent (5.20%), seed treatment with Mancozeb 75 WP @ 0.3 per cent + foliar spray with Tebuconazole 25 WP @ 0.025 per cent (5.58%), seed treatment with Mancozeb 75 WP @ 0.3 per cent + foliar spray with Myclobutanil 10 WP @ 0.05 per cent (6.18%), seed treatment with Carbendazim 50 WP @ 0.1 per cent + foliar spray with Mancozeb 75 WP @ 0.25 per cent (7.35%) and seed treatment with Mancozeb 75 WP @ 0.3 per cent + foliar spray with Mancozeb 75 WP @ 0.25 per cent (8.24%), respectively. Amongst the foliar sprays alone, foliar spray with Carbendazim 50 WP @ 0.05 per cent proved best followed by Tebuconazole 25 WP @ 0.025 per cent, Myclobutanil 10 WP @ 0.05 per cent and Mancozeb 75 WP @ 0.25 per cent in management of angular leaf spot disease of beans compared to other foliar sprays. In the experiment, disease intensity of foliar sprays was observed lowest in case of Carbendazim 50 WP @ 0.05 per cent (6.36%) followed Tebuconazole 25 WP@ 0.025 per cent (9.79%), Myclobutanil 10 WP @ 0.05 per cent (10.31%) and Mancozeb 75 WP @ 0.25 per cent (11.35%).

Further it was observed that amongst the seed treatments, treatment with Carbendazim 50 WP @ 0.1 per cent proved superior than other seed treatments. Disease intensity in case of seed treatments, was observed least in Carbendazim 50 WP @ 0.1 per cent (15.11%) followed by Mancozeb 75 WP @ 0.3 per cent

(16.86%).

The results regarding pod yield revealed that all the treatments had significant effect on pod yield of bean over control. Among the foliar sprays, foliar spray with Carbendazim 50 WP @ 0.05 per cent proved to be most effective as the average pod yield was recorded to 93.42 q ha⁻¹ indicating an increase of 28.5 per cent compared to control exhibiting on an average pod yield to 72.7 q ha⁻¹, followed by Tebuconazole 75 WP @ 0.025 per cent, Myclobutanil 10 WP @ 0.05 per cent and Mancozeb 75 WP @ 0.25 per cent exhibiting pod yield of 91.65, 89.01 and 86.64 q ha⁻¹ indicating an increase of 26.07, 22.43 and 19.17 per cent, respectively. Among the seed treatment Carbendazim 50 WP @ 0.1 per cent proved to be most effective over control as the average pod yield was recorded to 80.01 q ha⁻¹ indicating an increase of 10.05 per cent compared to check followed by Mancozeb 75 WP @ 0.3 per cent exhibiting on an average pod yield to 78.52 q ha⁻¹ indicating an increase of 17.24 per cent. A significant interaction existed between the foliar sprays and seed treatments. Among the interactions foliar spray with Carbendazim 50 WP @ 0.05 per cent + seed treatment with Carbendazim 50 WP @ 0.1 per cent proved to be most effective over control exhibiting on an average pod yield yield to 102.08 q ha⁻¹ followed by foliar spray with Carbendazim 50 WP @ 0.05 per cent + seed treatment with Mancozeb 75 WP @ 0.3 per cent (100.08 q ha⁻¹), foliar spray with Tebuconazole 25 WP @ 0.02% per cent + seed treatment with Carbendazim 50 WP @ 0.1 per cent (98.72%) and folair spray with Myclobutanil 10 WP @ 0.05 per cent + seed treatment with Carbendazim 50 WP @ 0.1 per cent (96.4 q ha⁻¹) indicating an increase of 40.41, 37.66, 35.79 and 32.5 per cent respectively. Foliar spray with Tebuconazole 25 WP @ 0.025 per cent + seed treatment with Mancozeb 75 WP @ 0.3 per cent exhibited on an average yield of 95.52 q ha⁻¹ followed by spray with Myclobutanil 10 WP @ 0.05 per cent + seed treatment with Mancozeb 75 WP @ 0.3 per cent (94.74 q ha⁻¹), folair spray with Mancozeb 75 WP @ 0.25% + seed treatment with Carbendazim 50 WP @ 0.1 per cent (92.25 q ha⁻¹) sand folair spray with

Mancozeb 75 WP @ 0.25 per cent + seed treatment with Mancozeb 75 WP @ 0.3 per cent (90.27%) indicating an increase of 31.38, 30.31, 26.89 and 24.17 per cent, respectively.

