Studies on Anthracnose Disease of Walnut

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(2012-A-892-M)

Division of Plant Pathology

Faculty of Post-graduate Studies

Sher-e-KashmirUniversity of Agricultural Sciences and Technology of Kashmir

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2014

Dedicated

to my beloved Parents

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Division of Plant Pathology, Shalimar Campus Srinagar– 190 025
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Certificate – I

This is to certify that the thesis entitled, **"Studies on Anthracnose Disease of Walnut"** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science in Agriculture (Plant Pathology),** to the **Faculty of Post-graduate Studies, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir** is a record of bonafide research work carried out by **Mr. Mudasir Hassan (Regd. No. 2012-A-892-M)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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

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Certificate– II

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Certificate – III

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ABSTRACT

Walnut anthracnose is one of the most important disease of walnut (*Juglans regia* L.) worldwide. The present study was, therefore carried out to know the status of the disease in Kashmir valley and to evolve an effective management strategy. The disease is prevalent in all the walnut growing areas of Kashmir valley surveyed, during the year 2013. The overall mean disease incidence and intensity ranged from 71.78 to 97.34 per cent and 19.56 to 51.66 per cent on leaves and twigs, respectively. The maximum disease incidence of 97.94 and 74.66 per cent and intensity of 55.76 and 22.77 per cent on leaves and twigs, respectively was observed in district Anantnag and minimum disease incidence of 96.22 and 68.41 per cent and intensity of 49.03 and 15.32 per cent, respectively was observed in district kupwara. The pathogen associated with the disease was identified as *Marssonina juglandis* (Lib.) Magnus. In earlier stages of the disease development, spots are circular light brown slightly sunken which later on turned dark brown in colour with irregular margins.Maximum disease development in the field was observed during Augustand spots attained maximum size of 27-29 mm. The disease symptoms on the twigs initially appeared as dark brown oval lesions which later on turned black in colour with irregular margins attained maximum size of 26-27 mm. The pathogen produced white glaborus raised colony, which later turns creamy white to grayish white with well-defined lobate margins and concentric zones. Acervulus (113.48- 190.49µm) appears in concentric rings having hyaline guttulated, crescent conidia (23.58 x 10.13µm). Maximum mycelial growth was achieved on corn meal agar, where as potato dextrose agar proved to be best sporulating media for the isolated causal pathogen (*M. juglandis*). The fungus requires incubation period of 14-18 days to cause infection on potted walnut seedlings. The disease development under field conditions on leaves was found to be highly correlated with mean minimum temperature, followed by mean maximum temperature and mean minimum relative humidity, where as positive correlation with average rainfall on leaves, negative correlation with maximum relative humidity was observed. The disease development on twigs was found to be highly correlated with mean minimum temperature, followed by mean maximum temperature, where as it showed negative correlation with rainfall and maximum and minimum relative humidity. The pathogen perpetuated in the form of acervuli on both leaves and twigs and produced viable conidia up to the end of May. Among the twelve fungitoxicants tested *in vitro*, metiram + pyraclostrobin 60WG, tebuconazole 25EC, flusilazole 40EC, mancozeb 75WP, captan 50WP, copper oxychloride 50WP proved significantly superior in inhibiting the spore germination as well as mycelial growth of test fungus.

Key words : Walnut, anthracnose, symptamotology, disease development, perpetuation, management

Signature of Student

Dated: ___________

Signature of Major Advisor

Dated: ________________

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Mr. Mudasir Hassan

Place: Shalimar, Srinagar

Dated:

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Chapter-1

INTRODUCTION

Walnut (*Juglans regia* L.) is economically an important dry fruit crop which belongs to family *Juglandaceae*. It originated in Iran from where it was distributed throughout the world (Arora, 1985). It is mainly grown in china, USA and Iran, whereas India stands seventh in production accounting upto 2.14 per cent of the world walnut production (Anonymous, 2010). In India, walnut is grown in Jammu and Kashmir, Arunachal Pradesh, Himachal Pradesh and Uttarakhand. In J&K, Walnut is grown in Badrawah, Poonch, Kupwara, Baramulla, Bandipora, Ganderbal, Budgam, Srinagar, Anantnag and other hilly areas occupying an area of 83,219 ha with an annual production of 20,873 tonnes (Anonymous, 2012). Jammu and Kashmir State has attained a special place in the international trade of walnuts contributing about 98 per cent of the total production in India (Sharma, 2012). Its cultivation plays a significant role in the economic profile of the farmers living in hilly and backward areas, where economic condition of the people is extremely fragile. Exports of walnut from Jammu and Kashmir State earns substantial foreign exchange upto 231.63 crore (Anonymous, 2012).

Walnut fruit is consumed as a dry fruit and is also used for preparation of bakery products, confectionary and oils. Walnut shells are used in glue and plastics as well as in dusting and solution making for cleaning and polishing surfaces (Bal, 2006). Walnut wood and even its leaves are usable in wood and veneer industry, dying, pharmaceutical and food industries (Zamani *et al.*, 2011). Among all nuts, walnut fruit is rich in protein, oils including omega-3 fatty acids, vitamins and minerals with excellent flavor and rich source of energy (Rana *et al.*, 2007). Its alpha linolenic acid has substantial cardio protective effects as it increases the ratio of high-density lipoprotein cholesterol to total cholesterol, reducing inflammation and improving arterial function (Hu *et al.*, 1999; Diousse *et al.*, 2001; Patel, 2005). It contains ‘melatonin’ an antioxidant produced by pineal gland and responsible for inducting and regulating sleeps (Reiter *et al.*, 2005). It also reduces the incidence of cancer and, delays neurodegenerative diseases of aging (McGranahan and Leslie, 2012).

Although the agro-climatic conditions of Kashmir valley are congenial for cultivation of the walnut, yet its productivity has been relatively low compared to other parts of the world owing to many biotic and abiotic factors that inflict huge loss to walnut industry. Among the major biotic factors, the important fungal diseases include walnut anthracnose (*Marssonina juglandis* (Lib.) Magnus), root and crown rot [*Phytopthora cactorum* (Lebert and Cohn) Schrot], branch wilt [*Hendersonula toruloidea* Nattras], ringspot [*Ascochyta juglandis* Blotshauser.), downy leaf spot [*Microstroma juglandis* (Berenger) sacc.)], heart rot [*Polyporus squamosa* Huds. ex Fr.) Fr.], powdery mildew [*Phyllactinia quttata* (Wallr. ex Fr.) Lev.;*Microsphaera extensa* (Cke and Peck)] besides stem canker and die back diseases caused by fungi like *Cytosperma leucosperma* (Pers. ex Fr.) Fr., *Nectria galligena* (Bres.), *Fusarium solani* (Mart. Sacc), etc (Sharma and Sharma, 1999; Anonymous, 2013). Amongst them, anthracnose is the wide spread foliar disease of *Juglans* spp. and the fungus attacks leaves, nuts and shoots of the current season growth (Berry, 1977; Belisario *et al.*, 2008). Symptoms develop on the on leaves and fruits as irregular necrotic areas that are often surrounded by small chlorotic halos. The disease causes premature defoliation, slows down plant growth, reduces quantity and quality of nut crops, thereby resulting in huge economic loss in the walnut cultivation regions of the world (David, 1997; Belisario *et al.*, 2001; Van Sambeek, 2003; Kalkisim, 2012).

Although different aspects of walnut anthracnose have been studied in India and abroad, which include causal pathogen, etiology, host susceptibility, predisposing factors, disease management etc., yet little study on these aspects of the disease has been undertaken in the valley. Therefore, the proposed study on walnut anthracnose will be undertaken with following objectives:

1) To study the status of anthracnose disease of walnut in Kashmir.
2) To isolate and identify the pathogen associated with the disease.
3) To study the perpetuation of the pathogen associated with the disease.
4) To establish the correlation of disease development with meteorological factors.
5) To evaluate the *in vitro* efficacy of different fungitoxicants against the causal pathogen.

Chapter-2

REVIEW OF LITERATURE

Walnut (*Juglans regia*) an important commercial dry fruit crop, is attacked by several diseases causing economic damage and amongst them walnut anthracnose caused by *Marssonina juglandis* (Lib.) Magnus has posed a serious threat to this crop in India and abroad.

Although the disease is of great economic importance but a very limited work has been conducted in India and abroad and almost no work in Kashmir. In the present review, attempts have been made to include only those aspects, which has direct bearing on problem under investigation. For the sake of convenience the text of the review has been made under various heads as follows:

2.1 Occurrence and economic importance

Walnut anthracnose or black spot/blotch has been reportedly considered as most serious fungal disease of black walnut (*J. nigra* L.) and Persian or English walnut (*J. regia* L.) as well as other species of genus *Juglans* throughout the walnut growing regions including North and South America, Europe, Iran and other Asian countries (Behdad, 1991; Belisario, 2002; Belisario *et al.,* 2008; Salahi *et al.,* 2009). In India, Kaul (1962) reported the occurrence of walnut anthracnose disease for the first time from Kashmir valley. Hassan (1979) reported the occurrence of walnut anthracnose in Iraq while as, Werner (1994) reported from poland. It is indigenous to North America (Todhunter and Beineke, 1984), economically important in the main production areas of Italy and Hungary (Belisario, 1992; Pinter*et al.,* 2001) and most widespread and dangerous disease in Bulgaria (Tsanov and Roshev, 1976; Kalkism, 2012). Saremi and Amiri (2010) reported that this disease caused 60-80% yield losses in quality and quantity in Iran. Walnut anthracnose results in reduction in quantitative parameters such as size, mass and actual crop of nuts, failure in metabolic processes in leaves and change in biochemical indices (Shirnina and Kotljarova, 2000). Premature loss of leaves results in poorly-filled, low-quality, and darkened kernels (Black and Neely, 1978;Zamani*et al.,* 2011). Walnut anthracnose infection results in reduction in nut yield which varied from cultivar to cultivar (Pinter *et al.*, 2001; kalkism, 2012). Early infection on nuts results in premature fruit drop (Worste and Beineke, 2001).

2.2 Symptamotology

The symptoms of the walnut anthracnose are mainly observed on current year leaves, twigs, fruits and rarely on shoots. The disease initially appears on leaves as brown to black coloured circular to irregularly circular spots. These spots eventually enlarge and coalesce into large necrotic areas. Later on these infected leaves turn yellow and drop prematurely however severe infection and leaf drop usually occurs late in the season (Black and Neely, 1976;Todhunter and Beineke, 1984; Belisario, 2002; Kalkism, 2012). Berry and Frederick (1997) observed symptoms on leaves, fruits and branches which appear as dark brown spots, more or less circular, usually bordered by a yellow ring. In later stage, spots merge to form large dead areas which usually results in leaf defoliation. Saremi and Amiri(2010) observed the characteristics variation in the anthracnose diseased spot during leaf development. Spot shape and area varied from several mm to several cm, from oval to round shape and often surrounded by a yellow halo. They further observed that the infection of leaves was severe in late summer and some infected trees became defoliated. The disease also affected fruits and nut meat since nut from diseased trees showed dark and shriveled meat and necrotic spots.

Zamani *et al.* (2011) observed that the walnut anthracnose caused by*M. juglandis*may appear on green outer layer of fruits in the form of circular black or brown stains while as the disease spots on leaves appear as dark brown, more or less circular spots bordered by a yellow ring which vary from 1/16 to 5/16 inch in diameter. These individual spots later on coalesce and form large necrotic areas. Leaf infection usually results in defoliation but sometimes the infected leaflets remain attached to the tree for much of the growing season. This fungus also appears on thinner branches in the form of oval lesions or irregular circles with brown color tending to grey and with reddish brown peripherals. They further reported that at the middle of season, black points appear on the upper part of the infected leaves bearing reproductive organ of fungi. These organs produce bicellular spindle-shaped conidia somewhat tending to limber (embowed) shape. Worste and Beineke (2011) also observed that the symptoms of walnut anthracnose develop on current year leaves, nuts and stem as irregular necrotic areas, which are usually less than 5mm in diameter, and are often surrounded by small chlorotic halos.

2.3 Causal pathogen

Anthracnose of walnut has been reported to be caused by *Marssonina juglandis* (Lib.) Magnus,with *Gnomonia leptostyla* (Fr.) Ces. and de Not as its perfect stage (Sogonov *et al.,* 2008; Dastjerd and Hassani, 2009; Anonymous, 2013). Sharma and Sharma (1999) reported that acervuli produced by fungus appeared early in the season as small black specks on the lower surface of diseased leaves. Conidiophores were hyaline, short, simple, elliptical, and one celled packed together in a small layer bearing conidia at the tips. The conidia were variously shaped being straight, ovoid, falcate or with only one end rounded and the other pointed. There was one septa, and two cells are unequal with prominent oil globules and measured 15- 26 × 2-5 µm. They further reported that brown coloured perithecia developed on fallen leaves which were immersed in the leaf tissues while the beak protrudes considerably on to the leaf surface. These were amphigenous, solitary, scattered, globose, reddish brown with long cylindric beak. The beak measuring 140-170 µm and 25-40 µm in breadth, while the globose base has a diameter of 120-125 µm. The inner cavity of the perithecia was lined with club shaped to fusiode asci while as the asci were hyaline aparaphysate, 8 spored, measuring 56-62 × 14-16 µm. The ascospores were hyaline, fusiode, straight to slightly curved septate and measured 15-19×4-5 µm.

Saremi and Amiri (2010) isolated *M. juglandis* on potato dextrose agar (PDA), corn meal agar (CMA) and nutrient agar (NA) fromleaves, fruits and foliage. They also observed that isolated fungus produces minute black fruiting bodies called acervuli in which conidia were colorless, usually crescent-shaped, and divided by a cross-wall into two approximately equal cells. Perithecium with 380- 450 µm in length and 150-260µm breadth neck like structure with 19.5-24.5 × 7.2-8 µm ascospore size. Salahi *et al.*(2007) isolated as a streak single spore on oat meal agar while Jamshidi *et al.*(2012) isolated *M. juglandis* from the leaf discs bearing acervuli by transferring germinating macroconidia from 2% water agar to 39% potato dextrose agar added with 7g/litre of oatmeal. Dastjerdi *et al.* (2009) isolated *G. leptostyla* (anamorph: *M. juglandis*) on oat meal agar and corn meal agar media. Kalkism (2012) isolated *G. leptostyla* on potato dextrose agar (PDA) from the walnut (*Juglans regia* L.) leaves showing typical symptoms and identified as *G. leptostyla* according to optimal growth of *G.leptostyla* occurred at 22^{o}C and pH 5.4.

