

Studies on Scab Disease of Almond (*Prunus amygdalus* Batsch.) in Kashmir Valley

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(2008-227-D)



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**Studies on Scab Disease of Almond (*Prunus amygdalus*
Batsch.) in Kashmir Valley**

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Thesis

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Dedicated:

To the memory of my father, who, would have been the happiest one to see me with doctorate degree as this was his dream for his daughter.

To my mother whose sacrificed everything to give her children best life possible.

To my husband - Mr. Mohammad Hussain Akhoun whose support have been the most precious thing to me throughout this journey and who helped me in many facets of my research programme

Finally, to my children Nisar and Zahra who someday when they read their name in the manuscript may feel proud of their mother.

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Faculty of Horticulture, Division of Plant Pathology

Certificate – I

This is to certify that the thesis entitled “**Studies on Scab Disease of Almond (*Prunus amygdalus* Batsch.) in Kashmir Valley**” submitted in partial fulfilment of the requirements for the award of the degree of **Doctor of Philosophy in Plant Pathology**, to the **Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir** is a record of bonafide research work carried out by **Ms. Nassreen Fatima (Regd. No. 2008-227-D)** 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 – III

This is to certify that the thesis entitled, “**Studies on Scab Disease of Almond (*Prunus amygdalus* Batsch.) in Kashmir Valley**” submitted by **Ms. Nassreen Fatima (Regd. No. 2008-227-D)** to the **Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir** in partial fulfilment of the requirements for the award of the degree of **Doctor of Philosophy in Plant Pathology** was examined and approved by the Advisory Committee and external examiner on

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ABSTRACT

Almond scab is one of the most important diseases of almond(*Prunus amygdalus* Batsch.) worldwide. The characteristic symptoms initiated on twigs in first week of May as indistinct, olive green, minute water soaked spots, measured 1.0-2.0 mm in size. Later on, these lesions coalesced to form dark reddish brown irregular patches. On leaves, the spots appeared on lower side in the second week of May as minute translucent, indistinct to somewhat circular and light yellow in colour with an average size of 0.61 mm. Later on, brownish black circular to irregular patches of 8.53 mm size were observed due to coalescing of numerous lesions which resulted in premature defoliation. On fruits, the symptoms were noticed in third week of May as small superficial, circular, olive green lesions on the upper exposed side measuring with an average of 1.22mm in size. The lesion later on coalesced to form grey to black patches giving sooty appearance with an average of 7.98 mm in size. Finally, severely infected fruits shrivelled and develop cracks. The pathogen constantly associated with the disease was isolated and pathogenicity test established on two year old almond (cv. Makhdoom) sapling in pots. On the basis of symptomatology, morphological characters, pathogenicity test and comparison to authentic description, the pathogen was identified as *Cladosporium carpophilum* Thum. and later on its identification was confirmed by Division of Plant Pathology, IARI, New Delhi (ITCCref.No.9279.13). The pathogen exhibited maximum colony growth and excellent sporulation on potato dextrose agar medium with pH 5.5 at 25±1°C. Twelve almond cultivars were screened under controlled conditions to determine their resistant/susceptibility

against the disease. It was observed that only cultivar Primorskij exhibited resistant reaction against the disease whereas Waris and Shalimar were highly susceptible. Host range studies revealed that the pathogen also infects apricot and peach while cherry and plum did not show any symptoms. The fungitoxicants tested for their efficacy against the disease proved significantly effective as compared to check. The minimum leaf scab intensity of 1.92 percent and twig scab intensity of 2.48 per cent as compared to check (26.35 and 28.92%, respectively) were recorded in treatment T₁ where copper oxychloride 50 WP (0.3%) was applied at dormant stage beside carbendazim 50WP (0.05%), chlorothalonil 75 WP (0.3%), difenaconazole 25 EC (0.03%), captan 50 WP (0.3%) and pyraclostrobin 60 WG (0.025%) applied at pink bud, petal fall, fruitlet, 15 days after fruitlet and 30 days after fruitlet stages, respectively. Maximum leaf scab intensity of 16.83 per cent and twig intensity of 18.37 per cent were recorded in case of treatment T₄ where only carbendazim 50WP (0.05%), chlorothalonil 75 WP (0.3%) and difenaconazole 25 EC (0.03%) were applied at respective phenological stages.

Key words: Almond, Scab, *Cladosporium carpophilum*, Kashmir

Signature of Student
Dated: _____

Signature of Major Advisor
Dated: _____

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Chapter – 1

INTRODUCTION

Almond (*Prunus amygdalus* Batsch.), belongs to family Rosaceae, is one of the most important nut crop mostly cultivated in temperate regions of the world. The probable origin of almond is believed to be the Mediterranean region (Ladizinsky, 1999). It was further spread by traders in ancient times along the shores of Mediterranean into northern Africa and southern Europe, and more recently distributed to other parts of world, notably California in United States (Zohary and Maria, 2000). It possesses wide spread popularity and is even considered as the golden crop of California (Rogers, 1974). Its cultivation is mainly confined to the countries lying between latitude 36° and 45° N (Ragini and Monastera, 1991), and is mainly grown in USA, Spain, Syria, Italy, Iran and China. The total world production of almond was estimated 2.51 million MT in 2010 (FAOSAT, 2013). Nutritionally, almond kernel is a rich source of energy (576 calories per 100 g fresh weight) and contains protein (21.22%), fat (49.42%), carbohydrates (21.6) and other minerals and vitamins (Blomhoff *et al.*, 2006). Almond kernel is highly delicious and its oil is used in confectionery, pharmaceutical and cosmetic preparation.

In India, almond is mainly grown in Jammu and Kashmir (J&K) and Himachal Pradesh (H.P.) over an area of 23.81 thousand ha yielding 11.47 thousand MT (Kumar, 2010). However, commercial cultivation of this nut fruit has been mainly confined to the Kashmir Valley. In the Valley it is mainly grown in district Budgam, Pulwama, Shopian, Ganderbal, Bandipora and other hilly areas occupying an area of 15.93 thousand ha with annual production of 8.21 thousand MT (Anonymous, 2014). The productivity of almond in the state is 0.73 MT per ha which compared to global productivity of 1.15 MT per ha (Anonymous, 2011). Almond is a high value crop and also used as a filler crop in saffron fields. However, its yield as well as area under cultivation have shown a declining trend during the recent past. This reduction is attributed to several biotic

and abiotic constraints which include diseases, occurrence of spring frost, poor pollination during cool, cloudy or rainy weather or by low temperature as the main abiotic factors (Qureshi and Dalal, 1985; Connel, 2002).

Among the various disease fungal pathogens mainly have been posing a serious threat to the crop. The prominent fungal disease include twig blight (*Cryptosporiopsis* sp.), die back (*Gloeosporium amygdalinum*) [Lele et al., 1981]; leaf spot (*Cercospora rubrotincta*) [Chona et al. 1959]; shot hole (*Wilsonomyces carpophilus*) [Kaul, 1966]; leaf blotch (*Polystigmina rubra*) [Anonymous, 1984]; in addition leaf curl (*Taphrina deformans*) [Padwick, 1945]; powdery mildew (*Phyllactinia gullata*), rust (*Tranzschelia discolor*) [Kaul, 1966]; seedling blight (*Rhizoctonia solani*) are also prevalent, however they are sporadic and of less economic importance [Puttoo and Razdan, 1988; Sharma, 1978], besides nut and kernel rots caused by *Mucor janseni* (Mehrotra et al., 1969); *Cephalothecium roseum*, *Aspergillus flavus*, *Fusarium moniliforme*, *Cladosporium* sp., *Penicillium* sp., *Alternaria alternate*, *Rhizopus stolonifer* and *Curvularia lunata* (Madan, 1981) also have been reported.

During the last few years, however, the almond orchardists of the Valley have been facing scab like serious disease problem on almond plant (unpublished). The disease affects fruits and lead to premature leaf fall resulting in low productivity and poor fruit quality. The disease has been reported to be caused by *C. carpophilum* Thum. and occurs in other parts of the world (Pammel, 1893; Anderson, 1956). In India the fungus *C. carpophilum* was first reported on peach and nectarine from Kullu Valley of H.P. (Khosla et al., 2009). Various studies have been carried out abroad on other *Prunus* spp. particularly on peach scab. Scanning of literature indicates that very little work has been documented with regard to almond scab throughout the world.

Keeping in view the significance of disease in limiting almond production, based on feedback from growers and field observations the present investigation was undertaken with following objectives:

- ↪ To Isolate and identify the pathogen responsible for the disease.
- ↪ To study the physiology of the pathogen.
- ↪ To screen out the available almond germplasm against the disease.
- ↪ To study the host range of the pathogen.
- ↪ To study the chemical management of the disease.

Chapter –2

REVIEW OF LITERATURE

The literature available on almond scab is very scanty. However, an attempt to review the available literature on scab of almond, and other stone and nut fruit plant has been made under the following heads:

2.1 History

Scab on peach caused by *Cladosporium carpophilum* Thum., a Dematiaceous hyphomycetes fungus, has been recognised worldwide since the early 1900's (Keitt, 1917; Anderson, 1956). The pathogen in the conidial state was first reported from Klostemenberg, Australia by Thumen in 1877, who observed its occurrence on peach fruit and briefly described the associated fungus as *C. carpophilum*, the name still accepted at present. Towards the end of the nineteenth century, the fungus infecting almond, apricot, plum and peach was reported from USA, Canada and Australia (Smith, 1889; Pammel, 1893; Smith, 1924). Later on, the disease on cherry caused by *C. cerasi* Aderh. (Teleomorph: *Venturia cerasis* Aderh) was also reported from USA and New Zealand (Bensaude and Keitt, 1928; Dingley, 1965). Hughes (1953) proposed that the name *C. carpophilum* Thum. be changed to *Fusicladium* (Thum.) Oudem, which was generally not accepted. However, in some European countries, *F. amygdyle* Docolet was used for the almond scab fungus (Ogawa and English, 1991). The sexual state of the fungus was found on over-wintered apricot leaves in Australia and named *V. carpophila* (Fisher, 1961).

The disease on almond, has been reported to occur in sporadic nature in various almond growing areas especially in California and USA (Ogawa *et al.*, 1955a; 1955b; French, 1987). The disease reportedly occurs on various commercially grown cultivars of almond and is especially severe on varieties viz., Ne Plus Ultra, Carmel, Marced, Price and Peerless (Ogawa and English, 1991; Mosz, 2002). In India *C. carpophilum* was first reported by Khosla *et al.* (2009) on

peach and nectarine from Kullu Valley (H.P.) and they recorded disease incidence of 5 to 25 and 3 to 53 per cent, respectively.

2.2 Symptomatology

Warren (1996) noticed that symptoms of almond scab on leaves appeared as small, indistinct, somewhat circular, greenish yellow blotches or lesions, first on undersurface of leaves which enlarged up to 10 mm or more in size and sporulating lesions turned olivaceous and eventually became yellow brown to dark brown in colour. He further mentioned that the symptom on fruit as dark grey to black sooty colour lesions and on twigs, lesions were superficial, brown and elliptical. The symptoms of scab on almond leaves appeared as indistinct green-yellow lesions initially on the underside and later on both sides of the leaves, which can cause premature defoliation and lead to yield loss, on fruit the symptoms appeared as small green to olive-coloured circular spots which later on became darker in colour (Connel, 2002; David, 2012).

Ogawa and English (1991) observed that symptoms of scab on almond leaves, fruits and twigs are similar to those of scab disease of peach. Keitt (1917) described the symptoms of peach scab on fruit, as small greenish circular spots gradually enlarge and with the production of spore colour turned to black. He further reported fruit lesions were most common on shoulder of the fruit, but can occur anywhere on the surface of fruit, coalescing of lesion led to cracking of fruit hence paving way for entry of rot causing microorganism. In 1989, Miller and Bertrand described the symptoms on fruit as small, round, olive green spots about 2 to 4 mm in diameter developed on the surface of the fruit and on twig developed yellowish brown, oval to round, slightly raised blotches about 4 to 7 mm in diameter with dark grey or bluish bordered. They further described the symptoms on leaves as small, round, pale green spots under the leaf surface which later turned yellowish brown, finally becoming brownish black and long narrow dark brown lesions developed along the midrib.

Khosla *et al.* (2009) reported that lesions on peach fruits appeared when the fruits were about one half of their final size as small olive green circular spots which gradually enlarged to form large irregular blotches that later turned velvety dark olive green or black due to sporulation while on nectarine fruits, the spots are somewhat depressed and distinctly marked by light brown ring. They further noticed that the lesions were most common on the shoulders of the fruit but also occurred anywhere on the fruit surface and where the lesions are numerous, they often coalesced and led to cracking of the fruit.

Bocket *et al.* (2011) reported that the symptoms of scab on peach fruit should not be confused with shot hole, which cause raised spots on the fruit surface, while scab lesions were pale green and remained flush with fruit surface. They further reported that leaf infection appeared as sooty or olive blotches on the underside of leaves and in severe infection caused defoliation but in some cases little or no leaf infections were found even when fruits were badly affected.

2.3 Disease development

The pathogen *C.carpophilum* overwintered as mycelia on one year old twig lesions (Keitt, 1917; Haris, 1941). Conidiophores arose from the fungal stroma and produce dark brown elliptical conidia and these conidia serve as primary inoculum for scab epidemic and were disseminated during rainy periods to newly formed fruits, current season vegetative shoots and foliage but most of the lesions were not completely developed and become infectious until the following year (Mathee and Thomas, 1981; Hendrix, 1995; Schubert *et al.*, 2003). Cook and Scherm (1999) reported that foliar infections were not common, and conidia produced later in the season and secondary inoculum from fruit lesions did not contribute significantly to disease development because the latent period was quite long, 42-77 days.

Lan and Scherm (2003) reported that relative humidity, infrared radiation, vegetative wetness were the major factors that influence the discharge

of conidia in peach scab. Scherm *et al.* (2008) found the range of temperature for disease infection by *C. carpophilum* in stone fruits were between 20 and 25°C and observed that new twig lesions may produce conidia later in that season were of little importance in the disease cycle. David (2012) observed that the incremental increase in disease intensity was correlated with high humidity due to rains and sprinkle irrigation. Lalancette *et al.* (2012) observed that decrease in relative humidity and increase in temperature accompanied by solar radiation, increased the concentration of air borne conidia.

Many workers while conducting experiment on almond scab reported that the pathogen did not survive on two year old wood and conidia overwintered on lesions of one year old almond twig served as primary inoculums for epidemic of scab, which sporulate in late spring (Mosz, 2002; Horsefield *et al.*, 2010).

2.4 Pathogenicity

While conducting pathogenicity test on premature apricot fruits, by spraying with spore suspension of *C. carpophilum*, Bottomley (1944) observed typical scab lesions measuring 0.5-1 mm dia. after five weeks of inoculation, which turned brown and velvety with maturity of fruit. Fisher (1961) established the pathogenicity test of *C. carpophilum* Thum. on young healthy leaves of apricot and symptoms appeared as circular brown spots on the lower surface of the leaves after eight weeks of inoculation. He also noticed linear lesions approximately 2 mm long on the veins and petioles. However, no literature was found on pathogenicity of *C. carpophilum* on almond.

2.5 Morphology

2.5.1 On host

Schubert *et al.* (2003) reported that mycelium of *C. carpophilum* was subepidermal, hyphae branched, 3-6 µm wide septate, olivaceous, forming a pseudoparenchyma below the cuticle. He also observed that conidiophores were solitary or in loose fascicles, erumpent, erect, straight or somewhat flexuous,

unbranched, $25-100 \times 3-6 \mu\text{m}$, septate, slightly, constricted at the septa, olivaceous or medium to dark brown, paler towards the apex, smooth, sometimes swollen at the base walls.

