STUDIES ON MARSSONINA LEAF BLOTCH OF APPLE

THESIS

by

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CERTIFICATE-I

This is to certify that the thesis entitled **"Studies on Marssonina leaf blotch of apple",** submitted in partial fulfilment of the requirements for the award of degree of **MASTER OF SCIENCE** in **MYCOLOGY AND PLANT PATHOLOGY** to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Solan (H.P.) is a bonafide research work carried out by **Ms. Renu Bala (H-97-29-M)** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of investigations have been fully acknowledged.

Place : Nauni, Solan
Dated : December 22 ,1999

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CERTIFICATE-II

This is to certify that the thesis entitled **"Studies on Marssonina leaf blotch of apple"** submitted by **Ms. Renu Bala (H-97-29-M)** to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Solan (H.P.), in partial fulfilment of the requirements for the award of degree of **MASTER OF SCIENCE** in **MYCOLOFY AND PLANT PATHOLOGY** has been approved by the Student's Advisory Committee after an oral examination of the same in collaboration with the external examiner.

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Dean
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Dedicated

to

my Parents

and

my Brother

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JAI DHINGU MATA

In the name of Lord Shiva, the most beneficient, the most merciful. All Praise and Glory belong to Him and Him alone.

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(*Renu Bala*)

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INTRODUCTION

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INTRODUCTION

Apple (*Malus domestica* Borkh.), the premier table fruit of the world, has been under cultivation since time immemorial. It is a temperate tree fruit grown extensively in temperate parts of the world as well as cooler high hills of sub tropical areas. 'An apple a day keeps the doctor away', this old adage focuses man's attention on the importance of apple in daily diet. The fresh apple fruit is a rich source of vitamins, minerals, proteins, fats, carbohydrates etc.

In India, apple is commercially cultivated in the states of Jammu & Kashmir, Himachal Pradesh, Sikkim, Arunachal Pradesh and hills of Uttar Pradesh. It is the number one commercial fruit crop of Himachal Pradesh both in terms of area and productivity. Presently, there are more than 1,50,000 apple growers in the state and more than 90,000 ha area is under apple cultivation (Anonymous, 1998a). Although the area under apple cultivation is increasing every year but the fruit production per unit area is decreasing despite the use of plant protection measures. The fruit production which made rapid strides in '80s ranging from 3.42 lakh tonnes in the year 1981-82 to 4.60 lakh tonnes in the year 1989-1990, maintained a lead in quality standard as well. However, in the '90s, not only the quality of fruit had deteriorated but the production too had declined down to 1.71 and 3.12 lakh tonnes in the year 1994-95 and 1995-96, respectively whereas, during the corresponding period, the area under fruit production had nearly doubled (Anonymous, 1999a). The reasons for low production are many but one of the major reasons is the losses caused by insect-pests and diseases.

Of the various diseases attacking apple, Marssonina leaf blotch commonly known as premature leaf fall is one of the most destructive diseases wherever apple is grown in Himachal Pradesh. This disease appeared in small portions in Kullu valley during 1994,has now assumed epiphytotic proportions in almost all apple growing areas of Himachal Pradesh. The annual loss to the tune of Rs. 300 crores has been estimated during the year 1998 due to this disease in Himachal Pradesh (Anonymous, 1998b). The disease has also been recorded from other apple growing states of India such as Jammu & Kashmir and Uttar Pradesh, besides a score of countries such as Japan, Canada, Korea, China, Romania and Brazil.

The disease results in early defoliation leading to reduction in photosynthetic area and fruit size, impairing fruit colour development, deteriorating fruit quality and excessive preharvest fruit dropping. Besides causing leaf blotch, the disease also develops spots on fruits of apple and thus lowers their marketability. In severely affected trees, the vegetative and floral buds sprout just after fruit harvest which leads to the development of weak spurs and thus adversely affects the fruit set in the following season. Due to repeated infection over the years, affected plants become weak and ultimately die (Anonymous, 1999b; Sharma and Verma, 1999).

Keeping in view the destructive nature of the pathogen and extent of loss caused to apple industry in the state, the present investigations were undertaken with the following objectives :

i) to find out the nutritional requirements of the apple leaf blotch pathogen and

ii) to device a suitable strategy for the management of Marssonina leaf blotch of apple.

REVIEW OF LITERATURE

2

REVIEW OF LITERATURE

Leaf blotch of apple caused by *Marssonina mali* (P. Henn.) S. Ito was reported by Miyake in 1907 as a new disease of apple in Japan. In 1971, Parmelee reported *Marssonina coronaria* (Ell. et J.J. Davis) J.J. Davis as the correct name of the causal fungus and *Marssonina mali* was regarded as its synonym.

2.1 DISTRIBUTION

Several species of *Marssonina* causing different diseases such as scorch, leaf blotch or leaf spot or premature leaf fall on different agricultural, horticultural, ornamental and forest crops have been reported time to time from various countries of the world. However, the occurrence of *Marssonina mali* on apple causing leaf blotch was first reported from Japan (Miyake, 1907). Subsequently, the occurrence of leaf blotch of apple was reported from Azores (Bensaude, 1926), Romania (Savulescu and Eliade, 1960), Canada (Parmelee, 1971) and Brazil (Leite Junior *et al.*, 1986).

Besides apple, *Marssonina secalis* was first reported on barley from Holland (Van Poeteren, 1925), *M. potentillae* on strawberry from New South Wales (Birmingham, 1925), *M. salicicola* on weeping willow from New Zealand (Murray, 1926), England (Nattrass, 1927), Argentina (Jauch, 1952) and Brazil (Figueiredo and Hennen, 1995) and *M. martini* on Oak from USA (Van Hook, 1925). In 1950, another species of *Marssonina* i.e. *M. quercina* (Wint.) n. comb. (Syn. *Gloeosporium septorioides* Sacc.) was also reported on oak from United States (Lentz, 1950). *Marssonina* species causing brown discoloration

of the veins of cotton leaves was reported from Belgian Congo (Staner, 1928). *M. juglandis* causing anthracnose and defoliation of walnut trees was reported from France (Gard, 1928), Czechoslovakia (Tomsa, 1929) and Yugoslavia (Sutic and Klijajic, 1954). Leaf spot of *Daphne mezereum* caused by *M. daphnes* in Great Britain was reported in 1934 by Green.

Other species of *Marssonina* reported on different host crops were *M. panattoniana* on lettuce from New Zealand (Taylor and LI, 1944), *M. melonis* on melons from New York (Dolan, 1947), *M. rosae* on rose from Europe, America and other countries (Baker, 1948), *M. celtidis* on *Celtis tournefortii* from Turkey (Bremer and Petrak, 1950), *M. agaves* on sisal from Venezuela (Ciferri, 1951) and *M. artocarpi* was reported on jak tree leaves from Brazil (Batista and Vital, 1954).

On Poplars, *Marssonina brunnea* was reported from Netherlands (Gremmen, 1965). Later on, its occurrence was also reported from Czechoslovakia (Urosevic, 1973), Bulgaria (Naiclerov, 1975), Yugoslavia (Gojkovic, 1971a,b), France (Pinon and Poisonnier, 1975), USA (Jokela *et al.*, 1976), Greece (Kailides, 1973) and Australia (Simpson, 1990). However, Cellerino (1979) reported thirteen species of *Marssonina* on poplars from Italy.

In India, occurrence of *Marssonina coronaria* causing leaf blotch in apple was first reported in 1994 from Kullu valley of Himachal Pradesh (Sharma and Bhardwaj, 1994). Since then the disease has been reported from all over wherever apple is being grown in the state. Studies carried out in Scab Monitoring Research Laboratory, Kotkhai (Himachal Pradesh) have conclusively proved that leaf blotch in apple is primarily due to fungus *Marssonina coronaria* (Sharma and Kaul, 1997). Its prevalence has also been reported from other apple growing states of India such as Uttar Pradesh, Jammu & Kashmir, Arunachal Pradesh and Sikkim. Besides this, high incidence of

Marssonina leaf blotch in apple was also reported from neighbouring countries like Bhutan and Nepal (Personal Communications).

2.2 ECONOMIC IMPORTANCE

Marssonina leaf blotch was reported to cause direct losses in economic gains. The pathogenic fungi, *Marssonina coronaria* caused severe defoliation in apple plants thereby affecting fruit size, colour, quality and quantity besides affecting the tree vigour and the next year's crop adversely (Leite Junior *et al.*, 1986; Sharma and Bhardwaj, 1994; Sharma and Kaul, 1997).

Sharma and Kaul (1997) reported that during the year 1996 the Marssonnina leaf blotch in apple (*Marssonnina coronaria*) was so severe that only fruits were left on the trees and there was no foliage as a result of which huge crop loss was witnessed in the state of Himachal Pradesh. Harada *et al.* (1974) reported that the fungus *M. coronaria* not only reduced the photosynthetic area, fruit size, fruit colour development, fruit quality but affected the productivity level adversely.

During the cropping season of 1998, the Govt. of Himachal Pradesh estimated a bumper yield of 25 million boxes of apple but due to severe outbreak of the disease, the estimate was slashed to less than 10 million boxes (Anonymous, 1998a).

2.3 SYMPTOMATOLOGY

The symptoms of the disease are mainly confined to leaves however, symptoms on fruits were uncommon. The disease in the form of blotch first appeared on the upper surface of the leaves. The blotch are were 5-10 mm in diameter, grayish brown and often tinged purple at the periphery. Later small, black acervuli visible with naked eye are were formed on upper leaf surface. When

lesions coalesced, the surrounding tissue turned chlorotic and defoliation occurred. Severe defoliation was observed in early summer that resulted in failure of the crop in the following season.

On the fruits, clear brown to black, circular depressed spots were formed. Later on black acervuli of the pathogenic fungus were formed in the lesions (Miyake, 1907; Parmelee, 1971; Leite Junior *et al.*, 1986; Sharma and Kaul, 1997).

2.4 ISOLATION

Various workers have devised different techniques for the isolation of *Marssonina* spp. from the affected plant parts. Dolan (1947) isolated *Marssonina melonis* causing anthracnose of melons, on potato dextrose agar medium from the diseased tissues. Gremmen (1962) isolated *M. brunnea* from the ascospores derived from poplar leaves on malt agar medium, while Simpson and Hayes (1978) isolated *M. brunnea* on minimal salts medium. The fungus, *M. salicicola* attacking weeping willow was isolated on potato dextrose agar (2%) and carrot agar (2%) media (Figueiredo and Hennen, 1995). However, the incitant of apple leaf blotch pathogen, *M. coronaria* was isolated by transferring the germinating ascospores with germ tube 40-60 um long, from water agar onto potato saccharose agar medium (Harada *et al.*, 1974) whereas, Sharma and Kaul (1997) isolated *M. coronaria* on potato dextrose agar and later on modified sucrose agar medium (Sharma, 1999).

2.5 PATHOGENICITY

To prove the pathogenicity of the *Marssonina* spp. different methods have been used by different workers. Some used the conidial or ascospore suspension while, others used brushing with mycelial fragments. Jauch (1954) proved the pathogenicity of *Marssonina salicicola* on weeping willow by

spraying the leaves with inoculant either in the form of spore suspension or mycelial fragments. However, Harada *et al.* (1974) proved the pathogenic nature of *M. coronaria* causing leaf blotch of apple, on young leaves of potted apple plants by spraying ascospore suspension. Sharma (1999) proved the pathogenic nature of *M. coronaria* by spraying conidial suspension on potted apple plants. The pathogenic nature of *M. panattoniana* and *M. salicicola* attacking lettuce and weeping willow respectively, was also proved by spraying conidial suspension (Moline and Pollack, 1976; Figueiredo and Hennen, 1995).

2.6 MORPHOLOGY

Bensaude (1927) studied the morphological characters of *Marssonina mali* and reported that the conidia of *M. mali* causing leaf blotch in apple measured 5x3.5 um in size. Harada *et al.* (1974) reported that the conidia of *M. coronaria* measured 20-24 x 6.5-8.5 um in size. Acervuli were subepidermal, discoid, lens shaped and 100-200 x 35-45 um in size. The conidia borne on small clavate conidiophores (3-8 um long) were hyaline, one septate and constricted at the septum.

Sharma (1999) reported that the fungus *Marssonina coronaria* causing leaf blotch in apple produced abundant, hyaline, bicelled conidia (17-26 x 6-9 um) on small clavate conidiophores in acervuli on affected leaves.

2.7 NUTRITIONAL STUDIES

2.7.1 Media selection

Several workers have reported very slow growth of different *Marssonina* spp. on cultural media. Simpson and Hayes (1978) reported minimal salts medium as the best medium for the growth of *Marssonina brunnea*, the causal agent of poplar leaf blight whereas, least grwoth was on Czapek Dox agar, intermediate on potato dextrose agar, tomato juice and malt agar. However,

Galea *et al.* (1986) reported best growth of *M. panattoniana* causing ring spot of lettuce, on malt extract agar, intermediate on potato dextrose agar and least on water agar. Sharma (1999) isolated *M. coronaria*, causal agent of leaf blotch of apple on modified sucrose agar medium.

2.7.2 Temperature studies

Gard (1928) reported 10°C as the optimum atmospheric temperature for the development of anthracnose of walnut caused by *Marssonina juglandis*. The fungus, *M. rosae* causing black spot of rose was able to grow between 6-33°C with a sharp decline below 18°C (Melching, 1962). Palmer and Semeniuk (1963) studied the effect of temperature on germination of conidia of *M. rosae* and reported 70 per cent and 57 per cent conidial germination in rain water at 75°F and 55°F respectively, after 48 hrs of incubation ~~period~~. Harada *et al.* (1974) reported 20°C as the optimum temperature for the growth of *M. coronaria* under laboratory conditions. *M. brunnea* causing leaf blight of poplar could grow on a range of temperature between 2 to 30°C, optimum being 22°C whereas, no growth was reported at 37°C (Simpson and Hayes, 1978). Optimum temperature range for the growth of *M. panattoniana* was between 15-20°C, while the germination of conidia varied between 10-26°C after 24 hrs of incubation period (Galea *et al.*, 1986).

2.7.3 Carbon sources

Fungi require different carbon sources for growth and sporulation and their nutritional requirements vary from species to species. Dolan (1947) reported maximum germination of conidia of *Marssonina melonis*, the causal agent of anthracnose of melons, in a medium containing both mineral nutrients as well as dextrose while, no germination was osberved in mineral salt solution alone. Dhanvantri (1967) registered best growth of *Marssonina* (*Diplocarpon earlianum*) causing leaf scorch of strawberry on glucose and sucrose. Similar

results on the utilization of glucose, sucrose and cellulose by *M. brunnea* were also reported by Simpson and Hayes (1978) while fructose, lactose, maltose and starch were least preferred by the fungus.

2.7.4 Nitrogen sources

Nitrogen sources are used both for functional as well as structural purposes by fungi. Some fungi prefer inorganic nitrogen sources while, others like organic sources. Dhanvantri (1967) reported aspartic acid, glutamic acid and γ-aminobutyric acid as the best nitrogen sources for the growth of *Marssonina* [*Diplocarpon earlianum*] causing leaf scorch of strawberry. Simpson and Hayes (1978) reported best growth of *M. brunnea* on potassium nitrate and 4-amino-n-butyric acid. Besides this, ammonium nitrate, ammonium sulphate, ammonium chloride, sodium nitrate, alanine, aspartic acid, glutamic acid, asparagine, glycine, leucine and serine were also utilized efficiently by the fungus.

2.7.5 Vitamins

Importance of vitamins in fungal nutrition has been well documented in literature. Vitamins even in very small quantity were reported to be of paramount importance to nourishment (Cochrane, 1958). Simpson and Hayes (1978) reported that growth of *Marssonina brunnea* causing poplar leaf blight was totally dependent upon exogenous supply of vitamins like pantothenic acid, pyridoxine and riboflavin.

2.7.6 Relative humidity

Relative humidity is known to influence the growth and germination of various fungi. Dolan (1947) reported that spores of *Marssonina melonis* causing anthracnose on melons, could germinate at lower humidity level i.e. 88.0 per cent, however, maximum germination was reported at 100 per cent RH whereas,

conidia of *M. panattoniana* causing ring spot of lettuce, were reported not to germinate at lower humidity levels and germination at 100 per cent RH was very low. (Galea *et al.*, 1986).

2.8 DEVELOPMENT OF THE PERFECT STAGE OF THE FUNGUS

Several workers have reported that species of *Marssonina* overwinter in the form of ascigerous state while others reported that fungus survives in the form of conidial state. The perfect state of *Marssonina rhabdospora* associated with a brown spotting of the poplar leaves was reported to be *Pleuroceras populi* in North America (Thompson, 1954). Sexual stage of *Marssonina* sp. attacking poplars was described as *Drepanopeziza populorum* at Bosbouwprofest. The ascospores were reported to be air borne and caused primary infection (Vonica, 1970). In Japan, *Diplocarpon mali* sp. nov. was reported to be the perfect state of apple leaf blotch fungus *M. coronaria* which developed on the outdoor overwintering leaves. The asci were reported to be fusiform and measured 144-217 x 16-19 um in size. Ascospores were constricted at the middle septum, hyaline, 3-septate and measured 48-70 x 3.5-4 um in size (Harada *et al.*, 1974).