2.4 Physiological studies

Fayret and Parguey (1976) reported that production of ascospores of *G. leptostyla* occoured at 10^{o}C. Perithecia remain immature at 20^{o}C in which the multinucleate pro-sporophyte grows normally and produces a syncytium from which the ascosporophyte originates, but differentiation of the ascogenic phase is heat inhibited. They further observed that the cold is necessary for the evolution of initial dicaryotic cells, which are the true carpospore cells. These cells and the ascosporophytic phase have special physiologic requirements as regards temperature. Matteoni and Neely (1979) reported that growth of *M. juglandis* was maximum at 22 ^{o}C and at pH 5.4 whileas sporulation was maximum at 26^{o}C with pH 6.8 on oatmeal agar and the optimum temperature for germination of ascospores and conidia were 26^{o}C and 24^{o}C, respectively. Microconidia were not produced at temperature less than 10^{o}C and did not germinate. They further observed that the light reduced vegetative growth, but promoted production of conidia and acervuli. Production of fertile perithecia were observed *in vitro* after incubating crosses of 2 mating types in darkness at 10^{o}C for up to 3 months. Belisario *et al.*(2008) while investigating the mean diameter of colonies grown *in vitro* at 22^{o}C and sporulation of 191 isolates of *G. leptostyla* grouped by site of collection, were compared observed that the isolates that grew significantly more slowly were from sites with colder early springs and higher altitudes. Acervular conidiomata were abundantly produced by all isolates at 22^{o}C in darkness after 21 days, while productions of protoperithecia were noticed within 2 months by most isolates, under the same conditions. Production of conidiomata was observed at 20 and 25^{o}C while as after 2 months, protoperithecia were present in most isolates at 20^{o}C, very few at 25 and 15^{o}C, and no production was recorded at 10 and 30^{o}C after 2 months. Fertile perithecia with asci and ascospores were produced, after 3 months at 10^{o}C in darkness. They further noticed that the ascocarp diameter, width of asci and length of ascopores of *in vitro*produced perithecia were larger than those of perithecia produced in nature. The latter showed a neck length longer than *in vitro*-produced perithecia. Salahi *et al.*(2009) reported that oat meal agar to be the best artificial media for growth and asexual reproduction of the *M. juglandis.* Dastjerdi *et al.*(2009) noticed that the isolates of *G. leptostyla* produced acervular type of conidiomata at 21°C temperature, under photo-period of 16-hours light : 8-hours darkness) after 18-21 days of incubation period. Jamshidi (2011) reported that colonies exposed to light produced acervuli faster and in a denser way on optimum pH of 5.8. He further reported that fertile perithecia with asci and ascospores were produced *in vitro* after 75 to 90 days at 4°C in darkness. Slow growths of different *Marssonina* spp. have also been reported by several workers. Zhao *et al.* (2010) reported that for rapid mycelial growth and sporulation of *Diplocarpon mali*, potato and carrot dextrose broth (PCDB) and potato and carrot sucrose broth (PCSB) were most favorable. The optimum temperature for mycelial growth and conidial production was 25 ^{o}C. Active mycelial growth occurred at pH 5-7, and pH 5-8 was favorable for sporulation.Galea*et al.*(1986) reported that lettuce anthracnose pathogen, *M. panattoniana*, grows at a wide temperature range at 3-26°C and pH 4-5.2. Colony growth was best on potato dextrose agar (PDA) at 20 ^{O}C and pH 5.2. Wolcan (1985) reported that sporulation of *Diplocarpon earliana* causing leaf scrotch of strawberry was best on malt extract agar with peptone and yeast, at 20 ± 2°C.

2.5 Pathogenicity

In order to prove the pathogenic nature of *Marssonina* spp. Different workers have adopted different methods. Figueiredo and Hennen (1995) proved the pathogenicity of *M. salicicola* in the laboratory by artificial inoculation with a conidial suspension brushed on to healthy detached young leaves of *Salix babylonica* and left in the greenhouse. When maintained in wet conditions, leaf symptoms were evident 12 days after inoculation, and threads of conidia coming out from acervuli were produced 15 days later. Nadroo (2006) proved the pathogenic nature of *M. coronaria* by spraying the conidial suspension on one year old budded potted plant of red delicious apple cultivar. Cline and Neely (1983) proved the pathogenic nature of the of *Gnomonia leptostyla* byspraying conidial suspension on mature leaves of *Juglans nigra*and observed macroscopic lesions at 240 h and acervuli formation after 240 h. Dastjerdi *et al.* (2009) proved the pathogenicity of *M. juglandis* by spraying the conidial suspension on mature, fully expanded leaflets. Macroscopic brown spots were observed on leaves 16 days after inoculation while acervuli were observed after 24 days while as Neely (1986) proved the pathogenic nature of *G. leptostyla* by spraying the conidial suspension on mature leaves of walnut seedlings and noticed the development of lesions on leaf surface after approximately two weeks.

2.6 Epidemiological studies

Black and Neely (1976) observed that infection of anthracnose disease on leaves occurred at relative humidity above 95 per cent and severity of infection was not influenced by temperature between 10-32^{o}C but was significantly reduced below 10^{o}C. Rosnev and Naidenov (1986) also reported that *M. juglandis* requires temperature of 15-30°C, frequent precipitation, and humidity over 65 per cent. Matteoni and Neely (1977) observed that incubation period was directly related to infection frequency. Infection was more severe on older leaves and 10 times more frequent with adaxial inoculation.

Vonica (1970) reported that conidia reportedly cause secondary infections and intensify the disease during summer. Low temperature, rainfall and high relative humidity at the start of growth promoted infection by the pathogen (Andrievskii and Rikhter, 1976; Jamshidi and Salahi, 2009). Kessler (1984) reported that, after initial lesions arising from infections by ascospores in the month of May, the lesion numbers increased through early summer as a result of secondary leaf infection by conidia. He further observed that disease development was maximum in late July and early August, when most defoliation of previously infected leaflets occurred. Hashemi (2005) noticed that relative humidity of 80 percent for 24 hours and temperature in the range of 10-20°C under windy conditions resulted in more than 80 per cent of ascospores released, whereas high humidity and wind during growing season were necessary for occurrence of secondary infections and dispersal of conidia. Belisario *et al.*(2001) reported that increase in disease incidence during late August to ending September could be related to the increasing number of leaflets bearing fertile acervuli for secondary infections, leading to progressive senescence of leaves.

2.7 Perpetuation

Different *Marssonina* spp. attacking different crops overwinter by different means either by forming sexual fruiting bodies on overwintering leaves, shoots and fruit debris or as conidia. Vucinic (1977) reported the formation of apothecia of*M. brunnea*at the end of winter on fallen leaves while Sokolova (1975) observed maturation by late march to early april. Harad *et al.* (1974) reported the formation of apothecium of *M. coronaria* on overwintering apple leaves infected with blotch, containing ascospores which served as primary inoculums in spring. Anselmi (1979) reported that *M. salicicola* infecting *Salix babylonica*overwinters by means of small stromata on the edges of cankers or as conidia in the acervuli on branches. Milicevic*et al.*(2002) reported that *Diplocarpon earliana* (anamorph *M. fragariae*), the causal organism of leaf red spot or leaf scorch of strawberry, overwinters in the form of mycelium and produces two types of fruiting bodies (apothecia and acervuli) in early spring. Primary infections are caused by ascospores and conidiospores from the acervuli while as secondary infections are caused only by conidiospores.

The pathogen(*G. leptostyla*) reportedly overwinters primarily on infected leaf debris, and ascospores produced in perithecia act as the primary inoculum during spring (Vonica, 1970; Black and Neely, 1978; Dimova and Arnaudov, 2008). Veghelyi and Penzes (1990) observed that the fungus overwintered in infected leaves and occasionally in the epicarp of the fruit. Asci were formed by the end of February and ascospores developed during March. Incubation period took 3-5 weeks. After the appearance of the first symptoms, the development of conidia and secondary infections occurred almost continuously until late autumn.

Jamshidi*et al. (*2009) reported the perithecia from fallen leaves. Perithecia had one beak on leaves and up to four beaks on culture media. Perithecium in homothallic isolates had significantly higher diameter and longer beaks than non-homothallic isolates. Saremi and amiri (2010) reported that the fungus commonly overwintered in fallen walnut leaves, infected during the preceding summer in walnut orchards

2.8 Disease management

Cultural as well as chemical management has been suggested by various research workers for the management of walnut anthracnose.

2.8.1 Cultural practices

Burying (ploughing in) the fallen leaves in autumn and winter to a depth of 10-15 cm, pruning of infected twigs and branches and adequate nitrogen fertilization has been recommended for the management of walnut anthracnose(Rosnev and Tsanova, 1980; Neely, 1981; Pscheidt and Ocamb, 2014).Zakhov (1980) recommended rouging and planting healthy material. Shevchenko (1981) advocated that the best control in rugged terrain where the chemical control is difficult is the selection of local immune forms of *Juglans regia* and production of hybrids with *J. nigra, J. cinerea* etc. Kessler (1985) found that an over winter cover of autumn olive leaves reduced the number of ascospores, the primary inoculum, discharged from infected fallen walnut leaves. He further observed nitrogen fixed and released from autumn olive increases the walnut foliage nitrogen content and reduces walnut susceptibility to secondary infections initiated by conidia released from the primary infections. Neely (1986) suggested that the incidence or severity of anthracnose can be altered through site modification and increase in foliage nitrogen content. Van Sambeek *et al.* (2003) reported that under planting herbaceous legumes in walnut plantations could potentially reduce the severity of anthracnose either in response to increased soil nitrogen or by disrupting ascospore dispersal, while as under planting walnut saplings with annual and perennial legumes has been shown to increase foliage nitrogen content. Saremi and Amiri (2010) recommended that eradication of walnut plant residue, especially fallen leaves is very beneficial in reducing disease. Kalkasim (2012) recommended leaf extracts of *Corcus mas* and *Morus nigra* against *M. juglandis*. Neely (1986) suggested that the incidence or severity of anthracnose can be altered through site modification and increase in foliage nitrogen content.

2.8.2 Resistance of different cultivars

Black and Neely (1978) while artificially inoculating *juglans* species with *G. leptostyla* conidia observed that the Hinds (*J. hindsii*) and Arizona (*J. major*) walnuts were more susceptible than black walnut (*J. nigra*). Little walnut (*J. microcarpa*), Japanese walnut (*J. ailantifolia*), butternut (*J. cinerea*), heartnut *(J. ailantifolia var. cordiformis*), and assorted hybrids, were less susceptible than black walnut while as the clones of English walnut (*J. regia*) showed the greatest range in susceptibility. Dimova (2007) reported that Izvor-10 is medium susceptible and the index of infesting could reach up to 52.5 per cent in the leaves and 34.5 per cent in the fruit. Belisaro *et al.*(2008) observed *J. sieboldiana* and *J. cinerea* to be highly resistant and both *J. nigra* and *J. hindsii* to be highly susceptible to the disease while as *J. regia* showed an intermediate response of susceptibility to anthracnose. Salahi and Jamshidi (2009) reported that Z67 and K73 cultivars showed more resistance in comparison with others and Z67 was the most resistant one. The cultivars Ser, Vina, Hartley, Ronde de Montignac, Lara and Franquett had moderate to weak resistance and the cultivars Z63, Z60 and Pedro were all susceptible to the disease. While evaluating 15 walnut cultivars for susceptibility to *G. leptostyla* infection, Arnaudov and Gandev (2009) recorded that only one cultivar “Chandler” was resistant and five cultivars were slightly susceptible, whereas rest were either susceptible or highly susceptible (cvs. “Sheinovo”, “zvor 10” and “Slivenski”) or very highly susceptible (cvs. “Alososzentivani” and “Seer”) with disease intensity ranging from 4.9 to 62.4 percent.

2.8.3 Chemical management

To manage the disease caused by various *Marssonina spp.* in different host crops, the use of chemicals has been suggested by various workers. Rimfeldt (1979) suggested that effective control of *M. salicicola* can be achieved with copper oxychloride at 2500ppm, captafol at 1800ppm, and benomyl at 600 ppm while as Anselmi, (1979) recommended Benomyl, maneb and mancozeb against *M. salicicola* infecting *Salix babylonica.* Sharma (2000) recommended protective sprays with broad spectrum fungicides, mancozeb (0.3%) and carbendazim (0.05%) reduced the disease significantly. Further he observed that benomyl, thiophanate methyl, propineb, chlorothalonil, dithianon and ziram were also effective in controlling the disease.

Milicevic *et al.*(2002) suggested that fungicide Folicur M 50 WP (tebuconazole + tolylfluanid) and Kidan SC (iprodione) were the most effective against the leaf spot disease of strawberries (*M. fragaria*). Devappa*et al.(*2006) suggested that chlorothalanil (0.2%), mancozeb (0.2%), hexaconazole (0.1%), propiconazole (0.1%), carbendazim (0.1%) copper oxychloride (0.3%) were highly effective in controlling the black spot of rose (*Diplocarpon rosae*). Thakur and Nirupma (2010) recommended that Indofil M-45 (0.3%), Antracol (0.3%), Indofil Z-78 (0.3%), Bavistin (0.05%), Kocide (0.3%), Tohfa (0.075%) and Copter (0.3%) against premature leaf fall of apple caused by (*M. coronaria*).

Vonica (1970) reported that (3-6 treatments per year) of zineb, dodine, zinc-metiram, phaltan, PMC, maneb, captan and thiram gave effective control of walnut anthracnose while as Reznikova (1977) observed that 2-6 sprays of combined treatment with Bordeaux and urea gave the best control than treatments with 1% Bordeaux alone. Various workers reported that fungitoxicants like carbendazim, benomyl, DNOC, dodine, cupric oxide, zineb, maneb and chlorothalonil proved to be effective against *M. juglandis* and significantly reduced the disease (Berry, 1977; Kleiner and Bulatova, 1978; Zamani *et al.*, 2011). Neely (1977) reported that soil application of benomyl, reduced the the incidence and severity of the anthracnose of black walnut for several years. Movsesyan (1978) recommended spraying with 0.5% copper oxychloride and 0.4% zineb during the growing period for control of walnut anthracnose. Rosnev and Tsanova (1980) recommended that Bordeaux mixture 1%, Dithane M-45 0.3%, and Dithane cupromixin 0.6% against walnut anthracnose while as Zakhov (1980) recommended that spraying with Bordeaux, at 2% during winter and 1% before flowering and once after flowering against *G. leptostyla*. While testing various fungitoxicants on 8-year old walnut trees in Poland, Cimanowski *et al.*(1991) observed that different formulations of mancozeb, dithianon, flusilazole and copper fungicides controlled anthracnose and leaf spot (*Xanthomonas campestris* pv. *juglandis*). Nakova and Dimova (2003) while investigating the effects of 22 fungicides on the mycelial growth and ascospore germination of *G. leptostylain vitro* observed that the inhibition of both parameters was greatest with the contact fungicides Kuprozine Super (copper oxychloride), Dithane (mancozeb) and Ronilan (vinclozolin), and with the systemic fungicides Corzate (simoxanile), Rubigan (fenarimol), Topsin M (thiophanate-methyl), Anvil (hexaconazole) and Fundasol (benomyl). In field tests conducted in Bulgaria, the efficacy of Dithane, Corzate, Topsin M, Anvil and Fundasol against *G. leptostyla* in walnut was evaluated. The most effective were Anvil and Fundasol (control of more than 90%), followed by Topsin M (control of more than 80%).Zamani *et al.*(2011) advocated that application of Bordeaux solution in winter and copper fungitoxicants in early spring could be highly effective for controlling the walnut anthracnose.