Hashemiet *al.* (2013) while studying the *Fusicladium carpophilum*, on host observed that conidiophores were solitary, erect, flexuous or straight, unbranched, $29-105 \times 4-6.2 \mu\text{m}$, septate, medium brown, smooth, sometimes smaller at the base and conidiogenous cells integrated the terminal, $19-26 \mu\text{m}$ long, with 3-7 denticulate loci, $1-2 \mu\text{m}$ wide, neither thickened nor darkened. They further studied that conidia were catenate, cylindrical to fusiform, straight, $12.5-20 (-22) \times 4-5 \mu\text{m}$, aseptate, pale olivaceous and smooth.

2.5.2 In culture

While studying the culture characteristics of *C. carpophilum* isolated from peach and grown on PDA at 25°C temperature, Khosla et *al.* (2009) observed that the mycelial colony was olivaceous green in colour which turned grey after 25-30 days of incubation and produced, upright, single or clustered conidiophores which are dark brown in colour and branched around apex. They also noticed that conidia were 1-2 celled, ovoid with diameter of $1.6 \mu\text{m}$, light grey in colour, lemon shaped or oblong with dimension of $8.3-128.26 \times 2.49-3.32 \mu\text{m}$ and borne in short chains of 2-5 conidia.

Partridge and Morgan (2003) observed that conidia of the pathogen were mostly in simple or branched chains, rarely solitary, cylindrical to fusiform, sometimes short obclavate, straight, $12-20 \times 4-5 \mu\text{m}$, aseptate or single septate, then slightly constricted at the septum, pale olivaceous, smooth to verruculose, $1.5-2 \mu\text{m}$ wide, neither thickened nor darkened.

Various workers have also reported that conidia of various species of *Cladosporium* were solitary or catenate, in simple or branched as acropetal chains, amento-, didymo- to phragmo-sporous, ellipsoid to ovoid, fusiform, obclavate, subcylindrical, straight to curved, hyaline to medium brown but mostly

olivaceous, usually non-constricted at the septa, smooth to verruculose, ends pointed or rounded to truncate, wall thin to somewhat thickened, hila unthickened, occasionally somewhat darkened(David, 1997; Braun *et al.*, 2003; Braun *et al.*, 2008).

2.6 Physiology

Schweizer (1958)observed that potato dextrose agar(PDA)and oat meal agar media were the best media for growth and sporulation of *C. carpophilum*.Lawrence and Zehr (1982)also reported that mycelia growth and sporulationof *C.carpophilum*was maximum attemperature range of 20-30°Cand relative humidity of 98 to 100 per cent respectively. They further reported that theconidial germinationand sporulation was also maximum at 98 to 100 per cent relative humidity.Lalancette *etal.* (2012) reported that the sporulation of*F. carpophilum*occurred at temperaturerange of 5 to 30°C after 72 hours of incubation.

Viruega*et al.*(2011) found that growth and sporulation of*F.oleaginum*, causing scab in olive, varied significantly with temperature and was optimum at25°C temperature.In case of pecan scab fungus (*C.effusum*), Payne (2011) observed that PDA amended with lactic acid (50%)was best medium followed by malt extract agar and potato carrot agar for growth and sporulation of the fungusat 20°C temperature. Domigues*et al.* (2013) reported that the *F.eriobotrya*ecausing scabin loquat was able to grow between 5 and 25°C temperature and observed radial growth of the colony on potato extract agaroccurred at15°C(averageof 0.35 mm/day)and 25°C (0.36 mm/day), with maximumat 20°C (0.49 mm/day) and at 5 and 10°C, the growth of the colonies was slower (0.05 and 0.19 mm/day, respectively). They further noticed that any isolate of thefunguswas able to grow at 30 and 35°C and the colonies die after one month.

Physiological studies conducted on other fungi causing scab of other crops also indicated thatoptimumtemperature and pH for mycelial growth and

sporulation of *C. cucumerinum* (cucumber scab) on PDA medium was at 20°C and 4.5 of pH (Lee *et al.*, 1997) and maximum mycelia growth of *C. herbarum* (strawberry scab) was at 25°C temperature on PDA (Kwon *et al.*, 2001).

2.7 Screening

Post (1984) reported that almond varieties such as Carmel, Merced, Ne Plus Ultra, Peerless, Price and Ruby were susceptible to almond scab, while as Butte, Fritz, Mission, Monterey, Wood, Colony Thompson were moderately susceptible and Non-Pareil moderately resistant to the disease under natural condition. Later on, other workers also found that scab occurred on most of the commercially grown cultivars of almond and disease was especially severe on Ne-Plus-Ultra and Drake cultivars (Ogawa and English, 1991; Brent and Hoffman, 2002).

2.8 Host range

Ogawa and English (1991) concluded that scab was found in most of the cultivated species of *Prunus*, but it was not known with certainty whether the fungus on all host was *C. carpophilum*. Earlier, Bensaude and Keitt (1928) indicated that the pathogen isolated from peach (*P. persica*) and plum (*P. americana*) also infected apricot (*P. armeniaca*) but not cherry (*P. cerasus*) or European plum (*P. domestica*). Schweizer (1958) reported that fungi causing scab of stone fruit exhibited differences in pathogenicity as *F. cerasi* had limited host range and appeared to be almost completely specific to cherry, whereas *F. carpophilum* was found on various stone fruits including peaches, plums and apricots. Hashemi *et al.* (2013) reported that almond scab pathogen had broader host range but rarely occurred on *P. cerasus* compared to cherry scab pathogen which had narrow host range but mostly occurred on *P. cerasus*.

2.9 Chemical management

Many workers have attempted the chemical management of scab of almond and other stone fruits. English *et al.* (1955) observed that fungicidal treatment five weeks after petal fall reduced leaf and fruit infection of rust, shot-hole, twig blight and scab of almond.

Keitt (1917) observed that the fruit infection of scab in peach could be controlled effectively, with sulphur applied at 4-weeks after petal fall, followed by one or two additional sprays at 3 to 4 week interval after the initial treatment. Zolam and Klonsky (1985) reported that fungicidal sprays conducted at petal fall for the management of shot hole disease also gave protection against scab disease. Research in Argentina had shown that susceptibility of nectarine to scab was significantly reduced by the foliage application of monosodium phosphate plus urea (Montero and Esposito, 1984). Huang *et al.* (1993) observed that bitertanol mixed with summer oil one week before blossom followed by two applications of bitertanol at an intervals of 15-20 days, reduced scab incidence in apricot and increased fruit yield. Panday and Tripathi (1998) reported that Bordeaux mixture, copper oxychloride, and carbendazim gave best results with 90.6 per cent disease control of peach scab in U.P. hills.

The current recommendation for peach scab control comprised a series of sprays beginning at petal fall or calyx split and containing 2-week interval until 3 to 4 weeks before harvest (Horton *et al.*, 2000). Weekly applications of sulphur based products were also found to control peach scab effectively whether disease incidence was low or high (Schnabel and Hitzler, 2003). Cragolini *et al.* (2005) observed that combination of lime sulphur with chlorothalonil or with dodine reduced the peach scab infection up to 76 or 66 per cent, respectively. The use of anti-sporulant fungicides at bloom has been considered an effective strategy for reducing primary inoculum of scab for the subsequent infection during fruit development period of peach. In a study on influence of trifloxystrobin on primary inoculum and progression of scab epidemics on stone fruit, Lalancette *et al.* (2015) demonstrated that incorporation of trifloxystrobin and

azoxystrobin fungicide improved the efficacy of current protectant programmes used for scab control by providing season long control of *F. carpophilum* sporulation on twig lesions.

Various fungicides reported to be effective against scab of stone fruits were ziram, captan, wettable sulphur, benomyl, thiophanatemethyl, azoxystrobin, difenoconazole, penconazole, chlorothalonil and benomyl, copper oxychloride (Taylor *et al.*, 1972; Chandler, 1974; Scherm and Savelle, 2001; Dzhafarov, 2003; Schnabel and Layne, 2004). Other fungitoxicants viz., azoxystrobin, pyraclustrobin, captan and propiconazole significantly reduced scab infection of almond with limited difference between the fungicides and formulation (Horsefield, 2010; Khosla and Bhardwaj, 2010).



Plate 1: Defoliation of almond trees due to scab disease

Chapter – 3

MATERIALS AND METHODS

The present investigations on scab disease of almond were conducted in the Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K), Shalimar, Srinagar. The field experiments were conducted at Central Institute for Temperate Horticulture (CITH), Rangreth, Srinagar in 2012-13 and 2013-14. The methodology used during the investigation is presented below.

3.1 Symptomatology

The symptomatology of the disease was studied on three randomly selected plants of almond cv. 'Makhdoom' at the almond orchard block of Central Institute for Temperate Horticulture (CITH), Rangreth, Srinagar. The selected plants were tagged and kept unsprayed throughout the growing season to study the symptoms of the disease under natural epiphytotic conditions. First observation was taken as soon as the disease appeared and periodic observations were recorded with respect to size, shape, coalescing and colour of the lesion on leaves, twigs and fruits. Size of the lesions was recorded in terms of range and average lesion size in mm.

3.2 Isolation, purification and maintenance of culture

3.2.1 Isolation of the pathogen

Almond leaves and twigs showing typical scab symptoms, collected from almond orchard were brought to laboratory in paper bags and were used repeatedly for isolation of pathogen. The diseased leaves were first examined for associated fungus by teasing the diseased portion with the help of a teasing needle and observed under microscope (100x). For the isolation of fungus, small segments of disease tissue (5mm²) along with some healthy leaf portion were cut with sterilized razor blade and surface sterilized in 0.1 per cent mercuric

chloridesolution for 30 seconds (Johnston and Booth, 1983). The tissue were then rinsedin distilled sterilized water to removethe last trace of mercuric choloride solution, blotted dry andplacedon potato dextrose agar (PDA)medium in sterilized petriplates. Three pieces of sterilized specimen were placed in each petriplates and incubated for 5-7 daysat $25\pm 1^{\circ}\text{C}$. The composition of potato dextrose agar was:

Peeled potato : 200 g

Dextrose : 20 g

Agar agar : 20 g

Distilled water to make the volume 1000 ml.

3.2.2 Purification and maintenance of pathogen

The culture was purified by hyphal tip method (Pathak, 1972)and single spore technique (Johnston and Booth, 1983).As soon as the mycelial growth was observed in petriplates advancing hyphal tips growing out of tissue segments were cut offwith sterilized inoculation needle and transferred to PDA slants for further growth. In case of single spore technique, 2-3 drops of spore suspension made from 7 days old culture in a sterilized test tube containing sterilized water were aseptically pipetted into petri-plates containing thin layer of 2.0 per cent water agar previously mixed with 2.0 drops of antibiotic chloromphenicol as a bacteriostate. The plates were then incubated at $25\pm 1^{\circ}\text{C}$ for 72 hours.Small specks of the colonies formed from single spores were later transferred aseptically to PDA slants, identified and preserved at 4°C for further experimentation. The colonies were re-cultured at an interval of 10-12 weeks. To maintain the virulence and vigour of the pathogen, spore suspension of the culture was sprayed on leaves under growth chamber conditions and the pathogen was re-isolated from fresh lesions and maintained again at 4°C .

3.3 Morphological and cultural characteristics

Morphological and cultural characters of the causal fungus, isolated from

diseased spots, on its host as well as in pure culture were studied for its identification.

3.3.1 In culture

Colony characters such as colour and status of the mycelium were studied from the medium visually. For morphology of the fungus, mycelium from marginal portion of actively growing colonies, an average of 40 observations of conidia and conidiophores from 30 day old culture were examined under a microscope and the observations with respect to shape, size and colour as well as septation of mycelium, conidia and conidiophores was recorded.

3.3.2 On host

The leaf and fruit samples exhibiting typical symptoms of almond scab were observed under microscope for the presence of mycelium, conidiophores and conidia. The mycelium, conidiophores and conidia thus located were picked up with the help of a teasing needle, placed separately in a drop of water in cavity slides and studied for shape and size. They were also placed in a drop of lactophenol, pressed gently under cover slip and examined under microscope. Observations for overall morphology of the fungus were recorded with respect to the following characters:

Colony	:	Shape, colour and size
Mycelium	:	Colour, shape, septation, branching and width
Conidiophore	:	Colour, shape, size and septation
Conidia	:	Colour, shape, size and septation

3.4 Pathogenicity test

To ascertain whether the fungus isolated was primary cause of the disease, pathogenicity test was conducted on two year old almond seedlings cv. “Makhdoom” grown in 40 cm diameter pots containing sterilized soil. One set of

plants was given injuries with the help of sterilized teasing needle while as other two sets kept uninjured.

The pots were kept in artificially created humid chambers and maintained at room temperature. High humidity inside the chamber was maintained by frequent spraying the plant with sterilized water. The plants were constantly observed for one month to rule out any latent infection prior to inoculation. Spore suspension was made from young and vigorous culture of tested fungus *Cladosporium carpophilum* with sterilized distilled water and diluted to get a concentration of 1×10^6 conidia/ml (Dingra and Sinclair, 1985). The spore suspension was automated on to the test plants to run-off while as, an equal volume of sterile distilled water was used in third set served as check. Plants were examined daily for symptom development. The symptoms developed were microscopically examined for presence of conidia. Re-isolation was made from the lesion produced by artificial inoculations and comparison was made with the inoculated fungus to satisfy Koch's postulate.

3.5 Identification of the pathogen

The pathogen was identified on the basis of colony characters viz., colour, margins, growth and morphological characters of its mycelium, conidiophores and conidia produced on host and in culture. Further, the identification of the isolated pathogen was confirmed by Indian Type Culture Collection, Division of Plant Pathology, Indian Agriculture Research Institute (IARI), New Delhi.

3.6 Physiological studies

To ascertain the best medium, optimum temperature and most favourable pH of the medium for the growth and sporulation of *C. carpophilum* following studies were undertaken.

3.6.1 Effect of media

Five different solid media viz., potato dextrose agar, corn meal agar,

Czapek's dox agar, oat meal agar, and Richard's synthetic agar were evaluated with respect to their support for growth and sporulation of the test fungus. Each of the media to be evaluated were prepared as per their composition (Appendix-I) and sterilized in an autoclave at 15 lbs pressure per square inch for 15 minutes. Each test media (30 ml) was poured into sterilized petriplates under aseptic conditions. The culture discs (3 mm dia.) were cut with a sterilized cork borer from a 30 days old actively growing fungus culture and were aseptically transferred to a petriplates containing different test media. The inoculated plates were incubated at $25\pm 1^{\circ}\text{C}$. The experiment was laid out in a completely randomized design (CRD) having three replications for each treatment. The observations for colony diameter and sporulation were recorded after 30 days of incubation respectively. The sporulation was studied by homogenizing thoroughly 1 mm of mycelial mat in 1 ml of distilled water. The suspension, thus obtained was used for counting the number of spores with the help of haemocytometer.

The medium supporting the best growth as well as sporulation of the test fungus was selected as a basal medium for further studies.

3.6.2 Effect of temperature

Effect of temperature on the growth and sporulation of the fungus was studied on the medium, which supported best growth and sporulation (potato dextrose agar medium). The medium was prepared by mixing the ingredients as mentioned 3.2. The petriplates each containing 20 ml of sterilized basal medium (PDA) were inoculated with a 3 mm culture disc of test fungus and then incubated at five different temperatures viz., 15, 20, 25, 30 and 35 ($\pm 1^{\circ}\text{C}$). Three replications of each treatment were maintained in complete randomized design (CRD). Methods adopted for recording observations on colony diameter and scale adopted for grading sporulation were same as described in section 3.6.1.

3.6.3 Effect of pH

In order to determine the optimum pH level for better growth and

sporulation of the test fungus, the pH of basal medium was adjusted at desired levels viz., 4.5, 5.5, 6.5, 7.5 and 8.5 with the help of pH meter using N/10 hydrochloric acid (HCl) and N/10 sodium hydroxide (NaOH) solutions. Inoculation and observation were made in similar manner as described in section 3.6.1.

