EPIDEMIOLOGY AND MANAGEMENT OF ANTHRACNOSE FRUIT ROT IN STRAWBERRY

Thesis

Submitted to the Punjab Agricultural University
in partial fulfillment of the requirements
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MASTER OF SCIENCE
in
PLANT PATHOLOGY
(Minor Subject: Entomology)

By

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(L-2014-A-122-M)

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CERTIFICATE – I

This is to certify that the thesis entitled, **“Epidemiology and management of anthracnose fruit rot in strawberry”** submitted for the degree of **M.Sc.** in the subject of **Plant Pathology** (Minor Subject: **Entomology**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Simran Jeet Kaur (L-2014-A-122-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

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

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CERTIFICATE – II

This is to certify that the thesis entitled, **"Epidemiology and management of anthracnose fruit rot in strawberry"** submitted by **Simran Jeet Kaur (L-2014-A-122-M)** to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **M.Sc.** in the subject of **Plant Pathology** (Minor subject: **Entomology**) has been approved by the Student's Advisory Committee along with Head of Department after an oral examination on the same, in collaboration with an External Examiner.

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ABSTRACT

Among the strawberry diseases, anthracnose (*Colletotrichum gloeosporioides*) plays a significant role in causing yield losses. During the surveillance it was observed that the disease is prevalent in Barnala, Gurdaspur, Hoshiarpur, Ludhiana, Patiala and Ropar districts with highest disease index in Gurdaspur (22.3 %) and lowest in Barnala (14.8 %). Chandler variety was found most susceptible. The disease produce brown to black necrotic lesions on leaves, green fruits and ripe fruits resulting blight and fruit rot. For pathogenicity test, pin prick method of inoculation was better than spray method. The pathogen was identified as *Colletotrichum gloeosporioides* on the basis of colony colour, shape and size of conidia and acervuli production. Acervuli are not produced in the culture but on plant tissues. Black and erect setae are produced in acervuli. A temperature of 25°C was found significantly effective in favouring the mycelial growth, sporulation, spore germination and development of disease symptoms on leaves (5 days), green fruits (6 days), mature fruits (3 days) and strawberry plants (5 days). The inoculum concentration of 10^7spores/ml was found significantly better in producing disease symptoms on plant parts. A wetness duration of 24 hours significantly favoured the development of more disease symptoms. *In vitro*, propiconazole systemic fungicides and mancozeb non-systemic fungicide were significantly effective in inhibiting the growth of pathogen. ED_{50} value of propiconazole was < 5 ppm and mancozeb was<25 ppm. Three sprays of propiconazole and mancozeb were found significantly better in controlling fruit rot of strawberry.

Keywords: Strawberry, *Colletotrichum gloeosporioides*, Prevalence, Pathogenicity, Symptomology, Epidemiology, Management

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ਸਾਰ

ਸਟ੍ਰਾਬੇਰੀ ਐਂਥਰਾਕਨੋਜ਼ (*ਕੌਲੀਟੋਟ੍ਰਾਈਕਮ ਗਲੀਓਸਪੋਰੀਓਇਡਸ*) ਇਕ ਭਿਆਨਕ ਰੋਗ ਹੈ ਜੋ ਝਾੜ ਤੇ ਮਾੜਾ ਅਸਰ ਪਾਉਂਦਾ ਹੈ।ਪੰਜਾਬ ਦੇ ਵੱਖ-ਵੱਖ ਜ਼ਿਲ੍ਹਿਆਂ ਦੇ ਸਰਵੇਖਣ ਤੋਂ ਪਤਾ ਚੱਲਿਆ ਕਿ ਇਹ ਰੋਗ ਬਰਨਾਲਾ, ਲੁਧਿਆਣਾ, ਹੁਸ਼ਿਆਰਪੁਰ, ਗੁਰਦਾਸਪੁਰ, ਪਟਿਆਲਾ ਅਤੇ ਰੋਪੜ੍ਹ ਵਿੱਚ ਆਮ ਪਾਇਆ ਜਾਂਦਾ ਹੈ।ਇਸ ਦਾ ਵੱਧ ਹਮਲਾ ਗੁਰਦਾਸਪੁਰ (22.3%) ਅਤੇ ਘੱਟ ਬਰਨਾਲਾ (14.8%) ਵਿੱਚ ਹੈ।ਚਾਂਦਲਰ ਕਿਸਮ ਤੇ ਸਭ ਤੋਂ ਵੱਧ ਬਿਮਾਰੀ ਪਾਈ ਗਈ।ਇਹ ਰੋਗ ਪੱਤਿਆਂ, ਹਰੇ ਫਲਾਂ ਅਤੇ ਪੱਕੇ ਫਲਾਂ ਤੇ ਗੂੜ੍ਹੇ ਭੂਰੇ ਜਾਂ ਕਾਲੇ ਧੱਬਿਆਂ ਦੇ ਰੂਪ ਵਿੱਚ ਜ਼ਾਹਿਰ ਹੁੰਦਾ ਹੈ ਅਤੇ ਫ਼ਲ ਦੇ ਗਲਣ ਦਾ ਕਾਰਨ ਬਣਦਾ ਹੈ।ਪੈਥੋਜੈਨੇਸਿਟੀ ਟੈਸਟ ਲਈ ਪਿੰਨ ਚੋਭ ਤਰੀਕਾ, ਸਪ੍ਰੇਅ ਤਰੀਕੇ ਨਾਲੋਂ ਵਧੀਆ ਪਾਇਆ ਗਿਆ।ਕਲੌਨੀ ਦੇ ਰੰਗ, ਕਣਾਂ ਦੇ ਆਕਾਰ ਅਤੇ ਸ਼ਕਲ, ਅਸਰਵੁਲੀ ਦੀ ਪੈਦਾਇਸ਼ ਅਤੇ ITCC, IARI, ਨਵੀਂ ਦਿੱਲੀ ਦੀ ਰਿਪੋਰਟ ਦੇ ਅਧਾਰ ਤੇ *ਕੌਲੀਟੋਟ੍ਰਾਈਕਮ ਗਲੀਓਸਪੋਰੀਓਇਡਸ* ਉੱਲੀ ਨੂੰ ਬਿਮਾਰੀ ਲੱਗਣ ਦਾ ਕਾਰਨ ਪਾਇਆ ਗਿਆ।ਅਸਰਵੁਲੀ ਦੀ ਪੈਦਾਵਾਰ ਕਲਚਰ ਵਿੱਚ ਨਹੀਂ ਹੁੰਦੀ ਬਲਕਿ ਪੌਦੇ ਦੇ ਤੰਤੂ ਵਿੱਚ ਹੁੰਦੀ ਹੈ।ਪੱਤਿਆਂ (5 ਦਿਨ), ਹਰੇ ਫਲਾਂ (6 ਦਿਨ), ਪੱਕੇ ਫਲਾਂ (3 ਦਿਨ) ਅਤੇ ਸਟ੍ਰਾਬੇਰੀ ਪੌਦੇ (5 ਦਿਨ) ਤੇ ਰੋਗ ਪੈਦਾ ਕਰਨ ਦੀ ਸ਼ਕਤੀ 25°C ਤਾਪਮਾਨ ਤੇ ਸਾਰਿਆਂ ਨਾਲੋਂ ਵੱਧ ਪਾਈ ਗਈ ।ਬਿਮਾਰੀ ਨੂੰ ਪੈਦਾ ਕਰਨ ਲਈ 10^7 ਕਣ/ਮਿ:ਲੀ ਦੀ ਮਾਤਰਾ ਪੱਤਿਆਂ (5 ਦਿਨ), ਹਰੇ ਫਲਾਂ (5 ਦਿਨ), ਪੱਕੇ ਫਲਾਂ (3 ਦਿਨ) ਅਤੇ ਪੂਰੇ ਪੌਦੇ (5 ਦਿਨ) ਵਿੱਚ ਪ੍ਰਭਾਵਸ਼ਾਲੀ ਰਹੀ। ਬਿਮਾਰੀ ਦੇ ਵਾਧੇ ਲਈ 24 ਘੰਟੇ ਦਾ ਗਿੱਲਾਪਣ ਬੇਹਤਰ ਪਾਇਆ ਗਿਆ।ਇਨਵਿਟਰੋ ਨਰੀਖਣ ਵਿੱਚ, ਸਸਟੈਮਿਕ ਉੱਲੀਨਾਸ਼ਕ ਵਿੱਚੋਂ ਪ੍ਰੋਪੀਕੋਨਾਜ਼ੋਲ ਅਤੇ ਨਾਨ-ਸਿਸਟੈਮਿਕ ਵਿੱਚ ਮੈਕੋਜ਼ੈਬ ਰੋਗ ਨੂੰ ਰੋਕਣ ਲਈ ਸਭ ਤੋਂ ਵਧੀਆ ਪਾਏ ਗਏ। ਪ੍ਰੋਪੀਕੋਨਾਜ਼ੋਲ ਅਤੇ ਮੈਂਕੋਜ਼ੇਬ ਦੀਆਂ ਤਿੰਨ ਸਪਰੇਆਂ ਸਟ੍ਰਾਬੇਰੀ ਫਰੂਟ ਰੌਟ ਦੀ ਰੋਕਥਾਮ ਲਈ ਬਹਿਤਰ ਪਾਈਆਂ ਗਈਆਂ।

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CHAPTER – I

INTRODUCTION

Strawberries (*Fragaria* × *ananassa* Duch) are the members of family Rosaceae, subfamily Rosoideae and genus *Fragaria*. These are grown in subtropical and temperate countries like USA, Japan, Turkey, Spain, Egypt, Mexico, Poland, Korea and Israel (FAO 2012). In India, it is grown in Himachal Pradesh, Jammu & Kashmir, Uttar Pradesh, Maharashtra, West Bengal, Punjab, Haryana, Delhi, Nilgiri hills and Tamil Nadu. It occupies an area of 0.83 thousand hectares, with annual production of 8.42 MT (Anonymous 2015).

Strawberry plants are attacked by number of diseases like anthracnose, grey mould, *Phytophthora* crown rot, leaf spots and leaf blight which effects its foliage, fruits and roots. Among these, anthracnose is economically important causing nearly 60-75 per cent fruit losses (Smith 2008). In the United States, losses attributed to fruit rot have been greater than 30 per cent when warm and humid conditions favor serious epidemics (Howard *et al* 1992). The term 'anthracnose' is defined as a disease that appears as black, sunken lesions on leaves, stems and fruits (Agrios 1988). Legard *et al* (2003) reported that anthracnose fruit rot is more common on green and ripe fruits by producing round, firm and sunken spots.

Anthracnose of strawberry is caused by *Colletotrichum* species namely *C. fragariae* Brooks, *C. gloeosporioides* (Penz.) Penz. et Sacc. and *C. acutatum*. These three species of *Colletotrichum* can infect most plant tissue and economically devastating phases are crown rot and fruit rot (Simmond 1965, Howard and Albregts 1984, Smith and Black 1990, Howard *et al* 1992 and Freeman and Katan 1997). *Colletotrichum gloeosporioides* (Teleomorph: *Glomerella cingulata*) have grey or olive-grey colonies, dark grey to dark olive in reverse, cylindrical conidia, and asci in culture (Smith and Black 1990). The presence or absence of setae has also been used for identification of *Colletotrichum* species (Gubler and Gunnell 1991). Conidia are straight, cylindrical and oval and borne on distinct well developed hyaline conidiophores (Sattar and Malik 1939). The size of conidia varied from 11-16 x 4-6 µm and 13.8 x 4.8 µm, broad oblong with rounded ends 14.0 x 3.7 µm (Simmonds 1965 and Bose *et al* 1973).

The pH between 6-7, temperature 25-30ºC and 4 hours wetness duration favor the conidial germination, appressorial production and secondary spread of *C. gloeosporioides* (Leandro *et al* 2003). Conidial concentration of 10^5 and 10^6 conidia/ml produces the most severe symptoms (Bertetti *et al* 2009).

For its management, spray of prochloraz-Mn, propiconazole, captafol or benomyl, azoxystrobin, pyraclostrobin and combination of pyraclostrobin + boscalid, cyprodinil + fludioxonil, captan + fenhexamid have been exploited under field and green house conditions (Wedge *et al* 2007). The causal agent of strawberry anthracnose and various factors favoring the disease development have not been systematically studied under Punjab conditions. Therefore, keeping in view the importance and losses caused by the anthracnose, present study was undertaken with the following objectives:

(i) To isolate and identify the pathogen(s) associated with anthracnose/fruit rot of strawberry.

(ii) Management of anthracnose fruit rot of strawberry.

CHAPTER - II

REVIEW OF LITERATURE

The relevant literature pertaining to the various aspects of epidemiology and management of anthracnose fruit rot of strawberry in Punjab has been reviewed under following subheads:

2.1 Historical account and distribution of disease

Anthracnose of strawberry caused by *Colletotrichum* species is a persistent problem in many strawberry growing regions causing huge losses in yield. Brooks (1931) reported that since 1930s, anthracnose crown rot caused by *Colletotrichum* species has been a destructive disease in strawberry nurseries and fields in the Southeastern United States. The fungus causing anthracnose fruit rot of strawberry had also been reported from Queensland, Australia and later from strawberry growing areas of the world (Simmonds 1965). The presence of the anthracnose fruit rot pathogen, *C. acutatum*, was first reported on strawberry in the U. S. by Smith and Black in 1986. The primary source of inoculum was from the annual commercial crop of strawberry (Howard *et al* 1992).

The pathogen infects all plant parts resulting in substantial losses of fruit production (Freeman and Katan 1997). *Colletotrichum fragariae* and *C. gloeosporioides* were responsible for causing a destructive disease of strawberry in nurseries and fields in the Southeastern USA (Smith 1998a). The disease has also caused yield loss in many strawberry producing regions like North and South America and Europe (Kososki *et al* 2001). Legard and Mackenzie (2003) reported that during 2001-02 anthracnose fruit rot incidence was more than 70 per cent.

Mertely *et al* (2005) reported that anthracnose fruit rot caused by *C. acutatum* Simmonds, was a major strawberry disease in Florida and worldwide affecting fruits, leaves, petioles, flowers, crowns and roots. Under favorable weather conditions, lesions were expanded and completely covered the surface of the fruits, especially on highly susceptible cultivars (Seijo *et al* 2008). In USA, anthracnose fruit rot was observed in states like Florida, California, New York, Ohio, Pennsylvania as well as others (Strand 2008).

Guerber and Correll (2001) described *G. acutata* as telomorphic stage of *C. acutatum*. Since 1980s, with the introduction of new cultivars of strawberry viz. Nyohou and Toyonoka, the disease became more serious in many areas of Japan. The disease severely damaged the strawberry industry of Tochigi Prefecture which is the largest producer of strawberry fruits from last 44 years and many farmers have stopped the cultivation.

2.2 Symptomatology

The anthracnose produce symptoms of sunken spots of various colors on leaves, stems, fruits or flowers causing wilting, withering and dying of infected plant tissues of strawberry (Hiremath *et al* 1993 and Vidyalakshmi and Divya 2013).