Chapter-3

MATERIALS AND METHODS

3.1 Status of anthracnose disease of walnut growing belts of Kashmir

Survey of main walnut growing areas in the districts of Kupwara, Anantnag and Budgam of the Kashmir valley was carried out during the second fortnight of August, 2013 to record the incidence and intensity of Anthracnose disease of walnut. In each district three locations were visited and in each location randomly six walnut trees were selected. In all 54 plants were observed at nine locations. Randomly 100 leaves and 100 current twigs from each plant at each of the site were collected to record the per cent anthracnose incidence and intensity.

Percent disease incidence and intensity was calculated by using the following formula:

(a) Per cent disease incidence was calculated as per the formula:

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

(b) The disease intensity was recorded using 0-5 scale of (Townsend and Henberger, 1943) with slight modifications. Six catagories were made on the basis of percent leaf involved (Plate 2) as per the following key:

Category	Grade	Criteria (% diseased leaf/twig area covered)
I.	0	No infection/ disease
II.	01	Upto 10.0
III.	02	10.1-20.0
I V.	03	20.1-30.0
V.	04	30.1-50.0
VI.	05	>50.0

Plate-1 : Walnut plantation at location Larnoo (Anantnag)

Plate-2: Scale (0-5) for assessment of walnut anthracnose intensity

Per cent disease intensity (PDI) was calculated as per the following formula:

$$\text{Per cent disease intensity} = \frac{\sum (n \times v)}{N \times G} \times 100$$

Where, $\sum$ = Summation

n = Number of diseased leaves/twigs in each category

v = Numerical value of the category

N = Total number of leaves/ twigs examined, and

G = Highest grade value

3.2 Symptamotology and microscopic examination of the fungus on the host

Detailed studies on the development of walnut anthracnose symptoms were carried out on three walnut plants selected out in the walnut orchard at Shalimar campus grown under natural epiphytotic conditions. All round the plants four branches bearing leaves and twigs were tagged and were kept under constant observation throughout the growing season. Observations were recorded with regard to the first appearance of disease symptoms and different stages of its development like size, shape and colour of the lesions. The diseased leaves and twigs collected from the orchards were further examined in the laboratory for the associated pathogen by teasing the diseased portion and observed under microscope. The leaves and twigs were also kept in moist chamber at 21^{o}C for 36 hours to get the acervuli bulged. These bulged acervuli were lifted with the help of teasing needle under stereoscopic microscope and temporary mounts in cotton blue, lectophenol and distilled water were prepared. Acervuli and conidia were examined for their colour, shape, size and septation.

3.3 Characterization of the pathogen causing walnut anthracnose.

3.3.1 Isolation of pathogen

Walnut leaves and twigs exhibiting typical anthracnose symptoms collected from orchard at Shalimar campus during survey were used for isolation of the pathogen. For isolation of the pathogen the diseased leaf was surface sterilized in 0.1 per cent mercuric chloride. These leaves were then kept in moist chamber and incubated at 20±1°C for 24 hours to get the acervuli swollen. For isolation of the pathogen these bulged acervuli were lifted and directly placed aseptically on oat meal agar medium and incubated at 21^{o}C for 30 days and also spore suspension was prepared in sterilized distilled water by picking the spore gelatinous tendrils which oozed from these swollen acervuli with the help of sterilized needle under stereoscopic microscope. The spore suspension thus obtained was used to flood the surfaces of plain agar medium in Petri plates and incubated at 21^{o}C for 48 hours (Jamshidi *et al.*, 2012). Single spore isolation were made by transferring aseptically the germinating spores to OMA (oat meal agar) medium and incubated at 21^{o}C and maintained at the alternate 12 hour cycle of light and darkness. The pure culture thus obtained was maintained by repeated sub-culturing at an interval of 30 days for further studies. The stock culture in oat meal agar (OMA) was stored in refrigerator at 4°C for further studies.

The composition of oat meal agar media was :

Oat meal	:	40 g
Agar agar	:	15 g
Distilled water	:	1000 ml

3.3.2 Morphological characteristics of the pathogen.

The morphological characters of the pathogen were studied on both host as well as culture in a laboratory. Semi permanent slides were prepared from 14 days old culture stained with cotton blue in lacto phenol. The slides were examined under microscope (40x) with respect to following characters of the causal organism.

Colony	:	Colour, shape and mycelial growth
Mycelium	:	Colour, breadth, septation and branching
Acervuli	:	Colour, shape and size
Conidia	:	Colour, shape, size and septation

3.4 Pathogenicity test

To ascertain whether the fungus isolated was primary cause of the disease, three sets of Pathogenicity testswere conducted on one year walnut seedling.

Three sets of plants with each set compromising of three plants were kept in 40 cm diameter pots containing sterilized soil. The plants pots were kept in artificially created humid chambers and maintained at room temperature. High humidity inside the chamber was maintained by timely irrigation and spraying the leaves with sterilized water. The plants were constantly observed for 7 days to rule out any latent infection prior to inoculation. One set ofplants was given injuries with the help of sterilized teasing needle while as another set kept uninjured. Inoculation were made by spraying spore suspension (1×10^{5} spores per ml) from young and vigorous culture of *Marsonina juglandis* on the abaxial and adaxial surface of both injured and uninjured leaves with the help of atomizer. The injured and uninjured plants of third set were sprayed with sterilized water and served as a check. These plants were maintained for three weeks and were examined daily for symptom development. The symptoms developed were microscopically examined for presence of acervuli and conidia. The pathogenicity was confirmed after satisfying Koch's postulate.

3.5 Effect of media on *in vitro* growth and sporulation of fungus

The growth of the fungus was studied on different solid media viz., Potato dextrose agar, Richards, Czapeks (Dox) agar, Corn meal agar, Malt extract agar and Oatmeal agar. These media were prepared according to the composition given in Appendix-I.

Each of the media to be evaluated was poured separately in 150 ml conical flasks, plugged with non-absorbent cotton and autoclaved at 15lbs pressure per square inch for 15 minutes. 30 ml of each test media was poured into sterilized petriplates under aseptic conditions. Inoculations were made with uniform culture bits (3mm diameter) from actively growing fungus culture. Each treatment was replicated thrice in completely randomized design (CRD). The inoculated petriplates were incubated at 21^{o}C for 30 days. The radial mycelial growth in all the three replications from each medium was recorded and average of the three replications was taken taken as final observation.

The sporulation was studied, by thoroughly homogenizing a 3mm mycelial disc in 5ml of sterilized distilled water. The spore suspension thus obtained was used for counting the number of spores with the help of heamocytometer.

Media supporting the best growth and sporulation of the test fungus was used as a basal medium for further studies.

3.6 Disease development with respect to weather parameters.

Periodic observations on disease development of walnut anthracnose with respect to disease intensity on walnut leaves and twigs were recorded to find the correlation between disease development and prevailing weather conditions viz; temperature, humidity and rainfall.

Five walnut plants (8-10years old) were randomly selected and earmarked in the last week of March 2013 at Shalimar campus of SKUAST-K. Marked plants were examined regularly for first appearance of disease and subsequently at 15 days interval beginning from mid April for recording disease intensity on leaves and twigs according to the scale described at I(b).

The meteorological data regarding mean maximum and minimum temperature, relative humidity that prevailed around the SKUAST-K were recorded by installing maximum- minimum thermometer and dry and wet blub thermometer during the period of investigation in the orchard itself. The data regarding rainfall and number of rainy days were obtained from meteorological observatory at SKUAST-K. The different parameters were then correlated with the disease intensity for drawing the inferences.The rate of disease progress was calculated by using the following formula (Vander plank, 1963)

$$R = \frac{2.303}{T_2 - T_1} \text{Log}_{10} \frac{X_2}{X_1}$$

Where,

R = rate of disease progress or infection rate

$T_2 - T_1$ = time interval

X_1 = disease at time T_1

X_2 = disease at time T_2

3.7 Perpetuation

The anthracnose diseased leaves and twigs were kept on the ground surface in autumn 2013 to study their role in producing viable acervuli and conidia or producing any perfect state for initiating primary infection during following spring 2014. Walnut leaves and twigs bearing typical symptoms of anthracnose disease were collected from walnut orchard during the month of November. The presence of fungus in the diseased tissue was ensured before hand. The material thus collected was put into different sets of nylon mesh bags. The diseased leaves and twigs (in separate bags) were kept on ground surface below the walnut trees for overwintering under natural conditions. The diseased material from each set was examined for viability of fungus at weekly intervals starting from Ist week of March.Randomly10 leaf discs of 1sq.cm taken from each sample were examined for the presence of acervuli. These were then crushed in 10 ml of sterilized distilled water and were centrifuged at 3000rpm for 15 minutes. After discarding the supernatant sterile water was added to the pellet to make 5ml of spore suspension and the number of conidia was counted with the help of haemocytometer. To check the conidial viability during spring at weekly intervals, three drops of spore suspension of each sample were placed at each cavity slide and were replicated thrice. These were incubated at 21^{o}C for 48 hours in a moisture chamber. Presence of spores on the overwintering anthracnose lesions and their viability was also studied by harvesting spores from lesions by simply washing them and counting the suspension on petriplates. Spores germination in water was tested as per slide germination technique (Wellman and Mccallan, 1943).

3.8 *In vitro* evaluation of fungitoxicants

The following six systemic and six non-systemic fungitoxicants were evaluated on a.i. basis in the laboratory for their efficacy against the pathogen causing walnut anthracnose by using poisoned food technique (Nene and Thapliyal, 1993) /slidegermination technique (Montgomery and Moore,1938).

Each test fungitoxicant was evaluated at four different concentrations including check as under:

S.No.	Common name	Trade name	Concentration(ppm)
	Systemic		
1.	Carbendazim 50WP	Bavistin	50, 100, 150, 200
2.	Thiophanate methyl 70WP	Intop	50, 100, 150, 200
3.	Flusilazole 40EC`	Governer	50, 100, 150, 200
4.	Difenoconazole 25EC	Score	50, 100, 150, 200
5.	Tebuconazole 25EC	Folicur	50, 100, 150, 200
6.	Metiram+Pyraclostrobin 60WG	Cabriotop	50, 100, 150, 200
	Non systemic		
1.	Copper oxy-chloride 50WP	Cuprex	250, 500, 1000, 1500
2.	Mancozeb 75WP	Kohinoor M-45	250, 500, 1000, 1500
3.	Propineb 70WP	Antracol	250, 500, 1000, 1500
4.	Chlorothalonil 75 WP	Kovach	250, 500, 1000, 1500
5.	Captan 50 WP	Kohicap	250, 500, 1000, 1500
6.	Ziram 80 WP	Ziride 80WP	250, 500, 1000, 1500

3.8.1 Effect of fungitoxicants on spore germination.

Spore suspension of 50000 spores per ml concentration in sterile water was prepared aseptically from twenty days old culture of *Marssonina juglandis*. Fungitoxicant suspension of double the concentration than the desired one was prepared separately in 150 ml conical flasks. One drop (50µl) 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 21^{o}C for 48 hours. Each fungitoxicant treatment was replicated thrice in a completely randomized design (CRD), with five moist chambers as a unit of replication. Observations regarding spore germination of test fungitoxicant were recorded with the help of a compound microscope (400x). These were compared with the check, where a drop of sterile water was mixed with spore suspension. Hundred spores were examined for each replication and average per cent inhibition of conidial germination for each fungitoxicant was calculated by employing the following formula of Vincent (1947)

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

3.8.2 Effect of fungitoxicants on mycelial growth.

Fifty milliliters of basal medium (PDA) was poured in 250 ml conical flasks, plugged with non-absorbent cotton and autoclaved at 15 lbs per square inch pressure. After semi cooling the medium, 50ml of fungitoxicant solution of double concentration than the desired one was incorporated in each flask except check. Medium from each flask was transferred to sterilized petriplates under aseptic conditions. Each treatment was replicated thrice in completely randomized design (CRD) and inoculated with 3 mm diameter mycelial disc taken from 20 days old culture of the test fungus and incubated at 21^{o}C for 15 days. The relative efficacy of the fungitoxicants was ascertained by taking the radial mycelial growth of the test fungus. The observations regarding the per cent mycelial growth inhibition of fungus over control was calculated by using the formula of Vincent (1947).

$$\text{Per cent growth inhibition} = \frac{\text{C-T}}{\text{C}} \text{ X } 100$$

Where,

C = Mycelial growth in check

T = Mycelial growth in treatment

3.9 Analysis of the data

The data of the various experiments were subjected to statistical analysis with the help of computer. The data was subjected to appropriate transformation wherever needed, as suggested by Gomez and Gomez (1984) before analysis.

Chapter-4

EXPERIMENTAL FINDINGS

4.1 Occurrence and severity

Survey was carried out in three districts viz., Anantnag, Budgam and Kupwara of Kashmir valley for prevalence of Anthracnose disease of Walnut during the year 2013. The data regarding disease incidence and intensity recorded on leaves and twigs are presented in Table 1.

4.1.1 Disease incidence

The results obtained (Table 1; Fig. 1) indicate that Anthracnose disease of walnut was prevalent in all the three districts surveyed. The disease incidence recorded on leaves was highest in district Anantnag (97.94%) which was statistically at par with district Budgam (97.83%) while as the district Kupwara (96.22%) exhibited the least disease incidence. The data further revealed that the disease incidence on leaves was highest (98.75%) in Saller location of district Anantnag followed by Uttersoo (98.00%) and Larnoo (97.52%) locations of same district which did not differ significantly from that of Chadoora (98.50%), Sarai (97.50%) and Magam (97.50%) locations of district Budgam and Dragmulla (97.66%), Langate (96.50%) locations of district Kupwara. Sogam (94.50%) of the district kupwara exhibited least disease incidence on leaves. The highest disease incidence on twigs was observed in district Anantnag (74.66%) followed by district Budgam (72.27%) and least in district Kupwara (68.421%). The data further reveals that disease incidence on twigs was highest (85.00%) in saller location of district Anantnag which differ significantly from that of chadoora (84.30%), Larnoo (71.60%), Langate (70.50%), and Dragmulla (69.50%) locations of the district Budgam, Anantnag and Kupwara, respectively. Sarai location of district Budgam exhibited disease incidence of 66.66 per cent which did not differ significantly from Magam (66.16%) of same district and Uttersoo of district Anantnag (66.00%), while as Sogam of district Kupwara exhibited the least disease incidence of 65.23 per cent.