3.7 Screening of germplasm

Twelve almond germplasms viz; Butte, IXL, Merced, Mission, Ne-Plus-Ultra, Non-Pariel, Peerless, Pranyaj, Primoskij, Makhdoom, Shalimar and Warisavailable with Central Institute for Temperate Horticulture, Srinagar, J&K were screened against the scab disease under controlled condition during the year 2013 in Division of Plant Pathology SKUAST-K, Shalimar Campus. Two year old threeseedlings of each test line were planted in pots and transferred to controlled conditions in the 1st week of March and kept for one month to observe any latent infection prior to inoculation. The inoculation was made as per procedure described in section 3.4. The disease intensity of each test line was recorded after 45 days of inoculation using the disease rating scale as adopted by Hunter Robert (1983) with slight modifications.

Disease Rating Scale

Category	Numerical value	Per cent leaf surface affected
I	1	0.0-5.0
II	2	5.1-10.0
III	3	10.1.-25.0
IV	4	25.1-50.0
V	5	>50

Disease intensity was calculated by adopting the following formula :

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

Where, n = No. Of diseasedleaves in category

v = Numerical value of each category

N = No. of leaves examined

S = Maximum numerical value

The germplasm was grouped into different reaction types as per the following scale given by Hunter Robert (1983):

Reaction category	Per cent disease intensity
Tolerant (T)	: 0-5
Moderately tolerant (MT)	: 5.1-10
Moderately susceptible (MS)	: 10.1-25
Susceptible (S)	: 25.1-50
Highly susceptible(HS)	: >50.0

3.8 Host range studies

Five stone fruit plant species viz., almond (cv.Makhdoom) apricot (cv.Halman) cherry (cv.Napoleon), peach (cv.Elberta) and plum (cv.Santa Rosa) were tested for the host range of the pathogen isolated from almond causing scab disease under greenhouse conditions in Division of Plant Pathology SKUAST-K, Shalimar Campus. The two year old plants of tested stone fruit crop were grown in 40cm dia pots and inoculated with the pathogen during first week of April. The procedures adopted for preparation of spore suspension and inoculation of test plants were same as described in section 3.4. The disease intensity of each plant species was recorded (as described in 3.7) three times at 15 days interval.

3.9 Management

3.9.1 *In vivo* evaluation of fungitoxicants

Six fungitoxicants, both systemic and non-systemic at standard concentrations were evaluated for their efficacy in the field under natural conditions of disease development during the year 2012-13 and 2013-14. The evaluation of the fungitoxicants was carried out on almond cultivar 'Makhdoom' at Central Institute for Temperate Horticulture (CITH), Rangreth, Srinagar. The experiment was laid in randomized block design (RBD) with four replications for each treatment. The data on disease intensity was recorded 15 days after the each spray. The experiments were laid out in 4 sets and details of each sets of the fungitoxicants evaluated at different phenological stages of almond crop is as under:

Detail of the fungitoxicants evaluated at different phenological stages of almond under field conditions

Phenological stages	Experimental sets			
	Treatment (T ₁)	Treatment (T ₂)	Treatment (T ₃)	Treatment (T ₄)
Dormant	Copper oxychloride 50 WP@ 0.3%	No Spray	No Spray	No Spray
Pink bud	Carbendazim 50 WP@ 0.05%	Carbendazim 50 WP@ 0.05%	Carbendazim 50 WP@ 0.05%	Carbendazim 50 WP@ 0.05%
Petal fall	Chlorothalonil 75 WP @0.3%	Chlorothalonil 75 WP @0.3%	Chlorothalonil 75 WP @0.3%	Chlorothalonil 75 WP @0.3%
Fruitlet	Difenaconazole 25 EC @ 0.03%	Difenaconazole 25 EC @ 0.03%	Difenaconazole 25 EC @ 0.03%	Difenaconazole 25 EC @ 0.03%
15 days after fruitlet	Captan 50 WP @ 0.3%	Captan 50 WP @ 0.3%	Captan 50 WP @ 0.3%	No Spray
30 days after fruitlet	Pyraclostrobin 60 WG @0.025%	Pyraclostrobin 60 WG@0.025%	No spray	No spray

3.9.2 Observations recorded

3.9.2.1 Disease intensity

Disease intensity on leaves and twigs was recorded by adopting slightly modified rating scale given by Thind and Kour (2005).

3.9.2.1a On leaves (Plate 2a)

Category	Numerical value	Per cent leaf surface affected
I	0	No disease
II	1	0.1-10.00
III	2	10.1-20.00
IV	3	20.1-40.00
V	4	40.1-50.00
VI	5	>50

3.9.2.1b On twigs (Plate 2b)

Category	Numerical value	Per cent twig surface affected
I	0	No disease
II	1	Upto 25
III	2	25 to 50
IV	3	50 to 75
V	4	>75

Disease intensity was calculated by adopting the following formula :

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

(a)



(b)



Plate-2 : Scale for assessment of almond scab

a) Scale (0-5) in leaves

b) Scale (0-4) in twigs

Where, n = No. of diseased twigs/leaves in category

v = Numerical value of each category

N = No. of twigs/leaves examined

S = Maximum numerical value

3.10 Statistical analysis

The data generated on various aspects of research were subjected to statistical analysis as per the methods determined by Gomes and Gomes (1984).

Chapter-4

EXPERIMENTAL FINDINGS

4.1 Symptomatology

The detailed symptomatological studies of almond scab under natural condition were carried out during 2013 in the almond orchard block of Central Institute for Temperate Horticulture (CITH), Rangreth, Srinagar. The periodical symptomatological development were observed on twigs, leaves and fruits of susceptible almond cultivar 'Makhdoom'.

4.1.1 Symptoms on twigs

The symptom of disease on twigs was first noticed in first week of May, as slightly raised water soaked, indistinct olive green specks (Plate.3a) measuring 1.0-2.0 mm in dia. with an average of 1.43 mm. Periodic changes in size, shape and colour of the lesions were recorded and are presented in Table 1.

The lesion enlarged up to first week of June with an average of 11.57 mm and the colour changed to reddish brown with darkened periphery and started the production of spore. The shape of the lesion was circular to elliptical up to fourth week of May (Plate 3b) beyond which due to coalescing of 3-5 spots, irregular patches were formed (Plate 3c). In fourth week of June, colour of lesion changed to dark purplish brown which resembles with the colour of bark (Plate 3d).

4.1.2 Symptoms on leaves

On leaves the disease was first observed in second week of May and reached its peak during June (Table 2). Disease symptoms initially appeared as minute translucent, indistinct to somewhat circular and light yellow in colour. The initial size of the lesions were recorded with an average of 0.61 mm (Plate 4a). Later in third week of May the lesions turned into circular with an average of 1.15 mm in size and the colour changed to pale green. With the advancement of disease, the number and size of lesions increased ranging from 2.0-3.5 mm with

Table 1 : Symptomatological development of almond scab on twigs (cv. Makhdoom)

Period of observation		Symptom development	Lesion characters*			
			Shape	Colour	Size (mm)	Sporulation
Month	Week					
April	IV	Disease free	–	–	–	–
May	I	Slightly raised, water soaked specks	Indistinct	Olive green	1.0-2.0 (1.43)	–
May	II	Increase in the size of specks; specks get transformed into lesions	-do-	-do-	2-3.5 (2.63)	–
May	III	Increase in the number and size of previously formed lesions	Circular to elliptical	White center with brown periphery	3-7.5 (4.72)	–
May	IV	-do-	-do-	Reddish brown with darken periphery	7-12 (9.82)	+
June	I	Coalescing of 3-5 lesions emerging in patches	Irregular	-do-	10.5-15.0 (11.57)	+
June	II	-do-	-do-	-do-	–	+
June	III	Coalescing of more than 5 lesions	-do-	Dark reddish brown	–	+
June	IV	Dark purplish brown coloured lesions resembling with the colour of bark				

* Value in parenthesis are mean of 30 observations;

- = absent ; += present

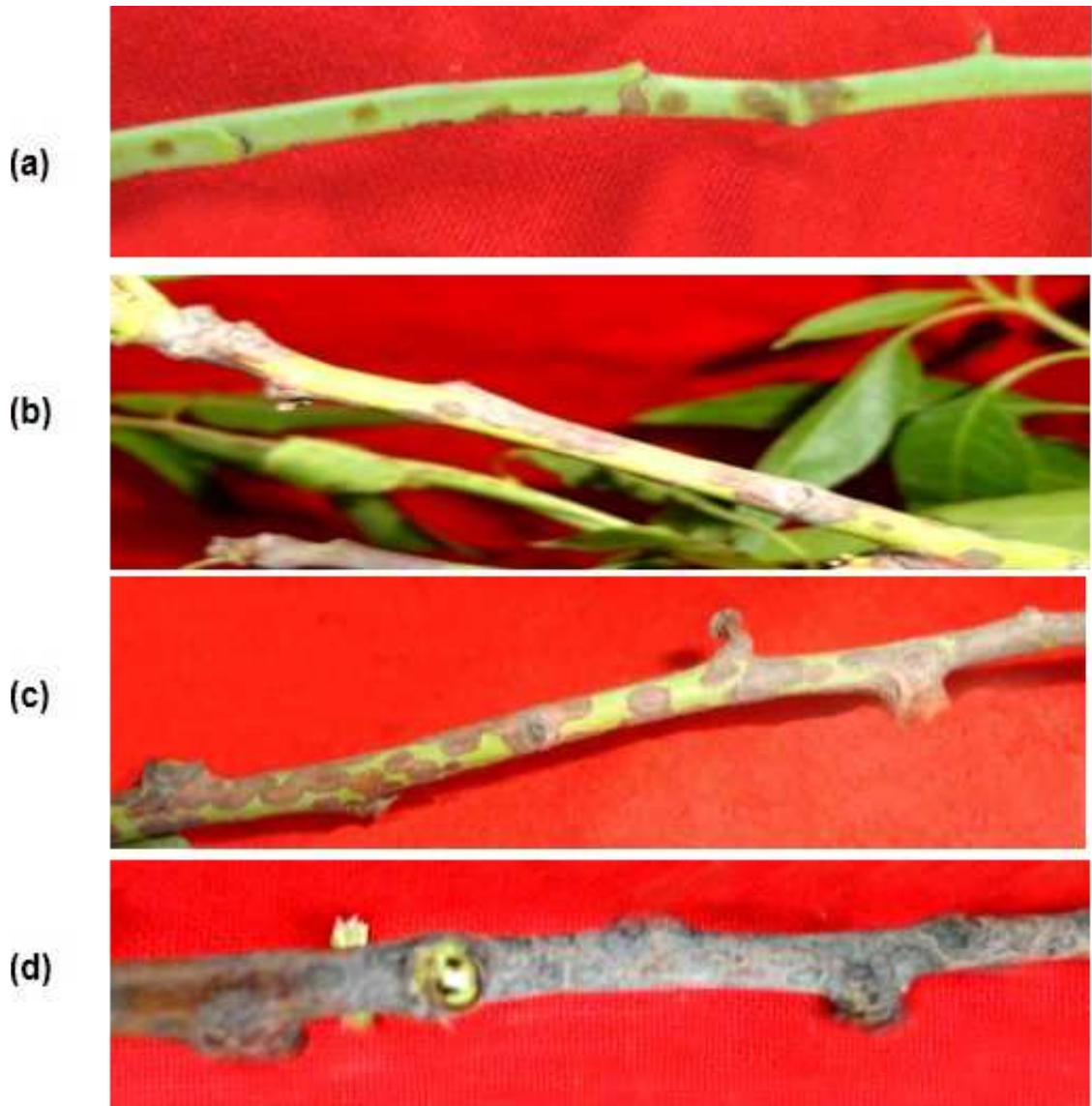


Plate-3 : Characteristic symptoms of scab on almond twigs

- a) **Indistinct light-yellow water soaked lesions**
- b) **Circular to elliptical light-brown with white center**
- c) **Irregular patches**
- d) **Dark purplish brown lesions resembling with the colour of bark**

an average of 2.22 mm in fourth week of May. In first week of June the colour of the lesions changed from olivaceous green to brownish black and the production of spore started, size increased to an average of 4.25 mm (Plate 4b). The development of olivaceous lesions were also seen on midribs in fourth week of May (Plate 4c). Later on, coalescing of numerous lesions were observed on lower side of the leaves during second week of June and formed brownish black circular to irregular patches attaining a maximum average size of 8.53 mm (Plate 4d). During second fortnight of June, irregular brownish black patches of coalesced lesions covering more than 50% of the leaf area are formed. Finally, 6.67 per cent pre-mature defoliation of severely infected leaves were observed in the first week of July and maximum pre-mature defoliation of 39.33 per cent was recorded in the third week of July (Plate 4e).

4.1.3 Symptoms on fruits

Symptoms on fruits were noticed in third week of May (Table 3) as small superficial, circular, olive green lesions developed on the upper exposed side to sun and measured 1-1.5 mm in diameter with an average of 1.21 mm. The lesions later on turned to velvety black and slightly raised in first week of June (Plate 5a). In second and third week of June, coalescing of lesions and formation of grey to black patches were observed with size ranging from 5.5 to 9.5 mm with an average of 7.98 mm (Plate 5b). During fourth week of June, the coalesced lesions giving sooty appearance on upper side of fruit (Plate 5c). Finally, severely infected fruits shrivelled and developed cracks on fruit surface during first week of July (Plate 5d).