The combined treatments, *viz.*, foliar spray + seed treatment were significantly superior over individual treatments. The results thus explain the viability of fungicides for the management of angular leaf spot of beans.



Plate-10: Management of angular leaf spot of beans during kharif 2017-18

Table -10: Management of angular leaf spot of beans during *kharif* 2017-18

S.No	Treatment Details	Disease Incidence (%)	Disease Intensity (%)	Pod Yield (q ha ⁻¹)
T1	Control	52.13 (7.29)*	26.22 (5.22)	72.7
T2	Foliar spray with Carbendazim 50 WP @ 0.05%	15.69 (4.08)	6.36 (2.71)	93.42
T3	Foliar spray with Tebuconazole 25 WP @ 0.025%	19.29 (4.5)	9.79 (3.28)	91.65
T4	Foliar spray with Myclobutanil 10 WP @ 0.05%	20.22 (4.61)	10.31 (3.36)	89.01
T5	Foliar spray with Mancozeb 75 WP @ 0.25%	27.45 (5.33)	11.35 (3.51)	86.64
T6	Seed treatment with Carbendazim 50 WP @ 0.1%	30.37 (5.6)	15.11 (4.01)	80.01
T7	Seed treatment with Carbendazim 50 WP @ 0.1% + Foliar spray with Carbendazim 50 WP @ 0.05%	9.48 (3.24)	3.26 (2.06)	102.08
T8	Seed treatment with Carbendazim 50 WP @ 0.1% + Foliar spray with Tebuconazole 25 WP @ 0.025%	11.15 (3.48)	4.39 (2.32)	98.72
T9	Seed treatment with Carbendazim 50 WP @ 0.1% + Foliar spray with Myclobutanil 10 WP @ 0.05%	12.35 (3.65)	5.2 (2.49)	96.4
T10	Seed treatment with Carbendazim 50 WP @ 0.1% + Foliar spray with Mancozeb 75 WP @ 0.25%	18.52 (4.42)	7.35 (2.89)	92.25
T11	Seed treatment with Mancozeb 75 WP @ 0.3%	31.14 (5.67)	16.86 (4.22)	78.52
T12	Seed treatment with Mancozeb 75 WP @ 0.3% + Foliar spray with Carbendazim 50 WP @ 0.05%	10.02 (3.32)	3.73 (2.17)	100.08
T13	Seed treatment with Mancozeb 75 WP @ 0.3% + Foliar spray with Tebuconazole 25 WP @ 0.025%	12.56 (3.79)	5.58 (2.56)	95.52
T14	Seed treatment with Mancozeb 75 WP @ 0.3% + Foliar spray with Myclobutanil 10 WP @ 0.05%	13.38 (3.79)	6.18 (2.68)	94.74
T15	Seed treatment with Mancozeb 75 WP @ 0.3% + Foliar spray with Mancozeb 75 WP @ 0.25%	19.02 (4.47)	8.24 (3.04)	90.27

C.D (p≤0.05)**0.09****0.033****1.21**

*Figures in parenthesis are square root transformed values

Chapter –5

DISCUSSION

Common bean (*Phaseolus vulgaris* L.) belonging to the family leguminaceae is the third most important food legume crop worldwide after soybean and peanut in the production (Mongi, 2016) and is popular due to its quality proteins and nutritional balance. But common bean cultivation also faces serious economic threats due to various diseases. Among all angular leaf spot disease is an important production constraint in the profitable cultivation of beans (*Phaseolus vulgaris* L.) in Kashmir. It is caused by an important fungal pathogen, *Phaeoisariopsis griseola* (Sacc.) Ferraris and occur in mild to severe form in different parts of the world and also in the Himalayan plains in India (Srinivasan, 1953; Weaver and Zaumeyer, 1956; Sohi *et al.*, 1963; Cole, 1966; Hagedorn and Wade, 1974; Saettler and Correa, 1988; Allen and Lenne, 1998; Melzer *et al.*, 2001). In the state of Jammu and Kashmir too the disease occurs consistently in one or the other part of the valley causing considerable yield losses (Kotwal, 1994). Keeping in view the economic importance of the disease, research gaps and meagre information available in literature on different aspects, it was thought worthwhile to investigate the disease with objectives as outlined in the introduction chapter. Establishing the disease status is pre-requisite to decide the adoption of management strategies. The lack of information on status, symptomatology, *in vitro* and *in vivo* evaluation of different fungicides made an impetus to undertake the present investigation.