2.9 EVALUATION OF GERMPLASM

In China, the apple cultivar 'Fu Shuai' was found highly resistant against *Marssonina mali* (Guo and Guo, 1984). The resistance in apple cultivars against *M. coronaria* was correlated with fruit cuticle thickness and N content (Xie and Leng, 1990). Sharma and Gautam (1997) while screening the apple cultivars for resistance against *M. coronaria* reported that none of the Delicious cultivars viz., Red Delicious, Royal Delicious, Rich-a-Red, Golden Delicious and Red Gold growing commercially in Himachal Pradesh was found resistant except Tydeman's Early Worcester which showed some tolerance reaction. In this cultivar the disease appeared 2 to 3 weeks later as compared to the

Delicious cvs. However, Stray plants of cv. Granny Smith were found to remain free from this disease throughout the season (Sharma and Verma, 1999).

2.10 DISEASE MANAGEMENT

For the management of Marssonina leaf diseases in the field both cultural as well as chemical means of disease control have been suggested by various research workers.

2.10.1 Cultural practices

Burning of affected plant parts and the avoidance of blotched plants for propagation were recommended for the management of leaf scorch of strawberry (Birmingham, 1925) and walnut (Tomsa, 1929). However, against ring spot of lettuce (*Marssonina panattoniana*), Taylor and LI (1944) reported the use of raised beds and removal of infected plants. For combating Marssonina leaf blotch of apple, (*M. coronaria*) orchard sanitation, pruning and the removal of overwintered leaves from the ground were recommended (Takahashi and Sawamura, 1990).

2.10.2 Chemical means

To combat the disease caused by various *Marssonina* spp. in different host crops, the use of fungicides have been suggested by various workers. Bordeaux mixture was recommended against *Marssonina* spp. causing diseases in strawberry (Birmingham, 1925), Walnut (Tomsa, 1929), rose (Parsons and Massey, 1933), lettuce (Taylor and LI, 1944), apple (Kwang and Chong, 1962) and poplars (Sokolova, 1975).

Downes (1932) reported effective control of black spot of rose (*Marssonina rosae*) with ammonium polysulphide and nicotine soap, while Parsons and Massey (1933) obtained best disease control with sulphur dust.

Fungicides like Colsul, Dithane Z-78, Flit 406, Captan, Ziram and Thirospray were reported highly effective against black spot of rose (Jacks *et al.*, 1955). Other promising fungicides against black spot of rose were maneb, zineb, glyodine and karathane (Massey, 1955; McClellan *et al.*, 1957; Rosen, 1959; Melching, 1962). Recently Baycor (bitertanol) was found highly effective against black spot of rose (Kolbe, 1981). For the control of lettuce ring spot (*M. panattoniana*), Cunnigham (1951) reported the use of copper compounds. Captan and thiram were also reported effective against ring spot of lettuce (Hurndell and Smith, 1958). Against strawberry leaf scorch caused by *Marssonina* [*Diplocarpon earlianum*], Teich and Ernstein (1961) obtained best control through the regular sprays of copper and zineb.

Metzner (1977) reported good control of black spot of walnut with copper fungicides, while Kleiner and Bulatova (1978) recommended the use of DNOC, carbendazim, benomyl, polyhom and zineb for its management.

Disease management trials carried out by Cellerino (1966) reported good control of poplar leaf blight (*Marssonina brunnea*) with Dithane M-45 and copper oxychloride. Other fungicides which limited the spread of poplar leaf blight were maneb, difoltan, and melprex (Castellani and Cellerino, 1967). Control of poplar leaf spot (*M. populi*) also worked out by Carlson (1972) who reported excellent disease control by spray application of benomyl or thiophenate methyl. However, Boudier (1983) recommended 4 to 5 application of triadimefon or propiconazole during vegetative period for the control of *M. populi*. In laboratory trials, dichlone, NIA9102, zineb and captan were found highly efficacious against *Marssonina* spp. causing leaf spot of poplars. Benomyl, Zn Fe-Maneb complex and triforine controlled infection by *Marssonina* sp. for 14 days and captafol, MBC and dodine for 21 days after spray application (Spiers, 1978).

For the management of leaf blotch of apple (*Marssonina coronaria*), many fungicides namely, Bordeaux mixture + $ZnSO_4$, ferbam, zineb, folpet, captan, tuzet (Kwang and Chong, 1962), mancozeb, propineb and carbendazim (Sharma and Kaul, 1997), Duokanqmeisu, Puhaiin (Jiang *et al.*, 1997) have been reported effective from India and abroad. Recently, Chang *et al.* (1998) reported best control of leaf blotch of apple with Shonqshenqjunsu + Boudeaux mixture, while Sharma (1999) reported best control of *Marssonina* leaf blotch of apple with carbendazim, mancozeb and dodine.

MATERIALS & METHODS

3

MATERIALS AND METHODS

The present investigations on apple leaf blotch pathogen *Marssonina coronaria* were carried out in the Fruit Pathology Laboratory of Department of Mycology and Plant Pathology, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan during the year 1997-99.

3.1 TECHNIQUE FOR HARVESTING CONIDIA OF *M. coronaria*

The conidia were harvested from the mature fruiting bodies (acervuli) of the fungus *Marssonina coronaria*, the incitant of leaf blotch of apple, formed on the upper surface of the infected leaves during the month of July through November. The infected leaves forming fruiting bodies were first washed under running tap water to remove the traces of dirt particles, thereafter, surface sterilized with 70 per cent ethanol and washed thoroughly with sterilized distilled water. These leaves were then put in the sterilized polypropylene bags and kept in BOD incubator at 25°C. The leaves were examined under stereobinocular microscope (Olympus make) after 24 hrs for the presence of conidial masses coming out from the fruiting bodies (acervuli) in the form of a ooze. The leaves showing conidial ooze of *M. coronaria* were cut into small pieces and were put in sterilized flasks (150 ml) containing 100 ml of sterilized distilled water. These flasks were then kept in the rotatory shaker (Orbitek make) and centrifuged at 150 r.p.m. for 30 minutes. The superanatant was taken out and the original volume was restored by adding sterilized distilled water. The conidia were given three such washings to remove any leaf fragment before being used for further studies. After final washing, the

concentration of conidia was adjusted to 100,000 per ml which was equivalent to 50 conidia per low power microscopic field (100 x).

All glasswares used were of corning make. The glasswares were first cleaned with cleaning solution, washed in running tap water followed by rinsing with distilled water before use. Sterilization of media and different solutions was done in an autoclave at 15 lbs pressure for 20 minutes and the glassware used were sterilized in hot air oven at 160-180°C for two hours.

3.2 ISOLATION

Isolation of leaf blotch fungus into pure culture was conducted under *in vitro* conditions by using different standard techniques. The diseased leaves were collected in paper bags from affected apple orchards located in the main campus, Nauni during July through October. The leaves were washed under running tap water and after soaking in the folds of blotting papers were cut into small bits (2-4 mm) from the junction of diseased and healthy portion with the help of a sterilized blade. These bits were then dipped in 0.1 per cent mercuric chloride ($HgCl_2$) solution for 20-30 seconds and then repeatedly washed with sterilized distilled water to remove the traces of mercuric chloride and placed on sterilized filter paper to remove the excessive moisture. These were then transferred to petriplates/flasks containing sterilized medium under aseptic conditions and incubated at 20±1°C. Both solid and liquid media were used for this purpose. The medium used were potato dextrose agar medium, sucrose asparagine agar medium, glucose asparagine agar medium, rose leaf extract and sucrose asparagine medium and glucose asparagine medium. The petriplates/flasks were observed daily for the fungal growth.

3.2.1 Isolation from germinating conidia

Isolation of the fungal pathogen, *Marssonina coronaria* was also undertaken from the germinating conidia under *in vitro* conditions. The conidia harvested from the fruiting structures (acervuli) of the blotch pathogen were

suspended in sterilized distilled water. They were allowed to germinate at 20°C in BOD incubator by slide germination technique (Anonymous, 1943), which were then poured into petriplates containing a thin layer of water agar. The petriplates, after the removal of excess water from agar surface, were incubated at 20±1°C. After four days of incubation, the germinating conidia were transferred onto sucrose asparagine agar medium slants, which were incubated at 20±1°C. The slants were observed daily for fungal growth.

3.3 PATHOGENICITY

Pathogenicity test was conducted on one year old healthy potted apple plants under *in vitro* conditions. The leaves of potted plants were surface sterilized with 70 per cent ethanol and washed thoroughly with sterilized distilled water. These were sprayed with conidial suspension (3.1) harvested from fruiting bodies of the leaf blotch pathogen *Marssonina coronaria* with the help of hand automizer. The potted apple plants after inoculation with conidial suspension of the pathogen were immediately covered with the polythene sheets for 48 and 72 hrs for the appearance of the disease symptoms. The uninoculated seedlings sprayed with distilled water served as control. Observations on the development of symptoms and incubation period were recorded.

3.4 NUTRITIONAL STUDIES

In order to find out the nutritional requirements of conidia of *Marssonina coronaria*, studies on conidial germination and germ tube formation were carried out under *in vitro* conditions by slide germination technique (Anonymous, 1943).

3.4.1 Liquid media

To ascertain the comparative suitability of different liquid media on the germination of conidia of *Marssonina coronaria*, eight different liquid media

(natural and synthetic) viz., apple leaf extract, walnut leaf extract, rose leaf extract, glucose asparagine medium, sucrose asparagine medium, Richards' medium, Coon's medium and Czapek's Dox medium were tested. In all cases, double strength media were prepared for conducting expreiments. The composition of these media are given in Appendix-I.

One ml of the conidial suspension containing 50 conidia per microscopic field was mixed with one ml of the medium, of which one drop was kept on the cavity slides. These cavity slides were then placed in the petriplates (9 cm) on the glass rods. To provide the humidity, sterilized filter papers wetted with distilled water were placed in the petriplates and were sprayed with water periodically to keep them moist. These petriplates containing the cavity slides were sealed with plastic films and then incubated at 20±1°C in BOD incubator. Conidial suspension without medium was kept as control for comparison. Three replications were kept for each treatment.

The observations on conidial germination and germ tube length (µm) were recorded after 24, 48, 72 and 96 hrs of incubation period. The percentage of conidial germination was calculated by the following formula.

$$\text{Per cent germination} = \frac{\text{No. of conidia germinated}}{\text{No. of conidia observed}} \times 100$$

The length of conidial germ tube (µm) was measured with the help of occular micrometer and this value was multiplied with calibration factor of the microscope to get the actual germ tube length of the conidia.

3.4.2 Temperature studies

In order to ascertain the optimum temperature required for the germination and germ tube formation of conidia of *Marssonina coronaria*,

sucrose asparagine medium was used as the basal medium. The basal medium was prepared in double strength. One ml of the conidial suspension (50 conidia per microscopic field) was mixed with one ml of the medium of which one drop was kept on the cavity slides. These cavity slides were then placed on the glass rods in the Petri plates (9 cm) lined with the wet blotting paper to provide the relative humidity greater than 95 per cent. The petriplates were sealed with the plastic films and incubated at different temperatures, viz., 10, 15, 20, 25, 30 and 35°C adjusted in the BOD incubators. Each treatment was replicated three times. The conidia were assessed for the germination and formation of germ tube after 24 h interval for four days. The observations on conidial germination and the germ tube length (µm) were recorded in the same manner as explained earlier in the text (3.4.1).

3.4.3 Carbon sources

To find out the best carbon source, the effect of different carbon sources was tested on germination and formation of germ tube of conidia of *Marssonina coronaria*. Six different carbon sources namely sucrose, glucose, mannitol, maltose, galactose and lactose were tested. The solutions of these chemicals were prepared in double strength and the final concentration used in each case was one per cent. To test the comparative utilization, one ml of the conidial suspension (50 conidia per microscopic field) was mixed with one ml of the sugar solution of which one drop was kept on the cavity slides. Each treatment was replicated thrice. The cavity slides were placed on the glass rods in petriplates (9 cm) containing wet blotting paper to maintain more than 90 per cent humidity. The petriplates were sealed with plastic films and incubated at 20±1°C and the conidia were assessed for germination after 24, 48, 72 and 96 hrs. The observations on the percentage of conidial germination and germ tube length (µm) were recorded in the same manner as explained earlier (3.4.1).

Of all the various carbon sources tested, the best evaluated carbon source was further tested at different concentrations to find out the optimum concentration required for the enhanced conidial germination and germ tube formation of *Marssonina coronaria*. The procedure and the method of recording data remained the same as explained above.

3.4.4 Nitrogen sources

Studies were carried out to find out the best nitrogen source for the germination and germ tube formation of conidia of *Marssonina coronaria*. Six different nitrogen sources namely, ammonium sulphate, ammonium nitrate, potassium nitrate, sodium nitrate, asparagine and urea were tested. The solutions of these chemicals were prepared in double strength and final concentration used in each case was one per cent except urea (0.5%). Each treatment was replicated three times. The remaining procedure and the method of recording data were the same as explained earlier (3.4.1).

The nitrogen source which was found most effective for conidial germination and germ tube formation of *Marssonina coronaria* was further tested at different concentrations (0.25%, 0.5%, 1.0%, 1.5%, 2.0% and 2.5%) to work out the optimum dose required for the enhanced germination and germ tube formation of conidia of *M. coronaria*. The procedure used in this case was the same as mentioned earlier and the observations on percentage conidial germination and germ tube length were recorded as explained earlier in the text (3.4.1).

3.4.5 C:N ratio

Various fungi have been known to utilize increasing or decreasing concentrations of carbon and nitrogen sources. To study the effect of different glucose and asparagine ratios as carbon and nitrogen sources, respectively, the

amount of carbon and nitrogen present in the basal medium was varied to get the different C:N ratios. Glucose in the carbon free basal medium was incorporated to give 28, 24, 20, 16 and 12 gm carbon per litre and the nitrogen source was kept constant (Appendix-II). Similarly, in case of nitrogen, asparagine was incorporated in the basal medium devoid of nitrogen so as to give 0.9325, 0.746, 0.5595, 0.3730 and 0.1865 g nitrogen per litre and carbon quantity was kept constant (Appendix-II).

One ml of the conidial suspension (50 conidia per microscopic field) was mixed with one ml of the above combinations. One drop from this mixture was placed on the cavity slides. The remaining procedure and the method of recording data were the same as mentioned earlier in the text (3.4.1).

3.4.6 Vitamins

To find out the best vitamin for the germination and germ tube formation of conidia of *Marssonina coronaria*, different vitamins viz., thiamine hydrochloride, meso-inositol, nicotinic acid, ascorbic acid, folic acid, biotin were tested. The solutions of these vitamins were prepared in double strength and the final concentration used in each case was one per cent. Three replications were kept for each treatment. The rest of the procedure and the method of recording data were the same as explained earlier (3.4.1).

3.4.7 Micro nutrients

The role of micro nutrients in the germination of conidia and formation of germ tube of fungal pathogen has been well documented in the literature. In the present study, the effect of different micro nutrients was tested on germination of conidia of *Marssonina coronaria* under *in vitro* conditions. Six different-micro nutrients used were zinc sulphate (0.25%), boric acid (0.5%), manganese sulphate (0.2%), calcium chloride (0.25%), ferrous sulphate

(0.001%) and ammonium molybdate (0.025%). The solutions of these trace elements were prepared in double strength and each treatment was replicated thrice. Rest of the procedure and method of recording data were remained the same as explained earlier in the text (3.4.1).

3.4.8 Relative humidity

The sucrose asparagine medium was prepared in double strength. One ml of this medium was mixed with one ml of the conidial suspension (50 conidia per microscopic field). One drop of this mixture was put on the cavity slides. Eight different levels of humidity viz., 100.0, 98.5, 96.1, 92.9, 88.5, 82.9, 75.6 and 56.8 per cent (Appendix-II) were maintained in dessicators as per the method of McLean and Cook (1941). Each treatment was replicated thrice. The remaining procedure and the method of recording data were the same as explained in the earlier experiments (3.4.1).

3.5 SURVIVAL OF THE FUNGUS

To study the survival of the apple leaf blotch pathogen, *Marssonina coronaria*, the diseased leaves of highly susceptive cv. 'Golden Delicious' collected from apple orchard of University of Horticulture and Forestry, Solan, durring the month of September were kept in the nylon bags. The nylon bags containing diseased leaves were then kept on orchard floor, laboratory and refrigerator in the month of October. Fifty leaves replicated thrice were kept in each treatment. The leaves were observed for the viability of the test pathogen *M. coronaria*. The viability of the fungus was tested after 3, 6, 9 and 12 months by germinating the conidia of the test fungus using the slide germination technique (Anonymous, 1943) explained earlier in the text. The formation of ascigerous state of the test fungus, if any, was also observed.

3.6 EVALUATION OF GERMPLASM

One hundered and one cultivars / advanced scab resistant lines of apple maintained at the Experimental Research Farm of Department of Fruit Breeding and Genetic Resources, UHF, Nauni, Solan were screened for resistance againt leaf blotch fungus *Marssonina coronaria* under natural epiphytotic conditions during the cropping season 1997, 1998 and 1999. The severity was recorded on hundred leaves selected at random from each cultivar / line in October by using the scale adopted by James (1974) the details of which is as under :

Per cent disease on leaves	Description of symptoms
0.0	Leaves completely healthy with no blotch symptom
0.1-25.0	Leaves show slight infection, disease mainly on the lower portion of the plant.
25.1-50.0	Upto 50% portion of the leaves infected.
50.1-75.0	About 75 per cent portion of the leaves infected and the leaves appear to be blotched.
75.1-100.0	Almost the whole of the leaves infected.