Brooks (1931) stated that symptoms of anthracnose fruit rot include sunken necrotic lesions with abundant conidia in acervuli. *Colletotrichum* crown rot was characterized by reddish-brown necrotic areas in the crown, which ultimately causes wilting and death of the plant. The later stages of lesion development are accompanied by eruption of pink, slimy spore masses through the plant surfaces as underlying acervuli matured (Jefferies *et al* 1990).

Agrios (1988) and Bailey *et al* (1992) defined anthracnose a disease that appears as black, sunken lesions on leaves, stems or fruit and is caused by fungi that produce their asexual spores in an acervulus. Anthracnose appears on developing and mature plant tissues and in some fields after planting, stunting and yellowing of plants also occurred.

Colletotrichum species causes stolon and petiole lesions, which are dark brown, black, sunken and often girdle the petiole or stolon (Howard *et al* 1992 and Heidenrich and Turechek 2013). Near the center of the lesions, pink masses of conidia are usually visible. Anthracnose crown rot (*C. gloeosporioides*) is characterized by wilting of the youngest leaves (Smith 1998a). *C. fragariae* and *C. gloeosporioides* caused black leaf spots which are characterized by grey or light black spots, peppered across the top surface of strawberry leaflets and also cause root lesions. *Colletotrichum* species also incite flower blights and fruit rots (Smith 1998b).

Fruit infection started as small, pin head sized spots on unripe fruits and gradually enlarged to attain a size of 5 to 6 mm in diameter. The spots were brown to black in color, sunken and bear minute black stomata in center of lesions. Under moist conditions, creamy spore masses are produced. Several spots coalesced to form bigger, hard and corky lesions which induce cracks.

On green fruits small, sunken, oval to round brown spots and on red fruits black spots develop which may expand to cover most or the entire fruit surface. The decayed tissues become firm and dry (Anonymous 2014). On mature fruits, softening of tissues occurs and lesions attain a size of 10 to 20 mm causing fruit malformation (Tandon and Singh 1969). The wilted leaves may appear to recover and become turgid in the evening; however, most will wilt and die after few days. A red discoloration appears within the crown tissue and pathogen can be isolated from the discolored tissue. *C. acutatum* may also cause crown death, typically a single side of the crown rather than the entire crown and infected plants are stunted but do not die.

2.3 Etiology of strawberry anthracnose

Nicholson and Moraes (1980) reported that *C. gloeosporioides* (Penz.) Penz. & Sacc. was disease causing organism (parasite), isolated from deteriorated plant parts (saprophyte) or living inside plant tissue asymptomatically as endophytic fungi.

Singh and Sharma (1982) reported *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk as a perfect stage of *C. gloeosporioides* (Penz.). Ishikawa (2013) reported that in

Japan, anthracnose caused by *G. cingulata* and *C. acutatum* Simmonds was the serious disease of strawberry (*Fragaria* × *ananassa*).

Grand *et al* (1990) reported that in North Carolina, anthracnose fruit rot or ripe rot of strawberry was caused by *C. acutatum*. Fruit rot was usually associated with *C. acutatum* (Smith and Black 1990). Three species of *Colletotrichum* namely *C. gloeosporioides*, *C. acutatum* and *C. fragariae* were responsible for anthracnose diseases of strawberries and *C. acutatum* was economically important (Howard *et al* 1992, Maas 1998 and Smith 1998a).

Crown rot was usually associated with *C. fragariae* and sometimes *C. gloeosporioides* and became the dominant species in the other regions of U. S. such as California (Eastburn and Gubler 1992) and Ohio (Ellis and Madden 1993).

Peres *et al* (2005) reported that anthracnose diseases of strawberry are caused by *Colletotrichum acutatum* J. H. Simmonds, *C. fragariae* Brooks and *C. gloeosporioides* (Penz.) Penz & Sacc. (teleomorph: *Glomerella cingulata* (Stoneham.) Schrenk & Spaulding). These fungi infected the most plant tissue but crown rot and fruit rot were devastating phases. The anthracnose was also serious disease of strawberry affecting foliage, runners, crowns and fruit (Truecheck *et al* 2006). The telomorphic stage of *C. acutatum* is *G. cingulata* (Stoneham.) Spaulding & Schrenk (Guerber and Correl 2001).

2.4 Morphology and taxonomy of pathogen

Gubler and Gunnell (1991) reported that isolates of *C. gloeosporioides* (Teleomorph: *G. cingulata*) have grey or olive-grey colonies, dark grey to dark olive in reverse, cylindrical conidia and asci in culture. Setae are usually long brownish coloured emerged from acervuli. Conidia are straight, cylindrical and oval and borne on distinct well developed hyaline conidiophores (Sattar and Malik 1939). Baxter *et al* (1983) defined *C. gloeosporioides* aggregate by using morphological methods and reported that conidia are cylindrical with rounded ends and less than 4.5 µm in diameter.

Conidia of three species of *Colletotrichum* causing anthracnose of strawberry are pink to orange in mass. Conidia of *C. gloeosporioides* are cylindrical and 92 per cent pointed on one end, hyaline, one-celled, ovoid to oblong, slightly curved or dumbbell shaped. Conidia are 10-20 µm long and 4.4-7 µm in width. Conidia of *C. fragariae* are either pointed at both ends or rounded at both ends with conidial size 12.4-15 µm long and 4.4-5.2 µm wide. Conidia of *C. acutatum* are either fusiformed or tapering to one end or rounded at both ends with average length 12.3 µm and width 4.4 µm. Appresoria of *C. gloeosporioides* are more lobed clavate than *C. acutatum*. *C. gloeosporioides* usually produces appresoia in 2 to 3 days. Setae may or may not be produced in cultures of *C. gloeosporioides*. The conidiomata are acervular, separate, composed of hyaline to dark brown septate hyphae. The fungus produces setae which are long, brown and septate. Variation in size and shape of conidia of *C. gloeosporioides* is dependent upon the host from which the pathogen is isolated and its area of origin (Simmonds

1965, Bose *et al* 1973, Smith and Black 1990, Sutton 1992 and Ivanovic *et al* 2007).

The mycelium of growing culture of *Colletotrichum* is hyaline, septate and branched. Normally the conidia may be oblong with obtuse ends. The waxy acervuli produced in infected tissues are sub epidermal, typically with setae and simple, short, erect conidiophores (Freeman *et al* 1998).

Setae are not produced in the culture but are present in diseased strawberry fruits which are long, brown and septate. Pigmented appressoria are produced after the germination of the conidia and vary in shape and size. There is no sexual stage formation under the laboratory conditions.

Colletotrichum gloeosporioides is an asexual facultative parasite belongs to the family Phyllachoraceae of the division Ascomycota. *Glomerella cingulata* is the sexual (perfect) teleomorph state of *Colletotrichum gloeosporioides*. *Glomerella cingulata* occurred on a broad range of host species and produces acervuli within the host tissue during asexual (mitotic) phase of their life cycle. The teleomorph state is known for their ability to cause serious disease (Cannon *et al* 2012).

2.5 Cultural studies of pathogen

The *C. gloeosporioides* grows well on PDA medium (Potato dextrose agar) and coconut watery endosperm (CWA) containing carbohydrates, proteins, minerals and lipid. *Colletotrichum gloeosporioides* produced good aerial mycelium in Richard's and Brown's agar and profusely sporulated on oat meal and corn meal agar along with abundant development of acervuli in rings and few setae. Glutamic acid and Alanine supported maximum growth and sporulation. Czapek's and yeast extract agar medium gave maximum growth. The growth was completely inhibited at 10°C. Light was not necessary but it enhanced sporulation and germination on a more acidic medium (Prakash and Srivastava 1987 and Santoso *et al* 1996).

The sporulation began after 4 days and the conidial production was lowest at 5 and 10°C and increased with increase in temperature from 15 to 25°C (King *et al* 1997). Sporulation generally decreased at or above 30°C. *Colletotrichum gloeosporioides* and *C. fragariae* produced about 10^3 and 10^4 conidia per fruit at 5°C.

The ambient temperature, pH, free water or relative humidity above 95 per cent was found ideal for conidial germination and aspersorium formation of *C. gloeosporioides* (Shih *et al* 2000 and Yakoby *et al* 2000).

Kumara and Rawal (2008) also reported maximum growth and sporulation of papaya anthracnose (*C. gloeosporioides*) at 28°C to 30°C temperature. High temperature (28°C) and humidity favored the growth of the pathogen and germination decreased below 97 per cent relative humidity (Tchatchou 2012). Spores are only released from acervuli when there is abundant moisture.

Colletotrichum remains inactive in dry season and become active under favorable environmental conditions. A pH of 6-7, temperature 25-30^{0}C and 12 hour light and 12 hour darkness showed the maximum mycelial growth of *C. gloeosporioides* (Hubballia *et al* 2011). Sharma and Kulshrestha (2015) also reported the best growth of *C. gloeosporioides* at temperature 25 - 28°C and pH 5.8 - 6.5.

2.6 Pathogenicity

Padilla *et al* (2002) proved the pathogenicity by inoculating crown of plants with sixty four isolates of strawberry anthracnose (60 of *C. gloeosporioides* and four of *C. fragariae*). All the isolates of *C. gloeosporioides* and *C. acutatum* produced typical symptoms of anthracnose rot on detached ripe fruit showing sunken necrotic lesions and orange coloured conidial masses within 4 days of inoculation. The twenty isolates of *C. acutatum* did not produce typical symptoms in crowns and have caused a slow decline in plant vigour, wilting and dying of older leaves and stunting.

The petiole isolates of *C. gloeosporioides* when inoculated to crowns of strawberry killed the plants within 4 weeks of inoculation (Mertely and Legard 2004).

The inoculated fruits produced symptoms as whitish, water soaked lesions (3 mm in diameter) which later become light tan to dark brown, eventually sunken and turn black within 2 to 3 days. After several days lesions were covered with salmon-coloured spore masses and infected fruits dried down to form hard, shrivelled mummies. The fruits challenged with distilled water did not develop lesions (Ivanovic *et al* 2007).

2.7 Epidemiology of disease

The pathogen (*G. cingulata*) overwintered in infected strawberry stocks and as perithecia on plant residues (Ishikawa *et al* 1989). In temperate regions, inoculum from plant debris initiated epidemics in spring (Eastburn and Gubler 1990).

The fungus preferred warm humid environment for spreading of anthracnose disease. The fungus invades the injured or weakened tissues of plants and produces conidia, acervuli, setae and appressoria during infection process. Conidia spread over short distances by rain splash or overhead irrigation and infected other healthy plant parts. The penetration into host tissues occurred by formation of appressoria. The whole infection process, the formation of conidia, acervuli, setae and appressoria resulted into tissue necrosis (Farr *et al* 2006).

The sexual stage of *Colletotrichum* species did not play any role in strawberry epidemics (Mass 1998). In South Eastern United States, *Colletotrichum* species does not survive between strawberry seasons in plant debris (Padilla *et al* 2002) or in soil (Horn and Carver 1968).

Wilson *et al* (1990) reported that immature fruits were less susceptible than mature fruits. Disease incidence on fruit increased with an increase in temperature (from 6 to 25^{o}C). The wetness durations of 0.5 to 51 hours and temperatures of 4 or 35^{o}C. The immature fruit did not favour the disease development but at 25^{0}C and after 25 hours of leaf wetness disease incidence reached 100 per cent. For mature fruit, disease incidence reached 97 per cent after 13 hours of wetness at 25^{0}C. The disease starts developing earlier on mature fruits (1 hour) than immature fruits (5 hours) wetness. Immature fruits (13%) and mature fruits (25%) showed disease symptoms after 50 hours of wetness duration at 6°C temperature. The incidence and severity of anthracnose fruit rot was increased in the humid conditions of the Southeast.

The conidial germination, appressorial production and secondary conidiation were favoured by longer periods of wetness than the 4 hrs wetness (Leandro *et al* 2001).

Leandro *et al* (2003) observed that a temperature of 17.6 to 27.7^{0}C was optimal for germination and appressorial development with continuous wetness periods. Development of anthracnose caused by *Colletotrichum* was favored at temperature 25-30°C (Hubballia *et al* 2011) and remains active in warm, wet and humid weather and became inactive in dry season (Sharma and Kulshrestha 2015).

2.8 Management of the disease

Application of benomyl effectively reduced the incidence of anthracnose crown rot and was intensively used by strawberry growers to control anthracnose and other diseases (Howard 1971 and Horn *et al* 1972).

Freeman *et al* (1997) reported the 50 per cent inhibition of mycelial growth (ED_{50}) with 0.05 mg/mL of prochloraz-Mn and single-dip treatment was effective and safe to manage the disease.

Freeman *et al* (2002) used soil disinfestation (solarization, fumigants, and steaming) for the management of anthracnose. Methyl bromide treatment completely controlled anthracnose when the infested mummified fruits were buried at depths of 10 and 20 cm in the field.

Spray of pyraclostrobin + boscalid, cyprodinil + fludioxonil, azoxystrobin, pyraclostrobin, captan + fenhexamid, and captan resulted less development of fruit rot on berries as compared to control (Wedge *et al* 2007 and Smith 2008).

Ivanovic *et al* (2007) also achieved the best fruit protection with metiram + piraclostrobin, captan and fludioksinil + ciprodinil.

Rahman *et al* (2009) demonstrated the spray programme in North Carolina and four applications namely one of Captan and Topsin M (thiophanate-methyl, Cerexagri-Nisso, King of Prussia, PA), two of Pristine, and one of Captevate (fenhexamid and Captan, Arysta, Cary, NC) provided a similar level of disease control as compared to eight applications

The weekly applications of a broad spectrum protectant fungicide (Captan) controlled the anthracnose disease in Florida under less favorable weather conditions. The rate of the protectant fungicide should be increased when anthracnose infected flowers and fruit were present in the fields during the blooming period. Fungicides containing two active ingredients (one for anthracnose and one for *Botrytis* fruit rot) viz. Captevate, Pristine or switch were recommended and Quadris 2.08F, Captan 50WP and Switch 62.5WG were the most effective (Mertely *et al* 2009).

Daugovish *et al* (2009) reported that the combination of Captan with premixtures of Boscalid + Pyraclostrobin or Cyprodinil + Fludioxonil were less effective in reducing the disease incidence as compared with single product application.

CHAPTER – III

MATERIAL AND METHODS

The present investigations were carried out in the Plant Pathology Laboratory of Department of Fruit Science, PAU, Ludhiana from 2014 to 2016. The field experiments were conducted in old orchard, pot house area and Department of Soil Water and Engineering, Punjab Agricultural University, Ludhiana. The nature of experiments, experimental procedures and requisites are given as follows:

3.1 Disease survey and collection of diseased samples

Strawberry growing areas in different districts of Punjab, viz. Barnala, Gurdaspur, Hoshiarpur, Ludhiana, Patiala and Ropar were surveyed during the months of January, February and March for two consecutive years 2015 and 2016 to record the prevalence of strawberry anthracnose and other fruit rot diseases. The observations on the development of strawberry anthracnose and other diseases were recorded on leaves, green fruits and mature fruits from different strawberry growing areas on the basis of 0-5 scale (Bowen 2007)

Scale	Disease severity range (%)
0	No symptoms
1	0-20
2	20-40
3	40-60
4	60-80
5	80-100

The per cent disease index (PDI) was calculated by the formula of McKinney (1923) given below:

$$\text{PDI} = \frac{\text{Sum of all numerical ratings}}{\text{Number of fruits examined} \times \text{Maximum grade}} \times 100$$

Samples of diseased leaves and fruits collected from different locations during survey were put in perforated polythene bags and brought to the laboratory for isolation of the causal fungus.