4.1.2 Disease intensity

Observation (Table 1; Fig. 2) regarding disease intensity on leaves revealed that highest intensity of 55.76 per cent was recorded in district Anantnag followed by district Budgam (50.11%), which was stastically at par with District Kupwara which exhibited disease intensity of 49.03 per cent. The data further reveals that disease intensity on leaves was highest at Saller (56.87%) of District Anantnag while as Larnoo of district Anantnag exhibited disease intensity of 55.54 per cent which was found to be stastically at par with the chadoora (55.05%) of district Budgam. Uttersoo of district Anantnag exihibited disease intensity of 54.89 per cent which differed significantly from Langate (52.02%) and Dragmulla (50.55%) of district kupwara. Magam of district Budgam exhibited disease intensity of 48.48 per cent which differed significantly from Sarai (47.80%) of the same district while as Sogam of district kupwara exhibited least disease intensity of 44.51 per cent. The per cent disease intensity on twigs was found to highest in district Anantnag (22.77%), followed by Budgam (20.58%) and Kupwara (15.32%). Saller location of district Anantnag recorded highest disease intensity of 35.00 per cent on twigs followed by Chadoora (29.90%) of district Budgam, which differed significantly from Sarai (17.97%) of same district. Larnoo of district Anantnag exihibited disease intensity of 16.92 per cent which differed significantly from Uttersoo (16.39%) of same district. Langate location of district kupwara exhibited disease intensity of 15.82 per cent which was stastically at par with Dragmulla (15.33%) of same district while as Sogam of same district exhibited least disease intensity of 14.81 per cent on twigs.

4.2 Symptomatology

Three walnut trees selected from walnut orchard in Shalimar campus grownunder natural epiphytotic conditions were kept under observation throughoutthe growing season and examinedforappearance ofthecharacteristicsymptoms of

Table-1 : Incidence and Intensity of Anthracnose disease of walnut at various locations in the Kashmir valley during the year 2013

District	Location	Per cent disease incidence		Per cent disease intensity	
		Leaves	Twigs	Leaves	Twigs
Anantnag	Larnoo	97.52	71.60	55.54	16.92
	Saller	98.75	85.00	56.87	35.00
	Uttersoo	98.00	66.00	54.89	16.39
	Mean	97.94[a]	74.66[a]	55.76[a]	22.77[a]
Kupwara	Dragmulla	97.66	69.50	50.55	15.33
	Langate	96.50	70.50	52.02	15.82
	Sogam	94.50	65.23	44.51	14.81
	Mean	96.22[c]	68.41[c]	49.03[c]	15.32[c]
Budgam	Sarai	97.50	66.66	47.80	17.97
	Magam	97.50	66.16	48.48	15.93
	Chadoora	98.50	84.30	55.05	29.90
	Mean	97.83[ab]	72.27[b]	50.11[b]	20.58[b]
Overall mean		97.34	71.78	51.66	19.56
CV		1.42	1.43	3.10	3.95
CD$_{(P\leq 0.05)}$					
District		0.93	0.69	1.10	0.52
Location		NS	0.69	1.10	0.52

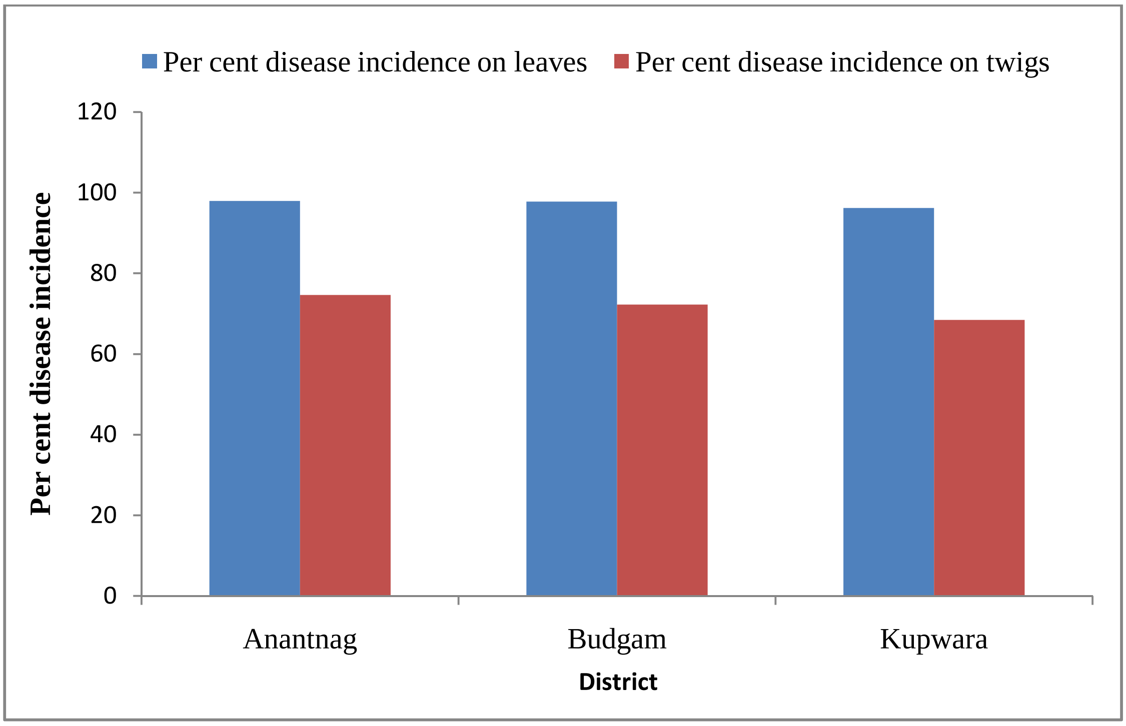


Fig. 1: Incidence of walnut anthracnose (*Marssonina juglandis*) in Kashmir valley during the year 2013

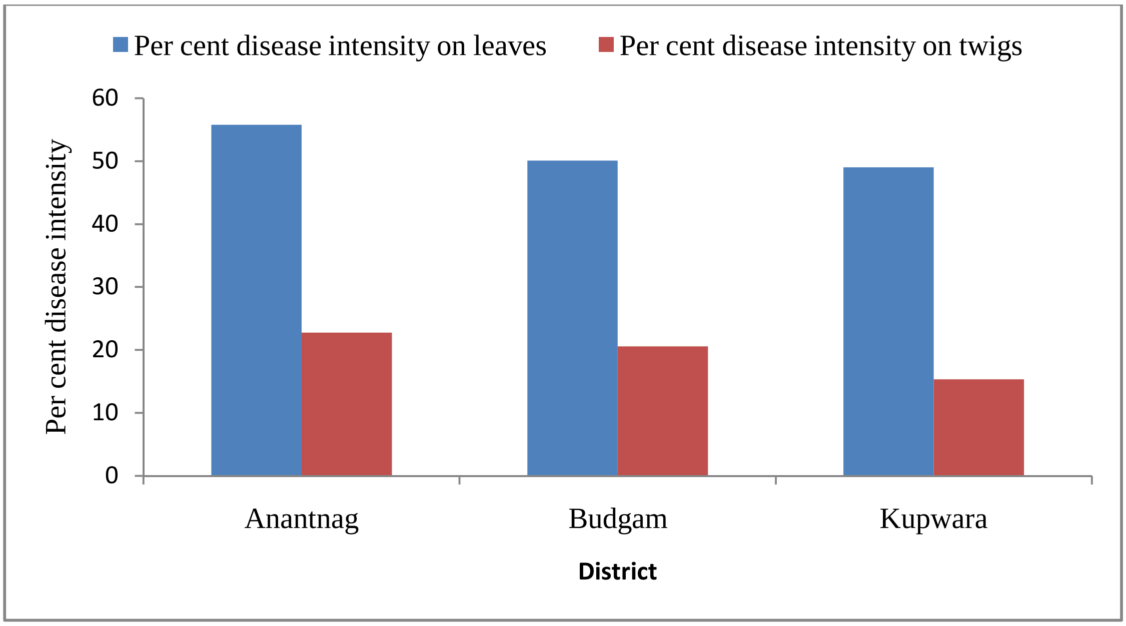


Fig. 2: Intensity of walnut anthracnose (*Marssonia juglandis*) in Kashmir valley during the year 2013

walnut anthracnose starting from last week of April, 2013. Periodical observations on symptomatological development were recorded at an interval of seven days on leaves and twigs. On the leaves disease was first noticed in the 1st week of May and reached at its peak during late August (Table 2). Disease symptoms initially appeared as small, scattered, slightly sunken light brown, circular spots ranging from 0.5-1.0 mm with an average of 0.7 mm in size (Plate 3a). In the last week of May circular dark brown spots with whitish centre were observed. In the Ist week of June black acervuli forming concentric rings appeared on undersurface of the leaves and size of the lesions ranged from 7.0-8.0 mm in diameter with an average of 7.4 mm. Later on lesions turned brown to dark brown in colour with irregular margins and size ranged from 10-12 mm with an average of 11.1 mm in the 3rd week of June (Plate 3 b,c,e). The characteristic yellow halos were observed around the leaf lesion in the second week of July (Plate 3d). Due to coalescing of lesions, the lesion size increased to 22-24 mm with average of 22.2 mm and large irregular patches of lesions were observed in the last week of July which was followed by chlorosis in the first week of August, ultimately resulted in defoliation in the last week of August (Plate 3 f,g).

On twigs the disease was first noticed in the 1st week of May and reached at its peak during August (Table 3). Initial disease symptoms initially appeared as small, dark brown lesions ranging from 1.0 to 1.5 mm with an average of 1.2 mm in size (Plate 4a). Later the lesions turned sunken in the second week of May and the size ranged from 1.5 to 2.5 mm in diameter with an average of 2.2 mm (Plate 4b). Black acervuli developed on the lesions during the second week of June. Coalescing of lesions was observed in first week of August, attaining size of 25-26 mm with an average of 25.1 mm which was followed by necrosis of the twigs in the third week of August. This was followed by drying up of severely infected twigs (Plate 4 c,d,e,f).

Table-2 : Symptomatological development of Anthracnose disease of walnut on leaves

Date of observation (Weekly)	Lesion on Leaf				
	Diameter (mm)		Shape	Colour	Other characters
	Range	Average			
April					
26	-	-	-	-	Disease free
May					
04	0.5-1.0	0.7	Circular	Light brown	Slightly sunken spots on leaves
12	1.0-2.5	1.5	-do-	-do-	-do-
20	2.5-6.0	3.8	-do-	-do-	Spots develop whitish centre
28	6.0-7.0	6.3	-do-	-do-	Circular dark brown spots on fruits
June					
06	7.0-8.0	7.4	-do-	-do-	Development of acervuli in concentric rings on undersurface of leaves
14	8.0 -10	8.9	-do-	Brown to dark brown	-do-
22	10-12	11.1	Circular to irregular	-do-	-do-
30	14-17	15.1	-do-	-do-	-do-
July					
07	17-19	18.0	-do-	-do-	-do-
15	19- 20	19.4	-do-	-do-	Yellow halos around leaf spots on ventral side.
23	20-22	20.8	Mostly irregular	-do-	Numerous Spots start coaleascing
31	22- 24	22.2	-do-	-do-	Formation of irregular Patches
August					
08	24-25	24.1	-do-	-do-	Chlorosis of highly infected leaves
16	25-26	25.2	-do-	-do-	-do-
24	26-27	26.5	-do-	-do-	Major portion of leaf gets involved
31	27-29	28.0	-do-	-do-	Defoliation

a)

b)

c)

d)

Plate-3: Characteristic symptoms of walnut anthracnose on leaves

a) Initial sunken light brown lesions
b) Circular to irregular dark brown lesions
c) Lesions with whitish centre
d) Yellow halos on ventral side

Continued…………

Plate-3 continued…………

(e)

(f)

(g)

(h)

e) Acervuli on undersurface of lesion

f) Formation of large necrotic patches

g) Necrotic patches on fruits

h) Defoliation

Table-3 : Symptomatological development of Anthracnose disease of walnut on twigs

Date of observation	Lesion on twig				
	Diameter(mm)		Shape	Colour	Other characteristics
	Range	Average			
April					
26	-	-	-	-	Disease free
May					
04	1-1.5	1.2	Oval	Dark brown	-
12	1.5 -2.5	2.2	-do-	-do-	Sunken
20	2.5-2.9	2.6	-do-	-do-	-do-
28	2. 5-4.5	3.4	-do-	-do-	-do-
June					
06	4.5 -7.5	5.7	-do-	-do-	-do-
14	7.5-8.0	7.7	-do-	-do-	Black pin point fruiting bodies(Acervuli)
22	8.0- 11.5	9.2	-do-	-do-	-do-
30	11.5-13.0	12.2	Oval to Irregular	Black	-do-
July					
07	13- 14.5	13.7	-do-	-do-	-do-
15	14.5-17.0	15.7	-do-	-do-	-do-
23	17.0- 19.0	18.0	-do-	-do-	-do-
31	19.0-22.0	20.3	-do-	-do-	-do-
August					
08	22.0-23.0	22.2	-do-	-do-	Coalescing of lesions
16	23.0- 25.0	24.0	-do-	-do-	-do-
24	25.0-26.0	25.1	-do-	-do-	Necrosis of twigs
31	26.0-27.0	26.5	-do-	-do-	Severely infected twigs dry up

(a)

(b)

(c)

(d)

(e)

(f)

Plate-4 : Characteristic symptoms of walnut anthracnose on twigs

a. Initial dark brown lesion
b. Sunken lesion with whitish centre
c. Coalescing of lesions
d,e. Formation of large necrotic patch
f. Necrosis of leaf and twig

4.3 Isolation of pathogen

During the present investigation, isolation was made from diseased leaves and twigs showing typical symptoms (Procedure mentioned in 3.3.1) yielded repeatedly a fungus which proved pathogenic and main cause of anthracnose disease under study. The fungal culture was raised on Oat meal agar for further studies.

4.4 Pathogenicity test

Observations regarding the pathogenicity of the test fungus revealed that the initiation of typical symptoms of the disease was recorded 14 days after inoculation on injured leaves and twigs of potted plants. However, in case of un injured leaves, the disease symptoms developed 18 days after inoculation. Re-isolation from infected leaves and twigs yielded the typical culture of the fungus, thus proved the Koch's postulates (Plate 5a,b).

4.5 Morphological characters

The morphological characters of the pathogen were studied both on host as well as from cultureand are presented in (Table 4).

4.5.1 On Host

Microscopic observations of the pathogen revealed that the mycelium was branched, septate, smooth and hyaline measuring 2-3 µm in width with an average of 2.41µm. Conidia were crescent shaped, hyaline, gutulated, one septate measuring 14.12-24.42 × 2.5-3.0 µm with an average of 17.82 × 2.26 µm. Fruiting bodies (acervuli) were sub-cuticular, discoid, pitch dark in colour and measured 105- 182µm with an average of 141.76µm.

4.5.2 In Culture

4.5.2.1 Colony characters

The petriplates inoculated with fungus, were critically observed for colony characters and the growth behaviour (Table 4). After 15 days of incubation at 21±1^{o}C, the colony appeared as circular whitish glabrous aerial mycelial tufts measuring (10-12.5 mm dia) (Plate 6a), which later turned creamy white, surrounded by flat white mass (23-24.4 mm dia) after 30 days (Plate 6b), and

(a) Symptoms on leaves

(b)Symptoms on twigs

Plate-5 : Establishment of pathogenicity of *Marssonina juglandis*on walnut seedling

finally grayish white, with appearance of characteristic concentric zones, well defined lobate margins and radial furrows (44.5-45.0 mm dia) were observed after 45 days of incubation (Plate 6c).