The per cent disease index (PDI) was calculated according to McKinney (1923).

$$\text{PDI (\%)} = \frac{\text{Sum of all disease ratings}}{\text{Total number of ratings} \times \text{Maximum disease grade}} \times 100$$

The disease reactions for different cultivars / lines were recorded by using the following scales :

Category	Disease severity (%)	Reaction
0	0-5	Resistant
1	5.1-15.0	Moderately resistant
2	15.1-25.0	Moderately susceptible
3	25.1-50.0	Susceptible
4	More than 50.0	Highly susceptible

3.7 DISEASE MANAGEMENT

3.7.1 *In vitro* evaluation of fungicides

To find out the most effective fungicide against *Marssonina coronaria*, different fungicides were evaluated *in vitro* by the slide germination technique (Anonymous, 1943).

The common as well as the trade name of the fungicides evaluated during the course of present investigation are as follows:

Trade	Common name	Formulation name
Rubigan	Fenarimol	5 EC
Contaf	Hexaconazole	5 EC
Kavach	Chlorothalomil	75 WP
Dithane	Mancozeb	75 WPM-45
Baycor	Bitertanol	25 WP
Bavistin	Carbendazim	50 WP
Captaf	Captan	50 WP
Bavistin +DM45	Carbendazim + mancozeb	50 WP + 75 WP

The double strength solutions of different concentrations of these fungicides were prepared individually and one ml of the solution was then mixed with one ml of the conidial suspension (50 conidia per microscopic field) so as to get the required concentration of the fungicide of which one drop was put in the cavity slides. The cavity slides were then placed on the glass rods in the petriplates (9 cm) containing the wet blotting paper to provide adequate moisture. These petriplates were sealed with plastic films and then incubated at 20±1°C. Three different concentrations (C_1, C_2 and C_3) were taken for each fungicide viz., Bavistin (100, 250, 500ppm), Dithane M-45 (1000, 2000, 3000ppm), Bavistin + Dithane M-45 (50+1000, 100+2000, 250+2500ppm), Captan (1000, 2000, 3000ppm), Kavach (500, 1000, 2000ppm), Baycor (500, 750, 1000ppm), Rubigan (100, 250, 500ppm) and Contaf (100, 250, 500ppm) and each treatment was replicated thrice. The slides were observed after every 24 hrs for four days for the conidial germination. The observations were recorded with the help of research microscope and per cent inhibition was calculated by the formula given by Vincent (1947).

$$I = \frac{C - T}{C} \times 100$$

where,

I = per cent inhibition

C = germination of conidia in control (per cent)

T = germination of conidia in treatment (per cent)

3.7.2 Field evaluation of fungicides

Fungicides found effective under *in vitro* conditions were further tested for their comparative effectiveness against *Marssonina coronaria* causing leaf

blotch of apple under the field conditions. The evaluation of fungicides was undertaken at the Experimental Research Farm of Department of Fruit Breeding and Genetic Resources, UHF, Nauni, Solan during the cropping season of 1998 under natural epiphytotic conditions. The field experiment was laid out in RBD with nine treatments including control (unsprayed) and each treatment was replicated thrice. The fungicides used were Bavistin (0.05%), Dithane M-45 (0.3%), Bavistin + Dithane M-45 (0.025% + 0.25%), Baycor (0.1%), Rubigan (0.05%), Contaf (0.05%), Kavach (0.2%) and Captan (0.3%). The first spray of different fungicides was given in the last week of May on a highly susceptible cultivar 'Golden Delicious' and the remaining three sprays were given at 15 days interval. The observations on leaf blotch incidence and disease severity were recorded 21 days after the last spray on hundred leaves collected at random from all the four sides of the tree. The leaf blotch incidence and per cent disease control were calculated with the help of following formulae.

$$\text{Leaf blotch incidence (\%)} = \frac{\text{No. of diseased leaves}}{\text{Total number of leaves}} \times 100$$

$$\text{Per cent disease control} = \frac{\text{Per cent leaf blotch incidence in control} - \text{Per cent leaf blotch incidence in treatment}}{\text{Per cent leaf blotch incidence in control}} \times 100$$

Observations on disease severity (3.6) were recorded by using the scale given by McKinney (1923).

The per cent disease control was also worked out by using following formula :

$$\text{Per cent disease control} = \frac{\text{Per cent disease severity in control} - \text{Per cent disease severity in treatment}}{\text{Per cent disease severity in control}} \times 100$$

The observations on number of leaves fell prematurely were also recorded on hundred leaves selected at random from all the four sides of the tree in October and the per cent leaf fall was calculated by the following formula:

$$\text{Per cent leaf fall} = \frac{\text{Number of leaves fell prematurely}}{\text{Total number of leaves}} \times 100$$

3.7.3 Field evaluation for the number of sprays of fungicides

In another experiment 2, 3 and 5 sprays of most effective fungicides i.e Bavistin (0.05%), Dithane M-45 (0.3%), Bavistin (0.025%) + DM-45 (0.25%) were evaluated under field conditions in Randomized Block Design with three replications during the cropping season of 1999. The rest of the procedure and method for recording data were the same as explained in the previous experiment (3.7.2).

3.8 STATISTICAL ANALYSIS

The data recorded from laboratory as well as field experiments were subjected to statistical analysis wherever required. The difference exhibited by the treatments in various experiments were tested for their significance, using standard procedures, as described by Gomez and Gomez (1983).

EXPERIMENTAL RESULTS

4

EXPERIMENTAL RESULTS

4.1 ISOLATION

Efforts were made to isolate the test pathogen, *Marssonina coronaria* but the fungus could not be isolated in pure culture despite the use of all possible methods of isolation.

4.2 SYMPTOMATOLOGY

Disease symptoms produced by *Marssonina coronaria* appeared both on leaves and fruits. The disease made its first appearance in late June and increased in intensity until it reached a peak in the latter part of August. The first symptoms to appear on older leaves were yellow and brown to dark brown necrotic spots of irregular shape which appeared most prominently on the upper surface of the leaves. The centre of the spots became grey brown and then greyish. Spots measured 5 to 10 mm in size and often tinged purple at the periphery (Plate 1). Numerous dot like black fruiting bodies (acervuli) were formed in the affected areas on the leaves (Plate 2). Later the spots coalesced and the surrounding tissue turned chlorotic. The infected areas presented a blotchy appearance whence the disease received its name. During late summer and the rainy season, severe premature leaf fall was observed on the diseased trees and in affected orchards apple trees were seen laden with fruits but having few or no leaves.

Infection on fruit was uncommon and restricted to trees with numerous leaf infections. On the infected fruit surface round, depressed, dark brown to

Symptoms of Marssonina leaf blotch of apple on leaves

Plate 1.

Leaf showing fruiting bodies (acervuli) of *Marssonina coronaria*)

Plate 2.

Plate 3. Symptoms of Marssonina leaf blotch of apple on fruits

Plate 4. Pathogenicity test on young potted apple plants

Plate 5. Initial stage of disease development

Plate 6. Advance stage of disease development

black spots appeared. Later on small, black acervuli were formed on the spots (Plate 3).

4.3 PATHOGENICITY

Pathogenicity test was conducted on healthy potted apple plants by inoculating the conidial suspension of the test fungus, *Marssonina coronaria* causing leaf blotch in apple under controlled conditions.

The results revealed that typical symptoms of the disease were produced by the pathogen within 10 to 12 days after inoculation, however, complete premature defoliation of the leaves was observed within 12 to 15 days after the appearance of first visible symptom. The first symptoms of the disease were formed on the older leaves of the plant, whereas the younger ones were the last to be infected by the pathogen. However, the development and spread of the disease were from bottom to top of the plants. The pathogen produced the fruiting bodies i.e. acervuli on upper leaf surface within 3 to 4 days after the symptom development (Plate 4 and 5).

The incubation period of the fungus was 12 days in potted apple plants which remained covered with the polythene sheets for 48 hours after inoculation. The first leaf in this case fell prematurely after three days of symptom development however, complete defoliation was observed 15 days after symptom development, while the incubation period was 10 days in potted apple plants which remain covered for 72 hours with polythene sheets after inoculation and premature defoliation began after two days of symptom development. Whereas, complete defoliation was registered after 12 days of symptom development (Plate 6).

4.4 MORPHOLOGICAL CHARACTERS OF THE LEAF BLOTCH PATHOGEN

Morphological characters such as shape and size of conidia and acervuli of the leaf blotch pathogen were studied in detail.

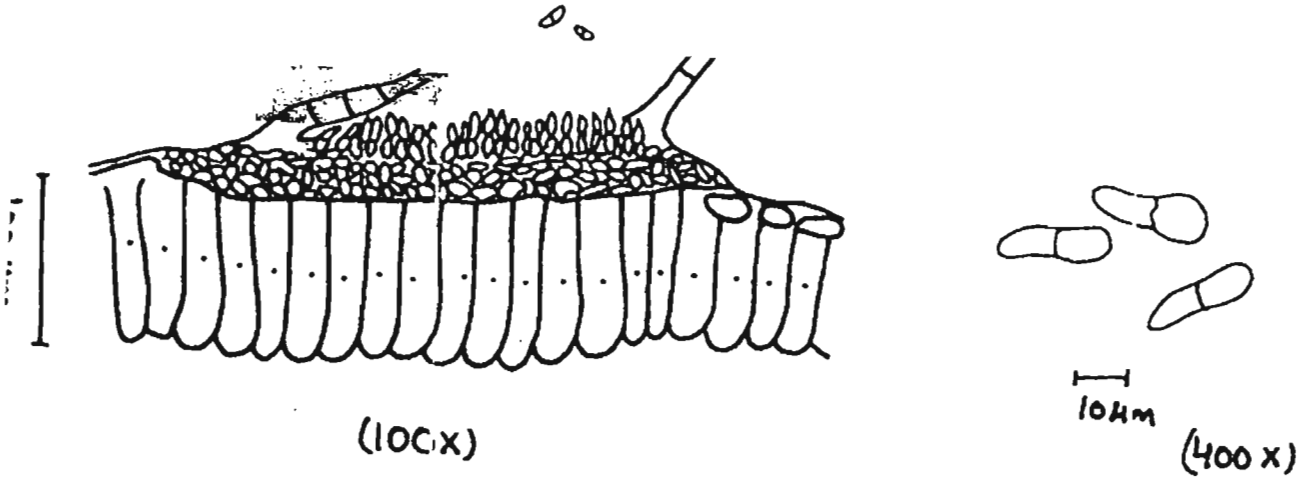

Plate 7. Camera lucida drawings of *Marssonina coronaria* showing acervulus and conidia formed on leaves

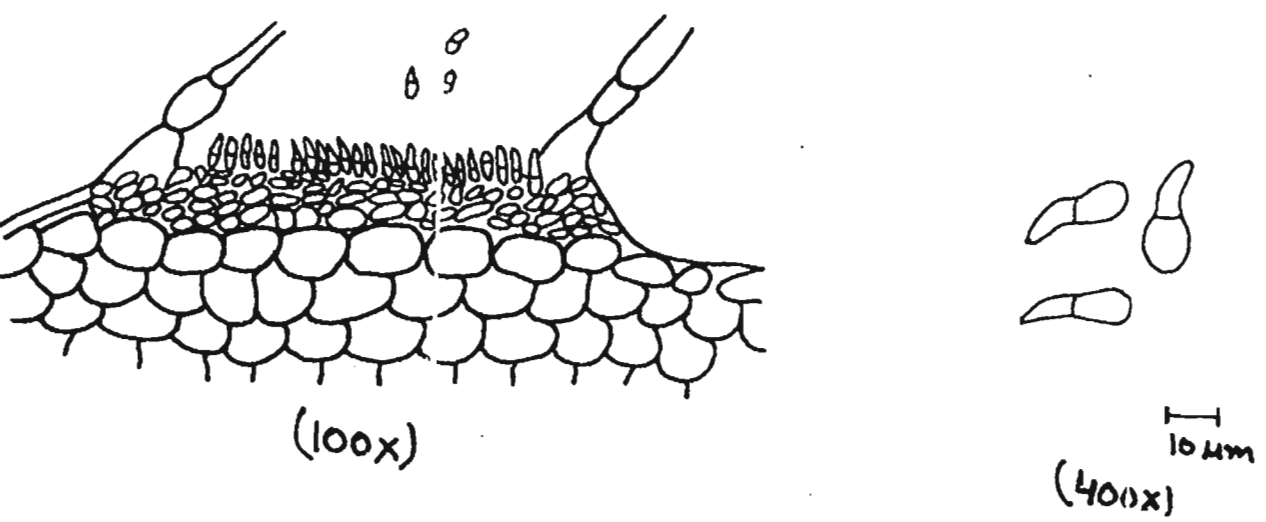

Plate 8. Camera lucida drawings of *Marssonina coronaria* showing acervulus and conidia formed on fruits

The fruiting bodies (acervuli) of the test pathogen were observed on the upper surface of the infected leaves as well as on the fruits. Acervuli were subcuticular, mostly in concentric rings, rarely scattered, lens shaped, pitch dark. The acervuli formed on leaves and on fruits measured 210.52-315.79 x 36.84-52.63 µm and 200-300 x 34.12-47.637 µm in size, respectively (Plate 7). Conidia borne on small clavate conidiophores were bi-celled, hyaline constricted at the septum, guttulate and measured 17.35-27.76 x 6.94 x 13.88 µm in size on leaves and 15.84-26.6 x 6.94-10.41 µm on fruits. Based on the characters the pathogenic fungus infected leaves and fruits of apple was identified as *Marssonina coronaria* (Ell. et. J.J. Davis) J.J. Davis (Plate 8).

4.5 NUTRITIONAL STUDIES

4.5.1 SELECTION OF LIQUID MEDIA

In order to ascertain the effect of different liquid media on the conidial germination and germ tube formation of the test fungus, *Marssonina coronaria*, nine different liquid media were tested and the data recorded in this regard are presented in Table 4.1.

It is evident from the data (Table 4.1) that the conidia of *Marssonina coronaria* germinated in all the liquid media tested, however, maximum conidial germination was observed in rose leaf extract (76.46%) followed by apple leaf extract (62.50%). The least conidial germination was observed in Czapek's Dox medium (16.41%) followed by Coon's medium (20.05%). Significant results were obtained when conidial germination was recorded at different incubation periods. The percentage of the conidial germination was found highest (63.60%) after 96 hrs and minimum (23.85%) after 24 hrs of incubation period. The results of the interaction between different incubation periods and liquid media were also found to be significant. All the liquid media tested registered a progressive increase in the conidial germination with the

Table 4.1. Effect of different liquid media on germination and germ tube formation of conidia of *M. coronaria*

Medium	Germination (%) after					Germ tube length (μm) after				
	24 h	48 h	72 h	96 h	Mean	24 h	48 h	72 h	96 h	Mean
Sucrose asparagine	22.92 (28.44)*	44.79 (42.00)	70.83 (57.36)	86.46 (68.60)	56.25 (49.10)^{c}	48.77	69.82	90.88	110.80	80.08^{c}
Glucose asparagine	16.67 (23.96)	26.04 (30.63)	48.96 (44.40)	62.50 (52.25)	38.54 (37.81)^{e}	31.03	50.99	76.49	89.77	62.07^{e}
Richards'	9.38 (17.67)	20.83 (27.06)	30.21 (33.30)	41.67 (40.17)	25.52 (29.55)^{f}	22.17	42.12	59.85	79.80	50.98^{f}
Coon's	7.29 (15.12)	16.67 (24.08)	22.92 (28.52)	33.33 (33.21)	20.05 (25.73)^{g}	13.30	32.14	49.87	66.50	40.45^{h}
Czapek Dox	4.17 (11.61)	15.62 (23.22)	19.79 (26.31)	26.04 (30.63)	16.41 (22.94)^{h}	8.867	22.17	38.79	59.85	32.42^{i}
Rose leaf extract	52.08 (46.20)	70.83 (57.32)	85.42 (67.74)	97.50 (81.07)	76.46 (63.08)^{a}	73.15	99.75	121.40	146.30	110.10^{a}
Apple leaf extract	41.67 (40.17)	54.17 (47.40)	69.79 (56.71)	84.37 (66.79)	62.50 (52.77)^{b}	55.42	75.37	98.64	119.70	87.28^{b}
Walnut leaf extract	37.50 (37.75)	50.00 (45.00)	64.58 (53.51)	80.21 (63.69)	58.07 (49.99)^{c}	42.12	59.85	80.91	103.10	71.49^{d}
Poplar leaf extract	29.17 (32.59)	42.67 (40.77)	60.42 (51.03)	75.00 (60.12)	51.81 (46.13)^{d}	28.82	53.20	74.26	95.32	62.90^{e}
Control (water)	17.71 (24.75)	35.42 (36.49)	42.71 (40.79)	48.96 (44.40)	36.20 (36.61)^{e}	24.38	39.90	48.75	63.17	44.05^{g}
Mean	23.85 (27.83)^{d}	37.70 (37.40)^{b}	51.56 (45.97)^{b}	63.60 (54.29)^{a}		34.80^{d}	54.53^{c}	74.98^{b}	93.43^{a}	

	$CD_{0.05}$	$CD_{0.05}$
Liquid media	2.57	2.54
Incubation period	1.63	1.61
Liquid media x Incubation period	5.15	5.07

* Figures in parentheses are arcsin transformed values
Means followed by the same letter do not differ significantly

increase in incubation period. The conidial germination in rose leaf extract increased from 52.08 per cent to 97.50 per cent as the incubation period was increased from 24 hrs to 96 hrs.