3.2 Isolation, purification and maintenance of the pathogen culture

3.2.1 Glassware Cleaning and Sterilization

The glassware of ‘Borosil’ brand was used during cultural studies. The glass apparatus were first cleaned with cleaning solution [concentrated Sulphuric acid (H_2SO_4) 25 ml, Potassium dichromate ($K_2Cr_2O_7$) 25 g and Water 150 ml] and then washed with running tap water followed by rinsing with double distilled water. The glass apparatus was finally dried in oven at 65°C for one hour for its further use in laboratory.

3.2.2 Preparation of substrate for growth of pathogen

The potato dextrose agar medium with following ingredients was used for the growth of pathogen.

Potato dextrose agar (PDA) medium

Peeled potato	:	200 g
Dextrose	:	20 g
Agar agar	:	20 g
Distilled water	:	1000 ml

Preparation: The peeled potatoes were cut into small pieces and boiled in water. The extract was taken by filtering through double layer of muslin cloth. The dextrose and agar were added and the total volume was made up to one litres with distilled water. Fifteen ml of each medium was poured into the sterilized Petriplates under aseptic conditions.

3.2.3 Isolation and raising of pure culture of the pathogen

Isolation of the causal agent was carried out from the diseased leaves, fruits and crown portion of strawberry plant. The samples were thoroughly washed under running tap water, air dried and sterilized by wiping with cotton swab dipped in absolute alcohol. Small bits (5 x 5 mm size) were cut and surface sterilized with 0.1 per cent mercuric chloride ($HgCl_2$) for one minute followed by four washings with sterile distilled water. These bits were then transferred to Potato dextrose agar (PDA) slants under sterile conditions in a laminar flow and incubated at 25±1°C in BOD incubator.

The culture of fungus was purified by single spore isolation technique. Ten ml of 2 per cent agar medium was poured in Petridishes, which were previously sterilized in hot air oven at 180°C for 60 minutes. The spore suspension was prepared in sterilized water containing 5-10 conidia per microscopic field (10x). It was poured uniformly on solidified medium and incubated at 25±1°C for few hours. The plates were examined frequently under the microscope for locating the germinating spores. The germinating spores were marked with marker on the glass surface of the plate. The marked agar area were cut with cork borer and transferred to PDA slants with the help of sterilized inoculation needle and incubated at 25±1°C. Ten such single spore cultures were raised and were compared for their growth characteristics. The isolates found to be identical were multiplied for further studies.

3.2.4 Morphological studies and confirmation of identity of pathogen

The cultures of the pathogens growing in test tubes as well as Petriplates were examined regularly for studying the characteristics of pathogens. The microscope used throughout study was calibrated to measure the size of the spore. The shape, colour, size and septation of conidia were measured from 15 days old culture and pathogen was identified on the basis of colony characters, size and shape of conidia.

3.3 Pathological studies

A large number of artificial inoculations were carried out with one fungal isolate on leaves, green fruits and mature fruits of strawberry under aseptic conditions.

3.3.1 Pathogenicity test on leaves and fruits

The leaves, green fruits and mature fruits of uniform size were collected and washed thoroughly under running tap water and air dried. The surface was disinfected by using cotton swab having absolute alcohol and after inoculation with pin prick and spray method all were placed on one cm thick moist cotton pads in the plastic trays. In pin prick method, the tip of sterilized pin was tapped lightly on 15 days older mycelium and then leaf surface was pricked. The inoculation sites were marked on the surface by making nearly circular marks with ball point pen. In spray method, 2×10^6 spores/ml inoculum concentration was prepared and sprayed over marked surface. Five sites were inoculated on each leaf, green fruit and mature fruit. After inoculation the trays were tightly covered with polythene sheets to create a moist chamber for maintaining humidity inside the trays and incubated at 25±1°C. The observations on the basis of number of lesions developed, size of lesions and time required for the development of anthracnose symptoms were recorded.

The lesions developed in pathogenicity test were again used for isolation on PDA and incubated at 25±1°C in BOD incubator and cultural characters of fungus were further compared with original culture under laboratory conditions. The same culture was further sent to Indian Type Culture Collection (ITCC), Division of Plant Pathology, Indian Agricultural Research, New Delhi for the identification to confirm the identity of the pathogen causing strawberry anthracnose disease.

3.3.2 Colony characteristics of fungus

The colony characters of fungus were recorded till 15 days of incubation starting from the day fungus start producing mycelium. The observations were recorded on the basis of colony colour, character of mycelium and presence or absence of acervuli in culture.

3.3.3 Measurement of spore size and acervuli

The calibration of the microscope was carried out using stage and ocular micrometre**.** The sizes of conidia were measured with the help of ocular micrometre. Five mycelial discs (5 mm size) from 15 days old culture were eluted in 5 ml of distilled water to obtain spore suspension. A drop of spore suspension placed on glass slide was covered with cover slip and observations on conidial length and breadth were made using ocular micrometre at 45x and 100x. In each case 50 conidia were observed and average size was calculated. The size of acervuli present in leaf tissue were measured with the help of ocular microscope. The leaf tissue having acervuli was cut into small bit and kept on glass slide and a water droplet was placed on bit of leaf. Then it was covered with cover slip. Twenty acervuli were measured and size was worked out at 45x.

3.4 Effect of temperature on development of pathogen under controlled conditions

3.4.1 Effect on radial growth of fungal mycelium

To determine the minimum and maximum range of temperature for the linear growth of the *C. gloeosporioides*, 20 ml of the basal medium (PDA) was poured in Petriplates, inoculated and incubated at different regimes of temperature viz., 5°C, 10°C, 15°C, 20°C, 25°C and 30°C maintained in different BOD incubators. Each treatment was replicated five times with single Petriplate per replication. The linear growth of the fungus was measured at 24 hrs intervals up to 9 days of incubation at different temperatures. The effect of factors "temperature" and "colony diameter" were analysed by completely randomized design in ANOVA (p = 0.05) using CPCS-I software.

3.4.2 Effect on extent of sporulation

The cultures of *C. gloeosporioides* grown on PDA were used for sporulation studies after 15 days of incubation at different temperatures viz. 5°C, 10°C, 15°C, 20°C, 25°C and 30°C. The sporulation in each isolate was measured with the help of haemocytometer. Spore suspension of each isolate was obtained by eluding five mycelia discs (5 mm size) of active culture in 5 ml of FAA (formaldehyde: absolute alcohol: glacial acetic acid: water 2: 10: 1: 7) contained in capped glass vials. Five vials were used for each isolate. The vials were thoroughly shaken for two to three minutes and eluded conidia were counted with the help of haemocytometer. The effect of the main factor "temperature" on "sporulation potential" was analysed by completely randomized design in ANOVA (p = 0.05) using CPCS-I software.

3.4.3 Effect on spore germination

To determine the suitable temperature for inducing the maximum spore germination of *C. gloeosporioides*, different temperatures viz. 5°C, 10°C, 15°C, 20°C, 25°C and 30°C were tested. The spore suspension was prepared in 2 per cent dextrose solution. The cavity slides with hanging drops of spore suspension placed on cover slips were kept in Petriplates and after creating moist chambers these were incubated at different temperatures in different BOD incubators and the data on spore germination was recorded at 4, 8, 12, 16, 20 and 24 hrs of intervals. Five replications for each temperature were kept keeping single unit per replication. The effect of factors "temperatures" and "time interval" and their interaction on per cent spore germination were analysed by two-way ANOVA (p=0.05) using CPCS-1 software.

3.5 Effect of temperature regimes on anthracnose on plant parts

The detached plant parts (leaves, green fruits and mature fruits) and whole plant were selected during February. After washing and sterilization with alcohol these were inoculated by pin prick method as described in section 3.3.1. After inoculation of leaves and fruits, humidity was provided to the inoculated plant parts and incubated at different temperatures viz. 15°C, 25°C and 30°C. Three replications were kept in each treatment keeping single unit per replication. The observations were recorded at 24 hours interval for 9 days. The effect of factors "temperatures" and "days" and their interaction on disease development were analysed by two-way ANOVA (p=0.05) using CPCS-1 software.

3.6 Effect of inoculum concentrations on disease development

Four inoculum concentrations viz. 10^4, 10^5, 10^6 and 10^7 spores/ml were prepared by counting conidia with the help of haemocytometer. The detached plant parts (leaf, green fruits and mature fruits) and whole plant were inoculated with each inoculum concentrations with pin prick method. Five sites on leaf and fruits were inoculated with 5 µl droplet of the inoculum suspension with micro pipette. Such inoculated detached plant parts (leaf, green fruits and mature fruits) and whole plant were incubated at 25 ±1°C after providing humid conditions. Five replications were kept in each treatment with single unit per replication. The observations were recorded at 24 hours interval for 9 days. The effect of factors "inoculum concentrations" and "days" and their interaction on disease development were analysed by two-way ANOVA (p=0.05) using CPCS-1 software.

3.7 Effect of wetness durations on disease development

The inoculated detached plant parts (leaf, green fruits and mature fruits) and whole plant were provided with 0, 8, 12, 16 and 24 hrs wetness. In 0 hr wetness duration the leaf and fruit surface was wet and then immediately dried with air flow. But in other treatments leaf and fruit surfaces were dried after specific hours of wetness durations. Such inoculated plant parts (leaf, green fruits and mature fruits) and whole plant were incubated at 25±1°C after providing sufficient relative humidity. Five replications were kept in each treatment with single unit per replication. The observations were recorded at 0, 8, 12, 16 and 24 hrs wetness duration for 9 days. The effect of factors "wetness duration" and "days" and their interaction on disease development were analysed by two-way ANOVA (p=0.05) using CPCS-1 software.

3.8 Periodical development of strawberry anthracnose in old orchard, PAU, Ludhiana

The periodical incidence of the strawberry anthracnose on 150 strawberry plants in old orchard, PAU, Ludhiana was recorded at weekly intervals starting from 1st meteorological week of January 2015 and 2016 to 12th meteorological week of March 2015 to 2016. The observations were recorded on the basis of per cent disease index. Weather data of each week was recorded from the observatory of the School of Climate Change and Agricultural Meteorology, PAU, Ludhiana and temperature, relative humidity, rainfall and number of rainy days were correlated with the development of disease. These weather parameters were also exploited for developing a forecasting model to predict the severity of strawberry anthracnose.

3.9 *In vitro* evaluation of fungicides against *C. gloeosporioides*

Four systemic and three non-systemic fungicides were evaluated *in vitro* against *C.*

gloeosporioides employing poisoned food technique (Nene and Thapliyal 1993).

Commercial name	Common name	Chemical name	Manufacturer
Amistar23 SC	Azoxystrobin	Methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrelate	Syngenta India Ltd
Tilt25 EC	Propiconazole	1-[[2(2,4-Dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]1-H-1,2,4-triazole	Syngenta India Ltd
Ditto 70 WP	Thiophanate methyl	1.2-alpha-(3-methoxycarbonyl-2-thioureido)benzene	Nippon Soda Co. Ltd, Japan
Bavistin 50WP	Carbendazim	Methyl-1H-benzimedazole-2yl carbamate	BASF India Ltd
Blitox50 WP	Copper oxychloride	Copper oxychloride	Rallis India Ltd
Indofil M 45	Mancozeb	Manganese ethylene bis-dithiocarbamate + 2% Zinc ion	Indofil Chemicals
Deviziram 27 SC	Ziram	(T-4)-bis(dimethyldithio-carbamato-S,S')zinc	Devidayal Agro Chemicals

Stock solutions of the fungicides (a.i. basis) were prepared in sterilized distilled water in 250 ml flasks containing PDA medium and the final concentrations 1, 5, 10, 25, 50 µg/ml of systemic and 10, 25, 50, 100, 200 and 500 µg/ml of non-systemic fungicides were prepared. The fungicide amended medium was poured in sterilized Petriplates of 90 mm size. After solidification of medium, 5 mm mycelial disc from actively growing culture of *C. gloeosporioides* were cut with a sterilized cork-borer and placed in the centre of each Petriplate in the inverted position. The Petridishes containing unamended medium served as control. Each treatment was replicated five times keeping single unit per replication. The inoculated Petridishes were sealed with cellophane film to minimize the chances of contamination and incubated at 25±1°C. Colony diameter was measured from two perpendicular sides and the average values were calculated after 7 days of incubation. The per cent inhibition in mycelial growth was worked out by the following of formula of Vincent (1947):

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

Where

Pi = Per cent inhibition in colony growth

C = Colony growth in control

T = Colony growth in test concentration

The effect of factors "fungicides" and "concentrations" and their interaction on per cent disease inhibition were analysed by two-way ANOVA (p=0.05) using CPCS-1 software. The ED_{50} value of each fungicide was also calculated by plotting per cent inhibition in colony growth against each concentration of the fungicide,

3.10 *In vivo* efficacy of fungicides in controlling strawberry anthracnose

The promising fungicides showing maximum mycelial growth inhibition *in vivo* were further exploited for field evaluation in controlling strawberry anthracnose on variety Chandler. Two systemic viz. propiconazole (0.1%) and carbendazim (0.1%) and one non-systemic mancozeb (0.25%) fungicides were used for field evaluation**.** Three sprays were given, first in January and thereafter two more sprays were given at 15 days interval. Unsprayed plants were kept as control. Each treatment was replicated thrice by keeping five plants per replication. The observations on the development of anthracnose disease on fruits were recorded and per cent disease index (PDI) was calculated. The effect of "fungicides" on per cent disease controlled was analysed by one-way ANOVA (p=0.05) using CPCS-1 software.

CHAPTER - IV

RESULTS AND DISCUSSION

4.1 Disease survey

To record the magnitude of strawberry anthracnose, systematic surveys of strawberry growing different districts of Punjab were conducted during the year 2015 and 2016. Three varieties of strawberry namely Camarosa, Chandler and Sweet Charlie were being grown by the growers at visited places (Plate 1). The data on disease incidence were recorded and presented in Table 1 and Fig. 1 which have indicated that disease was widespread in the strawberry growing areas of Barnala, Gurdaspur, Hoshiarpur, Ludhiana, Patiala and Ropar districts of Punjab.