4.5.2.2 Microscopic characters

Microscopic observations revealed that the pathogen produced branched, septate, smooth and hyaline hyphae measuring 2.5-3.0 µm in width with an average of 2.72µm (Plate 6d).Acervuli developed in concentric rings were discoid, pitch dark in colour measuring 113.48-190.49µm in size with an average of 157.91µm (Plate 6 e,f).Conidia were crescent shaped, hyaline, gutulated with one septate measuring 20.26-32.42 × 8.10-12.15µm with an average of 23.58 × 10.13µm(Plate 6h).

4.6 Identification of the pathogen

On the basis of the morphological characters, pathogenicity test and comparison with authentic description, the causal pathogen of Anthracnose disease of walnut was identified as *Marssonina juglandis* (Lib.)Magnus.

4.7 Effect of different media on growth and sporulation of the pathogen.

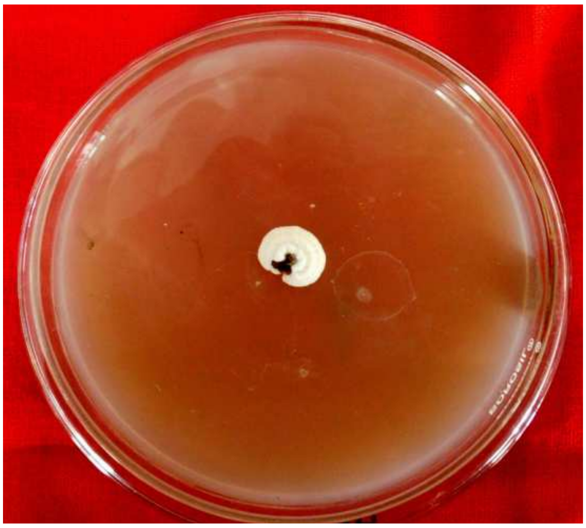
In order to ascertain the best solid media for the maximum growth and sporulation, the pathogen was grown on six different solid media viz.potato dextrose agar, Richards, Czapeks (Dox) agar, corn meal agar, malt extract agar and oat meal agar.The average radial growth (cm) was recorded in each media after 30 days of incubation at 20 ± 1^{o}C and the results obtained are presented in Table 5 and Fig. 3

The data reveals that fungus can utilize number of media for its growth. The maximum growth was recorded on corn meal agar (6.11cm) followed by oat meal agar (2.75cm) and potato dextrose agar (2.43cm). There was no significant difference between oat meal agar and potato dextrose agar and also between malt extract agar (1.78 cm) and Czapek (Dox) agar (1.42cm). Minimum growth (0.99 cm) was observed on Richard's Media (Plate 7).

Table-4 : **Morphological characters of *Marssonina juglandis* (Lib.) Magnus causing Anthracnose disease of walnut**

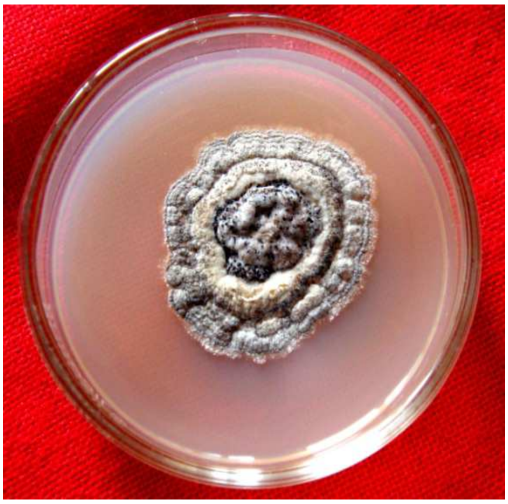
Propagule type		Colour	Size (µm)		Shape/structure	Septation
			In culture	On host		
Colony	15 days old	White	10-12.5 mm	-	Circular, whitish glaborus aerial tufts of mycelium in centre	-
	30 days old	Creamy white	23-24.4 mm	-	Circular, whitish glaborus aerial tufts of mycelium in centre, surrounded by flat white mass.	-
	40 days old	Greyish white	44.5-45 mm	-	Development of concentric zones, with well defined lobate margins, and radially furrowed.	-
Hyphae (width)		Hyaline	2.5-3 (2.72)*	2-3 (2.41)*	Smooth and branched	Septate
Conidia		Hyaline	20.26-32.42 × 8.10-12.15 (23.58×10.13)*	14.12-24.42 × 2.5-3.0(17.82×2.26)*	Crescent, guttulated, bicelled	One septa
Acervuli		Pitch dark	113.48-190.49 (157.91)*	105-182(141.76)*	Discoid, in concentric rings	-

*Figures in parenthesis are average values



a)

(a)



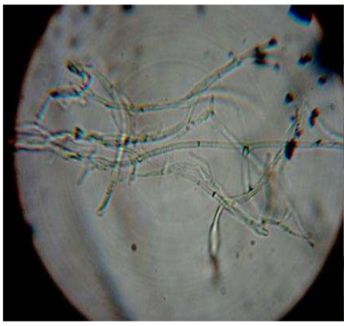
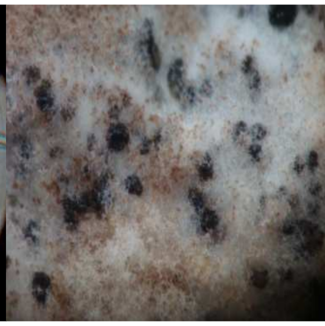
(c)

Plate-6 : **Morphological characters of *Marssonina juglandis*(Lib.) Magnus at 400x**

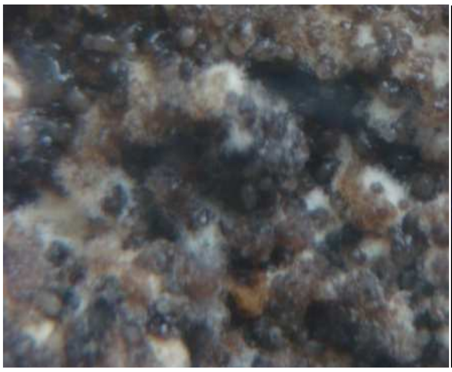
a) 15 days old white colony
b) 30 days old creamy white colony
c) 45 days old grayish white colony

Continued…………

Plate-6 Continued…………


d) **e)** **f)**




g) **h)**

d) **Mycelium**

e) **Acervuli in culture**

f) **Acervuli in concentric ring**

g) **Conidial ooze in culture**

h) **Hyaline conidia**

Table-5 : **Effect of different media on growth and sporulation of *Marssonina juglandis* at 21 ±1°C after 30 days of incubation**

Media	Radial Mycelial growth (cm)	Sporulation (10^6/ml)
Corn Meal Agar	6.11a	0.45
Oat Meal Agar	2.75b	0. 81
Potato Dextrose Agar	2.43bc	1.79
Malt Extract Agar	1.78cd	0.30
Czapek (Dox) Agar	1.42de	0.45
Richards	0.99ef	1.10
CD($p \leq 0.05$)		**0.775**

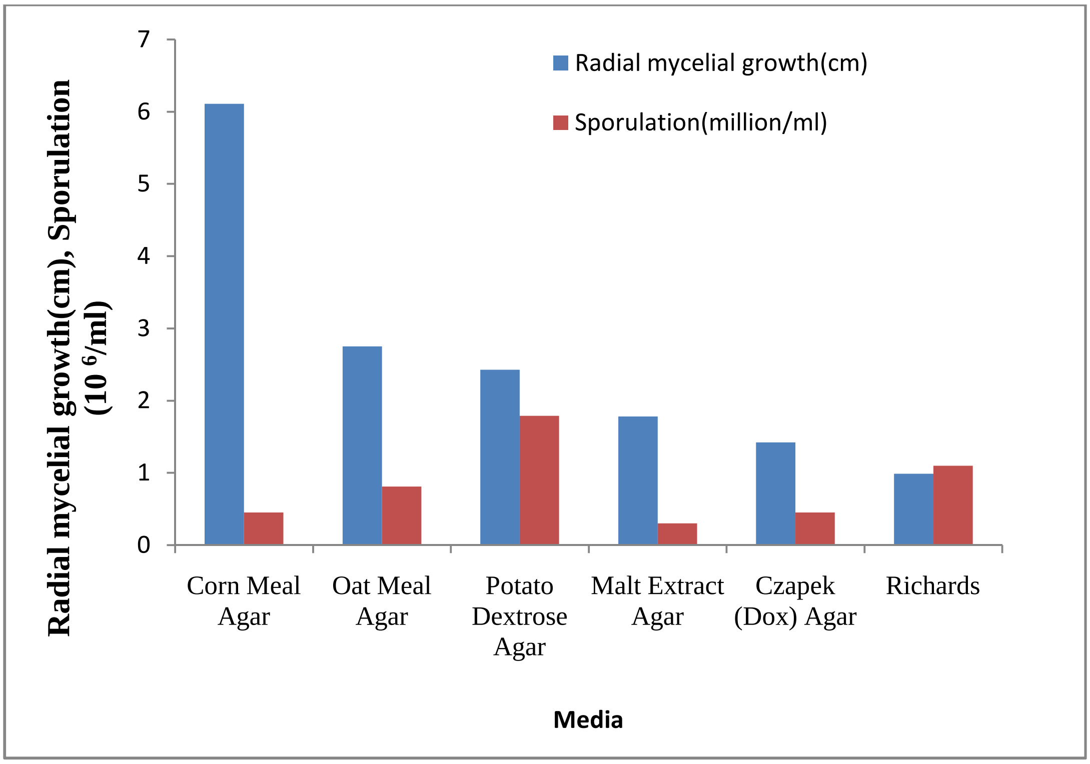


Fig. 3: Effect of different media on growth and Sporulation of *Marssonina juglandis* at 21± 1°C after 30 days of incubation

Plate-7: **Effect of different media on growth and sporulation of *Marssonina juglandis* at 21±1^{o}C after 30 days of incubation**

The data further reveals that sporulation was maximum on potato dextrose agar media 1.79 × 10^6 spores per ml followed by Richard's(1.10 × 10^6 spores per ml). Sporulation of 0.45 × 10^6per ml was recorded on both corn meal agar and Czapek (Dox) agar medium. Minimum sporulation was recorded on malt extract Agar (0.30 × 10^6 per ml).

4.8 Effect of weather parameters on development of walnut anthracnose

The intensity of walnut anthracnose was recorded on leaves and twigs at 15 days interval starting from the last week of April to last week of August during 2013. Dataobtained was correlated with the mean maximum and minimum atmospheric temperature, mean maximum and minimum relative humidityand mean rainfall during the corresponding period (Table 6) and illustrated in (Fig. 4 and 5).

The data revealed that the disease initiated under field conditions in the last week of May on both leaves as well as on twigs, when the mean atmospheric temperature both maximum and minimum were 21.4^{o}C and 8.4^{o}C, respectively and mean relative humidity both maximum and minimum were 84.66 and 56.40 per cent, respectively. Gradual increase in minimum temperature from 8.4^{o}C to 18.6^{o}C and mean minimum relative humidity from 56.4 to 65.0 per cent, coupled with moderate rainfall favoured the gradual spread of disease from 19.06 to 95.00 per cent on leaves and from 20.0 to 30.2 per cent on twigs. The maximum rate of disease progress was observed during the first fortnight of May on both leaves and twigs which coincided with the favourabletemperature and relative humidity for its progress.

The correlation coefficients of per cent disease intensity on leaves and twigs of walnut with mean maximum and minimum temperature, mean maximum and minimum relative humidity and mean rainfall are presented in (Table 7). The percent disease intensity on leaves showed positive correlation with mean minimum and maximum atmospheric temperature with correlation coefficients of 0.94 and 0.72, respectivelyfollowed by mean minimum relative humidity (r =

0.24) and mean rainfall (r = 0.03). However a negative correlation was observed with mean maximum relative humidity(r =-0.50).

The per cent disease intensity on twigs showed positive correlation with mean minimum and maximum atmospheric temperature with correlation coefficients of 0.88 and 0.77, respectively. However a negative correlation was observed with mean rainfall, mean maximumand minimum relative humidity with correlation coefficients of -0.17, -0.74 and -0.07, respectively on twigs.

4.9 Perpetuation

The diseased walnut leaves and twigs kept in the field of walnut orchard during winter were examined for the presence of acervuli, conidial production and viability. The weekly observations recorded from first week of March are presented in the (Table 8).

It is evident from the table that the presence of acervuli was observed on the leaves and twigs throughout the course of study. However, the acervuli were found immature on both leaves and twigs upto 1st week of April. These acervuli contained only mycelium upto the 1st week of april, while as formation of conidia was observed from 2nd week of April which coincided with the emergence of leaves in the walnut. However the average number of acervuli on leaves and twigs reached upto maximum of 4.2 and 5.8 per cm^2 of leaf and twig area, respectively in the 3rd week of May and decreased thereafter due to increase in sampling time. The average number of conidia on leaves was found to increase with every subsequent observation with maximumof 0.85×10^6 per ml in the 2nd week of May which decreased during subsequent observations upto 0.22×10^6 per ml in the 3rd week of June. The conidia were also examined for their viability. The conidia were found (51.60%) viable even on the first day of their appearance which reached maximum of 84.23 per cent and decreased

Table-6 :Effect of weather parameters on the development of anthracnose disease on walnut leaves and twigs

Date of observation(Fortnightly)	Average temperature (^oC)		Average Rainfall (mm)	Average relative humidity		Disease intensity(%)		Disease progress rate	
	Maximum	Minimum		RH1	RH2	Leaves	Twigs	Leaves	Twigs
30-04 -2013	20.10	8.20	6.45	92.26	61.60	00.00	00.00	0.000	0.0000
15-05-2013	21.40	8.40	1.78	84.66	56.40	19.06	20.00	0.195	0.1950
30-05-2013	26.53	9.99	2.01	75.12	42.62	20.30	20.13	0.003	0.0003
14-06-2013	29.73	14.36	4.46	79.93	52.26	30.36	21.21	0.026	0.0034
29-06-2013	29.13	15.45	2.68	78.86	48.00	46.93	22.91	0.028	0.0051
14-07-2013	28.02	16.84	3.57	79.26	59.33	71.98	24.82	0.028	0.0052
29-07-2013	31.75	18.60	0.80	74.12	47.62	89.31	27.98	0.014	0.0076
13-08-2013	29.01	18.48	9.81	81.26	59.20	92.36	30.20	0.002	0.0051
28- 08-2013	28.18	17.89	2.08	80.50	65.43	95.00	0.20	0.001	0.0000

RH1 = Maximum relative humidity

RH2 = Minimum relative humidity

Table-7 : Correlation coefficients between disease intensity on leaves and twigs with weather parameters during 2013