The results of the data with regard to the germ tube formation of the conidia of *M. coronaria* revealed almost similar trend. Maximum germ tube length (110.1 µm) was again recorded in rose leaf extract. The next best liquid media in order of their importance were apple leaf extract (87.28 µm), sucrose asparagine medium (80.08 µm), walnut leaf extract (71.49 µm), however, minimum germ tube length was recorded in Czapek's Dox medium (32.42 µm). It is also clear from the data that an increase in the incubation period from 24 hrs to 96 hrs has also resulted in the increase in the germ tube length from 34.80 µm to 93.43 µm. The interactions between different liquid media and incubation periods were also found significant.

4.5.2 EFFECT OF TEMPERATURE

With a view to determine the optimum, maximum and minimum temperature required for the germination and germ tube formation of conidia of *Marssonina coronaria*, six different temperatures ranging from 10-35°C were studied under *in vitro* conditions. Observations on conidial germination and germ tube formation were recorded and the data after statistical analysis are presented in Table 4.2.

It is evident from the data presented in (Table 4.2) that the conidia of *Marssonina coronaria* could germinate over a wide range of temperature (10-35°C), however, extreme low (<15°C) or high temperatures (>30°C) were found unfavourable for the germination of conidia. Maximum conidial germination was observed at 20°C (58.33%) and minimum at 35°C (13.28%). Significant results were also obtained when the conidial germination was tested at different incubation periods. The percentage of conidial germination was found highest

Table 4.2. Effect of different temperatures on germination and germ tube formation of conidia of *M. coronaria*

Temperature (^{0}C)	Germination (%) after					Germ tube length (µm) after				
	24 h	48 h	72 h	96 h	Mean	24 h	48 h	72 h	96 h	Mean
10	7.29 (15.12)*	14.58 (22.26)	25.00 (29.97)	35.42 (36.49)	20.57 (25.96)^e	17.73	29.92	38.79	47.66	33.53^d
15	16.67 (24.08)	30.21 (33.30)	43.75 (41.41)	55.21 (48.00)	36.46 (36.69)^c	42.12	54.29	66.50	74.26	59.29^b
20	23.96 (29.09)	48.96 (44.40)	72.92 (58.70)	87.50 (69.40)	58.33 (50.40)^a	50.98	65.39	86.45	106.40	77.31^a
25	21.87 (27.73)	41.67 (40.19)	68.75 (56.03)	82.29 (65.25)	53.65 (47.30)^b	47.66	62.07	82.02	99.75	72.87^a
30	13.54 (21.40)	23.96 (29.09)	32.29 (34.59)	38.54 (38.36)	27.08 (30.86)^d	36.57	47.66	56.52	66.50	51.81^c
35	4.17 (11.61)	11.46 (19.49)	17.71 (24.75)	19.79 (26.31)	13.28 (20.54)^f	13.30	24.38	32.14	43.22	28.26^e
Mean	14.58 (21.51)^d	28.47 (31.45)^c	43.40 (40.91)^b	53.13 (47.30)^a		34.73^d	47.29^c	60.40^b	72.97^a	

	$CD_{0.05}$	$CD_{0.05}$
Temperature	2.85	5.16
Incubation period	2.33	4.21
Temperature x Incubation period	5.70	10.31

* Figures in parentheses are arcsin transformed values
Means followed by the same letter do not differ significantly

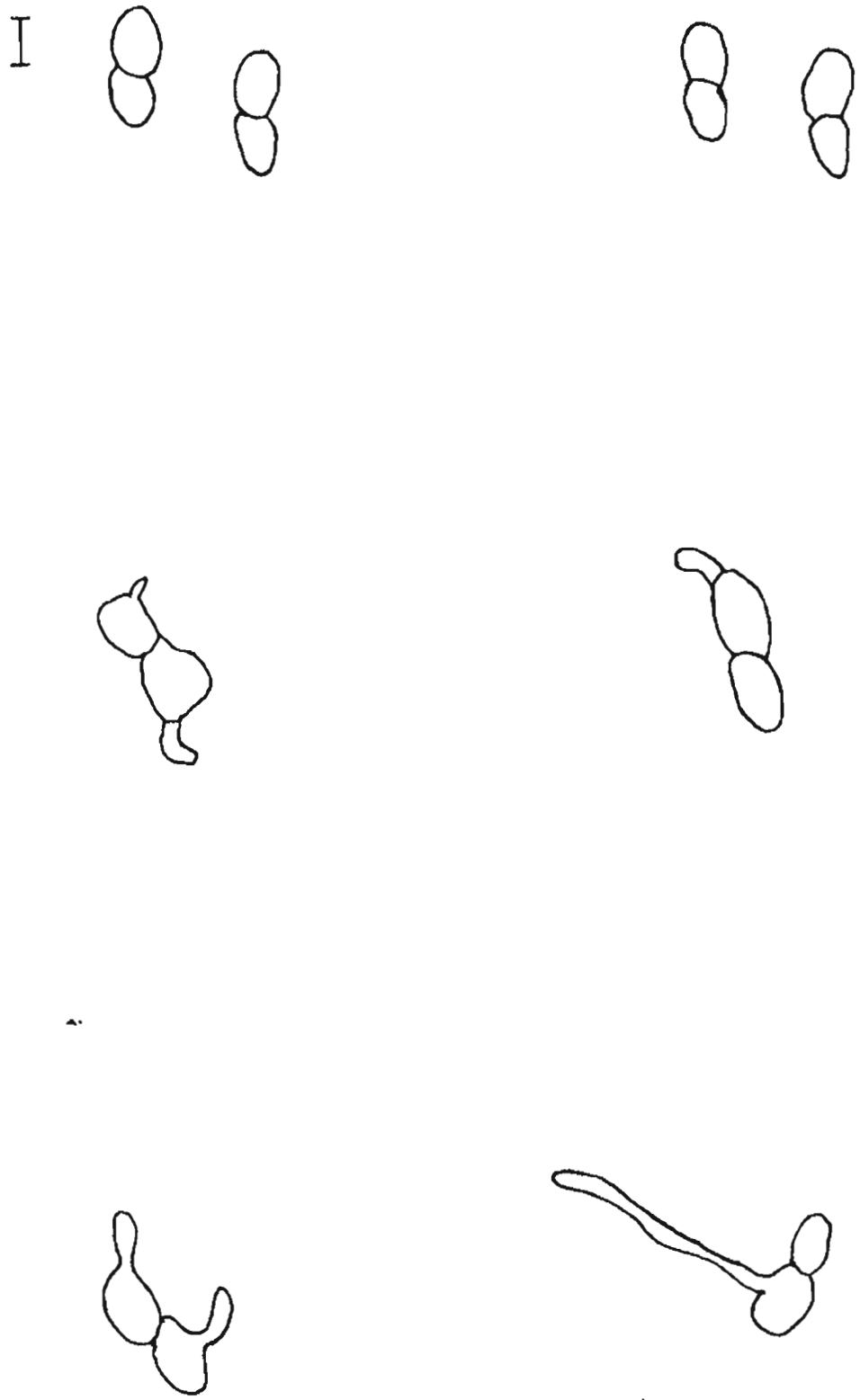

Plate 9. Camera lucida drawings showing conidial germination of *Marssonina coronaria*

(53.13%) after 96 hrs and minimum (14.58%) after 24 hrs of incubation period. The results of the interaction between different incubation periods and temperatures were also found to be significant. All the temperatures tested registered a progressive increase in the germination of conidia with the increase in incubation period. The conidial germination at 20°C increased from 23.96 per cent to 87.50 per cent as the incubation period was increased from 24 to 96 hrs.

The results of the data with regard to the germ tube formation of conidia of *M. coronaria* revealed almost similar trend. Maximum germ tube length (77.31 µm) was recorded again at 20°C followed by 25°C (72.87 µm), though statistically at par with each other. The next best in order of importance were 15°C (59.29 µm), 30°C (51.81 µm) and 10°C (33.53 µm), however, minimum germ tube length (28.26 µm) was recorded at 35°C. It is also clear from the data that an increase in the incubation period from 24 to 96 hrs has also resulted in the increase in the germ tube length from 34.73 µm to 72.97 µm. The results of interaction studies between different temperatures and incubation periods were also found significant (Plate 9).

4.5.3 EFFECT OF CARBON SOURCES

Carbon is one of the most essential elements for growth and sporulation of various fungi. In the present study, the effect of six different carbon sources were tested *in vitro* for the germination and germ tube formation of conidia of *Marssonina coronaria*. The data pertaining to percentage of conidial germination and germ tube length (um) were recorded and are presented in Table 4.3 after statistical analysis.

All the carbon sources tested did not increase the conidial germination of *Marssonina coronaria* except sucrose and glucose as percentage of germination in other sugar solutions was significantly much less than that of control (Table

Table 4.3. Effect of different carbon sources on germination and germ tube formation of conidia of *M. coronaria*

Carbon source	Germination (%) after					Germ tube length (μm) after				
	24 h	48 h	72 h	96 h	Mean	24 h	48 h	72 h	96 h	Mean
Sucrose	25.00 (29.97)*	47.92 (43.80)	85.42 (67.74)	91.67 (74.02)	62.50 (53.88)[a]	45.44	63.17	80.91	97.53	71.76[a]
Glucose	19.79 (26.31)	37.50 (37.75)	59.37 (50.43)	80.21 (63.69)	49.22 (44.55)[b]	35.47	53.20	69.83	85.34	60.96[b]
Mannitol	12.50 (20.28)	18.75 (25.61)	37.50 (37.75)	47.92 (43.80)	29.17 (31.86)[c]	24.38	43.22	57.63	73.15	49.60[c]
Maltose	9.38 (17.10)	11.46 (19.75)	25.00 (29.97)	38.54 (38.36)	21.09 (26.29)[d]	17.73	34.36	45.44	60.96	39.62[e]
Galactose	7.29 (15.12)	10.42 (18.06)	22.92 (28.44)	29.17 (32.59)	17.45 (23.55)[d]	15.50	24.38	36.47	48.77	31.28[f]
Lactose	4.17 (11.61)	8.33 (16.24)	15.62 (23.22)	18.75 (25.61)	11.72 (19.15)[e]	12.19	16.62	24.38	39.90	23.27[g]
Control (water)	15.62 (23.22)	25.00 (29.97)	40.62 (39.59)	42.71 (40.79)	30.99 (33.39)[c]	22.17	37.68	47.66	63.17	42.67[d]
Mean	13.39 (20.52)[d]	22.77 (27.31)[c]	40.92 (39.59)[b]	49.85 (45.55)[a]		24.70[d]	38.95[c]	51.76[b]	66.97[a]	

	$CD_{0.05}$	$CD_{0.05}$
Carbon source	3.15	2.31
Incubation period	2.38	1.74
Carbon source x Incubation period	6.29	4.62

* Figures in parentheses are arcsin transformed values
Means followed by the same letter do not differ significantly

4.3). In the present study, sucrose was found to be the best carbon sucrose where 91.67 per cent conidia germinated after 96 hrs of incubation period compared to 49.85 per cent in control (without carbon source). Glucose was the next best giving 80.21 per cent germination of conidia after 96 hrs of incubation period. While the percentage of conidial germination in maltose, galactose and lactose was significantly much less than that of control. When the germination of conidia was tested at different incubation periods, it was found maximum after 96 hrs (49.85%) followed by 72 hrs (40.92%) and 48 hrs (22.77%), whereas least conidial germination (13.39%) was registered after 24 hrs of incubation period. The interactions between incubation periods and different carbon sources were found significant. The percentage of conidial germination in sucrose increased from 25.00 per cent to 91.67 per cent with the increase in incubation period from 24 to 96 hrs.

The results with regard to the formation of germ tube show that only sucrose followed by glucose and mannitol increased the germ tube length of conidia of *M. coronaria* significantly over control, while all other carbon sources did not increase the germ tube length of conidia of the test fungus. While studying the length of the conidial germ tube at different incubation periods, it was observed that germ tube length increased significantly from 24.70 µm to 66.97 µm with the increase in incubation period from 24 to 96 hrs. The results of interaction between different carbon sources and incubation periods were also found highly significant with each other. After 24 hrs, the germ tube length of conidia was 45.44 µm in sucrose which increased to 97.53 µm after 96 hrs of incubation period. Results of similar nature were observed with other treatments in the present study.

4.5.4 EFFECT OF DIFFERENT CONCENTRATIONS OF SUCROSE

In earlier experiment, sucrose was adjudged the best carbon source both for the germination and germ tube formation of conidia of *Marssonina*

coronaria under *in vitro* conditions. In the present study, different concentrations of sucrose were tested to find out the optimum dose required for enhanced germination and germ tube formation of conidia of the test fungus. The data recorded in this regard are presented in Table 4.4.

From the analysis of data presented in (Table 4.4), it is clear that the conidia of *Marssonina coronaria* germinated well at all the concentrations of sucrose, however, the fungus preferred higher concentrations of sucrose for conidial germination with maximum (69.27%) being at 2.5 per cent concentration. The next best concentration of sucrose in order of importance was 2.0 per cent (64.32%) followed by 1.5 per cent (60.42%), however, least germination (45.05%) of the conidia was registered in 0.25 per cent concentration of sucrose. The conidial germination at different incubation periods was found highly significant. It was significantly higher (79.43%) after 96 hrs and least (22.62%) after 24 hrs of incubation period at 20°C. The data also reveal that with the progressive increase in the incubation period from 24 to 96 hrs the conidial germination also increased under all the treatments tested. After 24 hrs, the percentage of conidial germination at 2.5 concentration of sucrose was 36.46 per cent which increased to 93.75 per cent after 96 hrs of incubation period.

As regards, the germ tube length of conidia of *M. coronaria* is concerned, it was again found maximum (88.34 µm) at 2.5 per cent concentration of sucrose and minimum (54.03 µm) at 0.25 per cent. The data also show that after 96 hrs of incubation period, the germ tube length was highest (94.68 µm) followed by 72 hrs (75.84 µm) and 48 hrs (59.53 µm), while least germ tube length (41.61 µm) was registered after 24 hrs of incubation period. The interactions of different concentrations of sucrose and incubation periods were also found significant under all the treatments studied. The germ

Table 4.4. Effect of different concentrations of sucrose on germination and germ tube formation of conidia of *M. coronaria*

Sucrose (concentration %)	Germination (%) after					Germ tube length (μm) after				
	24 h	48 h	72 h	96 h	Mean	24 h	48 h	72 h	96 h	Mean
0.25	11.46 (19.75)*	28.12 (32.01)	65.62 (54.12)	75.00 (60.03)	45.05 (41.48)^{f}	28.82	44.33	63.17	79.80	54.03^{f}
0.50	15.62 (23.22)	34.37 (35.88)	72.92 (58.70)	80.21 (63.69)	50.78 (45.37)^{e}	35.47	53.20	70.93	86.45	61.51^{e}
1.00	21.87 (27.85)	41.46 (40.05)	78.12 (62.15)	85.42 (67.74)	56.72 (49.45)^{d}	42.12	60.96	78.69	95.32	69.27^{d}
1.50	26.04 (30.68)	46.87 (43.20)	81.25 (64.39)	87.50 (69.40)	60.42 (51.92)^{c}	47.66	66.50	83.12	103.10	75.09^{c}
2.00	31.25 (33.97)	50.00 (45.00)	85.42 (67.74)	90.62 (72.33)	64.32 (54.76)^{b}	53.20	72.04	88.67	110.80	81.19^{b}
2.50	36.46 (37.14)	56.25 (48.60)	90.62 (72.33)	93.75 (75.84)	69.27 (58.48)^{a}	59.65	79.80	96.42	117.50	88.34^{a}
Control (water)	15.62 (23.22)	25.00 (29.97)	41.46 (40.05)	43.54 (41.29)	31.41 (33.63)^{g}	24.35	39.90	49.87	69.82	45.99^{g}
Mean	22.62 (27.97)^{d}	40.30 (39.24)^{c}	73.63 (59.93)^{b}	79.43 (64.33)^{a}		41.61^{d}	59.53^{c}	75.84^{b}	94.68^{a}	

	$CD_{0.05}$	$CD_{0.05}$
Sucrose concentration	2.20	2.22
Incubation period	1.66	1.68
Sucrose concentration x Incubation period	4.39	4.44

* Figures in parentheses are arcsin transformed values
Means followed by the same letter do not differ significantly

tube length at 2.5 per cent concentration of sucrose was 59.65 µm after 24 hrs which became almost double (117.50 µm) after 96 hrs of incubation period.

4.5.5 EFFECT OF NITROGEN SOURCES

In order to determine the best nitrogen source for maximum conidial germination and germ tube formation of *Marssonina coronaria*, six different nitrogen sources were tested under *in vitro* conditions. Data pertaining to percentage of conidial germination and germ tube length (um) were recorded and the same are presented in Table 4.5 after statistical analysis.