Table 1: Disease index of strawberry anthracnose in different districts of Punjab during 2015 and 2016

Districts	Cultivars	Disease index (%)		
		2015	2016	Mean
Barnala	Camarosa	15.7	13.8	14.8
Gurdaspur	Chandler	23.3	21.2	22.3
Hoshiarpur	Chandler	21.4	19.5	20.4
Ludhiana	Camarosa	18.0	16.3	17.2
Patiala	Camarosa	16.7	15.0	15.9
Ropar	Sweet Charlie	19.0	17.5	18.3

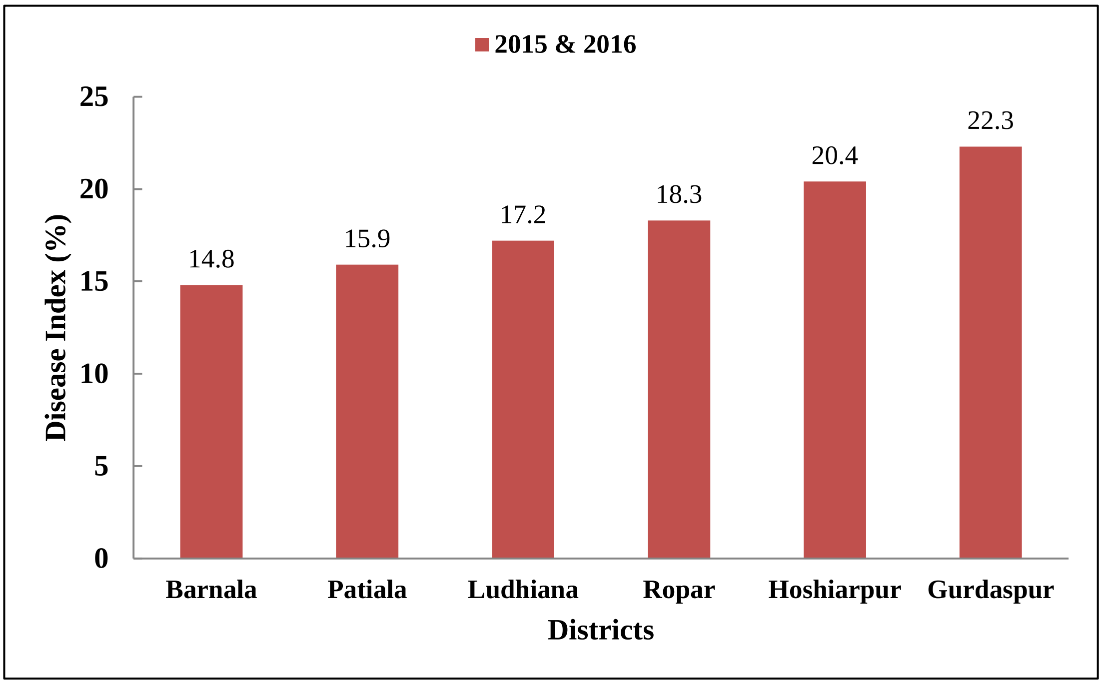

Fig. 1: Disease index of anthracnose of strawberry in different districts of Punjab

The mean disease index varied from 14.8 to 22.3 per cent in surveyed fields. The highest mean disease index 22.3 per cent was recorded in Gurdaspur and minimum 14.8 per cent in Barnala. The per cent disease index was more in sub mountainous districts; Gurdaspur, Hoshiarpur and Ropar as compared to central districts; Patiala, Ludhiana and Barnala.

Of the three strawberry grown varieties viz. Chandler, Camarosa and Sweet Charlie, Chandler was most susceptible to anthracnose with average disease index 21.4 per cent and Camarosa was least susceptible with mean disease index 16.0 per cent (Fig. 2).

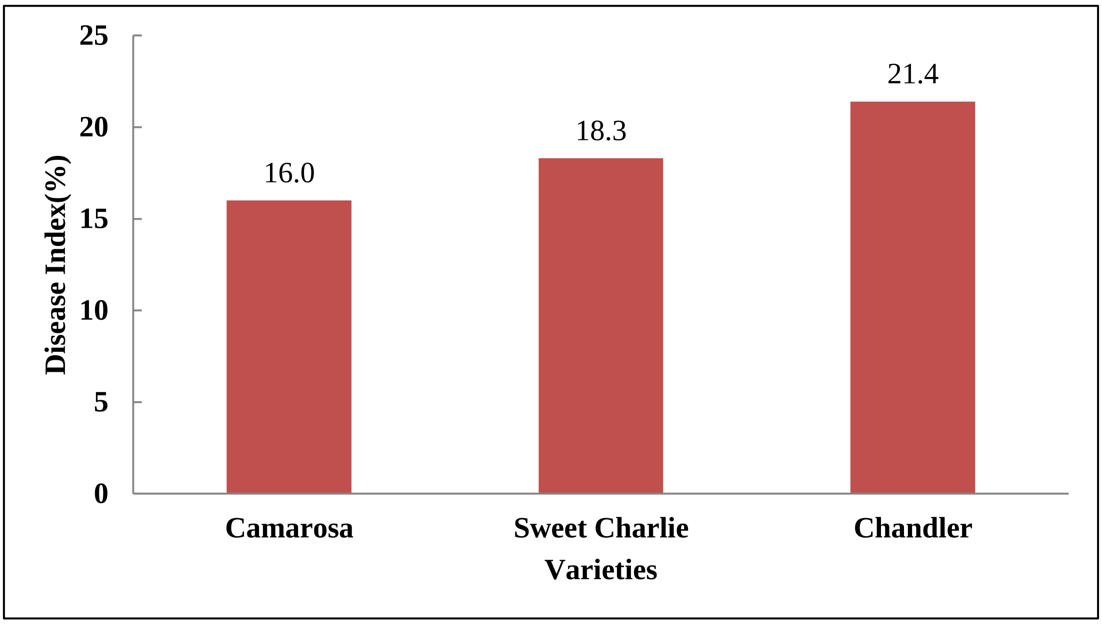

Fig. 2: Average disease incidence on strawberry cultivars grew by farmers

During surveillance, the presence of *Rhizopus* rot of strawberry was also recorded in the field but its incidence was low as compared to anthracnose. Disease index was highest in Gurdaspur (16.3%) and lowest in Patiala (5.4%) in 2015 and 2016 during January, February and March (Table 2).

Table 2: Disease index of *Rhizopus* rot in different districts of Punjab during 2015 and 2016

Districts	Disease index (%)		Mean
	2015	2016	
Ludhiana	11.2	10.5	11.1
Barnala	6.5	5.6	6.1
Ropar	13.5	12.6	13.1
Hoshiarpur	15.8	14.6	15.2
Gurdaspur	16.9	15.8	16.3
Patiala	6.4	4.5	5.4

4.2 Symptomatology of disease

During the surveillance, diagnostic symptoms of the strawberry anthracnose were observed on leaves and fruits which are described as below:

(a) Symptoms on leaves

Necrotic lesions at the tip and on the margins of leaves were observed (Plate 2). These lesions are earlier pin head sized and dark brown to back in color and later on expand (5-6 mm) to cover large area and become sunken straw and ashy colored in center with dark brown margins. Creamy spore mass is also produced in center of lesions (Plate 3). Light concentric rings are sometimes present or absent inside the lesions. Dark colored setae were also visible in these lesions when observed under microscope. Vidhyalakshmi and Divya (2013) also observed the appearance of sunken and water soaked spots on leaves of red brown to tan black color and it described as anthracnose disease. Similarly, Heidenreich and Truechek (2013) reported the small, dark spots on petioles which enlarged to become elongated dry and sunken and griddled the petiole and leaves. Jefferies *et al* (1990) also reported the production of slimy spore mass on the lesions in later stages of disease development.

(b) Symptoms on green fruits

The disease initiated as pinhead size small spots during the first fortnight of January, which gradually enlarge to attain size of 5.7- 6.2 mm in diameter. The spots were brown to tan in color, sunken, circular and have minute black stromata in the center of lesions, which produced spore masses in wet weather. Several spots coalesce to form bigger lesions. The infected area on green fruit become corky, hard and often develop cracks in severe infection (Plate 4 & 5). Similarly, Tandon and Singh (1969) reported that on green fruits infection starts as small, pin head sized spots and gradually enlarge to attain 5- 6 mm size in diameter. These spots are brown to black in color, sunken and bear black stromata in center of lesions.

(c) Symptoms on mature fruits

The infection on ripe fruits started as light to dark brown and black small depressed and water soaked lesions which later become soft, sunken and browning of tissues occurred (Plate 6 & 7). The size of lesions varied from 7- 8.5 mm. After few days, entire fruit is rotten. Similarly, Tandon and Singh (1969) found that on mature fruits softening of tissues occurs and lesions attain 10- 20 mm size. Mertely *et al* (2005) also reported that lesions on ripening fruits become brown that expand to slightly sunken lesions.

***Rhizopus* rot**

The symptoms of *Rhizopus* rot were also observed on ripe fruits. Earlier fruit surface was soft and secrete red juice from fruit tissues but later on it got rotten. After one day, white fuzzy mycelium appeared on fruit. Tiny black spherical structures appeared at end of mycelium (Plate 8 & 9). Similarly, Bolda (2012) reported that fruit either starts softening or leaks sticky red juice from infected fruit. Later on, affected fruits are covered with wispy, fuzzy black and white and black growth of pathogen with spherical structures on the end of white fungal strands.

4.3 Isolation and identification of the causal organism

The infected leaves and fruits were used for isolation of fungus on PDA slants and pure culture was obtained by single spore isolation method. The growth of fungus started within 24 hrs of incubation. Hyphae were septate, hyaline with several oil drops, initially cottony white and become grey in later stages (Plate 10). Similarly, Hiremath *et al* (1993) observed that the fungus produces circular, wooly or cottony colonies on culture media with characteristic color i. e. pale brown or greyish white. Similar findings were demonstrated by Ivanovic *et al* (2007) while working on that the colonies of GG-6A and GG-JUP isolates of *C. gloeosporioides* were first white later becoming orange, then turning into greenish grey as the cultures aged and became covered with pink to salmon conidial masses on PDA. Light orange spore masses were formed around the center of the colony. Similarly, Smith (2013) found that colonies of *C. gloeosporioides* were beige to olive to dark grey in color. Gubler and Gunnell (1991) also reported that isolates of *C. gloeosporioides* (Teleomorph: *Glomerella cingulata*) have grey or olive-grey colonies, dark grey to dark olive in reverse, cylindrical conidia and asci in culture.Microscopic examination of the culture revealed that conidia were cylindrical or oblong, straight with rounded ends, non-septate with oil droplets and were identical to those present on leaves and fruits (Plate 11). The conidia measured 12.9-16.9 µm × 3.01-4.5 µm at 45 x and 307.67-310.61 µm ×103.77 µm at 100x microscopic area (Plate 12 &13).

Similarly, Freeman *et al* (1998) also observed hyaline, one- celled, ovoid to oblong, slightly curved or dumbbell shaped conidia having size 10-15 µm × 5-7 µm. Similarly, Smith and Black (1990) reported 12.9-16.1 µm long and 4.4-5.4 µm width of conidia.

Acervulli were not produced inside the culture but were present inside the leaf tissues. Similar findings were also reported by Smith and Black (1990) who reported absence of setae inside the culture of *C. gloeosporioides* isolated from diseased strawberry tissues. Acervulli were round to irregular in shape, creamy in color with dark brown to black erect setae measuring 2.85 µm-10.52 µm (Plate 14 & 15). The mycelium of growing culture is hyaline, septate and branched. Similar findings were also reported by Freeman *et al* (1998) who reported that mycelium of growing culture is hyaline, septate and branched and the setae produced by fungus are long brown and septate. On the basis of mycelium (hyaline and septate), colony colour (white to grey), conidial shape (hyaline, non septate and straight condia with rounded ends having oil globule in center), conidial size (12.9-16.9 µm × 3.01-4.5 µm), presence of acervuli in leaf tissue and identification report from ITCC, IARI, New Delhi pathogen was proved as *C. gloeosporioides*.

4.4 Cultural characteristics of *Rhizopus* rot

Microscopic examination of culture revealed that the fungus has coenocytic hyphae,

Plate 1. During surveillance farmer field showing commercial cultivation of strawberry in Barnala district

Plate 2. Dark brown depresssed lesions **Plate 3. Creamy spore mass in centre of lesions**