In the present study, survey conducted in three districts of Kashmir valley during *kharif* 2017, indicated that angular leaf spot of beans was more or less prevalent in all the locations of the districts. The average disease incidence was highest in district Baramullah with 59.20 and 38.76 per cent on leaves and pods, respectively. The disease incidence at various locations revealed that maximum disease incidence was recorded at Sopore with 65.61 and 45.27 per cent on leaves

and pods, respectively, while lowest disease incidence was recorded in Kulgam with 25.90 and 14.86 per cent on leaves and pods, respectively. Among the different sites surveyed highest disease incidence was recorded at Arampora with 68.91 and 48.50 per cent, respectively, on leaves and pods, while as the lowest disease incidence was recorded at Banimullah with 22.50 and 12.50 per cent incidence, respectively, on leaves and pods.

Results about the disease intensity revealed that district Baramulla was having highest disease intensity of 30.94 and 18.14 per cent on leaves and pods, respectively, followed by Budgam with 24.89 and 12.62 per cent, respectively, on leaves and pods, while as lowest disease intensity was recorded in Kulgam with 20.07 and 9.63 per cent on leaves and pods, respectively. On comparing different locations it was observed that highest disease intensity was found in Sopore with 34.94 and 20.42 per cent on leaves and pods, respectively, while as lowest intensity was observed in Kulgam with 17.16 and 7.00 per cent on leaves and pods, respectively. Among the different sites surveyed highest disease intensity was recorded at Arampora with 38.33 and 21.86 per cent on leaves and pods, respectively, while as the lowest disease intensity was recorded at Banimullah site of district Kulgam with 15.14 and 6.31 per cent on leaves and pods, respectively. More disease incidence and intensity in North Kashmir could probably be attributed to use of contaminated seeds, use of susceptible local varieties, improper sanitary measures, no use of fungicidal sprays, frequent rains alternating with dry periods leading to more humid conditions which are essential for pathogen dispersal. Survey results are well supported with that of Kotwal (1994). He recorded 92.32 per cent incidence and 55 per cent intensity of angular leaf spot on French bean cultivar contender in Jammu and Kashmir State. The survey and surveillance studies conducted by Bhat (2005) in three districts of Kashmir valley revealed that disease incidence on pods during 2002 and 2003 cropping seasons was 48.06 and

60.06 per cent with intensities of 20.45 and 32.75 per cent, respectively. Similarly on leaves, the angular leaf spot incidence during 2002 and 2003 was 62.79 and 77.50 per cent with intensities of 29.06 and 38.78 per cent, respectively. The survey and surveillance studies conducted by Buruchara *et al.* (2007) in Kenya revealed the moderate (33%) to high (52.9%) disease severity of angular leaf spot of bean in the fields. Similar results were reported by Bassaniezi *et al.* (1998) and Mathew *et al.* (1998). Pamela *et al.* (2014) recorded the incidence and severity of angular leaf spot in five bean agro-ecologies within Uganda was between 40-99 per cent and 21-80 per cent, respectively. Kijana *et al.* (2017) in Eastern Republic of Congo, recorded severity and incidence of angular leaf-spot and other occurring diseases on common bean plants in farmers' fields. Angular leaf spot in these fields had an average severity index (PSI) of 49.9 per cent.

The symptoms of the disease appeared as angular light brown lesions surrounded by yellow halo on first trifoliate leaves. On other trifoliate leaves angular light brown lesions of small size appear initially confined to tissue between major veins with synnemata of the pathogen emerging from the abaxial surface of leaf. The symptoms of the disease first start appearing from the lower surface of both primary as well as trifoliate leaves. Later the lesions become darker and coalesce to cover larger areas on leaves resulting in excessive chlorosis and premature leaf fall. On pods the symptoms of the disease appeared first as small oval to circular brown spots which later become circular reddish brown in centre with well defined ashy black edges and coalesce to cover larger areas on pods. Lesions on stem were dark brown and elongated. The characteristic symptoms of disease as observed on leaves, stems and pods in present investigations are in accordance with those described by earlier workers (Miles, 1917; Skoric, 1930; Zaumeyer and Thomas, 1957; Barros *et al.*, 1958a; Hocking, 1967; Hagedorn and Wade, 1974; Sharma and Sohi, 1978; Monda *et al.*, 2001; Stenglein and Balatti, 2006). The isolation of the pathogen associated with angular leaf spot of French bean was made on V-8 agar juice media from necrotic

lesions of infected bean leaves and circular reddish brown lesions of pods under aseptic conditions.