It is apparent from the data (Table 4.5) that although nitrate forms of nitrogen, in general, were best utilized by the fungus for conidial germination than that of ammonical form, yet highest germination of conidia of *Marssonina coronaria* was registered in organic form of nitrogen as 77.08 per cent conidia germinated in asparagine after 96 hrs of incubation period compared to 41.67 per cent in control (without nitrogen). Potassium nitrate followed by ammonium nitrate were the next best giving 69.79 and 57.29 per cent conidial germination respectively, after 96 hrs of incubation period. Least conidial germination was however, observed in urea (15.36%) followed by ammonium sulphate (23.96%) which were significantly less than even control (29.17%). The percentage of conidial germination recorded at different incubation periods revealed maximum conidial germination (53.57%) after 96 hrs and minimum (9.97%) after 24 hrs of incubation period. The results further show that with the increase in the incubation period from 24 to 96 hrs, the percentage of conidial germination also increased significantly from 19.79 per cent to 77.08 per cent in case of asparagine. Similar results of increased germination at different incubation periods were also observed with other treatments.

The results with reference to the formation of germ tube show that all the nitrogen sources tested increased the germ tube length of conidia of *M.*

Table 4.5. **Effect of different nitrogen sources on germination and germ tube formation of conidia of *M. coronaria***

Nitrogen source	Germination (%) after					Germ tube length (μm) after				
	24 h	48 h	72 h	96 h	Mean	24 h	48 h	72 h	96 h	Mean
Ammonium sulphate	5.21 (13.05)*	13.54 (21.57)	29.17 (32.68)	47.92 (43.81)	23.96 (27.78)^{e}	13.30	25.49	34.36	43.22	29.09^{e}
Ammonium nitrate	10.42 (18.79)	21.87 (27.85)	41.67 (40.19)	57.29 (49.20)	32.81 (34.01)^{c}	29.92	42.12	55.42	68.72	49.04^{c}
Potassium nitrate	13.54 (21.57)	29.17 (32.68)	50.00 (45.00)	69.79 (56.67)	40.63 (38.98)^{b}	36.57	50.99	65.39	78.69	57.91^{b}
Sodium nitrate	7.29 (15.60)	16.67 (24.08)	35.42 (36.52)	52.08 (46.20)	27.86 (30.60)^{d}	22.17	35.47	47.66	58.72	41.00^{d}
Asparagine	19.79 (26.40)	35.42 (36.52)	58.33 (49.81)	77.08 (61.41)	47.66 (43.53)^{a}	39.90	54.31	69.82	82.02	61.51^{a}
Urea	2.08 (6.79)	10.42 (18.79)	19.79 (26.40)	29.17 (32.68)	15.36 (21.17)^{f}	11.08	15.52	24.38	32.14	20.78^{f}
Control (water)	11.46 (19.75)	22.92 (28.59)	40.62 (39.58)	41.67 (40.19)	29.17 (32.03)^{d}	23.27	35.47	42.12	60.96	40.45^{d}
Mean	9.97 (17.42)^{d}	21.43 (27.15)^{c}	39.29 (38.60)^{b}	53.57 (47.16)^{a}		25.17^{d}	37.05^{c}	48.45^{b}	60.64^{a}	

	$CD_{0.05}$	$CD_{0.05}$
Nitrogen source	1.60	2.06
Incubation period	1.21	1.55
Nitrogen source x Incubation period	3.20	4.11

* Figures in parentheses are arcsin transformed values
Means followed by the same letter do not differ significantly

Table 4.6. Effect of different concentrations of asparagine on germination and germ tube formation of conidia of *M. coronaria*

Asparagine (concentration. %)	Germination (%) after					Germ tube length (μm) after				
	24 h	48 h	72 h	96 h	Mean	24 h	48 h	72 h	96 h	Mean
0.25	14.58 (22.42)*	33.33 (35.26)	54.17 (47.40)	65.62 (54.12)	41.93 (39.80)^{c}	28.82	36.57	57.63	72.04	48.77^{d}
0.50	19.79 (26.40)	37.50 (37.70)	58.33 (49.81)	72.92 (58.65)	47.14 (43.14)^{b}	36.57	48.77	66.50	79.80	57.91^{b}
1.00	22.92 (28.59)	41.38 (40.03)	62.50 (52.25)	80.21 (63.60)	51.75 (46.12)^{a}	42.12	55.42	73.15	85.34	64.01^{a}
1.50	16.67 (24.08)	31.25 (33.97)	53.12 (46.80)	69.79 (56.67)	42.71 (40.38)^{c}	34.36	43.22	59.85	70.93	52.09^{c}
2.00	12.5 (20.61)	22.92 (28.59)	46.87 (43.21)	58.33 (49.81)	35.16 (35.55)^{d}	26.60	31.03	46.55	57.63	40.45^{e}
2.50	9.375 (17.67)	14.58 (22.42)	35.42 (36.52)	47.92 (43.81)	26.82 (30.11)^{f}	19.95	21.06	35.47	45.44	30.48^{f}
Control (water)	11.46 (19.75)	26.04 (30.68)	39.58 (38.98)	45.83 (42.61)	30.73 (33.00)^{e}	22.17	31.03	42.12	59.85	38.79^{e}
Mean	15.33 (22.79)^{d}	29.57 (32.67)^{c}	50.00 (44.49)^{b}	62.95 (52.75)^{a}		30.08^{d}	38.16^{c}	54.47^{b}	67.29^{a}	

	$CD_{0.05}$	$CD_{0.05}$
Asparagine concentration	1.62	2.10
Incubation period	1.22	1.59
Asparagine concentration x Incubation period	3.29	4.20

* Figures in parentheses are arcsin transformed values
Means followed by the same letter do not differ significantly

found significant with each other. The conidial germination in different concentrations of asparagine increased with increase in incubation period from 24 to 96 hrs. The conidial germination at 1.0 per cent concentration of asparagine was 22.92 per cent after 24 hrs which increased to 80.21 per cent after 96 hrs of incubation period.

Results of the data with regard to the formation of germ tube revealed that maximum germ tube length (64.01 µm) of conidia of *M. coronaria* was obtained in 1.0 per cent solution of asparagine followed by 0.5 per cent (57.91 µm) and 1.5 per cent (52.89 µm), while 2.5 per cent solution of asparagine supported least germ tube length (30.48 µm) of the conidia of test fungus. It is also clear from the data that an increase in the incubation period from 24 hrs to 96 hrs has also resulted in the increase in the germ tube length from 30.08 µm to 67.29 µm. The interactions between different concentrations of asparagine and incubation periods were also found significant.

4.5.7 EFFECT OF C:N RATIOS

Both carbon and nitrogen are the most important factors for the growth of fungi. In the present study, an attempt was made to determine the effect of a carbon (glucose) and a nitrogen (asparagine) source in varying ratios on the germination and germ tube formation of conidia of *Marssonina coronaria*. The data obtained are tabulated in Table 4.7.

The results show that glucose and asparagine ratio of 70:1 was the best (79.17%) for the germination of conidia of *Marssonina coronaria* followed by 60:1 ratio (73.18%). The next best C:N ratios in order of importance were 50:1 (65.10%), 40:1 (57.81%) and 30:1 (50.26%). Least germination (14.84%) of conidia was registered in 30:5 glucose-asparagine ratio followed by 30:4 (24.22%). The conidial germination at different incubation periods was found highly significant. The percentage of conidial germination was significantly

Table 4.7. Effect of different C:N ratios on germination and germ tube formation of conidia of *M. coronaria*

C:N ratio	Germination (%) after					Germ tube length (μm) after				
	24 h	48 h	72 h	96 h	Mean	24 h	48 h	72 h	96 h	Mean
30:1	22.92 (28.52)*	40.62 (39.59)	53.12 (46.80)	84.37 (66.79)	50.26 (45.42)^{e}	45.44	59.85	82.02	100.90	72.04^{e}
40:1	29.17 (32.68)	50.00 (45.00)	61.46 (51.65)	90.62 (72.33)	57.81 (50.42)^{d}	53.20	69.82	89.77	109.70	80.63^{d}
50:1	34.37 (35.88)	59.37 (50.41)	71.87 (58.00)	94.79 (77.27)	65.10 (55.39)^{c}	58.74	76.47	96.42	116.40	87.00^{c}
60:1	40.62 (39.59)	71.87 (58.00)	84.37 (66.79)	95.83 (78.39)	73.18 (60.69)^{b}	66.50	83.12	103.10	123.00	93.93^{b}
70:1	50.00 (45.00)	81.25 (64.39)	87.50 (69.40)	97.92 (83.21)	79.17 (65.50)^{a}	73.15	89.77	110.80	129.70	100.90^{a}
30:5	5.21 (13.05)	9.375 (17.67)	19.79 (26.40)	25.00 (29.97)	14.84 (21.77)^{f}	13.30	29.93	49.87	69.82	40.73^{i}
30:4	10.42 (18.53)	14.58 (22.36)	31.25 (33.97)	40.62 (39.59)	24.22 (28.61)^{h}	24.38	36.57	59.85	76.47	49.32^{h}
30:3	13.54 (21.57)	21.87 (27.85)	42.71 (40.81)	53.12 (46.80)	32.81 (34.25)^{g}	32.14	43.22	66.50	83.12	56.25^{g}
30:2	17.71 (24.87)	29.17 (32.68)	52.08 (46.20)	65.62 (54.12)	41.15 (39.47)^{f}	39.90	53.20	73.15	93.10	64.84^{f}
Control (water)	16.67 (24.08)	27.08 (31.35)	41.67 (40.20)	46.87 (43.21)	33.07 (34.71)^{g}	25.49	33.25	43.22	58.74	40.18^{i}
Mean	24.06 (28.38)^{d}	40.52 (38.93)^{c}	54.58 (48.02)^{b}	69.48 (59.17)^{a}		43.22^{d}	57.52^{c}	77.47^{b}	96.09^{a}	

	$CD_{0.05}$	$CD_{0.05}$
C:N ratio	1.94	2.53
Incubation period	1.23	1.60
C:N ratio x Incubation period	3.89	5.05

* Figures in parentheses are arcsin transformed values
Means followed by the same letter do not differ significantly

higher after 96 hrs (69.48%) of incubation period followed by 72 hrs (54.58%) and 48 hrs (40.52%), whereas least conidial germination was observed after 24 hrs (24.06%) of incubation period at 20°C. The data also reveal that with the progressive increase in the incubation period, the germination of conidia also increased under all the C:N ratios tested. In C:N ratio of 70:1, the conidial germination was 50.00 per cent after 24 hrs which became almost doubled (97.92%) after 96 hrs of incubation period.

As far as the germ tube length of conidia of *M. coronaria* is concerned, all the C:N ratios increased the germ tube length in comparison to control however, maximum (100.90 µm) was in C:N ratio of 70:1 and minimum (40.73 µm) in 30:5 ratio. Significant results were also obtained when formation of germ tube was tested under different incubation periods. The length of germ tube was found highest (96.09 µm) after 96 hrs and least (43.22 µm) after 24 hrs of incubation period. The interactions of different C:N ratios and incubation periods were also found significant. The germ tube length in C:N ratio of 70:1 was 73.15 µm after 24 hrs which increased to 129.7 µm after 96 hrs of incubation period. From the aforesaid studies it can be concluded that the rate of conidial germination of *M. coronaria* gradually decreased with the increase in nitrogen ratio and decrease in carbon content of the medium. The same was also found true for the germ tube formation of the conidia.

4.5.8 EFFECT OF VITAMINS

In order to determine the best vitamin for maximum germination and germ tube formation of conidia of *Marssonina coronaria*, six different vitamins were tested under *in vitro* conditions. Data pertaining to per cent conidial germination and germ tube length (µm) were recorded and tabulated in Table 4.8 after statistical analysis.

Table 4.8. Effect of different vitamins on germination and germ tube formation of conidia of *M. coronaria*

Vitamin	Germination (%) after					Germ tube length (μm) after				
	24 h	48 h	72 h	96 h	Mean	24 h	48 h	72 h	96 h	Mean
Thiamine hydrochloride	11.46 (19.75)*	13.54 (21.57)	17.71 (24.87)	22.92 (28.59)	16.41 (23.69)^{d}	16.63	32.14	47.66	56.51	38.24^{c}
Meso-inositol	1.04 (3.39)	2.08 (6.79)	5.21 (13.05)	9.38 (17.67)	4.43 (10.22)^{f}	9.98	14.41	16.62	17.73	14.69^{e}
Ascorbic acid	29.17 (32.68)	58.33 (49.81)	69.79 (56.67)	75.00 (60.03)	58.07 (49.80)^{a}	42.06	60.96	75.37	90.88	67.32^{a}
Nicotinic acid	6.60 (14.87)	10.42 (18.79)	12.50 (20.71)	14.58 (22.26)	11.02 (19.16)^{e}	13.30	24.38	32.14	43.22	28.26^{d}
Folic acid	19.79 (26.40)	32.29 (34.62)	39.58 (38.98)	41.67 (40.19)	33.33 (35.05)^{b}	31.03	48.77	60.96	73.15	53.48^{b}
Biotin	4.17 (11.61)	8.33 (16.55)	13.54 (21.57)	25.00 (29.97)	12.76 (19.93)^{e}	12.19	22.17	28.82	36.57	24.94^{d}
Control (water)	11.46 (19.75)	23.96 (29.23)	35.42 (36.52)	39.58 (38.98)	27.60 (31.12)^{c}	22.17	36.57	48.77	65.39	43.22^{c}
Mean	11.95 (18.35)^{d}	21.28 (25.34)^{c}	27.68 (30.34)^{b}	32.59 (33.96)^{a}		21.05^{d}	34.20^{c}	44.33^{b}	54.78^{a}	

	$CD_{0.05}$	$CD_{0.05}$
Vitamins	2.08	5.42
Incubation period	1.57	4.09
Vitamin x Incubation period	4.15	10.83

* Figures in parentheses are arcsin transformed values
Means followed by the same letter do not differ significantly

It is evident from the data (Table 4.8) that all the vitamins tested did not increase significantly the percentage of conidial germination of *Marssonina coronaria* except ascorbic acid and folic acid where the percentage of germination was significantly higher than that of other treatments including control (without vitamin). In the present investigation, ascorbic acid was found the best vitamin where 75.00 per cent conidia germinated after 96 hrs of incubation period. The percentage of conidial germination recorded at different incubation periods revealed maximum conidial germination (32.59 %) after 96 hrs and minimum (11.95%) after 24 hrs of incubation period. The interactions between different incubation periods and vitamins were also significant. With the increase in incubation period from 24 to 96 hrs, the percentage of conidial germination also increased significantly from 29.17 per cent to 75.00 per cent in case of ascorbic acid. Similar results of increased germination at different incubation periods were also observed with other treatments.

The data with reference to the formation of germ tube of *M. coronaria* show that ascorbic acid (67.32 µm) followed by folic acid (53.48 µm) increased the germ tube length of conidia significantly over other treatments. However, other vitamins had no significant effect on the increase in germ tube length of conidia of the test fungus. The length of the conidial germ tube also increased significantly from 21.05 µm to 54.78 µm with the increase in incubation period from 24 to 96 hrs. The interactions between different vitamins and incubation periods were found highly significant with each other. After 24 hrs, the germ tube length was 42.06 µm in ascorbic acid which increased more than two folds i.e. 90.88 µm after 96 hrs of incubation period. Results of similar nature were observed with other treatments.

4.5.9 EFFECT OF MICRO NUTRIENTS

In the present study, the effect of different micro nutrients was determined so as to find out the best micro nutrient needed for maximum

germination and germ tube length of the conidia of *Marssonina coronaria*. The data recorded in this regard are presented in Table 4.9.

The data presented in (Table 4.9) indicate that all the micro nutrients tested did not increase the percentage of conidial germination of *Marssonina coronaria* significantly over control except ferrous sulphate and ammonium molybdate. The percentage of germination of the conidia was significantly higher in ferrous sulphate (61.97%) followed by ammonium molybdate (52.08%), however, boric acid and zinc sulphate had negative effect on conidial germination as only 8.07 and 3.66 per cent conidia germinated as compared to 30.21 per cent in control. The percentage of conidial germination recorded at different incubation periods revealed maximum germination (41.66%) after 96 hrs and minimum (15.62%) after 24 hrs of incubation period. The interactions between different incubation periods and micro nutrients were significant. With the increase in incubation period from 24 to 96 hrs the percentage of conidial germination also increased significantly from 31.22 per cent to 82.29 per cent in case of ferrous sulphate. Similar results of enhanced conidial germination at different incubation periods were also observed with other treatments.

The results with reference to the formation of germ tube show that only ferrous sulphate (43.78 µm) increased the germ tube length of the conidia of *M. coronaria* significantly over control (38.51 µm). While, the remaining micro nutrients had no significant effect on the increase in germ tube length of conidia of the test fungus. The length of the conidial germ tube also increased significantly from 17.89 µm to 44.49 µm with the increase in incubation period from 24 to 96 hrs. The interactions between different micro nutrients and incubation periods were found highly significant with each other. After 24 hrs, germ tube length of conidia was 26.60 µm in ferrous sulphate which increased to 65.39 µm after 96 hrs of incubation period. Results of similar nature were observed with other interactions tested in the present study.