Plate 4. Anthracnose lesion on green fruit

Plate 5. Increase in lesion size on fruit

Plate 6. Anthracnose lesion on mature fruit

Plate 7.Lesion causing rotting of fruit in later stages

Plate 8. *Rhizopus* rot on mature fruit

Plate 9. Fruit showing fuzzy mycelium of*Rhizopus*

Plate 10.Grey colonies of C. gloeosporioides

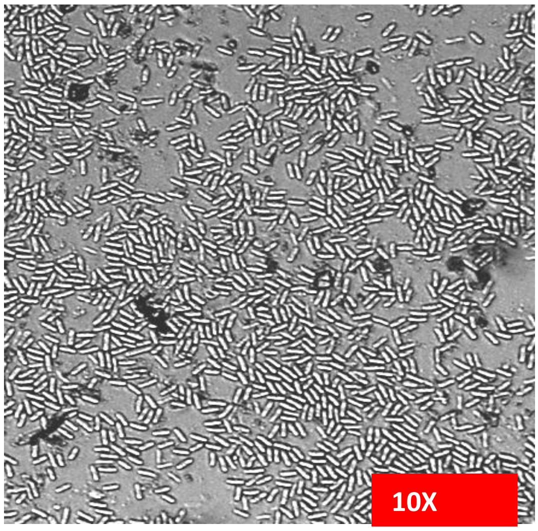

Plate 11. Shape and size of conidia

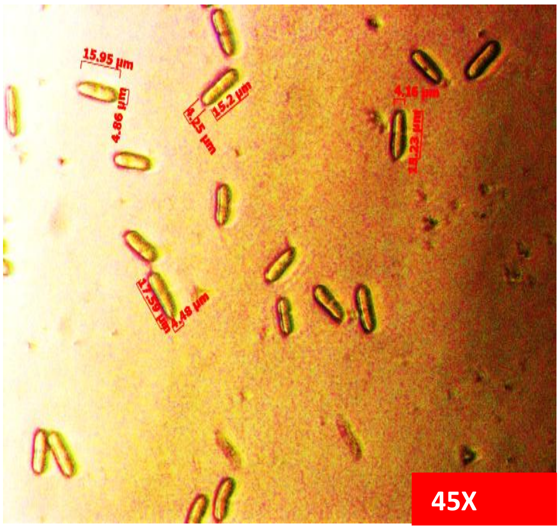

Plate 12.Shape and size of conidia

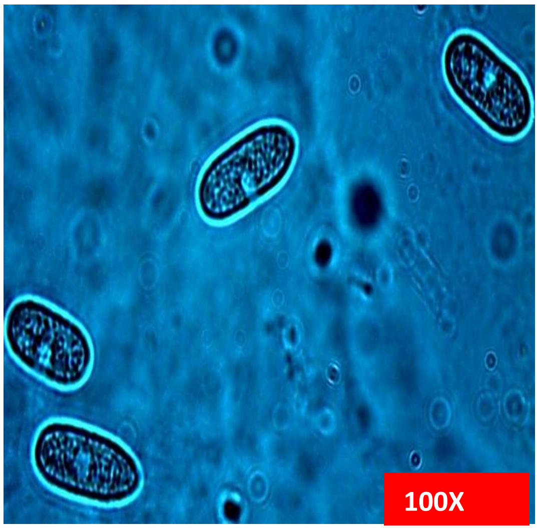

Plate 13.Shape and size of Conidia

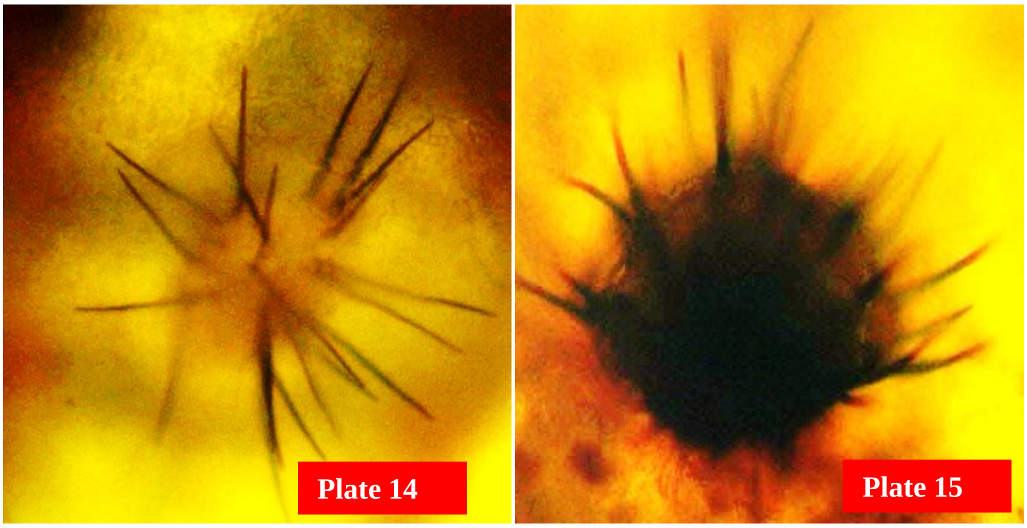

Plate14& 15.Acervulli and setae development on leaf tissues

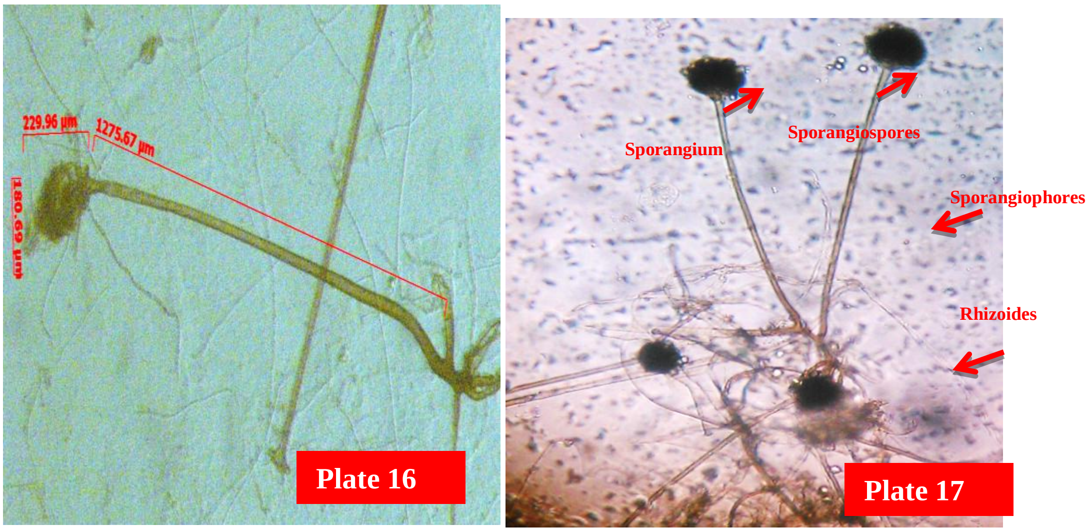

Plate 16& 17.Microscopic view of *Rhizopus* showingrhizoides, sporangia andsporangiospore.

rhizoids, stolon, erect sporangiophores and globose, black sporangia, 90-180 µm in diameter (Plate 16 & 17). Colonies on PDA were white-cottony in color. Sporangiophores were light brown and 10-12 µm in diameter. Sporangiospores were globose to oval and brown in color. Similarly, Kwon *et al* (2014) found that *Rhizopus* rot of strawberry caused by *Rhizopus oryzae* having white-cottony to brownish-black colony. Sporangia were globose, black and 40 -210 µm in diameter. Sporangiophores were light brown having size of 6-22 µm. Sporangiospores were globose to oval, brownish, streaked and 4-12 µm in length.

4.5 Pathogenicity test of the fungus

To determine the pathogenic potential of the strawberry anthracnose fungus, the inoculations were made with spore suspension by pin prick and spray method on detached leaves, green fruits and mature fruits of strawberry under *in vitro* conditions. The inoculated leaves, green fruits and mature fruits were observed for the appearance of visual symptoms (Table 3).

Table 3: Pathogenicity of *C. gloeosporioides* on strawberry plant parts

Method of inoculation	Plant parts	Incubation periods (days)	No. of lesions developed	Per cent lesions formed	Average lesion size (mm)*
Spray	Leaves	5	2	40.0	5.2
	Green fruit	6	2	40.0	5.7
	Mature fruit	3	4	60.0	7.0
Pin Prick	Leaves	4	4	80.0	6.0
	Green fruit	5	3	60.0	6.2
	Mature fruit	3	5	100.0	8.5

*Observation after 10 days of inoculation, No. of sites inoculated = 5

The spray method of inoculation produced symptoms on leaves, green fruits and mature fruits after 5, 6 and 3 days of inoculation whereas it was 4, 5 and 3 days in pin prick method of inoculation, respectively. On leaves and green fruits symptoms developed one day earlier in pin prick method as compared to spray method. On leaves brown to black necrotic spots having ashy grey center and dark brown margins were produced. On green fruits black pin head sized corky spots and on mature fruits brown water soaked spots were produced. The per cent lesion formation was also recorded more in pin prick method (60-80%) as compared to spray method (40-60%). Average lesion size on leaves, green fruits and mature fruits were also bigger (6, 6.2 and 8.5 mm) by pin prick as compared to spray method of inoculation (5.2, 5.7 and 7.0 mm). Similar finding was reported by Singh (2006) who reported that in guava, injured leaves, unripe and ripe fruits are more susceptible for disease development as compared to uninjured leaves and fruits. In unripe injured fruits, symptoms were developed earlier (3 days) as compared to uninjured fruits (4 days). Similarly, on ripe fruits, injured fruits induced symptoms after 2 days but uninjured induced after 3 days and on injured leaves symptoms appeared after 5 days but on uninjured leaves symptoms did not appeared.

Similarly, pathogenicity test of *Rhizopus* rot of strawberry produced white fuzzy mycelium on the rotten fruits. Similarly, Kwon *et al* (2014) reported that white mycelium grew from the primary infection site and gradually covered the fruit with tufted whisker-like grey sporangiophores and sporangia.

4.6 Effect of temperature regimes on mycelial growth of *C. gloeosporioides*

The data on the effect of different temperature regimes on the mycelial growth of *C. gloeosporioides* is presented in Table 4 and illustrated in Fig. 3. The growth (1.24 cm) started after 24 hours of inoculation at 25°C and 50 per cent area covered after 96 hours of inoculation. A temperature 25°C was found significantly effective in favoring the growth of the fungus (4.32 cm after 96 hours) as compared to other temperatures. Minimum growth was recorded at temperature 15°C and no growth was achieved at 5°C. In general, temperature between 25-30°C found ideal for mycelial growth and below 15°C failed to support the growth of pathogen. Similarly, Rahman *et al* (2003) reported that the temperature of 28-30°C was the most suitable for the growth of *C. gloeosporioides.* Srivastava and Tandon (1969) also reported that 25°C is optimum temperature for its growth. Leandro *et al* (2003) also reported

Table 4: Effect of temperature regimes on mycelial growth of *C. gloeosporioides*

Temperature (°C)	Mycelial growth (*cm)								
	Incubation (hours)								
	24	48	72	96	120	144	168	192	216
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
15	0.26	0.77	1.27	1.77	2.27	2.77	3.27	3.77	4.27
20	0.54	1.14	1.74	2.34	2.94	3.54	4.14	4.74	5.34
25	1.24	2.30	3.26	4.32	5.37	6.38	7.38	8.25	8.92
30	1.20	2.00	2.80	3.60	4.40	5.20	6.00	6.80	7.60
CD (p=0.05) Temperature = 0.65, Hours = 0.88, Temperature × Hours = 0.20									

* Observations based on average of five replications

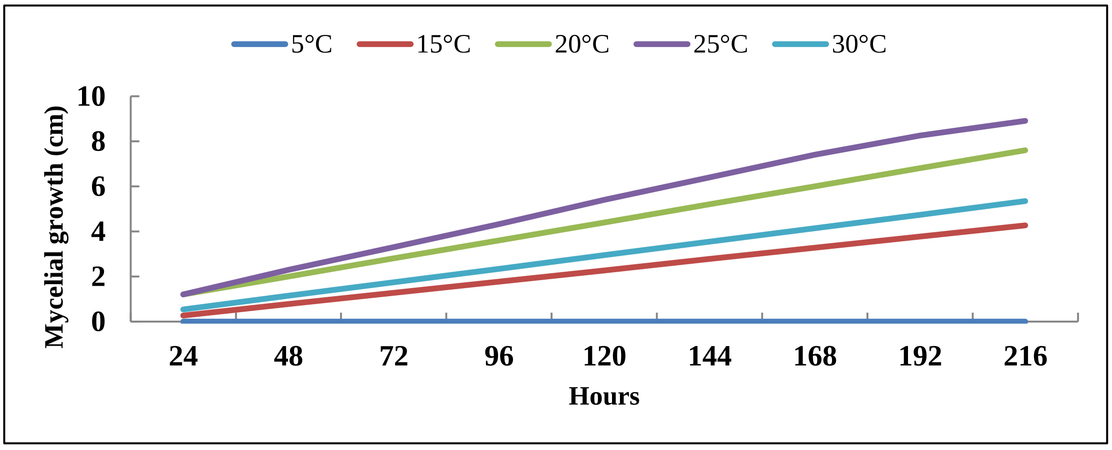

Fig. 3: Effect of different temperatures on mycelial growth of *C. gloeosporioides*

the optimal germination and appressorial development of *C. gloeosporioides* at temperatures of 17.6 to 27.7^{0}C with continuous wetness period. More development of disease was noticed between 20-25°C temperature and temperature > 30°C was not favorable for the disease Forcelini (2013).

4.7 Effect of temperatures regimes on sporulation

The data presented in Table 5 revealed that the highest sporulation (32.20 × 10^4 spore /ml) occurred at 25°C temperature in 15 days old culture which was significantly more than 30°C (27× 10^4 spore /ml) and 20°C (24.4× 10^4 spore /ml). These findings are in accordance with Singh (2006) who reported highest sporulation (34.50 ×10^4 spores/ml) of guava anthracnose (*C. gloeosporioides*) at 25°C followed by 30°C (26.30× 10^4 spore /ml). Similar finding were also reported by King *et al* (1997) while working on strawberry anthracnose and achieved maximum sporulation with increase in temperature from 15 to 25°C. Kumara and Rawal (2008) also noticed maximum growth and sporulation of papaya anthracnose (*C. gloeosporioides*) between 28°C to 30°C.

Table 5: Effect of temperature regimes on sporulation of *C. gloeosporioides*

Temperature (°C)	Sporulation (× 10^4spores/ml)
5	0.0
15	16.4
20	24.4
25	32.2
30	27.0
Mean	19.4
C. D (p=0.05)	1.86

4.8 Effect of temperature regimes on spore germination

The spores were suspended in 2 per cent dextrose solution and by using Hoffman nurse culture technique, the cavity slides were incubated at 5, 15, 20, 25 and 30° C to find the suitable temperature for spore germination. The data on effect of temperature on spore germination at different time intervals are presented in Table 6. Significantly maximum spore germination (22.4%) was recorded at 25°C temperature after 4 hours of incubation as compared to other temperatures and incubation periods. Cent per cent spore germination was observed at 25°C and 30°C after 16 hours of incubation which was significant to 5, 15 and 30 °C temperatures. The spores completely failed to germinate at 5°C even after 24 hours of incubation. In general, temperature between 25 to 30°C was more favorable for spore germination and below 20°C was unfavorable.

In the present investigation, it was observed that spores started germinating after 4 hours of incubation at 25°C. Germ tube usually emerged from the round end of spores but sometimes also from both ends (Plate 18 & 19). Similarly, Sattar and Malik (1939) also observed 25°C as optimum temperature for spore germination of *C. gloeosporioides.* The conidia of anthracnose of mango (*C. gloeosporioides*) form germ tubes within 3-8 hours at temperature between 25 to 30°C (Dodd *et al* 1991). The temperature between 25 to 30°C more favoured the development of anthracnose caused by *Colletotrichum* (Hubballia *et al* 2011).

Table 6: Effect of temperature regimes on spore germination of *C. gloeosporioides*

Temperature (°C)	Per cent spore germination					
	Hours					
	4	8	12	16	20	24
5	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)
15	0.0(0.0)	0.0(0.0)	0.0(0.0)	9.23(17.7)	19.3(26.02)	40.2(39.3)
20	0.0(0.0)	12(20.3)	41.1(39.9)	61.4(51.6)	71.1(57.5)	80.7(64)
25	22.4(28.3)	51.8(46)	93.7(75.5)	100(89.9)	100(89.9)	100(89.9)
30	0.0(0.0)	49.3(44.6)	82(64.9)	100(89.9)	100(89.9)	100(89.9)
C. D (p = 0.05) Temperature = 0.28, Time = 0.31, Temperature × time = 0.68						

Values in parenthesis are arc sine transformed

4.9 Effect of temperature regimes on disease development

To determine the effect of different temperature regimes on the disease development, the leaves, green fruits, mature fruits and whole plants were inoculated with pin prick method and subsequently incubated at 15, 25 and 30°C temperature for 9 days.