4.2 Isolation, purification and maintenance of pure culture

During the present investigation isolations were made from diseased leaves and twigs showing typical symptoms. After 7 days of incubation at $25 \pm 1^\circ\text{C}$, an olivaceous mycelial growth started emerging from the diseased leaf or twig tissues, inoculated on potato dextrose agar medium. The culture so obtained

Table 2 :Symptomatological development of almond scab on leaves (cv. Makhdoom)

Period of observation		Symptom development	Lesion characters*					
Month	Week		Shape	Colour	Size (mm)	Sporulation	Petiole infection	Defoliation (%)
May	I	Disease free						
May	II	Minute translucent lesions appear undersurface of leaf, best seen by holding the leaf towards light	Indistinct to somewhat circular	Light yellow	0.5-1.0 (0.61)	-	-	-
May	III	Increase in the number and size of lesions	Circular	Pale green	0.75-2.0 (1.15)	-	-	-
May	IV	Lesions also develop on midrib	-do-	Olivaceous	2.0-3.5 (2.22)	-	-	-
June	I	Increase in the number and size of lesions	-do-	Brownish black	3.0-7.0 (4.25)	+	-	-
June	II	Coalescing of numerous lesion on lower side and appearance of new lesions on upper surface of leaf	Circular to irregular	-do-	7.0-9.5 (8.53)	+	-	-
June	III	Coalesced lesions emerging in patches	Irregular	-do-	-	+	-	-
June	IV	Coalescing continued covering more than 50% area;	Irregular	-do-	-	+	-	-
July	I	Defoliation of severely infected leaves	-	-	-	+	-	6.67
July	II	-do-	-	-	-	-	-	17-0
July	III	-do-	-	-	-	-	-	39.33

* Value in parenthesis are mean of 30 observations - = absent ; += present

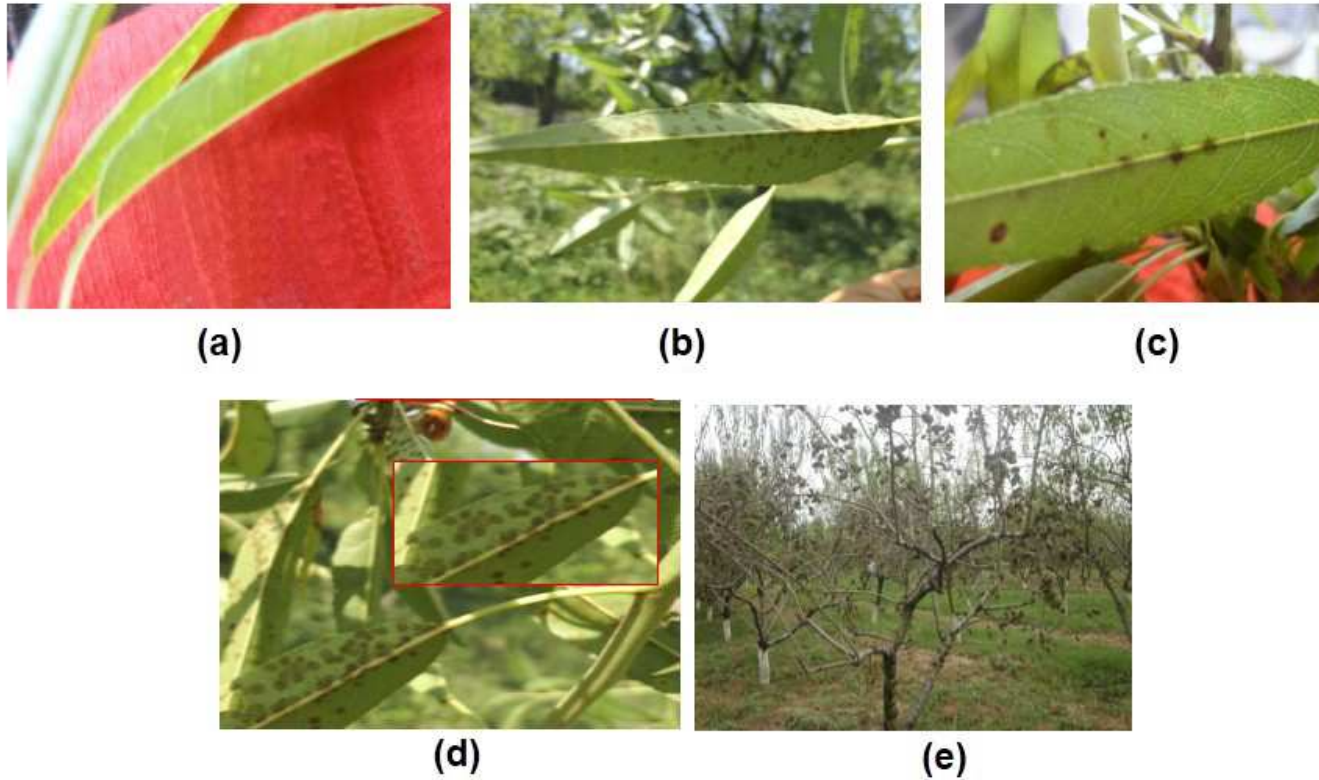


Plate-4 : Characteristic symptoms of scab on almond leaves

- a) Appearance of indistinct pale-green lesions on underside of leaves
- b) Brownish-black lesions on underside of leaves
- c) Brownish-black lesions on mid-rib
- d) Coalescing of lesions forming irregular patches
- e) Premature defoliation

Table 3: Symptomatology development of almond scab on fruits (cv. Makhdoom)

Period of observation		Symptom development	Lesion characters*			
			Shape	Colour	Size (mm)	Sporulation
Month	Week					
May	I	Disease free	-	-	-	-
May	II	Disease free	-	-	-	-
May	III	Small superficial lesions develop usually on upper exposed side to sun	Circular	Olive green	1.0-1.5 (1.21)	-
May	IV	Increase in the number and size of lesions	Circular	-Do-	2.0-3.0 (2.38)	-
June	I	Increase in the number and size of lesions and previously formed lesions become slightly raised	-do-	Velvety black	3.0-6.5 (4.26)	+
June	II	Coalescing of lesions emerging in patches	Irregular	Grey to Black	5.5-9.5 (7.98)	+
June	III	-do-	-do-	-do-	-	+
June	IV	Lesions coalesce together covering the upper side of fruit giving sooty appearance	-	Grey to Black	-	-
July	I	Severely infected fruits shrivelled while as some fruits cracks				

* Value in parenthesis are mean of 30 observations

- = absent ; += present



(a)



(b)



(c)



(d)

Plate-5 : Characteristic symptoms of scab on almond fruits

- a) Circular velvety black lesions**
- b) Coalesce of black lesions**
- c) Black sooty appearance on upper side of fruit**
- d) Cracking of fruit surface and shriveled fruits (in box)**

was purified by the hyphal tip and single spore isolation methods. The pure culture was maintained by sub-culturing at monthly intervals and stored in refrigerators for further studies.

4.3 Morphological characters

The morphological characters of the pathogen were studied both on host as well as in cultures are presented in Table 4.

4.3.1 On Host

Microscopic observation of the pathogen revealed (Table 4) that the mycelium was septate, branched, interwoven, smooth and blackish brown in colour measuring 2.32-4.96 μ m in width with an average of 4.13 μ m. Conidiophores with 3-5 septa were solitary, erect, somewhat flexuous, slightly constricted at septa and pale brown in colour. The size of conidiophores were ranging from 27.84-74.24 \times 6.96-11.60 μ m with an average of 48.60 \times 7.88 μ m. Conidia were hyaline, aseptate, mostly solitary, rarely catenate (Plate 6f), oval to cylindrical, straight and measured 16.60-20.88 \times 4.64-6.96 μ m with an average of 18.09 \times 5.85 μ m.

4.3.2 Inculture

4.3.2.1 Colony characters

The petriplates inoculated with fungus, was critically observed for colony characters and the growth behavior (Table 4). After 30 days of incubation at 25 \pm 1 $^{\circ}$ C on potato dextrose agar medium the colony was erumpent, rough blackish green surface with olivaceous black at reverse side, uneven margins measuring 18-20 mm diameter (Plate 6a).

4.3.2.2 Microscopic characters

The fungus in culture produced septate, branched, interwoven. Smooth, blackish brown mycelium measuring 2.32-6.96 μ m with an average of 5.04 μ m width (Table 4 and Plate 6b). The conidiophores were, pale brown, solitary,

erect,

Table 4: Morphological characters of *Cladosporium carpophilum* Thum. causing almond scab on host and in culture

Structure	Shape	Colour	Size*		Septation
			Length (μm) *	Width (μm)	
On host					
Mycelium	Branched, interwoven, smooth	Blackish brown	–	2.32-4.96 (Av. 4.13)	Septate
Conidiophores	Solitary, erect, somewhat flexuous, slightly constricted at septa	Pale brown	27.84-74.24 (Av.48.60)	6.96-11.60 (Av. 7.88)	3-5 septate
Conidia	Mostly solitary, oval to cylindrical, rarely catenate, erect	Hyaline	16.60-20.88 (Av. 18.09)	4.64-6.96 (Av. 5.85)	Aseptate
**In-culture					
Colony	Erumpent ,rough surface with uneven margin	Blackish green surface, oliveceous black at reverse side	18-20 mm (dia.)	–	–
Mycelium	Branched, interwoven, smooth	Blakish brown	–	2.32-6.96. (Av. 5.04)	Septate
Conidiophores	Solitary, erect, somewhat flexuous, slightly constricted at septa	Pale brown	30.16-83.52 (Av.53.36)	6.96-13.92 (Av. 8.01)	3-9 septate
Conidia	Solitary, oval to cylindrical, erect	Hyaline	16.60-23.20 (Av.20.40)	4.64-9.28 (Av. 7.64)	0-1 septate

* Average of 40 observations; **Incubated for 30 days at 25°C on PDA

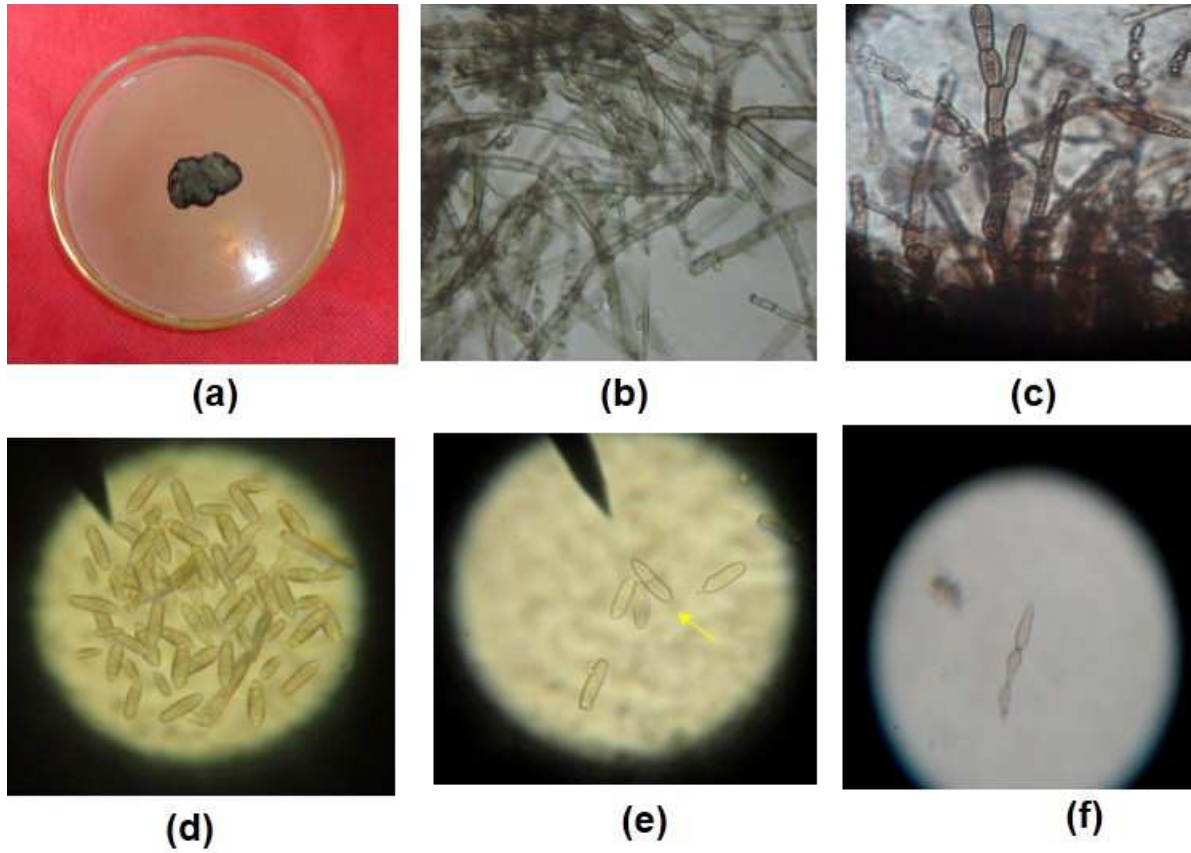


Plate-6 : Morphological character of *Cladosporium carpophilum* Thum. at 400X
 a) 30 days old raised blackish green colony b) Branched interwoven mycellium
 c) Pale brown upright conidiophore d) Oval to cylindrical hyaline conidia
 e) Septate conidia f) Conidia in chain (catenate)on

host

somewhat flexuous, with 3-9 septa, slightly constricted at septa (Plate 6c). They measured 30.16-83.52×6.96-13.92 µm with an average size of 53.36×8.01µm. The conidia were hyaline, solitary, ovate to cylindrical, having 0-1 septa measured 16.60-23.20×4.64-9.28 µm with an average of 20.40×7.64 µm. Catenate conidia were not found in culture (Plate 6d&e).

4.4 Pathogenicity test

Observations regarding the pathogenicity test of the fungus revealed that initial symptoms of the disease appeared 7 days after inoculation on almond (cv. Makhdoom) injured leaves and twigs of potted plants. However, in case of uninjured leaves and twigs, the disease symptoms developed 13 days after inoculation. Re-isolation from diseased leaves and twigs yielded the typical culture of the fungus, thus proving the Kitchin's postulates (Plate 7).

4.5 Identification of pathogen

On the basis of morphological characters, pathogenicity test and comparison with authentic description, the fungus was identified as *Cladosporium carpophilum* Thum. Further its final confirmation was obtained from Indian Type Culture Collection (ITCC), IARI, New Delhi under ITCC No. 9276.13 and reference No. 2810.

4.6 Physiological studies

4.6.1 Effect of media on growth and sporulation of *Cladosporium carpophilum*

In order to determine the best solid medium for growth and sporulation of *C. carpophilum*, the fungus was grown on five different media viz., corn meal agar, Czapek's dox agar, oat meal agar, potato dextrose agar, and Richard's synthetic agar. The average growth (mm) of the colony was recorded in each case after 30 days of incubation at 25±1°C.



Plate-7: Establishment of pathogenicity of *Cladosporium carpophilum* on two year old almond plants

The data presented in Table 5; Fig.1, indicates that the fungus *C. carpophilum* grows best on potato dextrose agar medium with a maximum colony diameter of 19.50 mm followed by oat meal agar (18.75 mm). There was no significant difference between potato dextrose agar and oat meal agar. However, these media were significantly different from corn meal agar (15.2mm), Czapek's Dox agar (11.25mm) and Richard's synthetic agar (8.75mm) media (Plate 8).

The results further indicate that the fungus gave best sporulation on potato dextrose agar (1.15×10^6 /ml) followed by corn meal agar (1.10×10^6 /ml) and oat meal agar (1.04×10^6 /ml). Minimum sporulation was recorded on Richard's synthetic (0.80×10^6 /ml) followed by Czapek's dox agar (0.96×10^6 /ml).

4.6.2 Effect of temperature regimes on growth and sporulation of *Cladosporium carpophilum*

There is a certain range of temperature best suited for each fungus. Therefore, the pathogen under study was grown on potato dextrose agar medium and incubated in at five different temperature regimes viz., 15, 20, 25, 30 and $35(\pm 1^\circ\text{C})$. The average colony diameter (mm) and sporulation produced was recorded after 30 days of incubation.

It is evident from the Table 6; Fig.2, that the fungus under study was able to grow on wide range of temperature. However, maximum colony diameter of 20.0 mm was observed at 25°C which was statistically at par with colony diameter of 19.50mm and 18.25mm observed at 20 and 30°C respectively. Minimum colony diameter of 8.75 and 3.75mm were recorded at 15 and 35°C , respectively.

Further a perusal of data revealed that the best sporulation was produced at 25°C (1.20×10^6 /ml) followed by 20 and 15°C (1.04×10^6 /ml) and (1.01×10^6 /ml) respectively. Least sporulation was recorded at 35°C (0.64×10^6 /ml) followed by 30°C (0.88×10^6 /ml).