Disease Intensity	Average temperature(°C)		Averagerainfall (mm)	Average relative humidity(%)	
	Max.	Min.		Max.	Min.
Leaves	0.72	0.94	0.03	-0.50	0.24
Twigs	0.77	0.80	-0.17	-0.74	-0.07

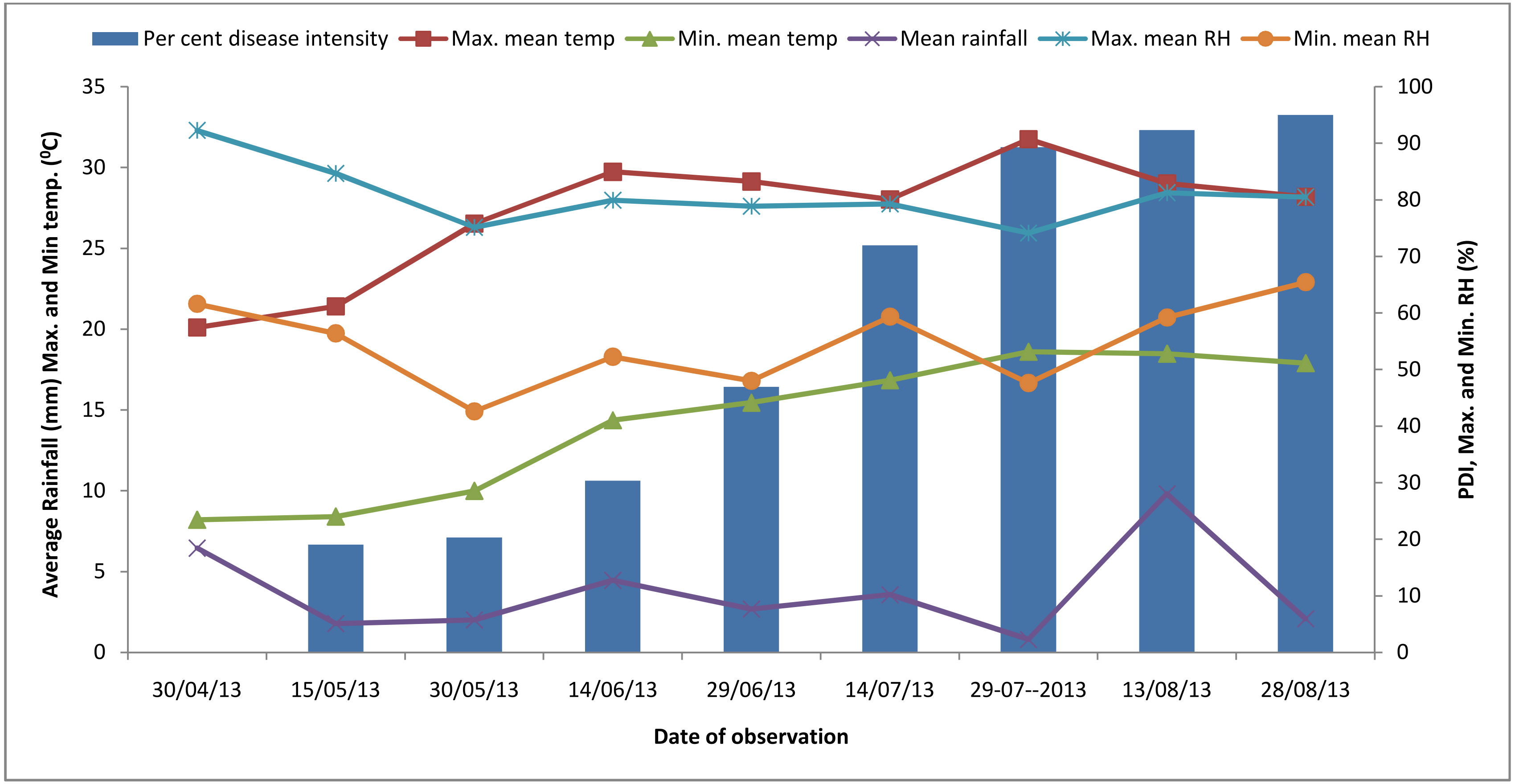


Fig. 4: Effect of weather parameters on the development of walnut anthracnose on leaves

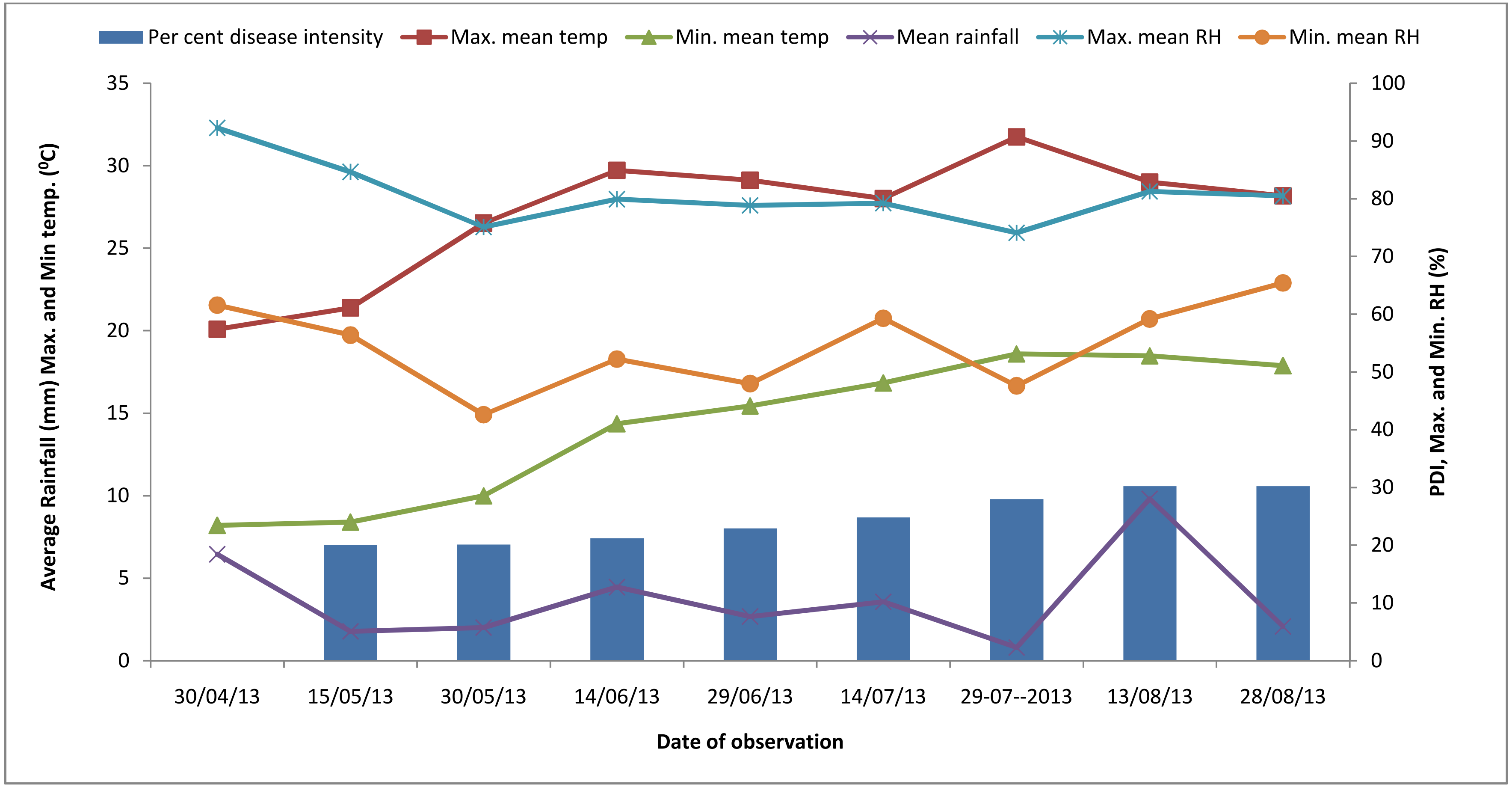


Fig. 5: Effect of weather parameters on the development of walnut anthracnose on twigs

thereafter with every subsequent observation with increase in sampling time. Similarly, the average number of conidia obtained from twigs was found to increase with every subsequent observations with maximum of 1.29 × 10^6 per ml in the 2nd week of May which decreased during subsequent observations upto 0.89 × 10^6per ml in the last week of June. The viability reached upto maximum of 85.08 per cent in the 2nd week of May and decreased thereafter with every subsequent observation with increase in sampling time.

However no perfect state of the pathogen was observed during the investigation.

5.0 *In vitro* evaluation of fungitoxicants

The efficacy of various systemic and non-systemic fungitoxicants against *Marssonina Juglandis* was evaluated *in vitro* by slide germination and poisoned food techniques.

5.1 Effect of fungitoxicant on spore germination

Six systemic and six non-systemic fungitoxicants at four different concentrations were evaluated for their comparative efficacy against spore germination of *M. Juglandis* by cavity slide germination technique.

Perusal of data (Table 9) revealed that all the six systemic fungitoxicants at all the test concentrationsinhibited spore germination. Among the systemic fungitoxicants tested, metiram + pyraclostrobin 60WG proved to be most effective as it provided 98.84 per cent spore germination inhibition over check followed by tebuconazole 25EC and flusilazole 40EC, exhibiting 93.16 and 89.50 per cent spore germination inhibition, respectively. However, carbendazim 50WP (87.45%) and thiophanate methyl 70WP (84.75%) were stastically at par. Difenconazole 25EC proved to be least effective with only 67.16 per cent germination inhibition.

On an overall basis the inhibitory effect of test fungitoxicants increased with increase in concentration. A minimum inhibition of 71.79 per cent was achieved at 50 ppm which increased gradually to 97.72 per cent at 200 ppm concentration.

Table-8 : Production and viability of conidia on overwintering diseased leaves and twigs of walnut

Date of observation	Leaves/ cm^2				Twigs/cm^2			
	Stage of acervuli	Average no of acervuli	Average no of conidia $\times 10^6$/ ml	Viability (%)	Stage of acervuli	Average no of acervuli	Average no ofconidia$\times 10^6$/ ml	Viability (%)
01-3-2014	I	2.2	Nil	Nil	I	2.4	Nil	Nil
07-3-2014	I	2.2	-	-	I	2.3	-	-
14-3-2014	I	2.3	-	-	I	2.5	-	-
21-3-2014	I	2.6	-	-	I	2.9	-	-
28-3-2014	I	2.5	-	-	I	3.0	-	-
04-4-2014	M	2.8	-	-	I	3.3	-	
10-4-2014	M	3.0	0.35	51.60	M	3.5	0.37	54.88
17-4-2014	M	3.0	0.45	70.32	M	3.6	0.53	69.66
24-4-2014	M	3.2	0.50	65.50	M	3.6	0.67	78.09
01-5-2014	M	3.4	0.65	73.04	M	4.0	0.98	74.88
08-5-2014	M	4.0	0.85	79.80	M	4.9	1.29	85.08
15-5-2014	M	4.2	0.55	84.23	M	5.8	1.21	78.64
22-5-2014	M	4.1	0.22	49.64	M	5.4	1.12	64.66
28-5-2014	E	3.0	Nil	Nil	M	3.6	0.89	40.44
04-06-2014	D	-	-	-	E	2.4	-	-

I = immatureM = mature, D = decomposed leaves, E= Empty,-= Nil

A non-significant interaction existed between test fungitoxicants and their concentration with a minimum inhibition of 20.66 per cent was recorded at 50ppm in difenconazole 25EC and cent per cent in metiram + pyraclostrobin 60WG beyond 50ppm. Metiram + pyraclostrobin 60WG at 100 ppm proved to be as effective as tebuconazole 25 EC at 200 ppm.

Perusal of the data table (10) revealed that all the non systemic fungitoxicants differ significantly among each other except mancozeb 75WP, copper oxychloride 50WP, and captan 50WP which showed non significant difference among each other. While making the overall comparison among the fungitoxicants, mancozeb 75WP was significantly superior with cent per cent spore germination inhibition followed by decreasing order of their efficacy captan 50WP, copper oxychloride 50WP, propineb 70WP, chlorothalonil 75WP, exhibiting 99.83, 99.41, 68.33, and 57.66 per cent germination inhibition, respectively. Minimum per cent inhibition of 55.08 per cent was recorded in ziram 80WP. Mancozeb at each respective concentration was found to be statistically superior to all test fungitoxicants.

The data further reveals that the mancozeb 75WP proved significantly most effective by providing cent per cent germination inhibition at all the test concentrations while as captan 50WP and copper oxychloride 50WP provided cent per cent inhibition at 500, 1000 and 1500ppm, respectively. It was followed by captan@ 250ppm(99.33%), copperoxychloride@ 250ppm (97.66%), cholorothalo-nil @ 1500ppm(95.33%), ziram@ 1500ppm(93.00), propineb @ 1000 ppm (90.00%),chlorothalonil @1000 ppm (70.00%), ziram@1000ppm(69.33%) and propineb@500ppm (56.66%) in decreasing order. The next best fungitoxicants were observed to be chlorothalonil@ 500 ppm (52.00%), Ziram@ 500ppm (46.66%) and propineb@250ppm (26.66%). However the chlorothalonil @ 250ppm (13.33%) and ziram @ 250ppm (11.33%) were least effective and were statistically at par with each other.

Table-9 : ***In vitro*** **efficacy of various systemic fungitoxicants in inhibiting spore germination of** ***Marssonina Juglandis***

Concentration (ppm) / Fungitoxicant	*Spore germination inhibition over check (%)				
	50	**100**	**150**	**200**	**Mean**
Carbendazim 50WP	76.00 (60.64)	85.53 (67.61)	92.29 (73.84)	95.99 (78.41)	**87.45 (70.12)[cd]**
Metiram+Pyraclostrobin 60WG	95.39 (77.57)	100 (89.39)	100 (89.39)	100 (89.39)	**98.84 (86.43)[a]**
Flusilazole 40 EC	81.40 (64.42)	86.00 (68.00)	93.30 (74.62)	97.33 (80.70)	**89.50 (71.93)[c]**
Tebuconazole 25 EC	86.66 (68.64)	92.66 (74.29)	93.32 (75.02)	100 (89.39)	**93.16 (76.83)[b]**
Difenconazole 25 EC	20.66 (27.02)	60.00 (51.12)	91.33 (72.87)	96.66 (79.56)	**67.16 (57.64)[f]**
Thiophanate Methyl 70 WP	70.66 (57.39)	84.00 (66.50)	88.00 (70.16)	96.33 (78.59)	**84.75 (68.25)[de]**
Mean	**71.79 (59.28)[d]**	**84.69 (69.48)[c]**	**92.99 (75.98)[b]**	**97.72 (82.73)[a]**	

$CD_{p \leq 0.05}$

Fungicide	2.65
Concentration	2.17
Fungicide × Concentration	5.31

Germination in check = 100%
*Average of three replications
Figures in parenthesis are arc sine transformed values

On an overall basis, the inhibitory effect of fungitoxicants increased with increase in concentration of fungitoxicants. A minimum inhibition of 58.05 per cent was achieved at 250ppm which increased gradually to 98.05 per cent at 1500 ppm concentration. The corresponding inhibition at 500 and 1000 ppm concentration was noticed to be 75.88 and 88.22 per cent, respectively.