The morphological characteristics of the fungus, studied on V-8 juice agar media, revealed that the mycelium was septate, irregularly branched, subhyaline measuring 3.1-4.0 μm in diameter, conidiophores were olivaceous brown in colour, synnematosus, filiform, septate and measured about 1.8-4.6 \times 95-159 μm , conidia were cylindrical to obclavate, olivaceous grey to olive brown in colour, 1-3 septate and about 2.7-5.3 \times 24.0-37.6 μm in size. The morphological characteristics of the pathogen are more or less, the same as described by Benlloch, 1944; Lianos, 1957; Hocking, 1967; Ellis, 1971; Sindhan and Bose, 1972; Karanja *et al.*, 1994. The test fungus *Phaeoisariopsis griseola* was used in spore cum mycelium inoculation to test pathogenicity that revealed peculiar disease symptoms appeared after 12 days of inoculation as angular shaped brown or grey lesions delimited by veins and veinlets, later becoming necrotic. On trifoliolate leaves, the symptoms appeared first on the lower leaf surface as light grey lesions. The pathogenicity studies of angular leaf spot disease on French bean conducted by Srinivasan (1953), Olave (1958) and Mathew (1999) have also revealed the similar symptom development on leaves. Isolation was made from the infected leaves. The culture was purified by single spore technique. On the basis of morphological and colony characteristics, the fungus was identified as *Phaeoisariopsis griseola* (Sacc) Ferraris.

Besides eradication, fungicides also provide a chemical toxic barrier against pathogens and thus are unavoidable means of controlling many plant diseases. *In vitro* evaluation of some new molecules of fungicides along with conventional fungicides was studied to work out the relative efficacies. In present investigation, six systemic and four non systemic fungitoxicants were screened *in vitro* by spore germination inhibition technique. The evaluated fungitoxicants

revealed that all the fungitoxicants inhibited spore germination of *Phaeoisariopsis griseola* at various concentrations.

Among the systemic fungitoxicants Carbendazim 50 WG proved to be significantly effective at each respective concentration by exhibiting mean germination inhibition of 97.41 per cent, followed by Tebuconazole 25 WP (88.14 %), and Myclobutanil 10 WP (81.95%). These fungitoxicants at 500 ppm were highly effective in spore germination inhibition. These fungitoxicants were also effective even at least concentration (100 ppm) and provided more than 60 per cent inhibition. Trifloxystrobin 25 WG + Tebuconazole 50 WG was least effective exhibiting spore germination of 69.05 per cent. Carbendazim 50 WP, Tebuconazole 25 WP and Myclobutanil exhibited cent per cent inhibition at 500 ppm, while as Carbendazim 50 WP exhibited cent percent inhibition even at 300 ppm.

All the non-systemic fungitoxicants inhibited spore germination of *Phaeoisariopsis griseola* in comparison to control. *In vitro* screening of non systemic fungitoxicants revealed that Mancozeb 75 WP was significantly effective at all the concentrations by exhibiting mean germination inhibition of 86.62 per cent followed by Chlorothalonil 75 WP, Copper oxychloride 50 WP and Captan 50 WP exhibiting 64.73, 58.22 and 51.26 per cent inhibition respectively. However, the effect of test fungitoxicants increased with increase in concentration. In overall comparison mancozeb 75 WP exhibited cent per cent germination inhibition at 1500 ppm. The available literature on different fungicide has also revealed the efficacy of Carbendazim, Mancozeb, Tebuconazole and Myclobutanil against the *Phaeoisariopsis griseola*. *In vitro* work done by Mathews (1999) on spore germination inhibition of *Phaeoisariopsis griseola* also revealed that Bavistin (Carbendazim 50 Wp) and Indofil M 45 (Mancozeb 75 WP) effectively caused reduction in spore germination. Gupta and Shyam (1996) while evaluating the *in vitro* efficacy of different fungicides against *P. griseola* reported

high inhibitory effect of Bavistin (Carbendazim 50 Wp) and Punch. Canteri *et al.* (1998a) found Difenconazole and Tebuconazole effective, with an efficiency of 95 and 74 per cent, respectively.