Table 5: Effect of different culture media on growth and sporulation of *Cladosporium carpophilum* after 30 days of incubation at 25±1°C

Medium	*Average colony diameter (mm)	**Sporulation (x10⁶/ml)
Potato dextrose agar	19.50(4.42) ^a	1.15
Oat meal agar	18.75(4.33) ^a	1.04
Corn meal agar	15.2(3.90) ^b	1.10
Czapeks Dox agar	11.25(3.35) ^c	0.96
Richards synthetic agar	8.75(2.89) ^d	0.80
SEm±	0.113	–
CD (p=0.05)	0.375	–

Values in parenthesis are square root transformation

Values superscripted by same letter(s) are statistically identical

*Average of 04 replications

**Average of 10 observations

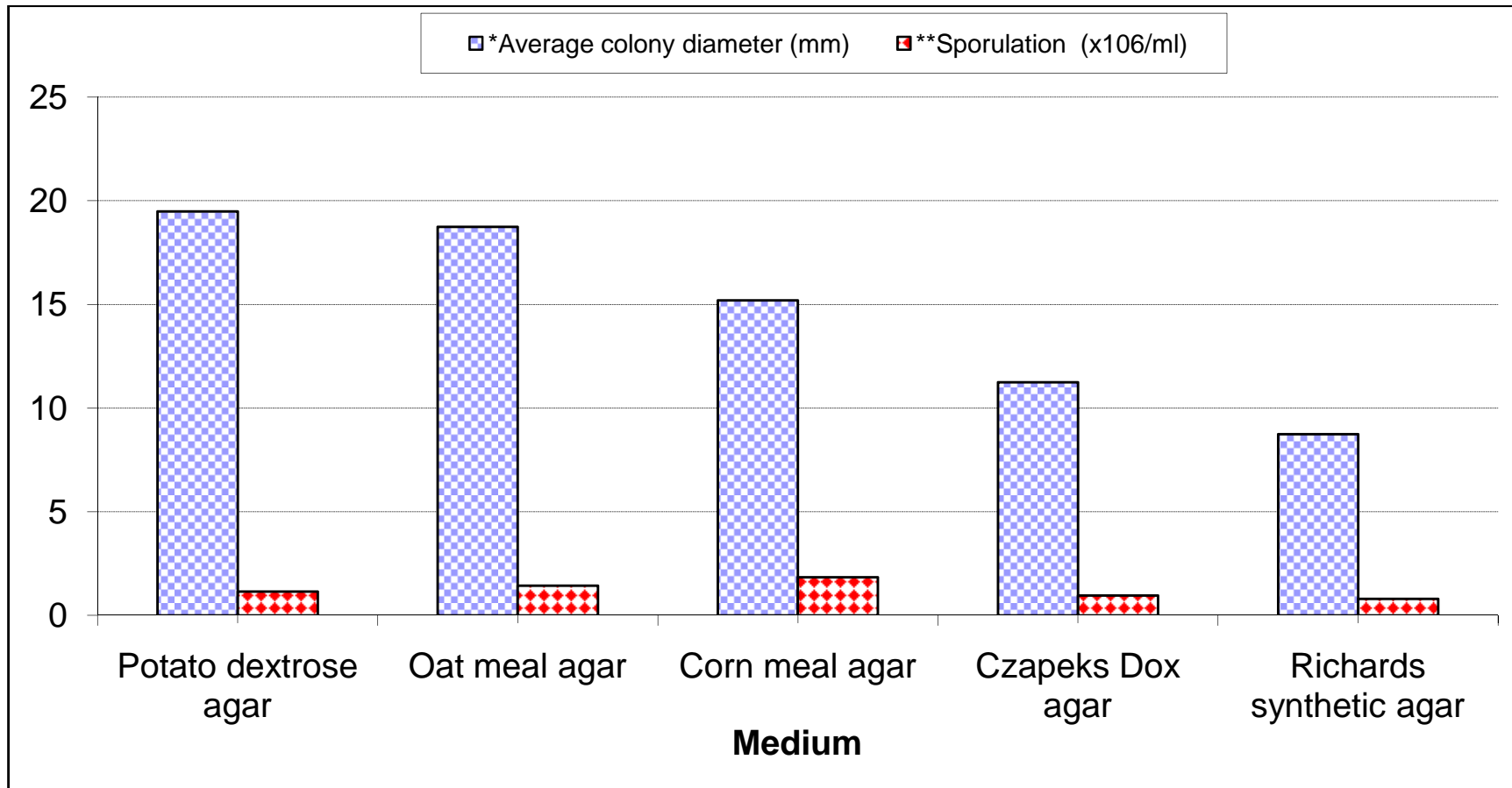


Fig. 1 : Effect of different culture media on growth and sporulation of *Cladosporium carpophilum* after 30 days of incubation at 25±1°C

*Average of 04 replications; **Average of 10 observations



Plate-8: Effect of different media on growth and sporulation of *Cladosporium carpophilum* at $25\pm 1^\circ\text{C}$ after 30 days of incubation

Table 6: Effect of different temperature regimes on growth and sporulation of *Cladosporium carpophilum* after 30 days of incubation on PDA

Temperature ± 1 ($^{\circ}\text{C}$)	*Average colony diameter (mm)	**Sporulation ($\times 10^6/\text{ml}$)
15	8.75 (2.96) ^b	1.01
20	19.50 (4.42) ^a	1.04
25	20.00 (4.47) ^a	1.20
30	18.25 (4.27) ^a	0.88
35	3.75 (1.94) ^c	0.64
SEm \pm	0.107	–
CD (p=0.05)	0.381	–

Values in parenthesis are square root transformation

Values superscripted by same letter(s) are statistically identical

*Average of 04 replications

**Average of 10 observations

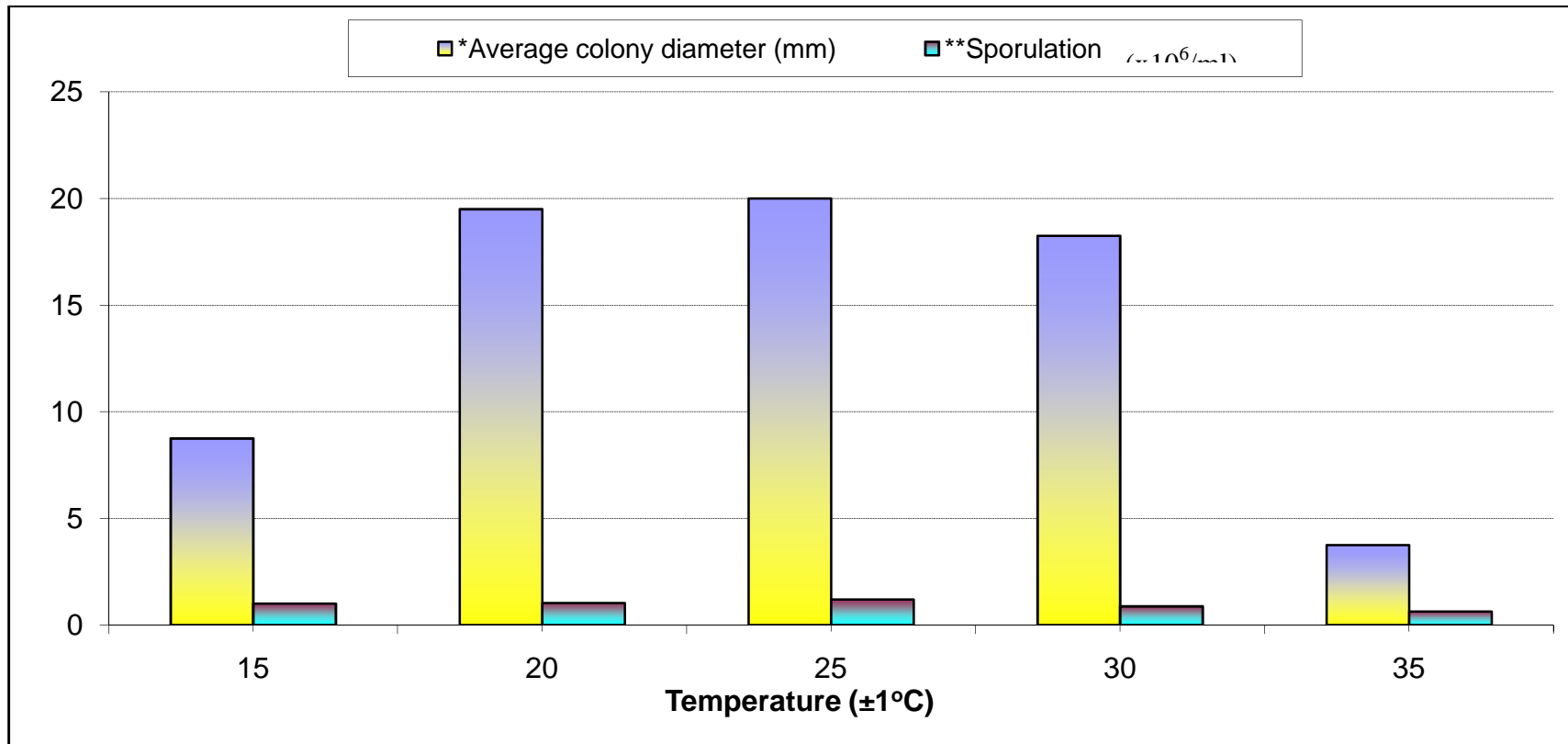


Fig. 2 : Effect of different temperatures regime on growth and sporulation of *Cladosporium carpophilum* after 30 days of incubation on PDA

*Average of 04 replications; **Average of 20 observations

4.6.3 Effect of pH regimes on growth and sporulation of *Cladosporium carpophilum*

In order to determine the optimum pH for growth and sporulation of *C. carpophilum*, the fungus was grown on potato dextrose agar medium at five different pH levels and incubated at 25(±1°C). The average colony diameter and sporulation were recorded after 30 days of incubation and the data is presented in Table 7; Fig.3.

It is obvious from the data that the fungus under study grew throughout the pH range of 4.5 to 8.5. However, maximum colony diameter of 19.25 mm and best sporulation of 1.18×10^6 /ml was recorded at pH 5.5 followed by 15.75 mm and 1.12×10^6 /ml at pH 6.5 and at pH 4.5 the colony diameter was 14.25 mm and sporulation was 0.86×10^6 /ml. Minimum colony diameter of 7.25 mm and sporulation of 0.30×10^6 /ml was recorded at pH 8.5 followed by pH 7.5 at which colony diameter was 11.25 mm and sporulation 0.70×10^6 /ml.

4.7 Screening of almond germplasm against *Cladosporium carpophilum*

Two years old twelve almond cultivars planted in pots were screened to assess their response to *C. carpophilum*. The evaluation study conducted under controlled conditions against disease revealed differential response to the disease (Table 8). All the test cultivars were susceptible to the disease to varying extent except Primorskij and IXL which were resistant and moderately resistant, respectively.

The perusal of data from Table 8 revealed that the disease intensity between the cultivars ranged from 4.67 to 54.33 per cent. Maximum disease intensity was recorded in cultivar Shalimar with an average intensity of 54.33 per cent followed by Warisand Makhdoom with an average disease intensity of 50.33 and 49.67 per cent, respectively. The disease intensity in Ne-Plus-Ultra followed by Peerless and Pranyaj were with an average of 45.67, 44.33, and 42.00 per cent, respectively. The disease intensity in Merced, Non-Pariel and Mission was recorded as 35.00, 32.67 and 27.50 per cent respectively. Average disease

Table 7: Effect of different pH levels on growth and sporulation of *Cladosporium carpophilum* after 30 days of incubation at 25±1°C on PDA

pH levels	*Average colony diameter (mm)	**Sporulation (x10⁶/ml)
4.5	14.25(3.77) ^c	0.86
5.5	19.25(4.39) ^a	1.18
6.5	15.75(3.97) ^b	1.12
7.5	11.25(3.35) ^d	0.70
8.5	7.25(2.69) ^e	0.30
SEm±	0.038	–
CD (p=0.05)	0.103	–

Values in parenthesis are square root transformation

Values superscripted by same letter(s) are statistically identical

*Average of 04 replications

**Average of 10 observations

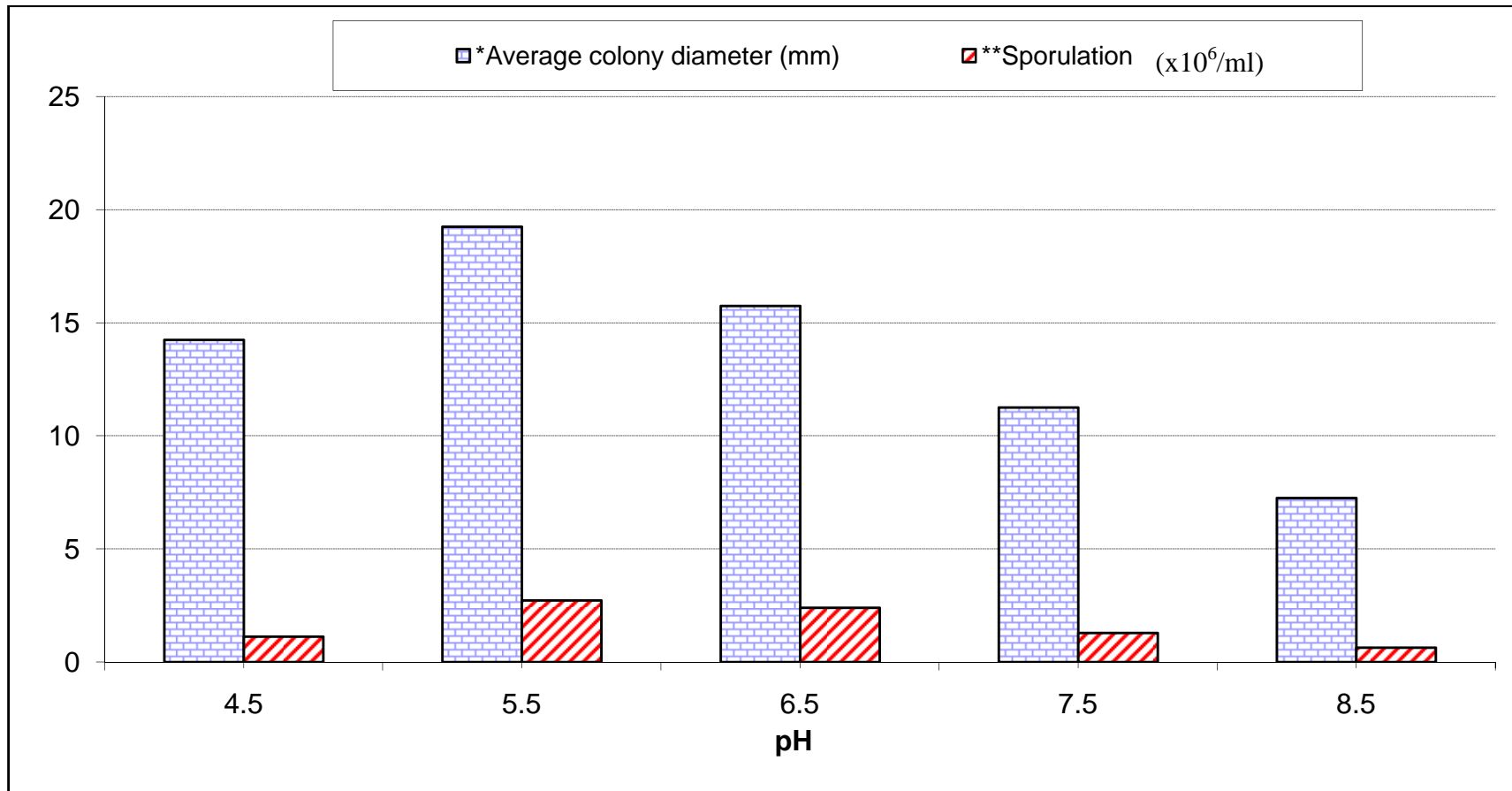


Fig. 3 : Effect of different pH levels on growth and sporulation of *Cladosporium carpophilum* after 30 days of incubation at 25±1 °C on PDA

*Average of 04 replications; **Average of 20 observations

Table 8: Screening of different almond germplasm against almond scab under controlled conditions

Cultivars	Incubation period (in days)	Per cent disease intensity*	Category	Disease reaction	Virulence index
Butte	19	23.33	3	MS	1.23
IXL	27	5.33	2	MT	0.26
Merced	15	35.00	4	S	2.33
Mission	18	27.50	4	S	1.50
Ne-Plus-Ultra	15	45.67	4	S	3.04
Non-pariel	17	32.67	4	S	1.92
Peerless	16	44.33	4	S	2.77
Pranyaj	17	42.00	4	S	2.47
Primorskij	31	4.67	1	T	0.15
Makhdoom	14	49.67	4	S	3.52
Shalimar	13	54.33	5	HS	4.18
Waris	13	50.33	5	HS	3.90

*Average of three replication

MT = Moderately tolerant, MS = Moderately susceptible, HS = Highly susceptible, T = Tolerant, S = Susceptible

intensity in Butte cultivar was recorded as 23.33 percent. Minimum disease intensity of 4.67 per cent was recorded in Primorskij followed by IXL with a disease intensity of 5.33 per cent. An insight into table revealed that the incubation period in twelve cultivars varied from 13 to 31 days, maximum was recorded in Primorskij (31 days) with virulence index of 0.15 while least incubation period was observed in Shalimar and Waris (13 days) with virulence index of 4.18 and 3.90, respectively. In the remaining cultivars virulence index were ranged between 0.26 to 3.52

Of the almond cultivars screened (Table 9) Primorskij was rated as tolerant (0-5PDI), IXL was rated as moderately tolerant (5.1-10PDI) while as Butte was rated as moderately susceptible (10.1-25% PDI). Seven cultivars viz., Marced, Mission, Ne-Plus-Ultra, Non-Pariel, Peerless, Pranyaj and Makhdoom were rated as susceptible (25.1-50 PDI). The remaining two cultivars Shalimar and Waris were rated as highly susceptible (>50 PDI).