5.2 Effect of fungitoxicants on mycelial growth

Analysis of the data (Table 11) revealed that all the systemic fungitoxicants significantly inhibited the mycelial growth of *M. Juglandis* at all the test concentration. Metiram + pyraclostrobin 60WG proved significantly superior to other test fungitoxicants in inhibiting mycelial growth over check. It was followed by tebuconazole 25EC, flusilazole 40EC, carbendazim 50WP and thiophanate methyl 70WP exhibiting mycelial growth inhibition of 98.72, 94.25, 91.14 and 89.83 per cent respectively. However, difenconazole 25EC proved least effective with mycelial growth inhibition of 77.51 per cent over check.

Tebuconazole, metiram + pyraclostrobin @ 100, 150, 200 ppm and flusilazole@ 150 and 200ppm while as carbandazim, thiophanate methyl, difenoconazole at 200 ppm concentration provided cent per cent mycelia inhibition over check. It was followed by metiram+pyraclostrobin@50ppm (98.42%),
carbendazim@150ppm(95.77%),difenoconazole@150ppm(95.20%),tebuconazole @50ppm(94.90%),flusilazole@100ppm(92.06%),carbendazim @ 100 ppm (88.60%),
flusilazole@50ppm(84.96%),thiophanatemethyl@50ppm(75.98%),carbendazim@ 50ppm(80.02%),thiophanatemethyl@50ppm(75.98%),anddifenoconazole@ 100ppm(65.36%). Difenconazole@50ppm provided least mycelial growth inhibition of 49.50 per cent over check.

Table-10 : ***In vitro*** **efficacy of various non-systemic fungitoxicants in inhibiting spore germination of *Marssonina Juglandis***

Concentration(ppm) / Fungitoxicant	*Spore germination inhibition over check (%)				
	250	**500**	**1000**	**1500**	**Mean**
Chlorothalonil 75WP	13.33 (21.07)	52.00 (46.13)	70.00 (56.97)	95.33 (77.93)	**57.66 (50.53)[e]**
Mancozeb 75 WP	100 (89.31)	100 (89.31)	100 (89.31)	100 (89.31)	**100 (89.31)[a]**
Copper oxychloride 50WP	97.66 (81.83)	100 (89.31)	100 (89.31)	100 (89.31)	**99.41[abc] (87.50)**
Captan 50 WP	99.33 (86.87)	100 (89.31)	100 (89.31)	100 (89.31)	**99.83[ab] (88.76)**
Ziram 80 WP	11.33 (19.52)	46.66 (43.07)	69.33 (56.58)	93.00 (74.62)	**55.08 (48.45)[ef]**
Propineb 70 WP	26.66 (30.98)	56.66 (48.81)	90.00 (71.53)	100 (89.31)	**68.33 (60.18)[d]**
Mean	**58.05 (54.95)[d]**	**75.88 (67.69)[c]**	**88.22 (75.54)[b]**	**98.05 (85.02)[a]**	

$CD_{p\leq 0.05}$

Fungicide	2.56
Concentration	2.09
Fungicide × Concentration	5.12

Germination in check = 100%
*Average of three replications
Figures in parenthesis are arcsine transformed values

A significant interaction existed between the test fungitoxicants at all the concentrations with minimum inhibition of 80.63 per cent at 50 ppm which gradually increased to cent percent at 200 ppm. The corresponding inhibition at 100 and 150 ppm concentration was noticed to be 89.10 and 97.62 per cent, respectively.

Among the non systemic fungitoxicants, analysis of data Table12) revealed that all the six test fungitoxicants significantly inhibited mycelial growth of *M. juglandis* as compared to check. All the fungitoxicants differed significantly from one another in their efficacy against the causal fungus. Captan50WP proved to be effective with per cent mycelial inhibition of 95.43 followed by mancozeb 75WP, copper oxychloride 50WP, propineb70WP, and chlorothalonil 75WP exhibiting per cent inhibition of 93.36, 90.20, 70.09 and 64.77, respectively while as ziram 80WP exhibited least mycelial inhibition of 58.69 per cent. Captan@ 1000and 1500ppm proved significantly superior by providing cent per cent mycelial inhibition over check, while as mancozeb and copper oxy chloride provided cent per cent inhibition at 1500ppm. It was followed by mancozeb@1000ppm (95.32%), copper oxy chloride@1000ppm(94.27%),propineb@1500ppm(91.33%),mancozeb@500ppm(90.20%),copper oxychloride@ 500ppm (89.76%), chlorothalonil@1500ppm(88.86%), and mancozeb@ 250ppm(87.93%). The next best fungitoxicants were propineb @ 1000 ppm (83.16%), copperoxychloride @ 250 ppm(76.80%),chlorothalonil@1000ppm(75.03%),ziram @ 1000 ppm (69.93%), propineb @ 500 ppm (63.90%), chlorothalonil @ 500 ppm (59.96%), ziram @ 500 ppm (54.90%), propineb @ 250 ppm (42.00%) chlorothalonil@ 250ppm(35.23%), and ziram@ 250ppm (25.03%) in decreasing order of their efficacy.A significant interaction was observed to exist between fungitoxicant and concentration. Irrespective of fungitoxicants, mean inhibition in mycelialgrowth increased significantly from 58.97 at 250 ppm to 94.18 per cent at 1500 ppm. The corresponding inhibition at 500 and 1000 ppm concentration was noticed to be 75.60 and 86.28 per cent, respectively.

Table-11 : ***In vitro*** **efficacy of various systemic fungitoxicants in inhibiting mycelial growth of *Marssonina Juglandis***

Concentration (ppm) / Fungitoxicants	*Mycelial growth inhibition over check(%)				
	50	**100**	**150**	**200**	**Mean**
Carbandazim 50WP	80.02 (63.42)	88.60 (70.23)	95.77 (78.08)	100 (89.9)	**91.14 (75.28)[d]**
Metiram + Pyraclostrobin	98.42 (82.25)	100 (89.39)	100 (89.39)	100 (89.39)	**99.60 (87.75)[a]**
Flusilazole 40EC	84.96 (67.16)	92.06 (73.61)	100 (89.39)	100 (89.39)	**94.25 (79.88)[c]**
Tebuconazole 25EC	94.9 (76.91)	100 (89.39)	100 (89.39)	100 (89.39)	**98.72 (86.27)[b]**
Difenconazole 25EC	49.50 (47.85)	65.36 (53.92)	95.2 (77.31)	100 (89.39)	**77.51 (64.24)[f]**
Thiophanate Methyl 70WP	75.98 (60.62)	88.59 (70.22)	94.78 (76.74)	100 (89.39)	**89.83 (67.12)[e]**
Mean	**80.63 (64.75)[d]**	**89.10 (74.46)[c]**	**97.62 (83.38)[b]**	**100 (89.39)[a]**	

$CD_{p\leq 0.05}$

Fungicide	0.24
Concentration	0.19
Fungicide × Concentration	0.48

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

Table-12 : ***In*** **vitro efficacy of various non-systemic fungitoxicants in inhibiting mycelial growth of *Marssonina Juglandis***

Concentration (ppm)	*Mycelial growth inhibition over check (%)				
Fungitoxicants	**250**	**500**	**1000**	**1500**	**Mean**
Chlorothalonil 75WP	35.23 (36.39)	59.96 (50.72)	75.03 (59.99)	88.86 (70.48)	64.77 (54.40)[e]
Mancozeb 75WP	87.93 (69.64)	90.20 (71.73)	95.32 (77.47)	100 (89.39)	93.36 (77.06)[b]
Copper oxychloride 50WP	76.80 (61.18)	89.76 (71.31)	94.27 (76.12)	100 (89.39)	90.20 (74.50)[c]
Captan 50WP	86.83 (68.69)	94.90 (76.91)	100 (89.39)	100 (89.39)	95.43 (81.09)[a]
Ziram 80WP	25.03 (30.01)	54.90 (47.79)	69.93 (56.72)	84.90 (67.10)	58.69 (50.40)[f]
Propineb 70WP	42.00 (40.38)	63.90 (53.04)	83.16 (65.75)	91.33 (72.87)	70.09 (58.01)[d]
Mean	**58.97 (51.05)[d]**	**75.60 (61.92)[c]**	**86.28 (70.70)[b]**	**94.18 (79.73)[a]**	

$CD_{p \leq 0.05}$

Fungicide	0.22
Concentration	0.18
Fungicide × Concentration	0.44

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

DISCUSSION

Walnut is economically important dry fruit crop mainly cultivated in temperate regions of the world. India stands seventh in production accounting up to 2.14 per cent of the world walnut production (Anonymous, 2010) where in Jammu and Kashmir alone accounts for more than 98 per cent of the India's total production with an annual production of 20.87 thousand MT from an area of 83,219 hectares (Anonymous, 2012; Sharma, 2012). Its cultivation plays a significant role in economic profile of the farmers living in hilly and backward areas, where economic condition of the people is extremely fragile.The walnut crop is prune to various diseases which inflicts considerable yield losses. Walnut anthracnose caused by *Marssonina juglandis* is one of the most severe disease both in abroad and India. The disease generally retards the size and mass of nuts, failure in metabolic processes in leaves and change in biochemical indices (Shirnina and Kotljarova, 2000).

Perusal of the literature revealed that the disease has also been reported from Kashmir but no work has been conducted on any aspect of this disease and hence the present study was undertaken.

In the present study, survey conducted in three districts of Kashmir valley during 2013 indicated that anthracnose disease of walnut was more or less prevalent in all the locations of three districts. Prevalence of the disease has also been reportedly observed in different walnut growing areas of abroad (Belisario, 2002; Kalkism, 2012; Salahi *et al*., 2009) and India (Sharma*et al.*, 2014).

During the course of survey, significantly highest mean disease incidence of 97.94 and 74.66 per cent and mean intensity of 55.76 and 22.77 per cent was recorded on leaves and twigs, respectively in district Anantnag. It was followed by district Budgam with corresponding incidence of (97.83 and 72.27%) and intensity (50.11 and 20.58%), respectively. The disease incidence of these two districts did not differ significantly. Significantly least disease incidence (96.22 and 68.41%) as well as intensity (49.03 and 15.32%) on leaves and twigs respectively, was observed in district Kupwara. These findings are in conformity with the estaiblished phenomenon of disease development i.e. more the disease incidence more will be the intensity of disease (Fry, 1988). So far as locations are concerned maximum disease incidence of 98.75 and 85.00 per cent with an intensity of 56.87 and 35.00 per cent on leaves and twigs, respectively was recorded at saller location of district Anantnag while as, the minimum disease incidence (94.50 and 65.23%) and intensity (44.51 and 14.81%) , respectively was recorded at Sogam location of district Kupwara. Higher disease at some locations of districts could be attributed to higher plant density leading to higher relative humidity in the microclimate of the plants, higher temperature during the growing season, besides non disposal of fallen diseased leaves. These observations are supported by the findings of Black and Neely (1976), Neely (1986), Rasnov and Naidenov (1986) and Pschedit and Ocamb (2014).

Observations recorded on disease development with respect to Symptamotology of the disease revealed that first appearance of disease was observed simultaneously on both twigs and leaves in the Ist week of May. The lesions on the leaves appeared as small, circular, light brown, slightly sunken lesions measuring 0.5 to 1.0 mm in dia. Similarly oval, dark brown lesions appeared on twigs measuring 1-1.5 mm in dia. The lesions gradually increased in size and attained maximum of 27 to 29 mm and 26 to 27 mm on leaves and twigs, respectively in the last week of August. Small black acervuli were visible on leaves and twigs in the first and second week of June, respectively. Yellow halos were observed around the leaf spots in the 2nd week of July. Lesions on the leaves turned irregular in shape due to coalescing of lesions, resulting in the formation of irregular patches in the third and fourth week of July and chlorosis of the highly infected leaves was observed in the first week of august while in case of twigs coalescing of lesions were observed in the Ist week of August, followed by drying up of the severely infected twigs. Similar type of symptoms on leaves and twigs caused by *M. juglandis* were also observed by earlier workers (Black and Neely, 1976; Belisario, 2002; Saremi *et al.,* 2011).

The causal organism (*M. juglandis*) involved in anthracnose disease of walnut was isolated from diseased walnut leaves and twigs collected during survey on oat meal agar medium and subsequently maintained for further studies. Morphological characteristics of *M. juglandis*observedon the host as well as in the culture (Oat meal agar) media were almost identical, except that size of conidia, Acervuli and hyphae were slightly larger in culture as compared to that of host. After 5 days of incubation the fungal colony appeared as circular whitish glabrous aerial mycelial tuft (10-12.5mm dia) which turned creamy white surrounded by flat white mass (23 -24.4 mm) after 30 days. Similarly after 45 days of incubation, the colony (44.5- 45.0 mm) appeared as grayish white with characteristic concentric zones, well defined lobate margins and radial furrows. The hyphae were branched, hyaline and septate measuring 2-3 µm in width with an average of 2.41µm on host and 2.5-3µm with an average of 2.72µm in culture. Conidia were hyaline, crescent, gattulated measuring 14.12-24.42 × 2.5-3.0µm with an average size of 17.82 × 2.26 µm (On host) and 20.26-32.42 × 8.10 -12.15 µm with an average of 23.58 × 10.13 µm (In culture) with one septa. Acervuli were subcuticular, in concentric rings, discoid and pitch dark measuring 105-182 µm in dia on host and 113.48- 190.49µm in culture. The above morphological characters of the pathogen resemble with the description given by Sogonov *et al.* (2008), Dastjerdi *et al.* (2009), Saremi and Amiri (2010) and Jamshidi *et al. (*2013).

On the basis of morphological characters, pathogenicity and comparison with the authentic description, the pathogen was identified as *Marssonina Juglandis*(Lib.)Magnus.

Several reports indicate that microorganisms differ in their nutritional requirements (Cochrane, 1958). In order to determine the best medium for growth and sporulation of *M. juglandis* the fungus was grown on six different solid media viz. corn meal agar,Richard's, oat meal agar, potato dextrose agar, malt extract agar, Czapek (dox) agar. The maximum radial mycelial growth was achieved on corn meal agar followed by oat meal agar, potato dextrose agar, malt extract agar and Czapek (Dox) agar medium, while the least growth was recorded on

Richard's. The fungus sporulated best on potato dextrose agar medium, followed by Richard's, oat meal agar while as corn meal agar exhibited same sporulation as Czapek (Dox) agar medium. The least sporulation was recorded in malt extract agar. These results are in agreement with Spiers (1981), who found corn meal agar as best media for *M. brunea* and those of Nadroo (2006) and Salahi*et al.* (2009) who observed oat meal agar and potato dextrose agar as best medium for growth and sporulation, respectively.