4.9.1 On leaves

The development of the disease on the inoculated leaves at different temperatures is presented in Table 7. A temperature of 25°C was found significantly better in developing anthracnose lesions after 5 days of inoculation as compared to 15 and 30°C. The temperature of

Table 7: Effect of temperature on development of disease on strawberry leaves

Temperature (°C)	Per cent disease index								
	Days								
	1	2	3	4	5	6	7	8	9
15	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
25	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	6.3 (14.5)	18.5 (25.1)	32.5 (34.4)	50.4 (44.9)	74.8 (59.7)
30	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3.5 (10.5)	24.5 (29.3)	28.5 (32.1)	31.7 (34.2)	40.5 (39.2)
C. D (p = 0.05) Temperature = 0.99, Days = 0.17, Temperature ×Days =0.31									

Values in parenthesis are arc sine transformed

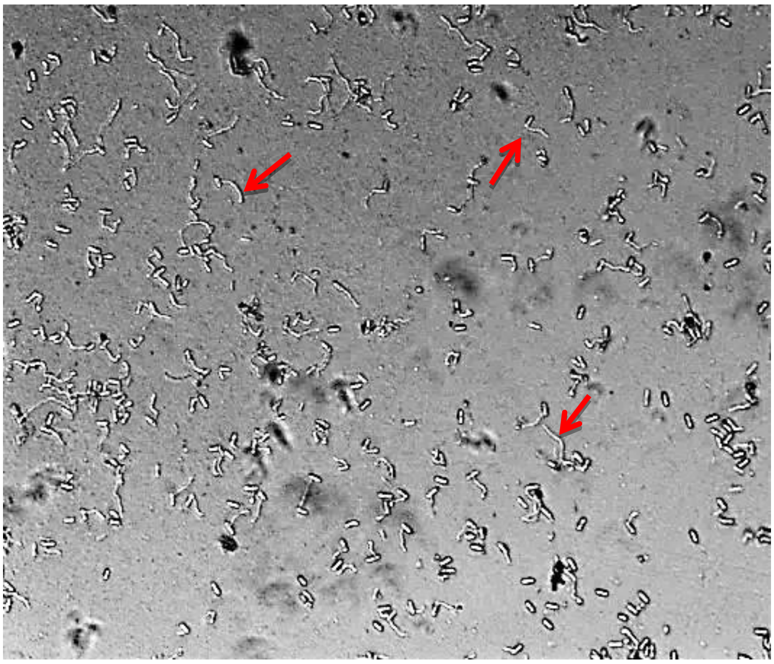

Plate 18. Small germ tubes of *C. gloeosporioides*after 8 hours at 25°C

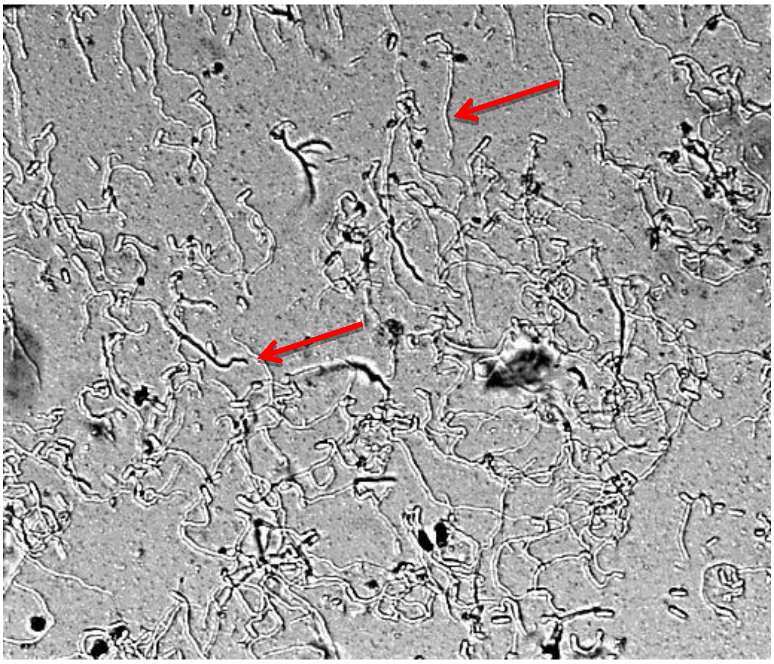

Plate 19. Long thread like germ tubes *C. gloeosporioides*after 24 hours at 25°C

25 and 30°C initiated the development of symptoms after 5 days of inoculation whereas at 15°C temperature there was no development of disease even after 9 days of inoculation. The per cent disease index was recorded significantly higher (74.8%) at 25°C as compared to 30°C (40.5%) after 9 days of incubation.

4.9.2 On green fruits

A temperature of 25°C was significantly better in developing disease symptoms on green fruits as compared to 15 and 30°C (Table 8). The initial symptoms appeared after 6 days at 25°C as compared to 30°C where it appeared after 7 days of inoculation. Disease index was significantly higher (50.7%) after 9 days of inoculation at 25°C. There was no development of symptom at 15°C even after 9 days of inoculation.

Table 8: Effect of temperature regimes on the development of disease on green fruits

Temperature (°C)	**Per cent disease index**					
	Days					
	4	**5**	**6**	**7**	**8**	**9**
15	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
25	0 (0.0)	0 (0.0)	10.7 (19)	20.7 (27)	30.7 (33.6)	50.7 (45.4)
30	0 (0.0)	0 (0.0)	0 (0.0)	9.3 (17.8)	19.3 (26.1)	29.3 (32.8)
C. D (p = 0.05) Temperature= 0.24, Days= 0.42, Temperature × Days = 0.73						

Values in parenthesis are arc sine transformed

4.9.3 On mature fruits

The data presented in Table 9 indicate that temperature of 25°C was significantly better in developing disease symptoms on mature fruits as compared to 15 and 30°C. Symptoms were developed after 3 day of incubation at 25°C where it was started after 4 days of inoculation at 30°C. At 15°C, symptoms initiated after 5 days of inoculation. Significantly

Table 9: Effect of temperature regimes on the development of disease on mature fruits

Temperature (°C)	**Per cent disease index**						
	Days						
	3	**4**	**5**	**6**	**7**	**8**	**9**
15	0 (0.0)	0 (0.0)	10.7 (19)	18.7 (25.5)	32 (34.4)	42 (40.4)	48.7 (44.2)
25	4.7 (12.5)	25 (29.9)	41.7 (40.1)	71.7 (58)	85 (63.3)	100 (89.9)	100 (89.9)
30	0 (0.0)	6 (14.1)	11.7 (19.9)	36.6 (37.2)	55 (47.9)	71.7 (57.8)	86.7 (68.8)
C. D (p = 0.05) Temperature = 1.33, Days = 2.3, Temperature × Days = 4.01							

Values in parenthesis are arc sine transformed

cent per cent area of the fruit was covered by the disease at 25 °C after 8 days of inoculation as compared to 15 and 30°C.

4.9.4 On whole plant

A temperature of 25°C was significantly better in developing disease symptoms on leaves as compared to 15 and 30°C (Table 10). Initial symptoms appeared after 5 days of incubation at 25°C (6.3%) and 30°C (5%). At 15°C disease development was not occurred even after 9 days of incubation.

Table 10: Effect of temperature regimes on development of disease on strawberry plants

Temperature (°C)	Per cent disease index				
	Days				
	5	**6**	**7**	**8**	**9**
15	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
25	6.3 (14.5)	12 (20.2)	22 (27.9)	32 (34.4)	42 (40.4)
30	5 (12.9)	10.6 (19)	20 (26.5)	30 (33.2)	40 (39.2)
C. D (p = 0.05) Temperature = 0.41, Days = 0.70, Temperature × Days =1.22					

Values in parenthesis are arc sine transformed

During the present investigation, 25°C temperature was found more favorable for the development of disease symptoms on leaves (5 days), green fruits (6 days), mature fruits (3 days) and strawberry plants (5 days) as compared to 15 and 30°C. Similar finding was also reported by Sharma and Kulshrestha (2015) who reported temperature between 20-30° C optimum for the growth of pathogen and development of symptoms. Davis *et al* (1987) also reported temperature between 20-30°C as the optimum for the growth and sporulation of *C. gloeosporioides* on mango. King *et al* (1997) also demonstrated less conidial production at 5 and 10°C and it increased with increase in temperature from 15°C to 30°C. Development of anthracnose caused by *Colletotrichum* is also Favored by temperature of 25-30°C (Hubballia *et al* 2011).

4.10 Effect of inoculum concentrations on disease development

To determine the effect of different inoculum concentrations on the development of disease, strawberry leaves, green fruits, mature fruits and whole plants were inoculated using 10^4, 10^5, 10^6 and 10^7 spores/ml by pin prick method and at 25±1°C temperature for 9 days.

4.10.1 On leaves

The data on the effect of different inoculum concentrations the development of the disease on leaves is presented in Table 11. The inoculum concentration of 10^7 spores/ml was significantly better than 10^4, 10^5 and 10^6 spores/ml in developing symptoms after 5 days of inoculation. The second best concentration was 10^6 spores/ml which was significantly better than 10^4 and 10^5 spores/ml concentration. Cent per cent disease was noticed at 10^6 and 10^7 spores/ml after 7 days of incubation.

Table 11: Effect of inoculum concentrations in disease development on leaves

Inoculum conc. (spores/ml)	Per cent disease index				
	Days				
	5	6	7	8	9
10^4	16.8 (24.2)	26 (30.6)	37.4 (37.7)	55.6 (48.2)	66.6 (54.7)
10^5	24 (29.2)	40.6 (39.5)	55.8 (48.3)	100 (89.9)	100 (89.9)
10^6	42.4 (40.6)	79 (62 .9)	100 (89.9)	100 (89.9)	100 (89.9)
10^7	55 (47.9)	86.7 (68.8)	100 (89.9)	100 (89.9)	100 (89.9)
C. D (p=0.05) Inoculum conc=0.64, Days = 0.96, Inoculum conc× Days=1.93					

Values in parenthesis are arc sine transformed

4.10.2 On green fruits

The inoculum concentration of 10^7 was found significantly better than 10^4, 10^5 and 10^6 in the development of anthracnose symptoms on green fruits (Table 12). Disease started appearing after 5 days of incubation at 10^6 and 10^7 spores/ml which was significantly better than 10^4 and 10^5 spores/ml. The 10^4 spores/ml did not induced symptoms even after 9 days of incubation.

Table 12: Effect of inoculum concentration in disease development on green fruits

Inoculum conc. (spores/ml)	Per cent disease index				
	Days				
	5	6	7	8	9
10^4	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
10^5	0 (0.0)	6 (13.9)	16 (23.4)	25 (29.9)	36 (36.8)
10^6	21.6 (27.6)	35.6 (36.6)	49.6 (44.7)	63.2 (52.7)	76.4 (60.9)
10^7	36.6 (37.1)	47.2 (43.4)	57.6 (49.4)	79.2 (63.1)	90 (71.6)
C. D (p = 0.05) Inoculum conc= 1.0, Days = 1.5, Inoculum conc × Days = 3.0					

Values in parenthesis are arc sine transformed

4.10.3 On mature fruit

Out of four different inoculum concentrations namely 10^4, 10^5, 10^6 and 10^7; 10^7 spores/ml was found significantly better for inducing symptom after 3 days of incubation (Table 13). The cent per cent development of symptoms was noticed at 10^7, 10^6 and 10^5 concentration after 5 days of incubation. It was interesting to note that all the inoculum concentrations produced symptoms after 3 days of incubation on mature fruits.

Table 13: Effect of inoculum concentration in disease development on mature fruits

Inoculum conc. (spores/ml)	Per cent disease index							
	Days							
	2	**3**	**4**	**5**	**6**	**7**	**8**	**9**
10^4	0 (0.0)	5.8 (13.9)	16.4 (23.9)	25.6 (20.4)	53.2 (46.8)	63.6 (52.9)	71.6 (59.1)	83.6 (66.2)
10^5	0 (0.0)	41.8 (40.2)	76.6 (61.1)	100 (89.9)	100 (89.9)	100 (89.9)	100 (89.9)	100 (89.9)
10^6	0 (0.0)	60 (50.8)	73.6 (59.9)	100 (89.9)	100 (89.9)	100 (89.9)	100 (89.9)	100 (89.9)
10^7	0 (0.0)	68 (55.6)	84.2 (66.7)	100 (89.9)	100 (89.9)	100 (89.9)	100 (89.9)	100 (89.9)
C. D (p = 0.05) Inoculum conc = 0.69, Days = 1.03, Inoculum conc × Days = 2.07								

Values in parenthesis are arc sine transformed

4.10.4 On whole plant

The data presented in Table 14 indicate the development of symptoms at 10^7, 10^6, 10^5 and 10^4 spores/ml concentrations. The 10^7 concentration was found significantly better in producing symptoms after 5 days of incubation. The disease development was 80.6 per cent at 10^7 inoculum concentration after 9 days of incubation as compared to 10^4 (45.7%), 10^5 (59.6%) and 10^6 (66.4%) concentrations.

Table 14: Effect of inoculum concentration on disease development on strawberry plants

Inoculum conc. (spores/ml)	Per cent disease index					
	Days					
	4	**5**	**6**	**7**	**8**	**9**
10^4	0 (0.0)	0 (0.0)	12.8 (20.9)	25.4 (30.2)	36.2 (36.9)	51.2 (45.7)
10^5	0 (0.0)	0 (0.0)	14.6 (22.3)	35 (36.2)	47.4 (43.5)	59.6 (50.5)
10^6	0 (0.0)	14.6 (22.3)	31.2 (33.9)	43 (40.9)	56 (48.4)	66.4 (54.6)
10^7	0 (0.0)	34.6 (35.9)	45.2 (42.2)	55 (47.9)	67.6 (55.3)	80.6 (63.9)
C. D (p = 0.05) Inoculum conc. = 0.69, Days = 1.03, Inoculum conc. × Days = 2.07						

Values in parenthesis are arc sine transformed

During present investigation 10^7 spores/ml inoculum concentration was found significantly better in producing disease symptoms on leaves (5 days), green fruits (5 days), mature fruits (3 days) and whole plant (5 days) as compared to other concentration. Bertetti *et al* (2009) also reported 10^5 and 10^6conidial concentration better for producing severe symptoms of strawberry anthracnose. Similarly, Forcelini (2013) showed an increase in anthracnose disease incidence by increasing the inoculum concentration from 0 to 10^6 spores/ml.

4.11 Effect of wetness duration in disease development

The data on the effect of different wetness durations on the development of disease on strawberry leaves, green fruits, mature fruits and whole plants is presented in Tables 15 to 18 with pin prick method of inoculation, incubated at 25±1°C temperature for 9 days.

4.11.1 On leaves

Out of the 0, 8, 12, 16 and 24 hours wetness duration, 24 hours wetness duration was significantly better in developing symptoms on leaves after 5 days of incubation (Table 15). The symptom did not appear after 5 days at 0 and 8 hours wetness duration. The development of disease was also more 86.0 per cent at 24 wetness duration after 9 days of incubation as compared to 12 hours (46.2%) and 16 hours (72.6%) of wetness duration.

Table 15: Effect of different wetness durations on development of disease on leaves

Wetness duration (hrs)	Per cent disease index					
	Days					
	4	5	6	7	8	9
0	0 (0.0)	0 (0.0)	46 (12.3)	9 (17.4)	15 (22.7)	23 (28.6)
8	0 (0.0)	0 (0.0)	9.2 (17.5)	17.2 (24.5)	24.6 (29.7)	33.4 (35.3)
12	0 (0.0)	5.2 (12.7)	11 (19.2)	20.4 (26.8)	34.6 (35.9)	46.2 (45.8)
16	0 (0.0)	13 (20.9)	24.2 (29.2)	40 (39.2)	55.8 (48.3)	72.6 (58.5)
24	0 (0.0)	23.4 (28.8)	37.6 (37.8)	52.8 (46.5)	70.8 (57.3)	86 (68.2)
C. D (p = 0.05) Wetness duration = 0.82, Days = 1.09, Wetness duration × Days = 2.46						

Values in parenthesis are arc sine transformed

4.11.2 On green fruit

A significant maximum disease index (14.2%) on green fruits was obtained at 24 hours wetness duration after 6 days as compared to other wetness durations (Table 16). Symptoms did not developed after 5 days of incubation at 0 and 8 hours wetness duration on green fruit. The disease index was 29.2 per cent at 24 hours of wetness duration after 9 days of incubation and differ significantly as compared to 0, 8, 12 and 16 hours of wetness durations.