4.8 Host range study of *Cladosporium carpophilum* on various stone fruits

Host range studies on almond (cv. Makhdoom), apricot (cv. Halman), cherry (cv. Napoleon) peach (cv. Elberta) and plum (cv. Santa Rosa) was conducted under controlled conditions.

The perusal of data from Table 10, revealed that during host range studies, symptoms were observed on almond, apricot and peach while no symptoms were observed on plum and cherry (Plate 9). The maximum disease intensity of 47.17 per cent was observed on almond cv. Makhdoom after 45 days of inoculation. In peach cv. Elberta disease intensity was 35.67 per cent while on apricot cv. Halman it was 23.50 per cent.

The minimum incubation period of 14 days was recorded in almond cv. Makhdoom followed by peach cv. Elberta (15 days) and the maximum incubation period of 21 days was recorded in apricot cv. Halman.

Table 9: Grouping of various almond cultivars into different reaction categories for scab disease based on per cent disease intensity

Reaction category	Per cent disease intensity (PDI)	Cultivar
Tolerant	0.0-5.0	Primorskij
Moderately tolerant	5.1-10	IXL
Moderately susceptible	10.1-25	Butte
Susceptible	25.1-50	Merced, Mission, Ne-Plus-Ultra, Non-Pariel, Peerless, Pranyaj, Makhdoom
Highly susceptible	>50.0	Shalimar, Waris

Table 10 : Host range studies of *Cladosporium carpophilum* on various stone fruits under controlled conditions

Host	Incubation period (days)	Per cent disease intensity		
		15 dpi	30 dpi	45 dpi
Almond (cv.Makhdoom)	14	0.67	3.33	47.17
Apricot (cv.Halman)	21	–	1.33	23.50
Cherry (cv.Napoleon)	–	–	–	–
Peach (cv.Elberta)	15	0.22	2.50	35.67
Plum (cv.Santa Rosa)	–	–	–	–

Average of three replications

dpi = Days post inoculation

– = No disease



Almond (cv. Makhdoom) Apricot (cv. Halman)

Cherry (cv. Napoleon)

Peach (cv. J.H. Hale)

Plum (cv. Santa Rosa)

Plate 9 : Host range study of *Cladosporium carpophilum* on different stone fruits

4.9 Management

4.9.1 Effect of various fungitoxicants on leaf scab intensity of almond

Field trials for the management of almond scab through fungitoxicants at different phenological stages were conducted in high density almond orchards at CITH, Rangreth, Srinagar, for two consecutive years 2012-13 and 2013-14. Six fungitoxicants both systemic as well as non-systemic namely copper oxychloride 50WP, carbendazim 50WP, chlorothalonil 75WP, difenaconazole 25EC, captan 50WP and pyraclostrobin 60WG at recommended concentrations were evaluated at different phenological stages of susceptible almond cultivar "Makhdoom" against the disease as described under section 3.9.

It is evident from the data presented in Table 11, that during the year 2013 all the fungitoxicant treatments significantly reduced the disease at different phenological stages to varying extent as compared to control. At 15 and 30 days after fruitlet stage, the disease intensity from different treatments ranged between 1.50 to 3.00 and 1.85 to 11.55 per cent, respectively compared to 6.85 and 19.60 per cent recorded in check. The overall scenarios of various treatments at different phenological stages were statistically significant except that of treatment T₂ and T₃ which were statistically at par with each other at 15 days after fruitlet stage. However, among the fungitoxicant treatments applied, treatment T₁ exhibited minimum disease intensity of 2.10 per cent followed by treatment T₂ (3.50%) and T₃ (7.75%) after 45 days of fruitlet stage. The maximum disease intensity of 17.70 per cent was recorded in treatment T₄ in comparison to 25.50 per cent recorded in check.

Perusal of the data from Table 12 reveals that during the year 2014, all the treatments significantly reduced the disease at different phenological stages as compared to check. However, varied levels of disease intensity were recorded from different treatments. At 15 days after fruitlet stage, the lowest disease intensity of 0.65 per cent was recorded in treatment T₁ followed by 1.15 (T₂), 1.01 (T₃) and 2.11 (T₄) per cent in comparison to 7.00 per cent in check. There was no statistical difference between treatment T₂ and T₃ at 15 days after fruitlet

Table 11: Effect of various fungitoxicants on leaf scab intensity of almond under field conditions during 2013

Treatments Stages	Percent disease intensity*						
	Dormant spray	Pink bud	Petal fall	Fruitlet	15 days after fruitlet	30 days after fruitlet	45 days after fruitlet
T ₁	–	–	0.00	0.00	1.50 (1.22) ^d	1.85 (1.10) ^e	2.10 (1.45) ^e
T ₂	–	–	0.00	0.00	2.05 (1.43) ^c	2.10 (1.45) ^d	3.50 (1.76) ^d
T ₃	–	–	0.00	0.00	2.10 (1.45) ^c	5.15 (1.77) ^c	7.75 (2.60) ^c
T ₄	–	–	0.00	0.00	3.00 (1.73) ^b	11.55(3.40) ^b	17.70 (3.96) ^b
Control	–	–	0.00	0.00	6.85 (2.20) ^a	19.60 (4.21) ^a	25.50 (5.05) ^a
CD (p=0.05)	–	–	–	–	0.182	0.266	0.272

Figures in parenthesis are square root transformed values; values superscripted by same letter(s) are statistically identical

– No leaves

* Average of four replication

Table 12: Effect of various fungitoxicants on leaf scab intensity of almond under field conditions during 2014

Treatments Stages	Percent disease intensity*						
	Dormant	Pink bud	Petal fall	Fruitlet	15 days after	30 days after	45 days after

	spray				fruitlet	fruitlet	fruitlet
T ₁	–	–	0.00	0.00	0.65(0.81) ^d	1.50(1.22) ^e	1.65(1.28) ^e
T ₂	–	–	0.00	0.00	1.15 (1.07) ^c	2.35 (1.53) ^d	2.95 (1.72) ^d
T ₃	–	–	0.00	0.00	1.01 (1.00) ^c	4.10 (2.02) ^c	6.05 (2.46) ^c
T ₄	–		0.00	0.00	2.11 (1.45) ^b	9.60 (3.10) ^b	15.55 (3.94) ^b
Control	–	–	0.00	0.00	7.00 (2.65) ^a	21.30 (4.62) ^a	27.20 (5.22) ^a
CD (p=0.05)	–	–	–	–	0.149	0.192	0.332

Figures in parenthesis are square root transformed values ; values superscripted by same letter(s) are statistically identical

– No leaves

* Average of four replication

stage. At 30 and 45 days after fruitlet stages the range of disease intensity varied from 1.50 to 15.55 per cent in comparison to 21.30 to 27.20 per cent in check and were statistically differ from each other.

Two year pooled data (2013 and 2014) presented in Table 13; Fig.4, revealed that all treatments at different phenological stages were significantly superior over control in reducing the disease intensity of almond scab. The disease intensity from different treatments ranged between 1.07 to 2.55 per cent at 15 days, 1.75 to 10.58 per cent at 30 days and finally 1.92 to 16.83 per cent at 45 days after fruitlet stage as against 6.98 to 26.35 per cent respectively, in check. The disease intensity among each treatments were significantly different from each other but only T₂ was statistically at par with that of T₃ at 15 days after fruitlet stage.

Among the fungitoxicant treatments applied, treatment T₁ exhibited minimum disease intensity of 1.92 per cent at 45 days after fruitlet stage where copper oxychloride 50 WP @ 0.3% was applied at dormant stage beside carbendazim 50 WP (0.05%), chlorothalonil 75 WP (0.3%), difenaconazole 25 EC (0.03%), captan 50 WP (0.3%) and pyraclostrobin 60 WG (0.025%) applied at pink bud, petal fall, fruitlet, 15 days after fruitlet and 30 days after fruitlet stages, respectively. Maximum disease intensity of 16.83 per cent as compared to 26.35 per cent in check was recorded in treatment T₄ where only three fungitoxicants viz., carbendazim 50 WP (0.05%), chlorothalonil 75 WP (0.3%) and difenaconazole 25 EC (0.03%) were applied at pink bud, petal fall and fruitlet stages, respectively. While as 3.75 per cent disease intensity was recorded treatment T₂ where dormant spray was not applied followed by treatment T₃ set (6.90%) where dormant and 15 days after fruitlet stage sprays was not sprayed.

Table 13: Effect of various fungitoxicants on leaf scab intensity of almond under field conditions (Pooled data)

Treatments Stages	Percent disease intensity*						
	Dormant spray	Pink bud	Petal fall	Fruitlet	15 days after fruitlet	30 days after fruitlet	45 days after fruitlet
T ₁	–	–	0.00	0.00	1.07 (1.03) ^d	1.75 (1.32) ^e	1.92 (1.39) ^e
T ₂	–	–	0.00	0.00	1.57 (1.25) ^c	2.72 (1.65) ^d	3.75 (1.94) ^d
T ₃	–	–	0.00	0.00	1.65(1.28) ^c	4.63 (2.15) ^c	6.90 (2.63) ^c
T ₄	–	–	0.00	0.00	2.55 (1.60) ^b	10.58(3.25) ^b	16.83 (4.10) ^b
Control	–	–	0.00	0.00	6.98 (2.64) ^a	20.45 (4.52) ^a	26.35 (5.13) ^c
CD (p=0.05)	–	–	–	–	0.173	0.229	0.302

Figures in parenthesis are square root transformed values; values superscripted by same letter(s) are statistically identical

– No leaves

* Average of four replication

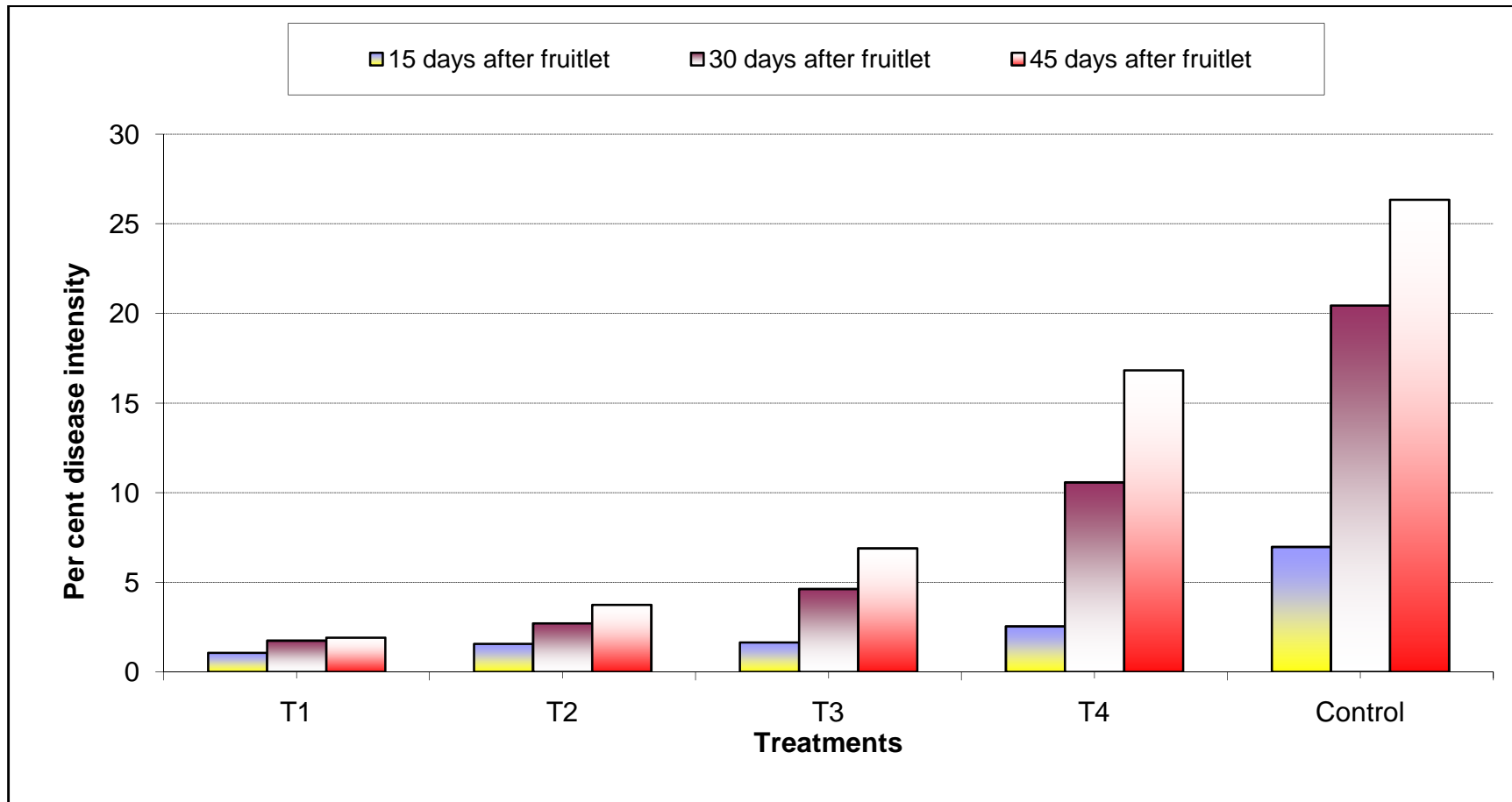


Fig. 4: Effect of various fungitoxicants on leaf scab intensity of almond under field conditions (Pooled data)

4.9.2 Effect of various fungitoxicants on twig scab intensity of almond

It is evident from the Table 14 that all the fungitoxicants at different phenological stages applied during the year 2013 proved effective in controlling the disease. There were statistically significant differences among the treatments. Minimum disease intensity of 2.19 per cent was recorded in Treatment T₁ followed by T₂ and T₃ where disease intensity recorded as 3.64 and 3.75 per cent, respectively and were statistically at par with each other, whereas in T₄ disease intensity was recorded 4.42 per cent at 15 days after fruitlet as compared to check (7.35%). At 30 and 45 days after fruit let stage, the disease intensity among the treatments ranged between 2.91 to 13.16 and 3.10 to 19.25 per cent, respectively compared to 21.39 and 28.64 per cent recorded in check. The overall scenarios of various treatments at different phenological stages were statistically significant except that of T₂ and T₃ at 15 days after fruitlet stage. However, at 45 days after fruitlet stage Treatment T₁ exhibited the lowest disease intensity of 3.10 per cent followed by Treatment T₂ (4.47%) and Treatment T₃ (10.15%). Maximum disease intensity of 19.25 per cent was recorded in treatment T₄ in comparison to 28.64 per cent recorded in check.