The disease initiated in the last week of May on both leaves and twigs, when atmospheric temperature both maximum and minimum were 21.4^{o}C and 8.4^{o}C, and maximum and minimum relative humidity were 84.66 and 56.40 per cent, respectively. It was interesting to note here that gradual increase in minimum temperature from 8.4^{o}C to 18.6^{o}C and minimum relative humidity from 56.4 to 65.0 per cent, coupled with moderate rainfall favoured the gradual spread of disease from 19.06 to 95.0 on leaves and 20.0 to 30.2 per cent on twigs. Correlation of disease development on leaves and twigs with meteorological factors revealed that the per cent disease intensity was highly correlated the mean minimum temperature (r = 0.94 and r = 0.80), followed by mean maximum temperature (r = 0.72 and r = 0.77) on leaves and twigs respectively, which was followed by mean minimum relative humidity (r = 0.24) and mean rainfall (r = 0.03) on leaves. The present investigation is in complete agreement with various workers who have also reported that moderate temperature, high humidity and high rainfall were necessary and favourable for its infection and disease development (Vonica (1970); Black and Neely (1978); Rosnev and Naidenov (1986); Hashemi (2005).

While studying the perpetuation of the fungus it was observed that fungus perpetuated in the form of acervuli on the fallen diseased leaves and twigs during winter. The leaves and twigs kept at ground floor of walnut orchard exhibited the presence of the acervuli and conidia upto third and fourth week of May. The maturation of the acervuli and conidiawere found in the first week of April which coincided with the appearance of leaves and twigs in the field. Average number of acervuli and conidia per centimeter square of area were observed to be more on twigs as compared to leaves. The maximum average number of acervuli (4.2 and 5.8) on leaves and twigs, respectively were recorded in the 2^{nd} week of May and declined thereafter till leaves and twigs were found to be decomposed. The maximum average number of spores 0.85×10^6 spores per ml and 1.29×10^6 spores per ml on the leaves and twigs, respectively were recorded in the second week of May and decreased till most of acervuli were found empty. Maximum spore viability of 84 and 85 per cent on leaves and on twigs, respectivelywas observed in the mid May and decreased gradually thereafter. The results of the present study suggest debris as primary source of inoculum. The present investigation are in complete agreement with the findings of Sharma *et al.*(2009) who reported Acervuli as source of primary infection for *Marssonina leaf blotch* of apple and also in agreement with the findings of Dimova and Arnaudov (2008), Saremi and Amiri (2010) and Veghelyi and Penzer (1990) who reported that the pathogen causing anthracnose disease of walnut overwinters primarily on infected leaf debris as acervuli and the development of conidia and secondary infection occurred almost continuously until late summer.

During the course of present study, no perfect state of the pathogen was observed and also no literature is available regarding its occurrence in India.

Fungicides are the frontline weapon against pathogen and are still one of the most widely used means of disease control. In present investigation, six systemic and six non systemic fungitoxicants were screened *in vitro* by spore germination and poisonfood techniques. The evaluated fungitoxicants revealed that all the fungitoxicants inhibited spore germination and mycelial growth of *M. juglandis* at various concentrations.

Among the systemic fungitoxicants metiram + pyraclostrobin 60WG proved to be significantly effective at each respective concentration by exhibiting mean germination inhibition of 98.84 per cent, followed by tebuconazole 25EC (93.16 %), and flusilazole 40EC (89.50%), which did not differ significantly from carbendazim 50WP (87.45%).These fungitoxicants at 200ppm were highly effective in spore germination inhibition. These fungitoxicants were also effective even at least concentration (50ppm) and provided more than 70 per cent inhibition. Difenconazole 25EC was least effective exhibiting spore germination inhibition of 67.16 per cent. Metiram + Pyraclostrobin 60WG and tebuconazole 25EC exhibited cent per cent inhibition at 100 and 200 ppm concentration, respectively.

All the non systemic fungitoxicants inhibited spore germination of *M. juglandis*excellently at 1500ppm.*In vitro* screening of the non systemic fungitoxicants revealed that the mancozeb 75WP, captan 50WP, copper oxy chloride 50WP, were also effective even at lower concentration (250ppm) exhibiting more than 90 per cent inhibition. However, the effect of test fungitoxicants increased with increase in concentration. In overall comparision mancozeb75WP exhibited cent percent mean germination inhibition at all the test concentrations followed by captan 50WP and copper oxychloride 50WP exihibiting 99.83 and 99.41 per centinhibition respectively, which were stastically at par. The other fungitoxicants in decreasing order of their efficacy were propineb 70WP (68.33%), chlorothalonil 75WP (57.66%) and ziram 80WP (55.08%).

All the fungitoxicants tested *in vitro* checked the mycelial growth of *M. juglandis* at various concentrations. The systemic fungitoxicants were highly effective in inhibiting mycelial growth at 200ppm by providing cent percent inhibition. It was observed that metiram + pyraclostrobin 60WG and tebuconazole 25EC @ 100, 150 and 200 ppm while as flusilazole 40EC @ 150 and 200ppm, carbendazim 50WP, thiophanate methyl 70WP and difenoconazole 25EC @ 200ppm proved to be highly effective by providing cent percent mycelial inhibition. The overall comparision among systemic fungitoxicants revealed a significant difference. Metiram + pyraclostrobin 60WG proved to be suprerior at each concentration exhibited mean mycelial inhibition of 99.60 per cent. It was followed by tebuconazole 25EC (98.72%), flusilazole 40EC (94.25%), carbendazim 50WP (91.14 %), and thiophanate methyl 70WP (89.83%), while as least mycelial inhibition of 77.51 per cent was recorded by difenconazole 25EC.

Among the non systemic fungitoxicants tested captan 50WP proved to be suprerior at each concentration exhibited mean mycelial growth inhibition of 95.43 per cent. It was followed by mancozeb 75WP (93.36%), copper oxy chloride 50WP (90.20%), propineb 70WP (70.09%), and chlorothalonil 75WP (64.77%), while as least mycelial inhibition of 58.69 per cent was provided by ziram 80WP. Various workers have also reported the efficacy of metiram + pyraclostrobin, tebuconazole, flusilazole, carbandazim, mancozeb, captan, copper oxy chloride for management of walnut anthracnose caused by *M. juglandis*(Vonica, 1970; Berry, 1977; Kleiner and Bulatova, 1978; Zamani *et al.*, 2011; Nakova and Dimova, 2003; Sharma, 2000).

Chapter– 6

SUMMARY AND CONCLUSION

The investigations on "Anthracnose disease of walnut in Kashmir" were carried out during 2013-2014. The results obtained during the course of this study are summarized as under:

The survey of the walnut growing areas for anthracnose disease in district Anantnag, Budgam and Kupwara revealed the prevalence of the disease in all the three districts surveyed with overall incidence irrespective of locations was 97.34 and 71.78 per cent, and overall intensity of 51.66 and 19.56 per cent on leaves and twigs, respectively. The maximum disease incidence of 97.94 and 74.66 per cent and intensity of 55.76 and 22.77 per cent on leaves and twigs respectively was observed in district Anantnag and minimum disease incidence of 96.22 and 68.41 per cent and intensity of 49.03 and 15.32 per cent, respectively was observed in district Kupwara.

Amongst the locations surveyed, Saller location of district Anantnag exhibited the highest disease incidence of 98.75 and 85.00 percent and intensity of 56.87 and 35.00 percent on leaves and twigs, respectively. Whereas, the least incidence of 94.50 and 65.23 per cent and intensity of 44.51 and 14.81 percent on leaves and twigs, respectively was observed in Sogam location of district Kupwara.

Disease under natural conditions was first noticed in first week of May and progressed steadily and reached its peak during late period of August. The symptoms first appeared on leaves as small, light brown, slightly sunken lesions, with somewhat circular margins. Small black acervuli in concentric rings were seen on undersurface of leaves. The Periodical changes in size, shape and colour of the lesions were noticed and finally enlarged upto 27 to 29 mm in dia and coalesced to form large necrotic patches, leading to premature leaf fall. The symptoms on twigs were noticed in the first week of April and reached its peak during late period of August. The symptoms first appeared as dark brown, oval lesions, which later on turned sunken with whitish centre. Black acervuli were visible on these lesions in the 2nd week of June. The periodical changes in size, shape and colour of the lesion were noticed and finally the lesions enlarged up to

26.0 to 27.0 mm which later on coalesce, resulting in necrosis and drying up of severely infected twigs.

The pathogen was isolated from the diseased walnut leaves and twigs collected during survey on oat meal agar and subsequently maintained for further studies. The pathogenicity of the isolated fungus was established by proving Koch's postulates. The characteristic disease symptoms were produced after14 days on injured leaves and twigs of potted plants while as on uninjured plant, the symptoms were produced after 18 days of inoculation.

The isolate on oat meal agar medium produce white mycelial colony with circular glabrous aerial tuft of mycelium in the center, which later on turned creamy white surrounded by white flat mass. Finally turned grayish white with characteristic concentric zones, lobate margins and radial furrows. Conidia were hyaline crescent shaped, gatulated with one septa, measuring 17.82 × 2.26 µm (on host) and 23.58 × 10.13µm (in culture).

On the basis of morphological characters, pathogenicity and comparison with authentic description, the isolate was identified as *Marssonina juglandis* (Lib.)Magnus.

Maximum mycelial growth of the fungus was achieved on corn meal agar medium while as potato dextrose agar (PDA) proved to be the best sporulating media.

The disease development in the field was found to be highly correlated with the meteorological factors. The disease first appeared during the first fortnight of May on both leaves and twigs and reached its maximum during the last fortnight of August. It was observed that the per cent disease intensity was highly correlated with the mean minimum temperature (r = 0.94 and r = 0.80), followed by mean maximum temperature (r = 0.72 and r = 0.77) on both leaves and twigs, respectively which was followed by mean minimum relative humidity (r = 0.24) and mean rainfall (r = 0.03) on leaves.

The pathogen perpetuated in the form of acervuli on fallen diseased leaves and twigs and produced viable conidia up to third and last week of June respectively. Thus infected plant debris on the ground proved to be primary source of inoculum in the coming season.

Twelve fungitoxicants both systemic and non-systemic were evaluated at different concentrations for their *in vitro* efficacy in inhibiting spore germination and mycelial growth of *M. juglandis*. Among systemic fungitoxicants metiram+pyraclostrobin 60WG proved to be highly effective in inhibiting the spore germination as well as mycelial growth of the test fungus. The other fungitoxicants in decreasing order of their efficacy were tebuconazole 25EC > flusilazole 40EC > carbendazim50WP >thiophanate methyl 70WP > difenoconazole 25EC. Among non systemic fungitoxicants mancozeb proved to be highly effective in inhibiting the spore germination while as in case of mycelial growth inhibition of test pathogen, captan 50WP proved to be highly effective. The other fungitoxicants in decreasing order of their efficacy were copper oxychloride 50WP > propineb 70WP > chlorothalonil 75WP > ziram 80WP. With the increase in concentration of fungitoxicants from 50 ppm to 200 ppm spore germination and mycelial growth inhibition of test fungus in case of systemic fungitoxicants increased from 71.79 to 97.72 per cent and 80.63 to 100 per cent, respectively. While as in case of non systemic fungitoxicants increase in concentration from 250 ppm to 1500 ppm spore germination and mycelial growth inhibition increased from 58.05 to 98.05 per cent and 58.97 to 94.18 per cent, respectively.

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***Original not seen**

APPENDIX – I

Composition of various cultural media used during the present investigation

1. **Potato Dextrose Agar(PDA)**
   Potato(peeled) = 200g
   Dextrose = 20.0g
   Agar = 20.0g
   Distilled water = 1000ml
2. **Corn Meal Agar (CMA)**
   Ground corn (maize) = 25.0g
   Agar = 20.0g
   Distilled water = 1000ml
3. **Czapek (Dox) Agar**
   Sodium nitrate = 2.0-3.0g
   Potassium phosphate (K_2HPO_4 or KH_2PO_4) = 1.0g
   Potassium Chloride(KCl) = 0.5g
   Magnesium sulphate ($MgSO_4.7H_2O$) = 0.5g
   Ferrous sulphate ($FeSO_4$) = 0.01g
   Sucrose = 30.0g
   Agar = 15.0g
   Distilled water = 1000ml

4.Malt Extract Agar (MA)

Malt extract = 20.0 Agar = 15.0g Distilled water = 1000ml

5.Oat Meal Agar (OMA)

Oat meal = 40.0g
Agar = 15.0g
Distilled water = 1000ml

6.Richard's

Pottasium nitrate = 10.0 g
Potassium dihydrogen phosphate (KH_2PO_4) = 5.0 g
Magnesium sulphate ($MgSO_4.7H_2O$) = 2.5g
Ferrous chloride($FeCl_3$) = 0.02g
Sucrose = 50.0g
Distilled water = 1000ml

APPENDIX – II

Mean weekly meteorological parameters during crop growth period

Standard meteorological weeks	Temperature (°C)		Rainfall	Mean relative humidity	Sun shine (Hours)
	Maximum	Minimum			
15	21.93	5.71	9.80	66.14	7.56
16	16.57	6.60	65.40	76.65	3.71
17	19.79	9.26	31.40	71.21	3.56
18	21.00	7.14	4.20	65.79	5.67
19	21.79	9.50	14.00	72.72	5.13
20	26.21	8.86	8.60	58.50	9.67
21	27.14	12.07	32.20	63.00	7.23
22	26.57	8.69	13.00	59.15	9.24
23	31.86	14.10	0.00	59.86	11.44
24	25.21	15.61	61.20	78.86	3.94
25	31.14	14.50	3.40	56.00	11.34
26	29.10	17.61	32.20	66.57	8.41
27	31.23	17.40	15.80	62.22	8.70
28	29.00	16.43	35.20	66.50	8.27
29	31.14	18.31	8.00	61.43	8.34
30	32.71	18.47	3.20	58.00	9.83
31	30.50	19.27	18.40	68.00	6.39
32	32.07	19.13	6.20	62.07	7.31
33	21.74	16.86	58.60	87.07	0.21
34	30.36	17.07	3.40	68.93	9.34
35	28.64	15.97	0.60	72.22	5.60
36	29.57	13.39	7.80	65.64	8.37
37	23.79	13.57	21.00	77.08	3.89
38	28.93	9.04	0.00	64.50	9.26
39	28.21	11.67	2.40	74.36	8.27
40	29.00	11.70	0.00	65.50	8.34
41	24.71	12.87	11.60	76.72	4.46
42	23.36	6.39	0.00	69.50	6.79

[Source : Meteorological Observatory, Division of Agronomy, SKUAST-Kashmir, Shalimar]

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CERTIFICATE

Certified that all the corrections/amendments as suggested by External Examiner Dr. Shahzad Ahmad, Associate Professor (Retired), Division of Plant Pathology, SKUAST-Kashmir, Shalimar, Srinagar during Viva-Voice examination held on 25th of October, 2014 have been incorporated in the manuscript entitled **"Studies on Anthracnose Disease of Walnut"** submitted by**Mr. Mudasir Hassan (Regd. No. 2012-A-892-M).**

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