Management of any disease is of paramount importance after *in vitro* evaluation. The *in vivo* studies of fungitoxicants revealed that all treatments significantly reduced the disease intensity as compared to control. However, the magnitude of reduction varied from treatment to treatment. The most effective fungicides evaluated *in vitro* were further evaluated under field conditions against angular leaf spot of beans. The present study undertaken to evaluate the efficacy of fungicides *viz.*, seed treatments and foliar sprays on angular leaf spot of beans were carried under field conditions using Carbendazim 50 WP @ 0.1 per cent and Mancozeb 75 WP @ 0.3 per cent as seed treatments and Carbendazim 50 WP @ 0.05 per cent, Tebuconazole 25 WP @ 0.025 per cent, Myclobutanil 10 WP @ 0.05 per cent and Mancozeb 75 WP @ 0.25 per cent as foliar sprays. It was observed that all the treatment combinations significantly reduced the disease incidence and disease intensity to a level significantly lower than that of control and enhanced pod yield significantly more than that of the control. Results revealed that the treatment combination of seed treatment with Carbendazim 50WP @ 0.1 per cent and foliar spray with Carbendazim 50 WP @ 0.05 per cent proved best, which gave least disease incidence (9.48%), disease intensity (3.26%), as against control, seed treatment and foliar spray with SDW, recording highest incidence (52.13%) and intensity (26.22%), respectively. Highest pod yield of 102.08 q ha⁻¹ was also recorded in treatment combination of seed treatment with Carbendazim 50 WP @ 0.1 per cent and foliar spray with Carbendazim 50 WP @ 0.05 per cent and lowest fruit yield of 72.7 q ha⁻¹ was recorded in case of control. Other treatment combinations, which proved effective, were seed treatment with Mancozeb 75 WP @ 0.3 per cent + foliar spray with Carbendazim 50 WP @ 0.05 per cent, seed treatment with Carbendazim 50 WP @ 0.1 per cent + foliar spray with Tebuconazole 25 WP @ 0.025 per cent, seed

treatment with Carbendazim 50 WP @ 0.1 per cent + foliar spray with Myclobutanil 10 WP @ 0.05 per cent ,seed treatment with Mancozeb 75 WP @ 0.3 per cent + foliar spray with Tebuconazole 25 WP @ 0.025 per cent, seed treatment with Mancozeb 75 WP @ 0.3 per cent + foliar spray with Myclobutanil 10 WP @ 0.05 per cent, seed treatment with Carbendazim 50 WP @ 0.1 per cent + foliar spray with Mancozeb 75 WP @ 0.25 per cent and seed treatment with Mancozeb 75 WP @ 0.3 per cent + foliar spray with Mancozeb 75 WP @ 0.25 per cent.

Amongst the chemicals used for foliar sprays, Carbendazim 50 WP proved best followed by Tebuconazole 25 WP, Myclobutanil 10 WP and Mancozeb 75 WP, respectively. Amongst seed treatments, seed treatment with Carbendazim 50 WP proved best followed by Mancozeb 75 WP. Bharadwaj and Thakur (1992) reported that seed dressing with Carbendazim followed by foliar sprays at 15 days interval as effective to manage angular leaf spot disease. Bhat (2002) revealed the high efficacy of Carbendazim (0.1%) sprayed at 10 days interval. Work done by Shukla and Sharma (2009) revealed that four sprays of Carbendazim (0.1%) at 10 days interval restricted the disease significantly. The studies of various workers have also reported the efficacy of these fungitoxicants for controlling angular leaf spot disease of beans (De-Castro *et al.*, 1991; Fortugno, 1977; Schwartz and Galvez, 1980; Sindhan, 1984; Srivastava and Gupta, 1994; Vierra *et al* 1998; Ploper *et al.*, 2002) thereby corroborate the present work.

Chapter – 6

SUMMARY AND CONCLUSION

The investigations on “Status and Management of Angular Leaf Spot of Beans (*Phaseolus vulgaris* L.) caused by *Phaeoisariopsis griseola* (Sacc.) Ferraris” was carried out at Faculty of Agriculture SKUAST-K during 2017. The present investigations on angular leaf spot of French bean (*Phaseolus vulgaris*) were undertaken with regard to survey, symptomatology, etiology, pathogenicity and management of the disease through chemical means.