Perusal of the data from Table 15 revealed that disease intensity during the year 2014 was significantly lower in all the treatments when compared to check and the range varied from 1.04 to 3.60 per cent in comparison to check where the disease intensity was recorded 9.77 per cent at 15 days after fruitlet stage. At 30 and 45 days after fruitlet stage the disease intensity among the treatments ranged between 1.71 to 17.50 per cent as against 23.39 to 29.21 per cent in check, respectively. An insight into data revealed that all the treatments were significantly differ from each other. However, T₂ (2.50%) was statistically at par with T₃ (2.40%) at 15 days after fruitlet stage.

Table 14: Effect of various fungitoxicants on twig scab intensity of almond under field condition during 2013

Treatments Stages	Percent disease intensity*						
	Dormant spray	Pink bud	Petal fall	Fruitlet	15 days after fruitlet	30 days after fruitlet	45 days after fruitlet
T ₁	–	–	0.00	0.00	2.19 (1.48) ^d	2.91 (1.71) ^e	3.10 (1.76) ^e
T ₂	–	–	0.00	0.00	3.64 (1.91) ^c	4.06 (2.01) ^d	4.47 (2.11) ^d
T ₃	–	–	0.00	0.00	3.75 (1.94) ^c	6.96 (2.64) ^c	10.15 (3.19) ^c
T ₄	–	–	0.00	0.00	4.42 (2.4) ^b	13.16 (3.35) ^b	19.25 (4.39) ^b
Control	–	–	0.00	0.00	7.35 (2.71) ^a	21.39 (5.23) ^a	28.64 (5.35) ^a
CD (p=0.05)	–	–	–	–	0.295	0.281	0.311

Figures in parenthesis are square root transformed values; values superscripted by same letter(s) are statistically identical

– No fresh twigs

*Average of four replication

Table 15: Effect of various fungitoxicants on twigs scab intensity of almond underfield conditions 2014

Treatments	Percent disease intensity*
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Stages	Dormant spray	Pink bud	Petal fall	Fruitlet	15 days after fruitlet	30 days after fruitlet	45 days after fruitlet
T ₁	–	–	0.00	0.00	1.04 (1.02) ^d	1.71 (1.31) ^e	1.87 (1.37) ^e
T ₂	–	–	0.00	0.00	2.50 (1.58) ^c	3.13 (1.77) ^d	3.33 (1.82) ^d
T ₃	–	–	0.00	0.00	2.40 (1.55) ^c	5.32 (2.32) ^c	8.69 (2.77) ^c
T ₄	–	–	0.00	0.00	3.60 (1.90) ^b	11.16 (3.34) ^b	17.50 (3.94) ^b
Control	–	–	0.00	0.00	9.77 (3.13) ^a	23.39 (4.84) ^a	29.21 (5.59) ^a
CD (p=0.05)	–	–	–	–	0.432	0.415	0.385

Figures in parenthesis are square root transformed values; values superscripted by same letter(s) are statistically identical

– No fresh twigs

*Average of four replication

Two year pooled data (2013 and 2014) presented in Table 16;Fig.5, revealed that all treatments at different phenological stages were significantly superior over control in reducing the disease intensity. At 15 and 30 days after fruitlet stage, the disease intensity ranged between 1.62 to 4.01 and 2.31 to 12.69 per cent, respectively compared to 8.56 and 22.39 per cent recorded in check. Overall scenarios of various treatments at different phenological stages were statistically significant from each other except that of T₂ and T₃ at 15 days after fruitlet stage. However, at 45 days after fruitlet stage the minimum disease intensity of 2.48 per cent was recorded in treatment T₁ where all six fungitoxicants were applied at six different respective phenological stages of the almond. This was followed by treatment T₂ (3.90%) where dormant (copper oxychloride 50 WP) sprays was skipped and treatment T₃ (9.42%) where dormant (copper oxychloride 50 WP) and 30 days after fruitlet stage (pyraclostrobin 60 WG) spray were skipped. Maximum disease intensity of 18.37 per cent was exhibited in treatment T₄ where only three sprays at pink bud (carbendazim 50 WP @ 0.05%), petal fall (chlorothalonil 75 WP @ 0.3%) and fruitlet (difenaconazole 25 EC @ 0.05%) stages were applied while rest three sprays were skipped.

Table 16: Effect of various fungitoxicants on twigs scab intensity of almond under field conditions (Pooled data)

Treatments Stages	Percent disease intensity*						
	Dormant spray	Pink bud	Petal fall	Fruitlet	15 days after fruitlet	30 days after fruitlet	45 days after fruitlet
T ₁	–	–	0.00	0.00	1.62 (1.27) ^c	2.31 (1.52) ^e	2.48 (1.57) ^e
T ₂	–	–	0.00	0.00	3.07 (1.75) ^b	3.59 (1.90) ^d	3.90 (1.97) ^d
T ₃	–	–	0.00	0.00	3.08 (1.75) ^b	6.14 (2.48) ^c	9.42 (3.06) ^c
T ₄	–	–	0.00	0.00	4.01 (2.00) ^b	12.69(3.56) ^b	18.37 (4.28) ^b
Control	–	–	0.00	0.00	8.56 (2.92) ^a	22.39 (4.73) ^a	28.92 (5.37) ^a
CD (p=0.05)	–	–	–	–	0.354	0.343	0.347

Figures in parenthesis are square root transformed values; values superscripted by same letter(s) are statistically identical

– No fresh twigs

*Average of four replication

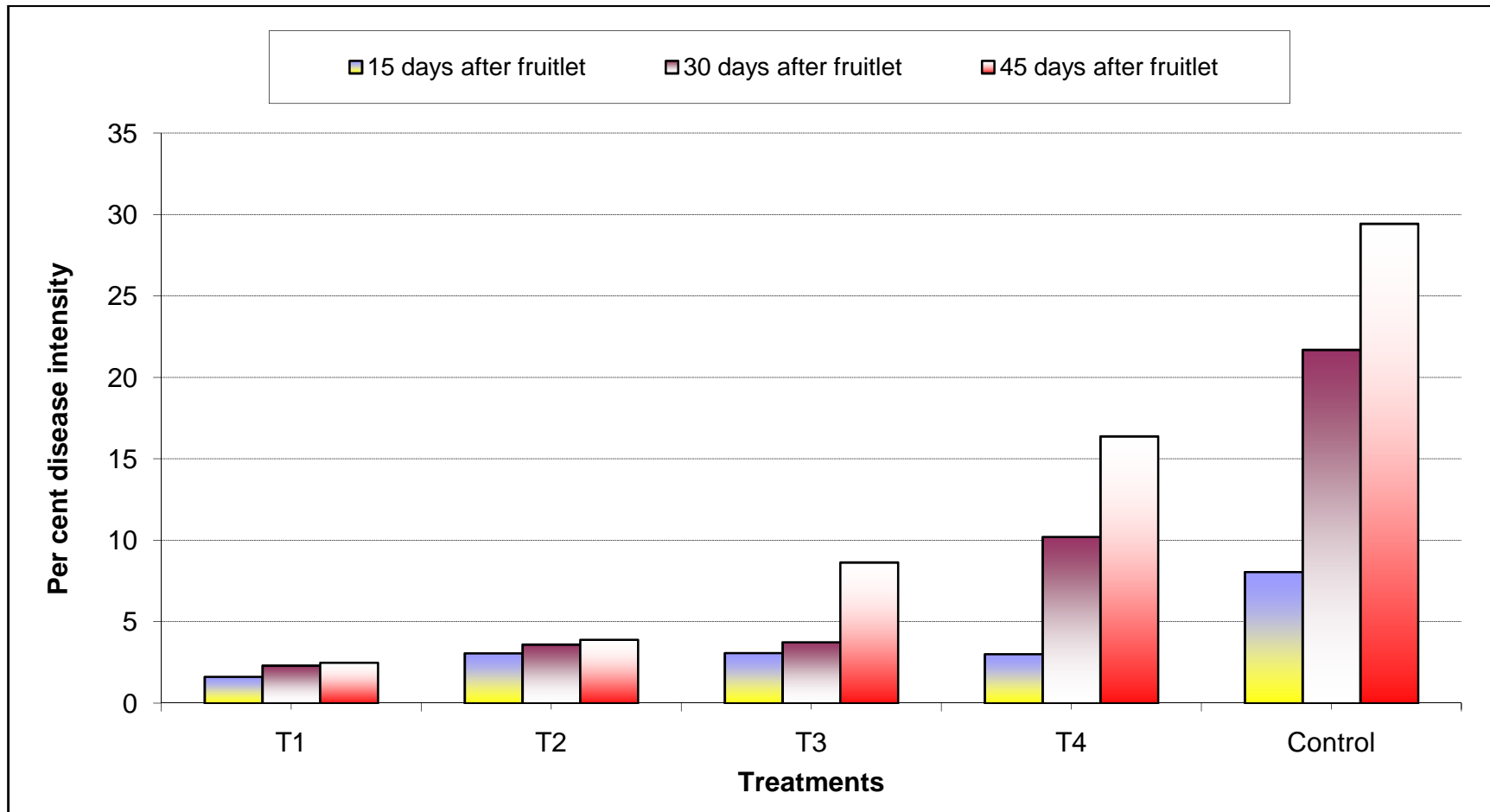


Fig. 5 :Effect of various fungitoxicants on twigs scab intensity of almond under field conditions (Pooled data)

Chapter-5

DISCUSSION

Almond (*Prunus amygdalus* Batsch.), is an important nut crop of temperate regions of the world. In India, Jammu and Kashmir state, produces about 90 per cent of total almond production of the country (Rather *et al.*, 2013).

However, various diseases have restricted the profitability of almond farming. The preliminary survey indicated the occurrence of almond scab in most of the commercial almond growing areas in Kashmir which necessitated to have an exact knowledge of disease development and its management. Retrieval of literature revealed that no systemic work has been conducted on any aspects of the disease in India and hence the present study was undertaken.

In present investigations, the scab symptoms were first noticed on twigs in starting May and then on leaves and fruits. Jones and Sutton (1984) also observed that scab lesion on current twigs of peach developed in early spring while on fruits and leaves it became evident after calyx-split. On current year twigs, the initial symptoms appeared as indistinct, raised, water soaked, olive green specks measuring an average of 1.43 mm in size and attained maximum size of 11.57 mm in first week of June. These symptoms are in agreement with the observations of Dzhafarov (2003) and Horst (2013) who reported the similar symptoms on peach twigs as a result of infection by the pathogen *C. carpophilum*. Later on, the number and size of lesions increased simultaneously whereas the shape turned circular to elliptical and colour became white with purple periphery. In third week of June lesions developed into patches due to coalescing of 3-5 spots, the colour turned dark purplish brown resembling with the colour of bark and the lesion lost their identity. These findings are more or less supported by the observations made by Adaskaveg (2013) on almond twigs and Khosla (2009) on peach twigs.

On leaves, the initial symptoms were observed in the second week of

May as a minute indistinct translucent, pale yellow lesions measuring an average of 0.61mm in size on undersurface of the leaves, best seen by holding the leaves towards the sun. Similar observation was made by Chernomorski (2010) in China on almond leaves. The size and number of the lesions were increased simultaneously whereas shape turned to circular and pale green in third week of May. The maximum average size of lesion attained 8.53mm in second week of June. Lesions become circular to irregular owing to coalescing and colour changed to brownish black on lower side of leaves and lesions also start to appear on upper surface of leaves and defoliation started from first week of July which reached up to 39.33 per cent in third week of July. Almost similar symptoms were noticed by Keitt (1917) on peach leaves and Connel (2002) on almond leaves. However, Iliev (1970) reported that in almond leaves infected by *C. carpophilum* total and bound water contents were lower while transpiration and concentration were higher which leads to defoliation which we have not done in our studies.

On fruit the initial symptoms of the disease were seen in third week of May, usually on upper exposed side to sun as small superficial, circular, olive green coloured lesions measuring 1.21mm in size which gradually increased and attained maximum average size of 7.98 mm in second week of June. Previously formed olive coloured lesions became slightly raised and changed to velvety black during first week of June. Irregular patches emerged due to coalescing of lesions and colour turned grey to black in second and third week of June. Maximum lesions in fourth week of June coalesced together covering the upper side of fruit giving sooty appearance and in severe infections cracking of fruit often occurred. These observations are supported by Gottwald (1983) and David (2012).

The casual organism was isolated from diseased leaves/twigs of almond cv. Makhdoom and was cultured on PDA medium for further studies.

The pathogenic behaviour of the isolated causal organism was established following Koch's postulates on healthy leaves of two year old potted

plants of almond cv. "Makhdoom". The typical symptoms of the disease on leaves were produced by the pathogen after 7 days and 13 days of inoculation on injured and uninjured leaves respectively under controlled conditions. Keitt (1914) found that 40 to 70 days elapse from the time of inoculation until the disease appeared in field. Bottomley (1944), also noticed that symptoms appeared after 5 weeks on the inoculated apricot fruit under natural condition. Similar observation was made by Fisher (1961) who noticed symptom development after 8 weeks of inoculation on apricot leaves under natural condition. However, no literature was found regarding the incubation period under controlled conditions.

The morphological characters of the pathogen were studied both on host as well as on potato dextrose agar medium. On the host the pathogen revealed that mycelium consisted of smooth, interwoven, branched, septate, blackish brown hyphal strands measuring 2.32 -4.96 μm wide with an average size of 4.13 μm . Conidiophores were solitary, erect, flexuous, slightly constricted at septa, pale brown having 3-5 septa and measured 27.84-74.24 x 6.96-11.60 μm in size. Conidia were aseptate, mostly solitary rarely catenate, hyaline, oval to cylindrical, straight and measured 16.60-20.80 x 4.64-6.96 μm . Similar observations were also made by Hashemiet al. (2013), who observed that conidiophores of *C. carpophilum* were solitary, erect, flexuous or straight, unbranched, measuring 29-105 x 4-6.2 μm while conidia were cylindrical to fusiform, straight, 12.5-22 x 4-5 μm in size, aseptate, and pale olivaceous in colour. During the studies conidiophores and conidia were recorded slightly larger in culture measuring 30.16-83.52 x 6.96-13.92 μm and 16.60-23.20 x 4.64-9.28 μm respectively, in comparison to host though possessing similar morphological characters. The morphological description of the pathogen almost corroborate with description given by many workers (Thumen, 1877; Keit, 1917 and Heuchert, 2005). The fungal colony of *C. carpophilum* on potato dextrose agar medium was erumpent, blackish green rough surface with an uneven margin and olivaceous black at reverse side which attained 18-20 mm diameter after 30 days of incubation at $25 \pm 1^\circ\text{C}$. Present

investigation are completely in accordance with the finding of Schweizer (1958) who observed similar characters of the colony in the pathogen *C. carpophilum* in peach and in *C. cerasi* causing scab disease in cherry. Almost similar observation were noticed by Domschet *et al* (1980) where colonies in *Cladosporium* sp. were rather slow growing, suede-like to floccose, olivaceous to blackish in color.

On the basis of symptomatology, morphological characters, pathogenicity test and comparison to the authentic description, the pathogen was identified as *Cladosporium carpophilum* Thum. and its identity confirmed by the Indian Type Culture Collection, Division of Plant Pathology, IARI New Delhi, under ITCC No. 9279.13.

In order to determine the best media for growth and sporulation of *C. carpophilum* the fungus was grown on five different solid media viz., potato dextrose agar, oat meal agar, corn meal agar, Czapek's dox agar and Richard's synthetic agar medium. Fungus grew and reproduced profusely on potato dextrose agar and oat meal agar media with minimum colony diameter and sporulation on Richard's synthetic agar medium as was also reported by Schweizer (1958) for *C. carpophilum* and *C. cerasi* causing scab disease in peach and cherry respectively. Sahan (2003) and Kata (2004), also observed that potato dextrose agar a preferred medium for growth and sporulation of *C. musae* responsible for leaf speckle in banana. Since carbon constitutes about half of dry weight of the fungus and is not only the main structural element but a chief source of energy as well (Cochrane, 1958). The fungus also sporulated best on corn meal agar and oat meal agar medium. These findings are in agreement with the observations made by Hall (1984), who obtained maximum sporulation of *C. allii-cepae* on corn meal agar. Since corn meal and oat meal agar have less easily digestible carbohydrates, most of the dematiaceous fungi sporulated better on corn meal agar and oat meal agar as observed by Charles (2012).