Table 4.9. Effect of different micro nutrients on germination and germ tube formation of conidia of *M. coronaria*

Micro nutrient	Germination (%) after					Germ tube length (µm) after				
	24 h	48 h	72 h	96 h	Mean	24 h	48 h	72 h	96 h	Mean
Ferrous sulphate	31.22 (33.95)*	62.50 (52.25)	71.87 (58.00)	82.29 (65.13)	61.97 (52.33)[a]	26.60	36.57	46.55	65.39	43.78[a]
Ammonium molybdate	22.92 (28.59)	48.96 (44.40)	63.54 (52.86)	72.90 (58.64)	52.08 (46.12)[b]	24.38	28.82	41.01	55.42	37.41[b]
Calcium chloride	19.79 (26.40)	29.17 (32.68)	37.50 (37.75)	41.67 (40.19)	32.03 (34.26)[c]	19.95	23.27	31.03	45.44	29.92[c]
Manganese sulphate	14.59 (22.26)	22.92 (28.59)	31.25 (33.97)	35.42 (36.52)	26.04 (30.34)[d]	13.30	18.84	25.49	36.57	23.55[d]
Boric acid	3.13 (8.22)	8.33 (16.71)	9.38 (17.67)	11.46 (19.75)	8.07 (15.59)[e]	11.08	15.52	18.84	28.82	18.56[e]
Zinc sulphate	1.04 (3.39)	4.17 (9.65)	4.17 (9.65)	5.21 (13.05)	3.66 (8.94)[f]	8.87	13.30	11.08	19.95	13.30[f]
Control (water)	16.67 (24.08)	25.00 (29.97)	36.46 (37.12)	42.71 (40.79)	30.21 (32.99)[cd]	21.06	31.02	42.12	59.85	38.51[b]
Mean	15.62 (20.99)[d]	28.72 (30.61)[c]	36.31 (35.29)[b]	41.66 (39.15)[a]		17.89[d]	23.91[c]	30.87[b]	44.49[a]	

	$CD_{0.05}$	$CD_{0.05}$
Micro nutrient	2.80	2.86
Incubation period	2.12	2.16
Micro nutrient x Incubation period	5.60	5.72

* Figures in parentheses are arcsin transformed values
Means followed by the same letter do not differ significantly

4.5.10 EFFECT OF RELATIVE HUMIDITY REGIMES

In order to find out the best relative humidity level for the germination and formation of germ tube of the conidia of *Marssonina coronaria*, the effect of different relative humidity levels was tested under the laboratory conditions. The data recorded in this regard is presented in Table 4.10.

The results of the experiment presented in (Table 4.10) clearly indicate that conidia of the fungus *Marssonina coronaria* germinated at all the humidity levels varying from 75.6 to 100 per cent except RH 56.8 per cent however, maximum conidia germinated (58.85%) at 100 per cent RH, followed by RH 98.50 (47.66 %), RH 96.1 (40.89%), RH 92.9 (31.25%) and RH 88.5 per cent (23.44%). Relative humidity below RH 88.5 per cent did not favour the conidial germination as only 17.45 and 13.02 per cent conidial germination was obtained at RH 82.9 and RH 75.6 per cent, respectively. No germination was however, recorded at RH 56.8 per cent. The data with regard to the conidial germination at different incubation periods were significant. Maximum conidial germination was recorded after 96 hrs (45.83%) followed by 72 hrs (37.11%) and 48 hrs (22.40%) whereas, least conidial germination (10.94%) was observed after 24 hrs of incubation period. The interactions between different incubation periods and relative humidity levels were significant with each other. The conidial germination at different relative humidity levels increased with increase in incubation period from 24 hrs to 96 hrs. After 24 hrs, the conidial germination at 100 per cent RH was 25.00 per cent which increased to 87.50 per cent after 96 hrs of incubation period. Similar findings were obtained with other interactions studied.

As regards the formation of germ tube is concerned, the data indicate that with the increase in relative humidity levels from 75.6 per cent to 100 per cent, the length of germ tube of conidia of *M. coronaria* also increased

Table 4.10. Effect of different RH levels on germination and germ tube formation of conidia of *M. coronaria*

RH level (%)	Germination (%) after					Germ tube length (µm) after				
	24 h	48 h	72 h	96 h	Mean	24 h	48 h	72 h	96 h	Mean
56.8	0.00 (0.00)*	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)^{h}	0.00	0.00	0.00	0.00	0.00^{h}
75.6	2.08 (6.79)	5.21 (13.05)	17.71 (24.75)	27.08 (31.26)	13.02 (18.96)^{g}	8.867	13.30	22.17	31.03	18.84^{g}
82.9	4.17 (11.61)	10.42 (18.06)	23.96 (29.09)	31.25 (33.97)	17.45 (23.18)^{f}	19.95	24.38	34.36	42.12	30.20^{f}
88.5	8.33 (16.24)	16.67 (24.08)	30.21 (33.30)	38.54 (38.36)	23.44 (27.99)^{e}	26.60	33.25	44.33	52.09	39.07^{e}
92.9	11.46 (19.75)	23.96 (29.09)	40.62 (39.57)	48.96 (44.40)	31.25 (33.20)^{d}	33.25	43.22	55.42	63.17	48.77^{d}
96.1	16.67 (23.96)	32.29 (34.59)	52.08 (46.20)	62.50 (52.25)	40.89 (39.25)^{c}	38.79	50.99	64.28	73.15	56.80^{c}
98.5	19.79 (26.31)	40.62 (39.57)	59.37 (50.41)	70.83 (57.36)	47.66 (43.41)^{b}	45.44	60.96	73.15	86.45	66.50^{b}
100	25.00 (29.97)	50.00 (45.00)	72.92 (58.70)	87.50 (69.40)	58.85 (50.77)^{a}	47.66	66.50	84.26	106.40	76.20^{a}
Mean	10.94 (16.83)^{d}	22.40 (25.43)^{c}	37.11 (35.25)^{b}	45.83 (40.87)^{a}		27.57^{d}	36.58^{c}	47.24^{b}	56.80^{a}	

	$CD_{0.05}$	$CD_{0.05}$
RH level	2.72	4.92
Incubation period	1.92	3.48
RH level x Incubation period	5.43	9.84

* Figures in parentheses are arcsin transformed values

Means followed by the same letter do not differ significantly

significantly with maximum (76.2 µm) being at 100 per cent RH and minimum (18.84 µm) at RH 75.6 per cent. It is also clear from the data that an increase in the incubation period from 24 to 96 hrs has also resulted in the increase in the germ tube length from 27.57 µm to 56.80 µm. The interactions between different relative humidity levels and incubation period were also found significant with each other.

4.6 SURVIVAL OF THE PATHOGEN

In order to study the survival of the pathogen under orchard, laboratory and refrigerated conditions, the viability of the test fungus, *Marssonina coronaria* in respect of conidial germination was tested by slide germination technique (Anonymous, 1943). The data recorded on conidial germination after 96 hrs of incubation period are given in Table 4.11.

Table 4.11. Effect of different storage conditions on the viability of pathogen (*M. coronaria*)

Storage condition	Per cent conidial germination after					Formation of sexual stage
	3 months	6 months	9 months	12 months	Mean	
Orchard	58.32	34.38	22.92	14.58	32.55	-
Laboratory	60.42	39.58	26.04	17.71	35.94	-
Refrigerated	56.25	33.33	23.96	16.67	40.72	-
Mean	58.33	35.76	24.37	16.32		

- Not formed

It is evident from the data (Table 4.11) that the pathogen, *M. coronaria*, survived on the infected leaves in the form of fruiting bodies (acervuli) under orchard, laboratory and refrigerated conditions as the spores remained viable for one year when the germination of conidia of *M. coronaria* was tested after 3, 6, 9 and 12 months of storage. It was interesting to note that percentage of conidial

germination decreased with increase in storage period from 3 to 12 months in all the storage conditions however, maximum germination of conidia was observed on leaves stored under laboratory condition followed by refrigerated and orchard conditions.

The formation of sexual (ascigerous) stage of the fungus was not observed on leaves stored in differemt storage conditions for one year.

The study concludes that the pathogen, *Marssonina coronaria* causing leaf blotch in apple is surviving in nature on the infected fallen leaves in the form of fruiting bodies (acervuli).

4.7 SCREENING OF APPLE CULTIVARS/ SCAB RESISTANT LINES

In order to ascertain the source of resistance if any, a collection of 101 cultivars/ scab resistant lines of apple planted at the Research Farm of Department of Fruit Breeding and Genetic Resources of Dr Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan were screened against *Marssonina coronaria* causing leaf blotch of apple during the cropping season of 1997 through 1999 under natural epiphytotic conditions. The observations on disease severity were recorded and per cent disease index (PDI) was calculated. Based on PDI, the genotypes were categorized into different reaction classes and the same are presented in Table 4.12.

From the data (Table 4.12) it is evident that none of the cultivar/ scab resistant line of apple was resistant to Marssonina leaf blotch, however, only two cultivars viz., Tydeman's Early Worcester and Granny Smith showed moderately resistant reaction with per cent disease index (PDI) varying between 8.89 to 10.57 per cent. Seven cultivars/ scab resistant lines viz., Alkene, Scab Resistant (P_7P_{23-22}), Red Fugi, Lord Lambourne (IRA 98-1), Doux P_1P_{24}, Gala and Antonovoka Obyknonevennaya were found moderately susceptible with

Table 4.12. Reaction of different apple cultivars/ scab resistant lines to *Marssonina coronaria* causing leaf blotch in apple

Sr. No.	Cvs./ scab resistant line	Per cent disease index (PDI)			Mean per cent disease index (PDI)	Reaction
		1997	1998	1999		
1	Granny Smith	8.75	7.92	10.00	8.89	MR
2	Tydeman's Early Worcester	13.75	10.00	7.92	10.57	MR
3	Red Fugi	20.00	12.00	22.00	18.00	MS
4	Antonovoka Obyknonevennaya	17.00	26.25	22.00	21.75	MS
5	Scab Resistent P_7P_{23-22}	20.00	24.42	26.25	22.14	MS
6	Doux P_1P_{24}	22.00	20.00	24.42	22.14	MS
7	Lord Lambourne (IRA 98-1)	20.00	22.00	26.25	22.75	MS
8	Gala	22.00	23.00	26.25	23.75	MS
9	Alkene	22.00	24.42	26.25	24.22	MS
10	Close	37.00	38.00	36.25	37.08	S
11	Cox's Orange Cortergrand	37.00	40.00	38.00	38.33	S
12	Exter Cross	40.00	42.00	38.75	40.25	S
13	Top Red	45.00	40.00	37.50	40.83	S
14	Spur type Red Delicious	42.00	45.00	38.75	41.91	S
15	EC 38683	42.00	40.00	45.44	42.48	S
16	Spartan	42.00	40.00	45.44	42.48	S
17	Hybrid No.1	40.00	38.75	50.00	42.91	S
18	Jonald	42.00	45.00	43.00	43.33	S
19	Elstar	42.00	40.00	50.44	44.14	S
20	Ve Red	42.00	40.00	51.00	44.33	S
21	Meghumi	40.00	42.00	50.44	44.14	S
22	Nema Red Delicious	45.00	40.00	48.85	44.61	S
23	EC 115671	40.00	45.00	51.47	45.49	S
24	Hardiman	48.00	45.00	47.50	46.83	S
25	$K_1R_{42}A_1$	42.00	54.00	45.00	47.00	S
26	Chahla	48.00	45.44	48.85	47.43	S
27	Skyline Supreme	50.00	42.00	50.37	47.45	S
28	EC $161287P_{20}$	40.00	50.00	53.50	47.83	S
29	Red Baron	50.00	50.14	45.00	48.38	S
30	Regent	48.00	50.00	48.85	48.95	S
31	Vance Delicious	50.00	45.00	53.00	49.33	S
32	Royal Delicious	48.00	50.00	51.47	49.82	S
33	Morspur	50.00	55.00	52.00	52.33	HS
34	Jonadel	45.00	50.00	65.00	53.33	HS
35	Stoyanovka Krasavist	60.00	55.00	45.00	53.33	HS
36	Semi Sweet Red	55.00	50.00	60.00	55.00	HS
37	Stark Red Rome	60.00	55.00	50.00	55.00	HS
38	X 6566	57.00	48.00	60.00	55.00	HS
39	$K_1R_{48}A_{41}$	60.95	55.25	48.85	55.35	HS
40	Fugi	63.00	48.55	55.00	55.51	HS

Sr. No.	Cvs./ scab resistant line	Per cent disease index (PDI)			Mean per cent disease index (PDI)	Reaction
		1997	1998	1999		
41	Mortan Worcester	55.95	45.35	65.75	55.68	HS
42	Spijon	60.00	50.00	65.00	58.33	HS
43	$P_{42}R_{9}A_{23}$	50.00	62.00	65.00	59.00	HS
44	$P_{42}R_{16}A_{11}$	68.00	50.00	60.00	59.33	HS
45	Ruspippin	65.25	50.00	63.15	59.47	HS
46	X 2755	60.00	55.00	65.00	60.00	HS
47	Royal Gala	58.00	55.00	67.75	60.25	HS
48	Thanedhar Early Flowering (Selection)	60.00	55.00	66.00	60.33	HS
49	Stark Cardinal	55.00	62.00	65.00	60.67	HS
50	$P_{42}R_{23}A_{25}$	60.00	65.00	62.00	62.33	HS
51	$K_1R_{32}A_{9}$	60.95	65.00	62.05	62.67	HS
52	Reinette-De-Canada	60.00	66.25	62.00	62.75	HS
53	Sinta	62.00	60.00	67.00	63.00	HS
54	$P_{42}R_{29}A_{22}$	60.00	65.00	67.00	64.00	HS
55	Crimson Gold	58.00	65.00	69.00	64.00	HS
56	Red June (Off Type)	68.00	60.00	65.00	64.33	HS
57	Golden Delicious (IRA 84-1-1)	68.25	62.00	63.00	64.42	HS
58	Royal Red	65.25	62.00	66.15	64.47	HS
59	$K_1R_{50}A_{29}$	62.00	67.00	65.00	64.66	HS
60	EC 161286 $P_1R_{23\text{-}22}$	65.00	60.00	70.00	65.00	HS
61	$P_{42}R_{19}A_{26}$	62.00	70.00	65.00	65.66	HS
62	Arlet	67.00	62.00	68.00	65.67	HS
63	Quinte	60.00	68.00	70.00	66.00	HS
64	Lodi	68.00	65.00	72.00	68.33	HS
65	$K_1R_{36}A_{1}$	70.00	65.00	72.00	69.00	HS
66	$K_1R_{35}A_{40}$	65.00	67.00	75.00	69.00	HS
67	Neomi	65.25	67.75	75.00	69.33	HS
68	$K_1R_{32}A_{53}$	66.15	67.00	75.00	69.38	HS
69	Summered	75.00	65.00	70.00	70.00	HS
70	$K_1R_{33}A_{25}$	70.25	65.00	75.15	70.13	HS
71	Skyline Supreme	65.00	76.00	70.00	70.33	HS
72	Tamma	70.25	67.75	73.25	70.42	HS
73	Tropical Beauty	70.00	68.00	76.00	71.33	HS
74	EC 43563	70.00	68.00	78.00	72.00	HS
75	Early McIntosh	70.25	68.00	78.00	72.08	HS
76	Maayan	65.00	70.00	82.00	72.33	HS
77	EC 57504	70.00	75.00	72.00	72.33	HS
78	Aziza	72.00	76.00	70.00	72.66	HS
79	Golden Delicious (IRA 13-1-1)	73.00	75.00	70.00	72.66	HS
80	Shlomit	75.15	73.50	70.25	72.97	HS
81	EC 10225	70.00	74.00	75.00	73.00	HS
82	Starr	76.00	74.00	70.00	73.33	HS
83	Ida Red	75.00	68.00	78.00	73.66	HS
84	EC 161283 $P_2R_{24\text{-}20}$	75.00	70.00	78.00	74.33	HS
85	Starkrimson	80.00	70.00	75.00	75.00	HS

Sr. No.	Cvs./ scab resistant line	Per cent disease index (PDI)			Mean per cent disease index (PDI)	Reaction
		1997	1998	1999		
86	Early Shanbury	76.00	80.00	70.00	75.33	HS
87	Cox Orange Pippin	82.00	70.00	75.00	75.66	HS
88	Mollie's Delicious	82.00	70.25	75.15	75.80	HS
89	EC 115666	76.00	80.00	72.00	76.00	HS
90	Red Gold	70.00	72.00	90.00	77.33	HS
91	Golden Delicious	80.00	85.00	72.00	79.00	HS
92	Parlin's Beauty	80.00	82.00	75.00	79.00	HS
93	EC 161283 P_7R_{24-39}	80.00	72.00	85.00	79.00	HS
94	Emperor	80.00	76.00	82.00	79.33	HS
95	Starkremtent	80.00	76.00	82.00	79.33	HS
96	Paragon	75.15	80.00	83.25	79.47	HS
97	Michal	85.00	75.00	80.00	80.00	HS
98	Stark Summer Gold	75.15	85.00	80.00	80.05	HS
99	EC 38727	85.00	76.00	80.00	80.33	HS
100	Anna	80.00	78.00	85.00	81.00	HS
101	Bright-N-Early	82.00	80.00	85.00	82.33	HS

PDI ranging from 18.00 to 24.22 per cent while, the remaining cultivars/ scab resistant lines exhibited susceptible to highly susception reaction.