Table 16: Effect of wetness duration on the development of disease on green fruits

Wetness duration (hrs)	Per cent disease index				
	Days				
	5	6	7	8	9
0	0 (0.0)	1.2 (6.2)	2.6 (9.2)	4 (11.4)	5.6 (13.7)
8	0 (0.0)	3.2 (10.2)	5.2 (13.1)	17.8 (16.2)	10.4 (18.8)
12	0 (0.0)	8 (16.2)	10 (18.2)	12 (20.1)	16 (23.5)
16	0 (0.0)	13.6 (21.6)	15.6 (23.2)	17.6 (24.8)	19.6 (26.3)
24	0 (0.0)	14.2 (22.1)	19.2 (25.9)	24 (19.2)	29.2 (32.7)
C. D (p = 0.05)	Wetness duration = 0.6, Days = 0.8, Wetness duration × Days = 1.7				

Values in parenthesis are arc sine transformed

4.11.3 On mature fruit

On the mature fruits, 24 hours wetness duration(50.6%) showed the significantly highest development of symptoms as compared to 0, 8, 12 and 16 hours wetness duration after 3 days of incubation (Table 17). The cent per cent fruit infection was observed at 16 and 24 hours wetness duration after 9 days of incubation.

Table 17: Effect of wetness duration on the development of disease on mature fruits

Wetness duration (hrs)	Per cent disease index							
	Days							
	2	3	4	5	6	7	8	9
0	0 (0.0)	16.4 (23.7)	26.4 (30.8)	36.4 (37.1)	46.4 (42.9)	56.4 (48.7)	66.4 (54.6)	76.4 (60.9)
8	0 (0.0)	20.4 (27)	30.4 (33.7)	40.4 (40)	50.4 (45.4)	60.4 (51)	70.4 (57)	80.4 (63)
12	0 (0.0)	30.6 (33.5)	40.6 (39.9)	50.6 (45.5)	60.6 (51.3)	70.6 (57.1)	80.6 (63.9)	90.6 (72.5)
16	0 (0.0)	40.6 (39.8)	50.6 (45.5)	60.6 (51.4)	70.6 (57.3)	80.6 (64.1)	90.6 (72.4)	100 (89.9)
24	0 (0.0)	50.6 (45.6)	60.6 (51.3)	70.6 (57.3)	80.6 (64)	90.6 (72.2)	100 (89.9)	100 (89.9)
C. D (p = 0.05) Wetness duration = 0.5, Days = 0.7, Wetness duration × Days = 1.5								

Values in parenthesis are arc sine transformed

4.11.4 On whole plant

The data presented in Table 18 indicate the development of the disease in different wetness durations on strawberry plants. A significant maximum disease index (14.2%) was recorded at 24 hours wetness duration after 5 days of incubation as compared to other treatments. The minimum disease development was noticed at 0 hours (5.6%) and 8 hours (12.4%) wetness duration after 9 days of incubation.

Table 18: Effect of wetness duration on the development of disease on plants

Wetness duration (hrs)	Per cent disease index					
	Days					
	4	5	6	7	8	9
0	0 (0.0)	0 (0.0)	1.2 (6.2)	2.6 (9.2)	4 (11.4)	5.6 (13.7)
8	0 (0.0)	3.2 (10.2)	5.2 (13.1)	17.8 (16.2)	10.4 (18.8)	12.4 (20.6)
12	0 (0.0)	5 (13.1)	8 (16.2)	10 (18.2)	12 (20.1)	16 (23.5)
16	0 (0.0)	9.6 (17.9)	13.6 (21.6)	15.6 (23.2)	17.6 (24.8)	19.6 (26.3)
24	0 (0.0)	14.2 (22.1)	19.2 (25.9)	24 (19.2)	29.2 (32.7)	34.6 (35.9)
C. D (p = 0.05) Wetness duration = 0.6, Days = 0.8, Wetness duration × Days = 1.7						

Values in parenthesis are arc sine transformed

During the present investigation, a wetness period of 24 hours was found significantly more favorable for producing the disease symptoms on strawberry leaves (5 days), green fruits (6 days), mature fruits (3 days) and whole plant (5 days) as compared to 0, 8, 12 and 16 hours wetness durations. These findings corroborate with Forcelini (2013) who also reported the increase in disease development with increase in wetness durations from 0 to 48 hours.

4.12 Epidemiology of strawberry anthracnose

4.12.1 Periodical disease development

The periodical development of the disease was recorded from 1st meteorological week of January to 12th meteorological week of March for two consecutive years 2015 and 2016 on the strawberry plants grown in old orchard, PAU, Ludhiana (Fig. 4 and Table 19). The disease showed variable development from January to March during the study period. Highest disease index (30.6%) was recorded in the 12th meteorological week of March when temperature was between 15.2 -29.0°C and relative humidity 53-96 per cent and lowest in 1st meteorological week of January (5.6%) when temperature was between 8.2 – 16.2°C and relative humidity between 74 – 97 per cent. The development of the disease was more in the month of March as compared to January and February. In general, temperature (22 - 28°C), relative humidity (63 - 96%), rainfall (8 - 36mm) and rainy days (3-6) were found favorable for disease development. The increase in anthracnose incidence was obtained with a continuous increase in temperature, rainfall and rainy days and decrease in relative humidity. The temperature >20°C was found more favorable and < 8°C was unfavorable for the disease development.

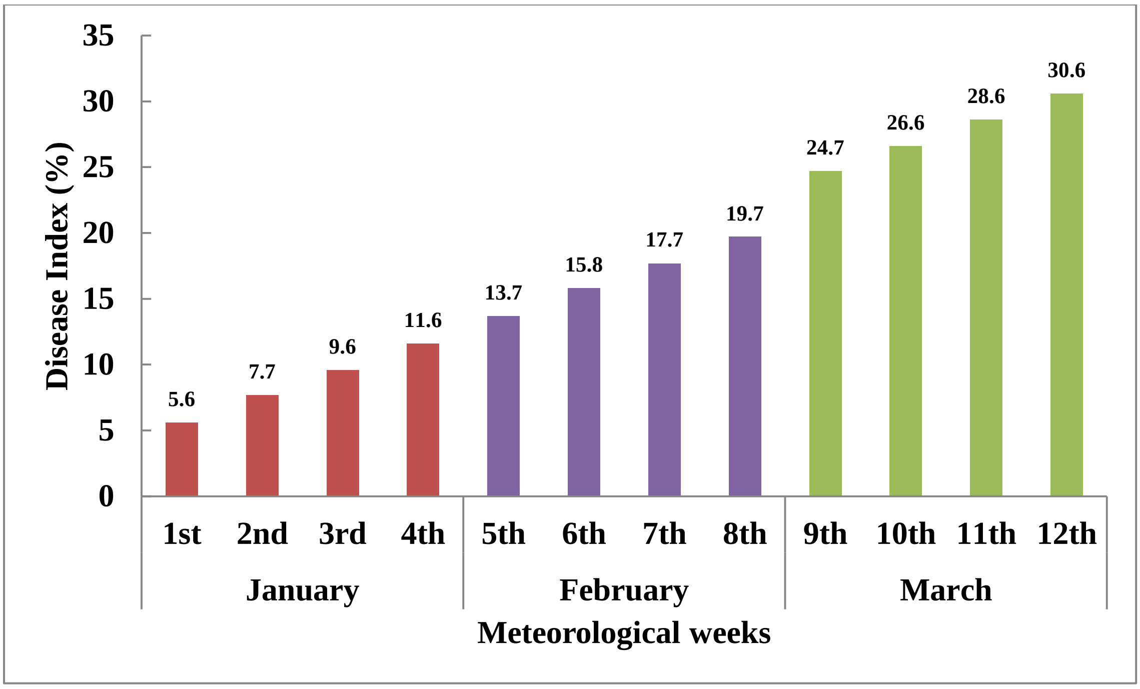

Fig. 4: Periodical development of strawberry anthracnose at PAU, Ludhiana

Table 19: Role of agrometeorological parameters in the development of strawberry anthracnose at PAU, Ludhiana

Meteorological weeks	Disease Index	Temperature (°C)		Relative humidity (%)		Rainfall (mm)	Rainy days
		Max	Min	Morning	Evening		
January							
1st	5.6	16.2	8.2	97	74	0.4	0
2nd	7.7	13.3	7.3	95	80	4.6	1
3rd	9.6	17.4	6.2	97	69	6.2	1
4th	11.6	14.7	7.7	97	77	14.6	1
Mean	9.0	15.4	7.35	96.5	75	25.8	3
February							
5th	13.7	18.6	7.1	92	61	11.6	1
6th	15.8	20.9	7.2	95	59	0	0
7th	17.7	23.9	11.2	93	62	8.4	1
8th	19.7	23.5	14.5	94	77	19	3
Mean	17.0	21.73	10	93.5	64.75	39.0	5
March							
9th	24.7	20.3	10.6	93	67	24.8	2
10th	26.6	22.5	9.2	94	61	8.2	1
11th	28.6	24.1	12.5	93	63	36.2	3
12th	30.6	29.0	15.2	96	53	0	0
Mean	28.0	23.9	11.86	94.0	61.0	69.2	6

Strawberry anthracnose severity depended on the prevailing weather parameters between January to March during which the disease normally appeared in strawberry growing areas of Punjab. High disease index in March may be due to active growth phase of strawberry plants and prevailing weather for infection. The congenial temperature, rainfall and rainy days during month of March resulted in the high disease development as compared to January and February months. The low disease in January to February may be due to the presence of low temperature and less rainfall (dry weather).

4.12.2 Correlation matrix of the disease with agrometeorological parameters

The results presented in Table 20 show the correlation matrix of the strawberry anthracnose disease with prevailing weather parameters. A positive correlation of the disease with minimum temperature (+0.745), maximum temperature (+0.857), rainfall (+0.441) and rainy days (+0.354) and negative correlation with morning and evening relative humidity was observed. The disease was significantly increased with the increase in temperature, rainfall and number of rainy days. The minimum, maximum temperature and rainfall have shown their more effects on the development of strawberry anthracnose as compared to morning and evening relative humidity. The correlation was significant with minimum and maximum temperature and non-significant with relative humidity, rainfall and rainy days.

Table 20: Correlation matrix of strawberry anthracnose with weather parameters

Weather parameters	**Correlation coefficient**
Temperature(°C)	
Minimum	+0.745
Maximum	+0.857
Humidity (%)	
Morning	-0.447
Evening	-0.644
Rainfall (mm)	+0.441
Rainy days	+0.354
Expected disease severity	+0.930
LSD (p=0.05)	0.576

During the present investigation, positive correlation with minimum and maximum temperature was obtained. It indicates that the disease increases with increase in temperature, rainfall and rainy days. Similar observations were recorded by Farr *et al* (2006) who reported warm and wet weather favors the development of strawberry anthracnose. Forcelini (2013) also reported more development of disease between 20-25°C temperature and temperature >

30°C was not favorable for the disease development. Similarly, Kurozawa and Pavan (1997) have also correlated high incidence of anthracnose of green pepper (*Colletotrichum gloeosporioides*) in mild to hot climate and under high moisture condition. Leandro *et al* (2003) also reported the optimal germination and appressorial development of *C. gloeosporioides* at temperatures of 17.6 to 27.7^{0}C with continuous wetness period. Similar correlation matrix was also computed in ber powdery mildew (Thind and Kaur 2005), grape anthracnose (Rao 1992 and Thind *et al* 2001) and citrus scab (Thind and Kaur 2008).

4.12.3 Forecasting model

A Multiple Linear Regression model to predict the disease severity was computed by feeding the two years data of anthracnose severity and prevailing agrometerological parameters. The forecasting model was computed as Y = 2.3 +1.42 (Min. Temp.) -0.22 (Max. Temp.) -0.70 (Evening RH) +0.52 (Morning RH) +0.19(Rainfall) + 1.5 (Rainy days), R^2 = 0.86, where Y = estimated disease severity. The R^2 value (coefficient of determination) indicated that 86 per cent variation in strawberry anthracnose could be monitored by prevailing air temperature, relative humidity, rainfall and rainy days. The validity of the model was tested by feeding the weather data from January to March 2015 and 2016 as input parameters and the predicted disease severity was found significantly positively associated (+0.93) with the observed disease severity (r = 0.576). This model may prove valuable for devising effective and efficient spray schedule for strawberry anthracnose under prevailing weather conditions. Similar forecasting model have also been devised for grape anthracnose (Rao 1992 and Thind *et al* 2001), ber powdery mildew (Thind and Kaur 2005), later blight of potato (Krause and Massie 1975), citrus scab (Thind and Kaur 2008).

4.13 *In vitro* efficacy of systemic and non- systemic fungicides against *C. gloeosporioides*

The data presented in Table 21 and Fig. 5 describe the *in vitro* efficacy of systemic fungicides against *C. gloeosporioides*. All the treatments inhibited colony growth to varying degree as compared to control. Propiconazole was found to be significantly effective in checking the growth (33.4%) at 1ppm (Plate 20). Second best treatment was carbendazim giving 29 per cent growth inhibition at 1 ppm (Plate 21). ED_{50} value of propiconazole and carbendazim was <5 ppm where it was > 50 ppm for azoxystrobin and <50 ppm in thiophenate methyl.

Among the non-systemic fungicides mancozeb was significantly better at all concentrations as compared to copper oxychloride and dithiocarbamate (Table 22 and Fig. 6). Cent per cent growth inhibition was recorded at 50 ppm in mancozeb (Plate 22) as compared to copper oxychloride (48%) and dithiocarbamate (32.6%). ED_{50} value of mancozeb was <25 ppm.