Temperature not only effect the growth and sporulation of fungus but all the biological activities of the organism are influenced and the influence usually

varying between and within different genera and species and among different strains and/or geographical isolates of the same species (Cochrane, 1958). In the present study, the fungus under study grew on a wide temperature range of 15-35±1°C but maximum growth and sporulation was obtained at 25±1°C followed by that at 20±1°C. These findings are in agreement with the observation of Lawrence and Zehr (1992) who also obtained maximum radial growth and sporulation of *C. carpophillum* at 25°C. Similar observation was also noticed by Darell (1995) in *C. caryigenum* causing scab of pecan nut. Domigues *et al.* (2013) while conducting physiological study of *Fusicladium eriobotryae*, causing scab of loquat, the best growth and sporulation was obtained at 20°C. The fungus grew poorly at 35±1 which is in accordance with the finding of Mohammed *et al.* (2015) who reported that the fungus *C. herburum* did not grow at 35°C.

The hydrogen ion concentration of the substrate medium is known to control the degree of dissociation in inorganic ions in culture and hence their uptake into the fungal thallus, thus significantly influencing the fungal growth and proliferation. Sometimes complete inhibition of growth and sporulation may occur on media which are too acidic or too alkaline (Graffin, 1996). The fungus has been found to grow well within a pH range of 4.5-8.5 but the maximum growth and sporulation was achieved at pH 5.5 followed by pH 6.5 and 4.5. The optimum pH range for growth and sporulation of the fungus was 4.5-6.5 indicating that the fungus preferred an acidic medium for growth and sporulation. The results are in close association with the finding of Rafal *et al.* (2012) who also found good growth of *Cladosporium* sp. at pH range of 5-6. Shahzad (1994) and Williams and Helton (1971), also reported optimum pH range of 5.0 to 6.0 for growth and sporulation of *Stigmina carpophilla* and *Coryneum carpophilum*, respectively.

The use of resistant cultivars has been preferred method for plant disease management since long. Excessive use of chemical and environment concern compelled the researcher to divert his attention towards environmentally safe practices including use of resistant varieties. Natural resistance within the

germplasm exists as source that can be exploited every time when situation arises. Therefore, screening of existing germplasm for their tolerance is primary requirement for evolving resistant varieties against the disease. However, relative resistance varies depending upon geographic location and pathogen strains found there (Littrell and Bertrand, 1981). However, resistant mechanism in case of pecan nut for scab is mediated by the speed of plant cell wall modification that limit the growth of subcuticular hyphae of the scab fungus *C. caryigenum* (Yates and Cason, 1996; Yates *et al.*, 1996; Conner and Stevenson, 2004). Tissue age is another major factor that affects resistance to scab infection (Diehl and Graves, 1994, Turechek and Stevenson, 1998). This present study examined the virulence pattern resulting from the inoculation of twelve almond cultivars with *C. carpophilum*. The pathogen was virulent to most of the cultivars and avirulent to few. Attempt made to identify cultivars showing resistant to *C. carpophilum* under controlled conditions indicated that only cultivar Primorskij exhibited resistant with minimum disease intensity of 4.67 per cent after 45 days of inoculation, while IXL and Butte exhibited moderately resistant and moderately susceptible reaction with 5.33 and 23.33 per cent disease intensity, respectively. This may be in agreement with Diehl and Graves (1994) who reported that total number of phenolic compounds play significant role in reducing the growth and development of *C. caryigenum*. The remaining cultivars were either susceptible or highly susceptible in reaction. Wetzstein and Sparks (1983) showed that scab susceptible cultivars of pecan had a high variable size peltate trichomes and these trichomes frequently abutted the abaxial surface of scab susceptible cultivars leaves, and secretion were found adjacent to some trichomes. They suggested that high trichome densities might contribute to a microclimate favorable for scab development by supporting a humid environment and also secreted substances might promote development and germination of conidia. The above findings are also in agreement with Post (1985) and Ogawa and English (1991) who observed that Carmel, Drake, Merced, Ne-Plus-Ultra, Peerless, Price and Ruby to be more susceptible, whereas Non-Pariel showed moderately resistant to almond scab

under natural conditions. Gottwald (1983) observed that on peach leaves the latent period varies from 25-45 days whereas on twigs symptoms developed after 25 days of inoculation. Further research is needed to confirm the latent period in almond.

In the present host range studies it was observed that the pathogen has shown its ability to infect all the test hosts except cherry and plum under artificial inoculation conditions. These findings are well supported by Bensaud and Kiett (1928), where they found that the pathogen *C. carpophilum* from peach infected apricot but not cherry and plum. Hashemi *et al.* (2013) also reported that almond scab pathogen differs from cherry scab pathogen as almond scab pathogen has a broad host range rarely occurring on cherry. During our study it was observed that incubation period has been shortest (14 days) in almond, while maximum incubation period of 21 days was observed in apricot. Domingues *et al.* (2014) also found 21 days of incubation period in *F. erobotyrae*, causing scab of loquat.

During the course of present investigation in 2012-13 and 2013-14, six fungitoxicants at different phenological stages were tested for their efficacy in controlling the disease under natural field conditions. The almond cv. "Makhdoom" were sprayed with different systemic as well as non-systemic fungitoxicants alternately. Fungicides with a different mode of action are suitable to alternate in resistance management programme (Luo *et al.*, 2013). All fungitoxicants applied proved significantly effective in controlling the scab disease on almond.

Result of the two year experimentation revealed that the minimum leaf scab intensity of 1.92 percent and twig intensity 2.48 per cent were recorded in case of treatment T₁ set where copper oxychloride 50 WP (0.3%) was applied at dormant stage beside carbendazim 50 WP (0.05%), chlorothalonil 75 WP (0.3%), difenaconazole 25 EC (0.03%), captan 50 WP (0.3%) and pyraclostrobin 60 WG (0.025%) applied at pink bud, petal fall, fruitlet, 15 days after fruitlet and 30 days after fruitlet stages, respectively. In treatment T₂, T₃ and T₄ the leaf scab intensity

were recorded 3.75, 6.90 and 16.83 percent while it was recorded 3.90, 9.42 and 18.37 per cent on twig respectively. The treatment T₂ was significantly superior over T₃, where all the fungitoxicants at different respective phenological stages were applied except dormant spray (copper oxychloride 50 WP), while as in T₃ copper oxychloride 50 WP (dormant spray) as well as pyraclostrobin 60 WG (30 days after fruitlet) were missed. The treatment T₄ was significantly inferior over all other treatments where three fungitoxicants namely copper oxychloride 50 WP at dormant, captan 50 WP at 15 days after fruitlet and pyraclostrobin 60 WG at 30 days after fruitlet stage were missed. Hogmire (1994) and Teviotdale (2001) reported that dormant spray with any copper fungitoxicants followed by 2 or 3 additional sprays after pink bud managed the scab disease of *Prunes sp.* efficiently. Brannen and Hotchkiss (2003) found that scab disease in nectarine controlled up to 90 per cent with copper oxychloride and carbendazim when sprayed at different phenological stages. Horten *et al.* (2000) reported that a series of sulphur fungicide sprays beginning at petal fall and containing 2 weeks intervals controlled the scab disease of stone fruit effectively. Several workers reported that captan at petal fall, followed by pyraclostrobin and chlorothalonil at 2 and 4 weeks interval after the petal fall spray respectively reduced scab infection of almond (Brent and Hoffman, 2002; Flint, 2002; Horsefield, 2010). These findings are more or less substantiate the present investigation.

The study showed that the fungitoxicants which were effective in reducing the leaf infection also limited the twig lesions thereby reduced the inoculum carryover to the next season. Lalancette *et al.* (2008) and Murdy *et al.* (2006) have also recorded this phenomenon while experimenting on control of scab epidemics on stone fruits with trifloxystrobin. They further reported that though of limited economical concern, infection caused by *C. carpophilum* current season twigs are of great epidemiological importance as the primary infection of expanding twigs, leaves and developing fruits in spring results solely from conidia produced in 1-year old twig lesions. However, reduction in twig lesions has significant

impact on the densities of primary inoculums and on the onset of the diseases in the following season.

Chapter-6

SUMMARY AND CONCLUSION

The investigation on “scab disease of almond in Kashmir Valley” were carried out at Division of Plant Pathology, SKUAST-K, Shalimar laboratory and at almond block of Central Institute for Temperate Horticulture, Rangreth, Srinagar during 2012-2014. The results obtained during the course of the study are summarized hereunder.

Symptomatological studies of the disease on twigs revealed that the disease manifested as indistinct, raised, water soaked olive green lesion measuring about 1.43 mm in dia. in the first week of May. Periodical change in size, shape and color of the lesions were noticed and finally become circular to elliptical, and reddish brown in colour with darken periphery. Coalescing of several lesions under severe infection resulted in the formation of large (11.57 mm) irregular patches. In the fourth week of June, the dark purplish brown color of lesion resembling with color of bark. The symptoms on leaves first observed in second week of May and reached its peak during the early period of June. The symptoms appeared as translucent, small indistinct to circular lesions on the underside of leaves, measuring about 0.61 mm in size. The lesions later become circular to irregular owing to coalescing and colour changed from olivaceous to brownish black and produced the spores in first week of June. Severely infected trees defoliated prematurely. On fruits symptoms appeared in third week of May as light black superficial, circular spots on upper side exposed to sun, measuring about 1.21 mm in size. Extension of spots later on resulted in the formation of velvety black lesions averaging 4.26 mm in diameter. In third week of June grey to black lesions coalesced together giving sooty appearance on upper side of fruit. Finally, severely infected fruits shriveled and developed cracks on the fruit surface in the first week of June.

The causal fungus was isolated from infected leaves of almond on potato

dextrose agar medium incubated at $25 \pm 1^\circ\text{C}$. Pathogenicity test of the isolated fungus was established by proving Koch's postulates. The monoconidial culture of the pathogen produced characteristic disease symptoms after 7 days of inoculation on injured leaves and twigs of potted plants while as on uninjured plants, the symptoms were produced after 13 days of inoculation. The fungus on potato dextrose agar medium appeared as blackish green colony with olivaceous black at reverse side, having uneven margin and produced septate, branched, blackish brown mycelium. Conidiophores were pale brown, multiseptate, solitary, erect and measured average size of $48.60 \times 7.88 \mu\text{m}$ on host while, it was recorded $53.36 \times 8.01 \mu\text{m}$ in culture. Conidia were ovate or cylindrical in shape with an average size of $18.09 \times 5.85 \mu\text{m}$ on host and $20.40 \times 7.64 \mu\text{m}$ in culture..

On the basis of pathogenicity test, morphological characters and comparison with authentic description (Thumen, 1817), the pathogen was identified as *Cladosporium carpophilum* Thum. which was later on confirmed by ITCC, IARI, New Delhi.

The fungus exhibited maximum colony growth on potato dextrose agar medium (19.50 mm) followed by oat meal agar (18.75 mm) and corn meal agar (15.20 mm) while as best sporulation gave on potato dextrose agar medium ($1.15 \times 10^6/\text{ml}$) followed by corn meal agar ($1.10 \times 10^6/\text{ml}$) and oat meal agar ($1.04 \times 10^6/\text{ml}$). The pathogen grew in a temperature and pH range of $15-35 \pm 1^\circ\text{C}$ and 4.5-8.5 respectively, but highest growth 20.0 and 19.25 mm and sporulation of $1.20 \times 10^6/\text{ml}$ and $1.18 \times 10^6/\text{ml}$ were recorded on $25 \pm 1^\circ\text{C}$ and pH 5.5, respectively.

Twelve available almond cultivars were screened for their performance against the disease under controlled conditions. Among the cultivars screened Primoskij, showed resistant with minimum disease intensity (4.67%), IXL moderately resistant (5.33%) and Butte showed moderately susceptible (23.33%),

while all other nine cultivars susceptible to highly susceptible reaction against the disease.

From the host range studies conducted on different stone fruits (almond cv. Makhdoom, apricot cv. Halman and peach cv. Elberta) it was observed that all the cultivars of stone fruits were susceptible except cherry cv. Napoleon and plum cv. Santa rosa. The maximum disease intensity (47.17%) was recorded on almond cv. Makhdoom with minimum incubation period (14 days) while minimum disease intensity (23.50%) with maximum incubation period (21 days) was recorded in apricot cv. Halman.

An attempt was made to manage the disease through six different fungitoxicants at different phenological stages during the two cropping seasons. Studies revealed that among the treatments applied, treatment T₁ (copper oxychloride 50 WP @ 0.3% at dormant, carbendazim 50 WP @ 0.05% at pink bud, chlorothalonil 75 WP @ 0.20% at petal fall, difenoconazole 25 EC @ 0.03% at fruitlet, captan 50 WP @ 0.3% at 15 days after fruitlet and pyraclostrobin 60 WG @ 0.025% at 30 days after fruitlet) proved significantly superior in reducing leaf scab intensity to 1.92 per cent and twigs scab intensity to 2.48 per cent in comparison to 26.35 and 28.92 percent respectively observed in unsprayed check. Treatment T₄ was significantly inferior to over all other treatments where three fungitoxicants namely copper oxychloride 50 WP (dormant), captan 50 WP (15 days after fruit let) and pyraclostrobin 60 WG (30 days after fruit let) were not sprayed.

The conclusions drawn are presented as follows:

- Almond scab is a new disease to Valley.
- Disease appeared in the month of May on current twigs, leaves and fruits.
- The disease incitant was identified as *Cladosporium carpophilum* Thum.

- The best media for mycelia growth and sporulation was potato dextrose agar at $25\pm 1^{\circ}\text{C}$ with optimum pH of 5.5
- All almond cultivars selected for screening had showed symptoms, with different disease intensity.
- In host range studies disease was observed on almond, peach and apricot whereas no disease was observed on plum and cherry.
- All fungitoxicants effectively combat the disease. Dormant spray with petal fall, 2 and 4 weeks post to petal fall sprays seemed to be essential for the disease management.

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Appendix-I

Preparation of different culture media

Ingredients	g/l
1) Oat meal agar	
Oat meal	60.00
Agar	12.50
Distilled water to make the volume	1000 ml
2) Richard's Synthetic Agar	
Potassium nitrate	10.00
Monopotassium dihydrogen phosphate	5.00
Magnesium sulphate	2.50
Ferric chloride	0.02
Sucrose	50.00
Agar	15.00
Distilled water to make the volume	1000 ml
3) Corn Meal Agar	
Corn meal infusion form	50.00
Agar	15.00
Distilled water to make the volume	1000 ml
4) CzapeksDox Agar	
Sucrose	30.00
Sodium nitrate	2.00
Dipotassium phosphate	1.00
Magnesium sulphate	0.50
Potassium chloride	0.50
Ferrous sulphate	0.01
Agar	15.00
Distilled water to make the volume	1000 ml
5) Potato Dextrose Agar	
Peeled potato	200.00
Dextrose	20 .00
Agar agar	20
Distilled water to make the volume	1000 ml



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CERTIFICATE

Certified that all the corrections/amendments as suggested by External Examiner Dr. Nazir Ahmad Munshi, Former Professor, Division of Plant Pathology, SKUAST-Kashmir during thesis Viva-Voce examination held on 26.05.2016 have been incorporated in the final manuscript entitled “**Studies on Scab Disease of Almond (*Prunus amygdalus* Batsch.) in Kashmir Valley**” submitted by **Ms. Nassreen Fatima (Regd. No. 2008-227-D)**.

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