4.8 DISEASE MANAGEMENT

4.8.1 *In vitro* evaluation of fungicides

To find out the best fungicides for disease management, eight different fungicides were tested for their inhibitory effect on conidial germination of *Marssonina coronaria* under *in vitro* conditions. the results obtained after statistical analysis are presented in Table 4.13.

The results of *in vitro* studies presented in (Table 4.13) indicate that all the fungicides tested at three different concentrations inhibited the conidial germination of *M. coronaria* in comparison to control. Maximum inhibition (99.67%) of conidial germination was recorded with Bavistin plus Dithane M-45 followed by Dithane M-45 (99.14%) and Bavistin (88.66%), whereas least inhibition was recorded in case of Rubigan (91.60%) and Contaf (91.69%), though statistically at par with each other.

Of the various concentrations of fungicides tested, maximum inhibition (99.19%) of germination of conidia of *Marssonina coronaria* was registered in the highest concentration (C_3) followed by C_2 (95.25%) and C_1 concentration (92.38%). The data further reveal that with the increase in the concentration of fungicide, the percentage of conidial germination inhibition also increased. In case of Bavistin, the percentage of conidial germination inhibition was 96.60 per cent at C_1 (100 ppm) concentration which increased to 99.39 per cent and 100.00 per cent at C_2 (250 ppm) and C_3 (500 ppm) concentration, respectively. Similar results were obtained with other concentrations of different fungicides tested during the course of present investigation.

Table 4.13. ***In vitro*** **evaluation of fungicide against** ***M. coronaria*** **causing leaf blotch of apple**

Fungicides	Per cent inhibition at different concentrations (ppm)			Per cent germination inhibition after					Concent-ration (ppm)	Per cent germination inhibition after				
	C_1	C_2	C_3	24 h	48 h	72 h	96 h	Overall mean		24 h	48 h	72 h	96 h	Overall mean
Bavistin	96.60	99.39	100.00	100.00	99.56	97.89	97.21	98.66	C_1	99.29	92.90	89.46	87.87	92.38
	(9.83)*	(9.97)	(10.00)	(10.00)	(9.98)	(9.89)	(9.86)	(9.93)^{b}		(9.96)	(9.63)	(9.45)	(9.37)	(9.61)^{c}
Dithane M-45	97.62	99.81	100.00	100.00	99.56	98.79	98.23	99.14	C_2	99.33	95.84	93.60	92.28	95.26
	(9.88)	(10.00)	(10.00)	(10.00)	(9.98)	(9.94)	(9.91)	(9.96)^{ab}		(9.97)	(9.79)	(9.67)	(9.60)	(9.76)^{b}
Bavistin+Dithane M-45	99.01	100.00	100.00	100.00	100.00	99.70	98.99	99.67	C_3	100.00	99.83	99.34	97.57	99.19
	(9.95)	(10.00)	(10.00)	(10.00)	(10.00)	(9.99)	(9.95)	(9.98)^{a}		(10.00)	(10.00)	(9.97)	(9.88)	(9.61)^{a}
Captan	90.86	94.35	100.00	100.00	94.22	93.67	92.40	95.07						
	(9.53)	(9.71)	(10.00)	(10.00)	(9.70)	(9.68)	(9.61)	(9.75)^{d}						
Kavach	89.22	91.57	98.63	100.00	93.67	90.38	88.53	93.14						
	(9.44)	(9.57)	(9.93)	(10.00)	(9.68)	(9.50)	(9.41)	(9.65)^{c}						
Baycor	91.56	96.92	99.17	100.00	97.30	94.46	91.77	95.88						
	(9.56)	(9.84)	(9.96)	(10.00)	(9.86)	(9.72)	(9.58)	(9.79)^{c}						
Rubigan	87.70	89.67	97.43	98.10	92.64	89.04	86.63	91.60						
	(9.34)	(9.47)	(9.87)	(9.90)	(9.62)	(9.43)	(9.30)	(9.57)^{f}						
Contaf	86.44	90.35	98.26	98.21	92.58	89.12	86.83	91.69						
	(9.29)	(9.50)	(9.91)	(9.91)	(9.62)	(9.43)	(9.31)	(9.57)^{f}						
Overall mean	92.38 (9.61)^{c}	95.26 (9.76)^{b}	99.19 (9.96)^{a}	99.54 (9.98)^{a}	96.19 (9.80)^{b}	94.13 (9.70)^{c}	92.57 (9.62)^{d}			99.34 (9.98)^{a}	96.19 (9.80)^{b}	94.13 (9.70)^{c}	92.57 (9.62)^{d}	

	$CD_{0.05}$		$CD_{0.05}$		$CD_{0.05}$
Fungicide	0.037	Incubation period	0.026	Concentration x Incubation period	0.045
Concentration	0.023	Fungicide x Incubation period	0.073		
Fungicide x Concentration	0.063				

* Figures in parentheses are square root transformed values
Means followed by the same letter do not differ significantly

When the conidial germination inhibition of *M. coronaria* was tested at different incubation periods, it was found maximum (99.54%) after 24 hours of incubation period followed by 48 hours (96.19%) and 72 hours (4.13%) while minimum inhibition (92.57%) was recorded after 96 hours of incubation period. It is clear from the table that as the incubation period was increased from 24 hours to 96 hours, the percentage of conidial germination inhibition decreased. In Bavistin, the per cent conidial germination inhibition was 100.00 per cent after 24 hours which decreased to 97.21 per cent after 96 hours of incubation period. Other fungicides also showed similar tend of conidial germination inhibition.

The interactions between different concentrations of fungicides and incubation period reveals that with the increase in incubation period from 24 to 96 hours, per cent conidial germination inhibition of the fungus *M. coronaria* was decreased under all the concentrations tested. On the contrary as the concentration of fungicides was increased from C_1 to C_3 the per cent conidial germination inhibition was increased at all the incubation periods studied during the course of present study.

4.8.2 FIELD EVALUATION OF FUNGICIDES

In the present study, the fungicides found effective against *Marssonina coronaria* under *in vitro* conditions were further tested for their effectiveness under orchard conditions. The trial was laid out at the University Research Farm of Department of Fruit Breeding and Genetic Resources at Kalaghat (Nauni), Solan during the cropping season of 1998-99 with eight different fungicides sprayed at 15 days interval. The data obtained in respect of disease incidence, severity and per cent leaf fall are presented in Table 4.14 after statistical analysis.

Table 4.14. **Effect of fungicides on the control of *Marssonina* leaf blotch of apple**

Fungicide	Concentration (%)	Per cent leaf blotch incidence	Per cent disease control	Per cent disease severity	Per cent disease control	**Per cent leaf fall
Bavistin	0.05	22.69 *(28.44)[b]	74.87	18.33 (25.32)[b]	78.64	15
Dithane M-45	0.30	29.33 (32.79)[c]	67.52	26.25 (30.82)[c]	69.42	20
Bavistin + Dithane M-45	0.025 + 0.25	12.26 (20.50)[a]	86.42	8.75 (17.18)[a]	89.80	8
Captan	0.30	39.13 (38.72)[d]	56.67	35.00 (36.27)[d]	59.22	30
Kavach	0.20	46.08 (42.75)[e]	48.97	38.75 (38.50)[de]	54.85	45
Baycor	0.10	51.19 (45.68)[f]	43.31	40.83 (39.70)[e]	52.43	50
Rubigan	0.05	59.27 (50.34)[g]	34.36	48.33 (44.03)[f]	43.69	55
Contaf	0.05	56.00 (48.45)[h]	37.99	41.25 (39.96)[e]	51.92	50
Control (untreated)	-	90.30 (71.88)[i]		85.83 (67.92)[g]		95
$CD_{0.05}$		0.89		2.42		

*Figures in parenthesis are arcsin transformed values
** Recorded at the end of October, 1998
Means followed by the same letter do not differ significantly

The analysis of data presented in (Table 4.14) shows that incidence as well as severity of the disease were greatly reduced under all the treatments in comparison to control however, combined effect of treatment was much more than that of individual treatment. Maximum disease control (89.80%) was registered in Bavistin plus Dithane M-45 followed by Bavistin (78.64%) and Dithane M-45 (69.42%), though differed significantly amongst each other. Captan and kavach also provided more than 55 per cent disease control but the percetage of disease control was significantly much less than that of Bavistin, Dithane M-45 and their mixture. The EBI fungicides such as Baycor, Rubigan and Contaf were not found effective in the present study in comparison to other fungicides tested.

As far as the per cent leaf fall is concerned, maximum premature defoliation was registered in Rubigan (55%) followed by Contaf (50%), however, minimum was in Bavistin + Dithane M-45 (8%).

4.8.3 EVALUATION OF NUMBER OF SPRAYS OF FUNGICIDES

In this experiment, two three and five sprays of most effective fungicides i.e. Bavistin (0.05%), Dithane M-45 (0.3%) and Bavistin (0.025%) plus Dithane M-45 (0.25%) were evaluated for the management of leaf blotch of apple under field conditions during the cropping season of 1999. The data recorded on different parameters of the disease are presented in Table 4.15.

It is evident from the data (Table 4.15) that all the number of sprays of different fungicides tested during the course of present investigation were effective in controlling the disease over check (without spray) however, five spray of Bavistin plus Dithane M-45 were found significantly superior to all other treatments including five sprays of Bavistin and Dithane M-45 as 90.95 per cent disease control was achieved compared to 82.86 and 75.22 per cent with five sprays of Bavistin and Dithane M-45, respectively. The data further

Table 4.15. Evaluation of number of sprays of fungicides for the management of *Marssonina* leaf blotch of apple

Fungicide	Number of sprays	Per cent leaf blotch incidence	Per cent disease control	Per cent disease severity	Per cent disease control	**Per cent leaf fall
Bavistin (0.05%)	2	36.73 *(37.31)^{f}	60.00	29.17 (32.68)^{e}	66.66	20
	3	24.66 (29.77)^{c}	73.14	19.58 (26.25)^{cd}	77.62	15
	5	18.25 (25.29)^{b}	80.12	15.00 (22.78)^{b}	82.86	8
Dithane M-45 (0.30%)	2	39.06 (38.68)^{g}	57.46	32.92 (35.01)^{f}	62.38	30
	3	35.02 (36.28)^{e}	61.86	31.25 (33.98)^{ef}	64.29	20
	5	26.96 (31.28)^{d}	70.64	21.67 (27.73)^{d}	75.23	15
Bavistin (0.025%) + Dithane M-45 (0.25%)	2	24.32 (29.55)^{c}	73.51	18.75 (25.65)^{c}	78.57	12
	3	19.26 (26.03)^{b}	79.02	13.75 (21.75)^{b}	84.29	5
	5	14.19 (22.13)^{a}	84.54	7.917 (16.33)^{a}	90.95	5
Control		91.81 (73.39)^{h}		87.50 (69.31)^{g}		95
$CD_{0.05}$		0.82		1.98		

*Figures in parenthesis are arcsin transformed values
** Recorded at the end of October, 1999
Means followed by the same letter do not differ significantly

depict that three sprays of Bavistin plus Dithane M-45 were found at par with five sprays of Bavistin giving 84.29 and 82.86 per cent disease control respectively. This was followed by two sprays of Bavistin plus Dithane M-45 (78.57%) and five sprays of Dithane M-45 (75.23%), though differ significantly from each other whereas, three sprays of Bavistin (77.62%) were found statistically at par with both two sprays of Bavistin plus Dithane M-45 and five sprays of Dithane M-45. Least disease control (62.38%) was registered in two sprays of Dithane M-45 followed by three sprays of Dithane M-45 (64.29%) and two sprays of Bavistin (66.66%).

As regards the per cent leaf fall is concerned, minimum premature defoliation was registered in five and three sprays of Bavistin plus Dithane M-45 followed by five sprays Bavistin and two sprays of Bavistin plus Dithane M-45 however, maximum was in two sprays of Dithane M-45.

The study concludes that five sprays of Bavistin plus Dithane M-45 can be recommended for the management of Marssonina leaf blotch of apple.

DISCUSSION

5

DISCUSSION

Apple is one of the most important fruit crop of temperate region of the world. It is commercially grown in C.I.S. (erstwhile USSR), U.S.A., China, France, Italy, Turkey, Argentina, West Germany, Spain and Japan. In India, this fruit crop is grown in the state of Jammu & Kashmir, Himachal Pradesh, Sikkim, Arunachal Pradesh and hills of Uttar Pradesh at an altitude ranging from 1350-2600 m amsl. "An apple a day keeps the doctor away" this old saying itself speaks about the importance of apple in our daily diet. Apple being the principal fruit crop of Himachal Pradesh is commercially grown in 39 per cent of the total area under fruit crop (Anonymous, 1998a). The area under apple cultivation is increasing rapidly every year but the fruit production is decreasing inspite of using best plant protection measures. The reasons for low productivity are many but one of the foremost reasons is appearance of Marssonina leaf blotch of apple commonly known as premature leaf fall in small portion in Kullu valley during the year 1994. Since then, the disease has assumed serious epiphytotic proportions in almost all the apple growing areas of Himachal Pradesh. The annual loss to the tune of Rs. 300 crores has been estimated due to the disease in Himachal Pradesh during the year 1998 (Anonymous, 1998b). The disease has also been reported from other apple growing states of India, besides a score of countries such as Japan, Canada, Korea, China, Romania and Brazil. In view of the seriousness of the disease, the detailed investigations with regard to nutritional requirements of the pathogen including the disease management were planned and the results are documented in the text.

The symptoms of the disease produced by *Marssonina coronaria* on leaves as well as on fruits were 5-10 mm in diameter, grayish brown and often tinged purple at the periphery. Later on small black acervuli were visible on the surface of the spots. When lesions were numerous they coalesced, the surrounding tissue turned chlorotic and such leaves fell prematurely. During late summer and the rainy season, severe defoliation occurred on diseased plants and the affected orchards bore barren appearance where leafless plants were seen bearing small sized fruits.

Infection of the fruits was uncommon, however, under high disease pressure, clear brown spots appeared on the surface of the fruits, they were oval, depressed and became dark brown with age. Later on small, black acervuli were formed on the lesions. Similar types of symptom on leaves and fruits of apple caused by *Marssonina coronaria* were also observed by earlier workers (Miyake, 1907, Parmelee, 1971, Leite Junior *et al.*, 1986, Sharma and Kaul, 1997).

The pathogenicity tests of *Marssonina coronaria* conducted under *in vitro* conditions on healthy potted apple plants revealed that the typical symptoms of Marssonina leaf blotch on leaves appeared within 10-12 days after pathogen inoculation, however, complete defoliation of the leaves was observed 12 to 15 days after symptom development. The results of incubation period of the test pathogen *M. coronaria* causing leaf blotch in apple are in line with the findings of Sharma and Kaul (1997).

The morphological characters of pathogenic fungus causing leaf blotch of apple were studied in detail . Acervuli were subcuticular, mostly in concentric rings, rarely scattered, lens shaped and pitch dark. Conidia borne on small clavate conidiophores were bicelled, hyaline, constricted at the septum and guttulate. The acervuli on leaves measured 210.52-315.79 x 36.84-52.63 um and on fruits 200-300 x 34.12-47.37 um in size. The size of conidia varied

from 17.35-27.76 x 6.94-13.88 um on leaves and 15.84-26.6 x 6.94-10.41 um on fruits. Similar observations on morphological characters of *Marssonina coronaria* attacking apple have also been reported by Harada *et al.* (1974) and Sharma (1999).

The nutritional requirements of fungi vary from species to species. In the present study, the nutritional requirements of conidia of *Marssonina coronaria* were investigated with respect to the germination and germ tube formation. Of the various liquid media studied, maximum germination and germ tube length of conidia of *M. coronaria* were recorded on rose leaf extract followed by apple leaf extract, walnut leaf extract and sucrose asparagine medium, while least conidial germination and germ tube length were registered in Czapek Dox medium. Since no work of this kind has been reported on *M. coronaria*, therefore, the results of the present findings could not be compared. However, Sharma (1999) reported sucrose asparagine agar medium to be the best medium for mycelial growth of *M. coronaria*. Simpson and Hayes (1978) also recorded least growth of *Marssonina brunnea* causing poplar leaf blight on Czapek-Dox medium.

While investigating the effect of different temperatures on the germination and germ tube formation of conidia of *Marssonina coronaria*, maximum conidial germination and germ tube length were registered at 20°C followed by 25°C, while at 35°C, least conidial germination and germ tube length were observed. Results obtained in the present study are in agreement with the findings of Harada *et al.* (1974) who also reported 20°C as the optimum temperature for the vegetative growth of *Marssonina coronaria* causing leaf blotch of apple. Simpson and Hayes (1978) also registered no growth of *Marssonina brunnea* at temperature greater than 35°C.

Requirements for carbon source(s) vary from fungus to fungus and species to species. Of the various carbon sources tested, maximum germination

and germ tube length of conidia of *Marssonina coronaria* occurred on sucrose followed by glucose, while lactose supported least conidial germination and germ tube length. The results of the present findings are in conformity to those by Dolan (1947) on *Marssonina melonis* causing anthracnose of melons, Dhanvantri (1967) on *Marssonina* (*Diplocarpon earlianum*) causing leaf scorch of strawberry and Simpson and Hayes (1973) on *M. brunnea* causing poplar leaf blight.