Angular leaf spot is more or less prevalent in almost every vegetable field of Kashmir valley with varied incidence and intensity from place to place. The survey of the bean growing areas for the disease in district Baramulla, Budgam and Kulgam revealed that irrespective of location the prevalence of angular leaf spot in all districts surveyed with overall incidence of 42.44 and 27.62 per cent on leaves and pods and intensity of 25.30 and 13.46 per cent on leaves and pods, respectively. The maximum angular leaf spot incidence of 59.20 and 38.76 per cent on leaves and pods, respectively, and intensity of 30.94 and 18.14 per cent on leaves and pods, respectively, was observed in district Baramulla and minimum leaf spot incidence of 30.40 and 18.62 per cent on leaves and pods, respectively, and intensity of 20.07 and 9.63 on leaves and pods, respectively, was observed in district Kulgam.

Amongst the locations surveyed, Sopore of district Baramulla exhibited highest leaf spot incidence of 65.61 and 45.27 per cent on leaves and pods, respectively and intensity of 34.94 and 20.42 per cent on leaves and pods, respectively, whereas the least incidence of 25.90 and 14.86 per cent on leaves and pods, respectively and intensity of 17.16 and 7.00 per cent on leaves and pods respectively, was observed in Kulgam of district Kulgam.

Under natural conditions, the disease symptoms first appeared on primary leaves as circular greyish lesions with concentric rings and first trifoliolate leaves as angular greyish brown lesions surrounded by a yellow halo, starting from the second week of June. However, characteristic symptoms appeared from first week of July on other trifoliolate leaves as light brown lesions, initially confined to tissue between major veins, giving an angular appearance, measuring 0.1-0.3 cm in dia, with synnemata emerging from abaxial surface of leaves. The spots gradually enlarged in size and numerous spots coalesced to form larger irregular patches. The weekly changes in size, shape and colour of the lesions were noticed and lesions finally enlarged upto 2 cm in dia. Later coalescing of lesions result in excessive chlorosis and the premature defoliation of leaves. On pods usually smooth, circular, superficial spots having reddish brown centre with well defined ashy black borders were observed that attained maximum size of 2.25 cm. On stem elongated dark brown lesions appeared.

Fungus isolated from naturally infested bean leaves was tested for pathogenicity established through Koch's postulates. The culture of the pathogen produced characteristic disease symptoms after 12 days of inoculation. Therefore, pathogen was reisolated on V-8 juice agar medium and incubated at $25\pm 1^{\circ}\text{C}$. The pathogen was found pathogenic on beans and later identified as *Phaeoisariopsis griseola* (Sacc) Ferraris.

The fungus grows slowly and produced colonies that were irregular, erumpent with moderate aerial mycelium. The fungal colony was initially light grey then turned dark olivaceous grey and finally black after 15 days of incubation. The mycelium was irregularly branched, septate, subhyaline and measured 3.1-4.0 μm in width. Conidiophores were synnematos, septate, branched, olivaceous brown in colour, measuring 95-159 μm in length and 1.8-4.6 μm in width. Conidia were cylindrical to obclavate and size varied from 24.0-37.6 μm long and 2.7-5.3 μm wide. The septation of conida varied from 1-3. On

the basis of the morphological characteristics of conidiophores and conidia, the pathogen was identified as *Phaeoisariopsis griseola* (Sacc) Ferraris.

Fungitoxicants both systemic and non systemic were evaluated at different concentrations for their *in vitro* efficacy in inhibiting spore germination of *Phaeoisariopsis griseola*. Among systemic fungitoxicants Carbendazim 50 WP proved to be highly effective in inhibiting the spore germination. The other fungitoxicants in decreasing order of their efficacy were Tebuconazole 25 WP > Myclobutanil 10 WP > Metiram 55 WG + Pyraclostrobin 5 WG > Hexaconazole 5 EC > Trifloxystrobin 25 WG + Tebuconazole 50 WG. Among non systemic fungitoxicants Mancozeb 75 WP proved to be highly effective in inhibiting the spore germination. The other fungitoxicants in decreasing order of their efficacy were Chlorothalonil 75 WP > Copper oxychloride 50 WP > Captan 50 WP. With the increase in concentration of fungitoxicants from 100 to 500 ppm spore germination inhibition of test fungus in case of systemic fungitoxicants increased from 58.26 to 81.06 per cent. While as in case of non-systemic fungitoxicants with the increase in concentration from 500 to 1500 ppm, spore germination inhibition increased from 42.61 to 62.74 per cent.