Table 21: ***In vitro* evaluation of systemic fungicides against *C. gloeosporioides***

Fungicides	Per cent growth inhibition					
	Concentrations(µg/ml)					
	1	**5**	**10**	**25**	**50**	**ED_{50}**
Amistar (Azoxystrobin23 SC)	26.2 (30.8)	27.4 (31.5)	29.6 (32.9)	32.4 (34.7)	42.4 (40.6)	>50
Tilt (Propiconazole 25 EC)	33.4 (35.1)	68.6 (55.9)	76.8 (61.2)	84.0 (66.4)	100.0 (89.9)	<5
Ditto (Thiophenate methyl 70WP)	23.6 (28.9)	22.4 (28.2)	32.6 (34.8)	40.0 (39.6)	57.0 (49)	<50
Bavistin (Carbendazim 50 WP)	29.0 (32.6.6)	54.6 (476)	68.4 (55.8)	77.2 (61.5)	83.2 (65.8)	<5
C. D (p=0.05) Fungicides = 1.3, Concentrations = 1.5, Fungicides × Conc. = 2.9						

Values in parenthesis are arc sine transformed

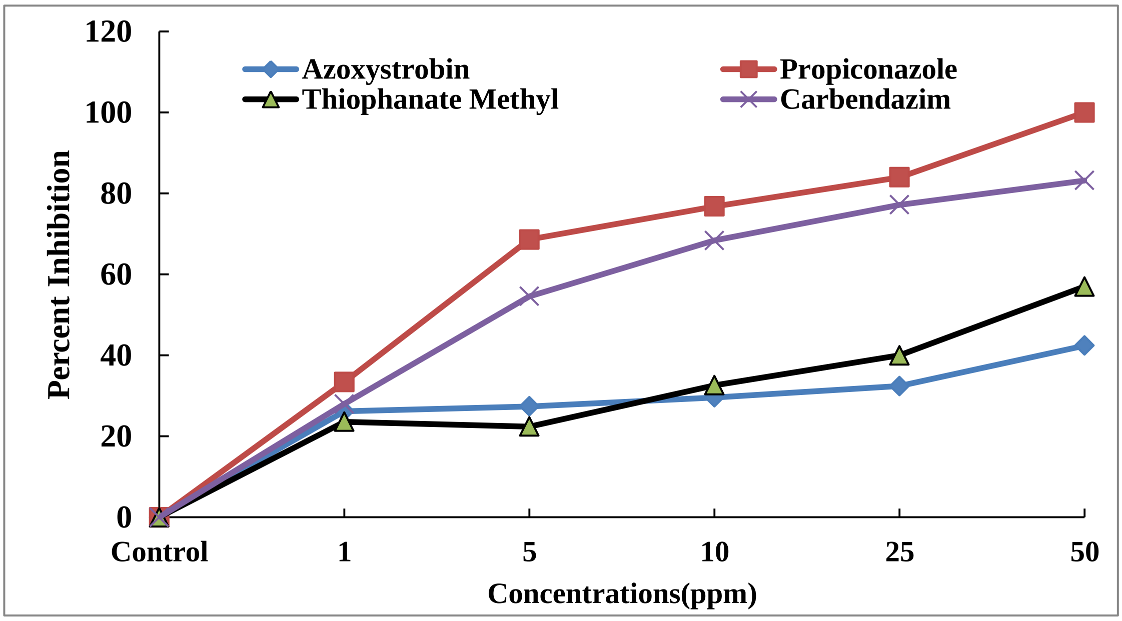

Fig. 5: ***In vitro* evaluation of systemic fungicides against *C. gloeosporioides***

Table 22: ***In vitro* evaluation of non-systemic fungicides against *C. gloeosporioides***

Fungicides	Per cent Growth Inhibition						
	Concentrations(µg/ml)						
	10	**25**	**50**	**100**	**200**	**500**	**ED_{50}**
Blitox (Copper oxychloride 50 WP)	10.2 (18.6)	17.8 (24.9)	48 (43.9)	76.4 (60.9)	100 (89.9)	100 (89.9)	>50
Indofil M- 45 (Mancozeb 75WP)	16.8 (23.9)	54.2 (47.4)	100 (89.9)	100 (89.9)	100 (89.9)	100 (89.9)	<25
Ziram (Dithiocarbamate27 SC)	19.2 (25.9)	19 (25.8)	32.6 (34.8)	39.6 (38.9)	49 (44.4)	69 (56.2)	>200
C. D (p=0.05) Fungicides = 0.76, Concentrations = 1.1, Fungicides × Conc. = 1.9							

Values in parenthesis are arc sine transformed

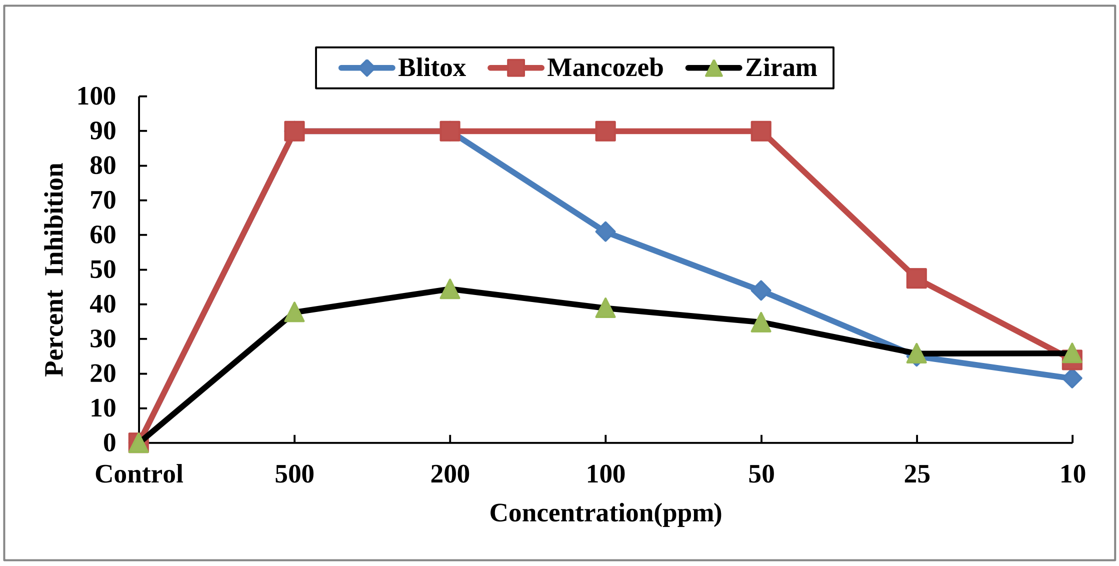

Fig. 6: *In vitro* evaluation of non-systemic fungicides against *C. gloeosporioides*.

4.14 *In vivo* efficacy of fungicides in controlling strawberry anthracnose

The results presented in Table 23 indicate the efficacy of propiconazole, carbendazim and mancozeb in controlling strawberry anthracnose under field conditions. Propiconazole was proved significantly better in controlling the disease (73.8%) as compared to mancozeb (59.6%) and carbendazim (52.4%). The efficacy of carbendazim and mancozeb was at par in checking the development of strawberry anthracnose on fruits.

Table 23: *In vivo* efficacy of fungicides in controlling strawberry anthracnose

Fungicides	Concentration (%)	Disease intensity (%)	Disease Control (%)
Tilt (Propiconazole 25 EC)	0.1	18.3	73.8
Bavistin (Carbendazim 50 WP)	0.1	33.3	52.4
Indofil M-45 (Mancozeb 75 WP)	0.25	28.3	59.6
Control	Water spray	70	-
C. D(p=0.05)		5.2	

During the present investigations, among systemic fungicides, propiconazole and non-systemic fungicides, mancozeb were significantly found better in inhibiting the growth of *C. gloeosporioides*. Of the three fungicides, propiconazole, carbendazim and mancozeb, three sprays of propiconazole and mancozeb were found significantly superior in controlling the fruit rot of strawberry. These fungicides are in accordance with Singh (2006) who reported the effective control of guava anthracnose with carbendazim and mancozeb. Similarly, Akhtar *et al* (1998) also reported that mancozeb was most effective under *in vitro* and *in vivo* conditions in controlling *G. cingulata.*

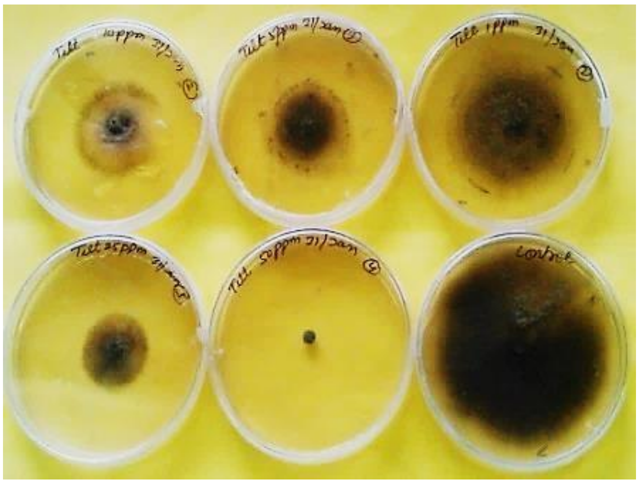

Plate 20. Mycelial growth inhibition by propiconazole

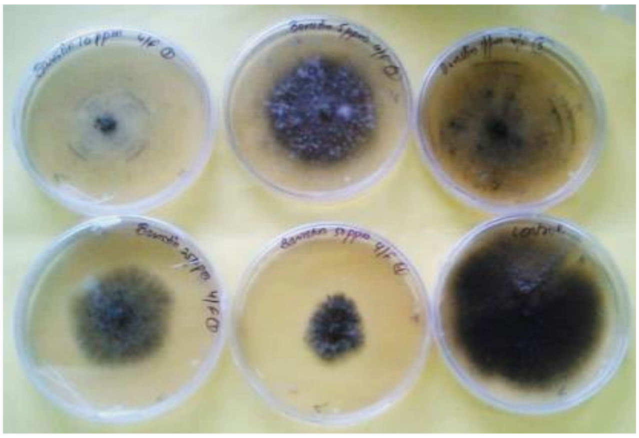

Plate 21. Mycelial growth inhibition by carbendazim

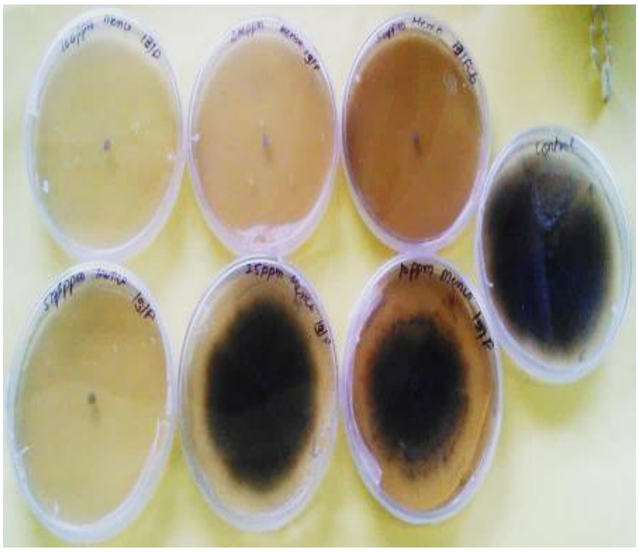

Plate 22. Mycelial growth inhibition by mancozeb

Kedar and Rehman (2001) also reported the effectiveness of propiconazole and carbendazim in inhibiting the mycelilal growth of *C. gloeosporioides*. The 50 per cent mycelial growth inhibition with 0.05 mg/ml Prochoraz-Mn was also reported by Freeman *et al* (1997) with 50 ppm prochoraz- Mn while working on anthracnose of strawberry. Similar results were also obtained by Mertely *et al* (2009), Horn *et al* (1972), Howard (1971), Ivanovic *et al* (2007) and Dovgovish *et al* (2009) while working on management of strawberry anthracnose with systemic and non- systemic fungicides.

CHAPTER – V

SUMMARY

Strawberry (*Fragaria* × *ananassa* Duch) is a perennial fruit crop grown in USA, Japan, Turkey, Spain and India. In Punjab, it is grown in isolated pockets of some districts like Barnala, Gurdaspur, Hoshiarpur, Ludhiana, Patiala and Ropar.

Among the various diseases, anthracnose caused by *C. gloeosporioides* is economically important disease of strawberry. It appears as black, sunken lesions on leaves, stems and fruit. The infected fruits fetch less market prices causing huge economic losses to growers. The present investigation entitled "Epidemiology and management of anthracnose fruit rot in strawberry" was conducted in Plant Pathology Laboratory of Department of Fruit Science and old orchard of Punjab Agricultural University, Ludhiana during the year 2014-2016. The salient features of the research findings are summarized below:

During the surveillance of strawberry growing districts of Punjab, it was observed that disease is present in Barnala, Gurdaspur, Hoshiarpur, Ludhiana, Patiala and Ropar with mean disease index varied from 14.8 to 22.3 per cent.

The highest disease index (22.3%) was noticed in Gurdaspur and lowest (14.8%) in Barnala.

Among the Camarosa, Chandler and Sweet Charlie strawberry grown varieties, Chandler was found most susceptible to the disease with 21.4 per cent disease index.

The anthracnose produces diagnostic symptoms on leaves, green fruits and mature fruits. On leaves, it produces pin head sized circular necrotic, straw coloured, brown to black lesions which later develops to large sunken spots with light concentric rings and produces creamy spore mass in moist weather. On green fruits, the spots are brown to tan in color, sunken, circular, corky, hard and had minute black stromata in the center of lesions. On ripe fruits, dark brown to black small depressed and water soaked lesions are produced which later on became soft and sunken. The ripe fruits infected with *Rhizopus* rot were soft, secrete red juice and bear white fuzzy mycelium.

Among the inoculation methods, pin prick was found better than spray method of inoculation on leaves, green fruits and mature fruits.

The isolation of fungus from diseased plant parts, pathogenicity test and report from ITCC, IARI, New Delhi confirmed the identity of the pathogen as *Colletotrichum gloeosporioides* (Penz.) Penz & Sacc.

Hyphae were septate, hyaline with several oil drops, initially cottony white and become grey in later stages. Conidia were cylindrical or oblong, straight with rounded ends, non-septate with oil droplets. The conidia measured 12.9-16.9 µm × 3.01-4.5 µm at 45 x and 307.67 -310.61 µm × 103.77 µm at 100x microscopic area. Acervuli were not produced in the culture but present on infected leaf tissues. Acervuli were round to irregular in shape, creamy in color with dark brown to black erect setae measuring 2.85 µm -10.52 µm.

A temperature of 25°C was found significantly effective than 5, 10, 15, 20 and 30°C for mycelial growth (8.92 cm) and sporulation (34.50×10^4 spores/ml) of pathogen.

Significantly maximum spore germination (22.4%) was recorded at 25°C after 4 hrs of incubation as compared to 5, 15, 20 and 30°C.

A temperature of 25°C was significantly found more favorable for the anthracnose symptoms expression on leaves (5 days), green fruits (6 days), mature fruits (3 days) and plants (5 days) than 15 and 30°C.

Inoculum concentration of 10^7 spores/ml was found significantly better in producing disease symptoms on leaves (5 days), green fruits (5 days), mature fruits (3 days) and plant (3 days).

Wetness duration of 24 hours induced significantly maximum disease on leaves (86%), green fruits (29.2%) and whole plant (34.6%) after 9 days and on mature fruits (100%) after 8 days of inoculation.

The periodical development of disease showed maximum index in March (28%) and lowest in January (9%).

A temperature of 17.9˚C, 77.5 per cent RH, rainfall 69.2 mm and 6 rainy days were found more conducive for the development of strawberry anthracnose at PAU, Ludhiana.

Correlation matrix of strawberry anthracnose with agrometeorological parameters showed a positive correlation with minimum and maximum temperature, rainfall and rainy days and negative correlation with morning and evening RH.

A multiple linear regression model fitted with various prevailing weather parameters was also devised for forecasting the disease.

In vitro, propiconazole systemic fungicide and mancozeb non- systemic fungicide were found significantly better in inhibiting the mycelial growth of *C. gloeosporioides*. ED_{50} value of propiconazole was < 5 and mancozeb was < 25 ppm.

Of the three fungicides viz. propiconazole, carbendazim and mancozeb, three sprays of propiconazole (0.1%) and mancozeb (0.25%) were found significantly effective in controlling the fruit rot of strawberry.

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