In order to find out the best dosage of sucrose for maximum germination and germ tube formation of conidia of *Marssonina coronaria*, different concentrations of sucrose were tested under *in vitro* conditions. The results of the study revealed that higher concentrations of sucrose favoured the maximum germination and germ tube length of conidia of *M. coronaria* while lower concentrations were unfavourable for conidial germination and germ tube formation. Since no work has been done earlier on this aspect, therefore, the results could not be compared. However, Dolan (1947) while working on *Marssonina melonis* reported that the percentage of germination of spores of *Marssonina melonis* increased as the sugar (dextrose) concentration was increased.

Nitrogen is one of the essential ingredients required for the growth and development of any fungus. Out of six nitrogen sources tested, asparagine followed by potassium nitrate were found to be the best nitrogen sources for the germination and germ tube formation of conidia of *Marssonina coronaria*, while urea supported the least conidial germination and germ tube length of the test fungus. These results are in conformity to those reported by Simpson and Hayes (1978) on *M. brunnea* inciting poplar leaf blight.

While studying the effect of different concentrations of asparagine on germination and germ tube formation of conidia of *Marssonina coronaria*, 1.0 per cent concentration of asparagine was found optimum for conidial

germination and germ tube length, an increase or decrease in this concentration resulted in the decreased germination and germ tube length of the conidia of *M. coronaria*. There is no reported work of this kind on *M. coronaria* or its related species, therefore, the results of the present study are reported in original.

It is well known that a proper balance between carbon and nitrogen is essential for various cellular activities of the different fungi. To see the effect of different C:N ratios on the germination and germ tube formation of conidia of *Marssonina coronaria*, the amount of carbon and nitrogen present in glucose asparagine basal medium was varied. Maintenance of carbon level constant and increase in the nitrogen concentration resulted in significant reduction in conidial germination and germ tube length of *Marssonina coronaria* in the present study. On the other hand, when the carbon concentration was moderately increased with constant quantity of nitrogen, it resulted in increased germination and germ tube length of *M. coronaria* used upto a certain level (70:1 ratio). Any further increase in carbon resulted in significant decrease of conidial germination and germ tube length. Since no research work has been carried out on this aspect by the earlier workers on *M. coronaria* and other related species, therefore, the results of the present study could not be compared. Hence, the findings reported are new to science.

Studies undertaken on the use of vitamins revealed that vitamins were not essential for germination and germ tube formation of conidia of *Marssonina coronaria*. However, exogenous supply of ascorbic acid and folic acid increased the conidial germination and germ tube length over control. The results of the present study could not be compared as there is no reported work of this kind on *Marssonina coronaria*. However, Simpson and Hayes (1978) reported that *Marssonina brunnea* causing poplar leaf blight was dependent on pantothenic acid, pyridoxine and riboflavin for optimum growth.

Fungal requirements of micro nutrients have received much more attention than the macro nutrients. Among the various micro nutrients tested, ferrous sulphate gave the highest germination and germ tube length of conidia of *Marssonina coronaria* followed by ammonium molybdate. However, zinc sulphate had inhibitory effect on the conidial germination of the test fungus. There is no reported work on the utilization of micro nutrients for conidial germination and germ tube formation by *M. coronaria* and its related species, therefore, the results of the present findings are reported in original.

In the present study, eight different relative humidity levels varying from 56.8 to 100.0 per cent were tested for determining the optimum RH level required for enhanced germination and germ tube formation of conidia of *Marssonina coronaria*. No conidial germination and germ tube formation was recorded at RH 56.8 per cent, while maximum was found at 100 per cent RH followed by RH 98.5 and RH 96.9 per cent. The results of the present findings are in conformity with those reported by Dolan (1947) while working on *M. melonis* causing anthracnose of melons.

Studies carried out on the survival of *Marssonina coronaria* causing leaf blotch of apple revealed that fungus overwintered on the infected fallen leaves in the form of acervuli. This was contrary to the findings of Harada *et al.* (1974) who reported that the fungus *M. coronaria* overwintered in the form of apothecia on fallen leaves. When the viability of conidia was tested after 3, 6, 9 and 12 months after storage, it was observed that the percentage of conidial germination declined with the passage of time. These findings are in agreement with the findings of Palmer *et al.* (1966) who also reported decline in the virulence of conidia of *M. rosae* causing black spot of rose with the passage of time.

One hundred and one apple cultivars/ scab resistant lines were screened for resistance against *Marssonina coronaria* causing leaf blotch of apple for

three consecutive years under natural epiphytotic conditions. Of these, only two apple cultivars i.e. Tydeman's Early Worcester and Granny Smith were found moderately resistant against *M. coronaria*, while the remaining were in between moderately susceptible to highly susceptible category including the commercially grown Delicious cultivars. The results of the present study were contrary to the findings of Sharma and Verma (1999) who reported that the stray plants of cultivar Granny Smith remained free from the disease throughout the season. However, the results of present findings on apple cultivars like Golden Delicious, Red Gold, Royal Delicious were in line with the findings of Sharma and Gautam (1997).

Fungicides are the frontline weapons against pathogens and still considered to be the best means of disease control. In the present investigations, eight different fungicides at three different concentrations were tested *in vitro* by slide germination technique for their inhibitory effect on the conidial germination of *Marssonina coronaria*. All fungicides and their concentrations tested were inhibitory to the conidial germination in comparison to control. Maximum conidial germination inhibition was obtained with Bavistin plus Dithane M-45 followed by Bavistin and Dithane M-45. Of the various concentrations of different fungicides tested, maximum inhibition was achieved with highest concentration and minimum with lowest concentration. The interaction between different concentrations of fungicides and incubation periods were found to be significant. Effectiveness of the fungicides such as Captan against *Marssonina* spp. attacking poplars have also been reported by earlier workers under *in vitro* conditions (Spiers, 1978).

For the management of Marssonina leaf blotch of apple caused by *Marssonina coronaria*, a field trial was laid out at Research Farm of Department of Fruit Breeding and Genetic Resources, Nauni under natural epiphytotic conditions. The results revealed that Bavistin (0.025%) plus Dithane M-45 (0.25%) gave highest disease control when sprayed four times at 15 days

interval during the cropping season. The next best fungicides found effective in order of their importance were Bavistin (0.05%), Dithane M-45 (0.3%), Captan (0.3%) and Kavach (0.2%). These fungicides reduced the leaf blotch incidence, disease severity as well as per cent leaf fall significantly over control (unsprayed). Various workers have also reported the effectiveness of fungicides including Bavistin, Dithane M-45, Captan for the management of leaf blotch of apple caused by *Marssonina coronaria* (Kwang and Chong, 1962; Jiang *et al.*, 1997; Sharma and Kaul, 1997; Chang *et al.*, 1998).

Studies were also conducted to find out the number of sprays required for effective management of leaf blotch of apple caused by *Marssonina coronaria*. Fungicides namely, bavistin (0.025%) plus Dithane M-45 (0.25%), Bavistin (0.05%) and Dithane M-45 (0.3%) found highly efficaceous under field conditions were further evaluated for their comparative effectiveness. The results of the field trial revealed that five sprays of Bavistin plus Dithane M-45 gave significantly highest control of the disease than five sprays of Bavistin and Dithane M-45. Even three sprays of Bavistin plus Dithane M-45 were found at par with five sprays of Bavistin. However, least disease control was recorded with two sprays of Dithane M-45. Information on this aspect of disease control is lacking in literature, therefore, results of present investigations are reported in original.

SUMMARY

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SUMMARY

The present investigations on "Studies on Marssonina Leaf Blotch of Apple" were conducted in the Fruit Pathology Laboratory of Department of Mycology and Plant Pathology UHF, Nauni during 1997-1999. The various aspects studied were:

i) Symptomatology
ii) Pathogenicity
iii) Nutritional requirements of the Marssonina leaf blotch pathogen
iv) Management of Marssonina leaf blotch of apple

The symptoms of the disease were studied in detail. Symptoms of the disease were formed mostly on the leaves, however, symptoms on fruits were uncommon.

Based on morphological characters i.e. shape and size of conidia and acervuli, the pathogenic fungus was identified as *Marssonina coronaria* (Ell. et J.J. Davis) J.J. Davis.

Pathogenicity test conducted under *in vitro* conditions revealed that the typical symptoms of Marssonina leaf blotch appeared on young potted apple plants within 10 to 12 days of inoculation and complete defoliation was observed 12 to 15 days after the appearance of first visible symptom.

Studies on nutritional requirements of conidia of *Marssonina coronaria* were carried out under *in vitro* conditions. Excellent germination of the conidia

of *M. coronaria* was recorded on natural media than on semi-synthetic and synthetic media. Amongst the nine liquid media tested, rose leaf extract, apple leaf extract, walnut leaf extract and sucrose asparagine medium were adjudged the best media for maximum conidial germination and germ tube formation of *M. coronaria*.

The conidia of the fungus could germinate over a wide range of temperature (10-35°C), however, extreme low (<10°C) and high temperatures (>35°C) were unfavorable for the conidial germination and germ tube formation of *Marssonina coronaria*. A temperature of 20°C was found optimum for germination and germ tube formation of conidia of *M. coronaria*.

Of the various carbon sources tested sucrose was adjudged the best carbon source for conidial germination and germ tube formation of *Marssonina coronaria*. The fungus preferred higher concentrations of sucrose (>1%) for conidial germination and germ tube formation with maximum being at 2.5 per cent concentration of sucrose.

Asparagine followed by potassium nitrate were the best nitrogen sources for the germination and germ tube formation of conidia of the test fungus. Lower dosages of asparagine (<1.5%) favoured the conidial germination and germ tube formation while, high dosages were unfavourable, and 1.0 per cent concentration of asparagine was found optimum for conidial germination and germtube formation of *Marssonina coronaria*.

When different carbon: nitrogen (glucose:asparagine) ratios were tested, best conidial germination and germ tube formation of *M. coronaria* were recorded in the ratio of 70:1.

Out of six vitamines, ascorbic acid enhanced the germination and germ tube formation of conidia of *Marssonina coronaria* by 58.07 per cent and 76.32 um over 27.60 per cent and 43.22 um in control, respectively.

Ferrous sulphate followed by ammonium modybdate proved to be the best micro-nutrient for maximum germination and germ tube length of the test fungus.

Maximum conidial germination and germ tube formation of *Marssonina coronaria* was recorded at higher relative humidity levels, however, optimum range for conidial germination and germ tube formation varied between 96.10 to 100 per cent relative humidity.

The fungus *Marssonina coronaria* overwintered in the infected leaves in the form of fruiting bodies (acervuli) under orchard, refrigerator and laboratory conditions. The conidia of the fungus in these conditions remained viable in the acervuli for one year.

Out of one hundred and one, cvs./scab resistant lines of apple screened for resistance against *Marssonina coronaria* causing leaf blotch of apple two i.e. Granny Smith and Tydeman's Early Worcester were found moderately resistant, seven moderately susceptible while the remaining cvs/lines were in susceptible to highly susceptible category under natural epiphytotic conditions.

Out of the various fungicides tested at different concentrations under *in vitro* conditions, Bavistin (500 ppm), Dithane M-45 (3000 ppm), Bavistin (100 ppm) + Dithane M-45 (2000 ppm) and captan (3000 ppm) completely inhibited the germination of conidia of *Marssonina coronaria*.

While testing the effectiveness of the different fungicides under field conditions, combination of Bavistin (0.025%) and Dithane M-45 (0.25%)

provided excellent control of Marssonina leaf blotch of apple followed by Bavistin (0.05%), Dithane M-45 (0.3%) and Captan (0.3%).

In order to find out the number of sprays of fungicides required to combat the disease, five sprays of Bavistin plus Dithane M-45 were found significantly superior in controlling the disease and premature leaf fall of apple caused by *Marssonina coronaria* followed by three sprays of Bavistin plus Dithane M-45 and five sprays of Bavistin.

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APPENDICES

APPENDIX-I

Rose leaf extract medium	
Rose leaves (crushed)	40.00 g
Distilled water	1000.00 ml
Apple leaf extract medium	
Apple leaves (crushed)	40.00 g
Distilled water	1000.00 ml
Walnut leaf extract medium	
Walnut leaves (crushed)	40.00 g
Distilled water	1000.00 ml
Poplar leaf extract medium	
Poplar leaves (crushed)	40.00 g
Distilled water	1000.00 ml
***Potato dextrose agar medium**	
Peeled potato	250.00 g
Distilled water	1000.00 ml
Glucose asparagine medium	
Glucose ($C_6H_{12}O_6$)	30.00 g
Asparagine ($N_2H_8C_4O_3$)	1.00 g
Potassium dihydrogen phosphate (KH_2PO_4)	1.50 g
Magnesium sulphate ($MgSO_4.7H_2O$)	0.50 g
Distilled water	1000.00 ml
***Sucrose asparagine medium**	
Sucrose ($C_{12}H_{22}O_{11}$)	30.00 g
Asparagine ($N_2H_8C_4O_3$)	1.00 g
Potassium dihydrogen phosphate (KH_2PO_4)	1.50 g
Magnesium sulphate ($MgSO_4.7H_2O$)	0.50 g
Distilled water	1000.00 ml
Richard's medium	
Potassium nitrate (KNO_3)	10.00 g
Magnesium sulphate ($MgSO_4.7H_2O$)	2.50 g
Potassium dihydrogen phosphate (KH_2PO_4)	5.00 g
Ferric chloride	0.02 g
Sucrose ($C_2H_{22}O_{11}$)	50.00 g
Distilled water	1000.00 ml
Czapek-Dox medium	
Sodium nitrate ($NaNO_3$)	2.00 h
Potassium dihydrogen phosphate (KH_2PO_4)	1.00 g
Potassium chloride (KCl)	0.50 g
Magnesium sulphate ($MgSO_4.7H_2O$)	0.50 g
Ferrous sulphate ($FeSO_4$)	0.01 g
Sucrose ($C_{12}H_{22}O_{11}$)	30.00 g
Distilled water	1000.00 ml
Coon's medium	
Potassium nitrate (KNO_3)	2.00 g
Magnesium sulphate ($MgSO_4.7H_2O$)	1.20 g
Potassium phosphate (KH_2PO_4)	2.70 g
Maltose ($C_{12}H_{22}O_{11}$)	7.20 g
Starch	10.00 g
Distilled water	1000.00 ml

* For preparing solid media 2% agar to be added

APPENDIX-II

Quantities of glucose asparagine (g)	C:N ratio
30:5	12.8686:1
30:4	16.0858:1
30:3	21.4477:1
30:2	32.1716:1
30:1	64.3432:1
40:1	85.7910:1
50:1	107.2386:1
60:1	128.6863:1
70:1	150.1341:1

APPENDIX-III

Relative humidity control by concentrated H_2SO_4

Per cent sulphuric acid	Per cent relative humidity at 25°C
0	100.0
5	98.5
10	96.1
15	92.9
20	88.5
25	82.9
30	75.6
40	56.8

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THESIS ABSTRACT

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Title of Thesis	:	Studies on Marssonina leaf blotch of apple
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ABSTRACT

Apple (*Malus domestica* Borkh.) is one of the most important fruit crop of temperate region of the world. Marssonina leaf blotch of apple has emerged as a serious threat to the successful apple cultivation in Himachal Pradesh since 1994. The present investigations were carried out to find out the nutritional requirements of leaf blotch pathogen, *Marssonina coronaria* and to devise a suitable strategy for management of leaf blotch of apple. The symptoms of the disease started appearing in mid-summer, as brown to dark brown spots on the mature leaves. These lesions coalesced together to form large spots (blotch) and the surrounding areas turned chlorotic followed by severe defoliation. Black to dark brown oval, depressed spots also appeared on fruits however, infection on fruit was uncommon. The pathogenic fungus produced fruiting bodies (acervuli) both on infected leaves and fruits. Based on morphological characters i.e. shape and size of conidia and acervuli, the pathogenic fungus was identified as *Marssonina coronaria* (Ell. Et J.J. Davis) J.J. Davis. The pathogenicity test of the fungus was conducted and the symptoms of the disease were observed on young potted apple plants 10-12 days after inoculation with conidial suspension of *M. coronaria* and defoliation started 15 days after the appearance of disease symptoms. Rose leaf extract medium, sucrose (2.5%), asparagine (1%), C:N ratio of 70:1, ascorbic acid (1%) and $FeSO_4$ (0.01%) enhanced the germination and germ tube length of conidia of *M. coronaria*. A temperature of 20°C and relative humidity of 100 per cent were found optimum for conidial germination and germ tube formation of *M. coronaria*. The fungus overwintered in the form of fruiting bodies (acervuli) on the infected fallen leaves. Out of 101 apple cultivars/scab resistant lines, two apple cultivars i.e. Granny Smith and Tydeman's Early Worcester were found moderately resistant to *M. coronaria*. Under *In vitro* conditions, Bavistin (500 ppm), Dithane M-45 (3000 ppm), Bavistin plus Dithane M-45 (100 ppm + 2000 ppm) and Captan (3000 ppm) completely inhibited the germination of conidia of *M. coronaria*. However, under orchard conditions, combination of Bavistin (0.025%) and Dithane M-45 (0.25%) provided excellent control of Marssonina leaf blotch of apple followed by Bavistin (0.05%), Dithane M-45 (0.3%) and Captan (0.3%). Five sprays of Bavistin plus Dithane M-45 were found significantly superior in controlling the disease than that of five sprays of Bavistin and Dithane M-45 followed by three sprays of Bavistin plus Dithane M-45 and five sprays of Bavistin.

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