Studies were conducted to control angular leaf spot of beans under field conditions with seed treatment and foliar sprays in combinations. It was observed that all the treatment combinations restrained disease incidence and intensity to a level significantly lower than that of control and enhanced pod yield. The treatment combinations of seed treatment with Carbendazim 50 WP @ 0.05 per cent proved best, which gave least disease incidence (9.48%) and disease intensity (3.26%) as against control, seed treatment and foliar spray with sterile distilled water, recording highest incidence (52.13%) and intensity (26.22%), respectively. Highest pod yield of 102.08 q ha⁻¹ was also recorded in treatment combination of seed treatment with Carbendazim 50 WP @ 0.1 per cent and foliar spray with Carbendazim 50 WP @ 0.05 per cent and lowest fruit yield of 72.7 q ha⁻¹ was

recorded in case of control. Other treatment combinations that proved effective, were seed treatment with Mancozeb 75 WP @ 0.3 per cent + foliar spray with Carbendazim 50 WP @ 0.05 per cent, seed treatment with Carbendazim 50 WP @ 0.1 per cent + foliar spray with Tebuconazole 25 WP @ 0.025 per cent, seed treatment with Carbendazim 50 WP @ 0.1 per cent + foliar spray with Myclobutanil 10 WP @ 0.05 per cent, seed treatment with Mancozeb 75 WP @ 0.3 per cent + foliar spray with Tebuconazole 25 WP @ 0.025 per cent, seed treatment with Mancozeb 75 WP @ 0.3 per cent + foliar spray with Myclobutanil 10 WP @ 0.05 per cent, seed treatment with Carbendazim 50 WP @ 0.1 per cent + foliar spray with Mancozeb 75 WP @ 0.25 per cent and seed treatment with Mancozeb 75 WP @ 0.3 per cent + foliar spray with Mancozeb 75 WP @ 0.25 per cent.

Amongst the chemicals used for foliar sprays, Carbendazim 50 WP proved most effective followed by Tebuconazole 25 WP, Myclobutanil 10 WP and Mancozeb 75 WP. Amongst seed treatments, seed treatment with Carbendazim 50 WP proved best followed by Mancozeb 75 WP.

The conclusions drawn from the present investigations are as follows:

- Angular leaf spot of beans is prevalent in all the vegetable growing areas of the three surveyed districts of Kashmir with varied degrees of severity.
- Disease appeared in the 2nd week of June and symptoms first appeared on primary and first trifoliate leaves.
- Disease appears around the time of pod formation (1st week of July) on other trifoliate leaves attaining maximum lesion size of 2 cm. Lesions were typically angular and brown in colour.
- The disease incitant was identified as *Phaeoisariopsis griseola* (Sacc.)

Ferraris.

- Amongst systemic fungitoxicants, Carbendazim 50 WP proved to be the most effective in inhibiting the spore germination of the test pathogen under *in vitro* conditions. Amongst non-systemic fungitoxicants, Mancozeb 75 WP proved to be the most effective in inhibiting the spore germination of the test pathogen under *in vitro* conditions.
- Under *in vivo* conditions seed treatment with Carbendazim 50 WP @ 0.1% of seed and foliar spray with Carbendazim 50 WP @ 0.05 per cent effectively managed the disease with lowest disease incidence of 9.48 per cent and intensity of 3.26 percent, respectively and highest pod yield of 102.08 q ha⁻¹, as compared to control plot showing highest disease incidence of 52.13 per cent and intensity of 26.22 per cent, respectively with lowest pod yield of 72.7 q ha⁻¹.
- Keeping in view the resistance developed by the pathogens against the systemic fungicides, it is recommended for the farmers to give seed treatment with carbendazim 50 WP @ 0.1% followed by two foliar sprays with Mencozeb 75 WP @ 0.25%. First foliar spray should be given with the appearance of disease and second foliar spray at an interval of 15 days.

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CERTIFICATE

Certified that all the corrections/amendments as suggested by external examiner Sajad-Un-Nabi, Scientist (Plant Pathology) ICAR-CITH (MOA & FW, GOI), Old Airfield Rangreth Srinagar (J&K) during viva-voce examination held on 29-01-2019 have been incorporated in the manuscript entitled “**Status and Management of Angular Leaf Spot of Beans (*Phaseolus vulgaris* L.) caused by *Phaeoisariopsis griseola* (Sacc.) Ferraris**” submitted by **Ms. Uzma Rashid (Regd. No. 2016-A-1114-M)**.

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