STUDIES ON ANTHRACNOSE OF POMEGRANATE CAUSED BY *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc.

Thesis submitted to the
University of Agricultural Sciences, Dharwad
in partial fulfillment of the requirements for the
Degree of

Master of Science (Agriculture)

in

PLANT PATHOLOGY

By

JAYALAKSHMI K.

DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OFAGRICUTURE, DHARWAD
UNIVERSITY OF AGRICULTURAL SCIENCES,
DHARWAD - 580 005

JUNE, 2010

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

1. INTRODUCTION

Pomegranate (*Punica granatum* L.), an ancient and commercially important fruit of both tropical and subtropical countries, belongs to the smallest botanical family punicaceae. Pomegranate is a native of Iran, where it was first cultivated in about 2000 BC, but spread to the Mediterranean countries at an early date. It is extensively cultivated in Spain, Morocco and other countries around the Mediterranean, Egypt, Iran, Afghanistan, Arabia and Baluchistan. It is also grown in Burma, China, Japan, USA, USSR, Bulgaria and Southern Italy. The fruit is very much liked for its cool and refreshing juice. The arils of the well matured fruits are consumed as such and also in processed form like juice or concentrate, syrup and jelly.

Pomegranate is a good source of carbohydrate and minerals such as calcium, iron and sulphur. It is rich in vitamin-C and citric acid is the most predominant organic acid in pomegranate (Malhotra *et al*., 1983). The fruits of pomegranate are known to posses pharmaceutical and therapeutic properties. It is a symbol of health, fertility, eternal life and also being valued as medicinal plant to treat diabetes, cancer, hypertension, gastric inflammation, heart and kidney diseases. Sweet varieties are mildly laxative, sour types are good for curing inflammation of stomach and heart ache. In India, there is a common adage 'Ek Anar Sau Bimar' meaning is one fruit cures hundred diseases. The flower buds are very useful in Ayurveda for managing bronchitis. The bark and pericarp of fruit is used for slimming, control of dysentery, diarrhea and killing tape worms. Extract of pomegranate fruit has antiviral activity. The bark of the stem and root contains number of alkaloids belonging to pyridine group. Rind of the fruit contains tannins, which are used as dyeing material for cloth and leather (Patil and Karle, 1990).

Seeds with fleshy portions of sour pomegranates are dried and marketed as "Anardana", which is being used as a condiment for curries.

The most popular varieties suitable for processing and table use are Ganesh, Mridula, Arakta, Bhagwa (Kesar), G-137 and Khandar. Area under pomegranate is increasing worldwide because of its hardy nature, wider adaptability, drought tolerance, higher yield levels with excellent keeping quality and remunerative prices in domestic as well as export market. It thrives well in dry tropics and sub-tropics and comes up very well in soils of low fertility status as well as on saline soil (Sheikh, 2006).

Pomegranate is regarded as the "Fruit of Paradise". It is one of the most adaptable subtropical minor fruit crops and its cultivation is increasing very rapidly. In India, it is regarded as a "vital cash crop", grown in an area of 1.5 lakh ha with a production of 11.0 lakh tons (Jadhav and Sharma, 2009). Among the different states growing pomegranate, Maharashtra is the largest producer occupying 2/3rd of total area in the country followed by Karnataka, Andhra Pradesh, Gujarat and Rajasthan. Karnataka state has the distribution of cultivating pomegranate under tropical condition in an area of 12,042 ha with a production of 1,29,547 tons (Anon, 2009) In Karnataka this crop has spread across different districts *viz*., Bangalore, Bijapur, Bellary, Bagalkot, Belgaum, Chitradurga, Davangere, Gadag, Gulbarga Koppal, Raichur, and Tumkur (Anon,2005). Successful cultivation of pomegranate in recent years has met with different problems such as pests and diseases. Among the various fungal diseases, anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. is one of the most serious disease of pomegranate. In India, anthracnose disease was first reported by McRae (1924). Many of these pathogenic fungi remain latent during maturity on the fruit and express disease symptoms during storage. Further, conidia of the pathogenic fungus which are abundant in the atmosphere of tropical plantation may lodge on the surface of the fruits as the fruits approach maturity. Propagules of these pathogens cause lesions and decay of the fruit.

The production of pomegranate has declined from a high of 1.8 lakh metric tons per annum in 2003-04 to less than 10,000 metric tons in 2007 – 08, there by causing a revenue loss of about Rs. 200 crore (at an average price of Rs. 50,000 per ton) in Karnataka (Giridhar, 2008). Anthracnose of pomegranate is one of the contributing factors for this low productivity. The systematic documentary research work on studies on anthracnose disease in Karnataka

Fig. 1: Pomegranate

Fig1: Pomegranate

is few and far between. The pomegranate growers of northern Karnataka are in need of a package for the management of this disease.

Survey on the disease in the field gives information about the extent of anthracnose disease affecting the crop and quality of the fruits in different locations. Anthracnose of pomegranate is widespread particularly in rainy season / high moisture conditions; however, systematic survey on the incidence and severity in northern parts of Karnataka is lacking. Considering the above facts, the survey was undertaken to know the incidence and severity of anthracnose disease in Bagalkot, Bijapur, Gadag, Koppal, and Raichur districts.

Studies on cultural, morphological, and physiological features of the pathogen are of immense use in understanding the nature of the pathogen.

There is little information available on management of anthracnose of pomegranate, but there is large number of chemicals available in the market as fungicides and their bioefficacy and suitability needs to be verified by *in vitro* and field studies, Fungicides are heterogeneous group of compounds used to manage loss of fruits due to fungal pathogens. Hence, screening of fungicides to manage anthracnose disease is most essential within the reasonable limit of fungicidal residues permitted by the importing countries, so as to incorporate the effective ones in the management package.

In recent years, there has been a major thrust on pesticide residue free organic pomegranate production. Taking the task into consideration, efficient botanicals and bioagents need to be explored to fit into the management schedule. Use of bioagents for the management of various diseases of crop plants is eco-friendly and environmentally safe. Therefore the fruits harvested using bioagents will fetch high price in the market without any hazardous effect. Plant derivatives possessing pesticide properties are generating worldwide interest as an alternative or as better supplements to the existing pesticides. These products will help in reducing the cost, environmental hazards and development of resistance by pathogen to fungicides. Continuous use of fungicide impose selective pressure on pathogen population and helps in acquiring resistance. There is an increased awareness among the people about health hazards of chemical residues. Hence, botanicals and bioagents were included in the present investigation.

The screening for disease resistance is essential to identify resistant variety / source. Resistant variety is one of the best ways in reducing loss due to disease. However, there is need to screen the genotypes against anthracnose of pomegranate. Hence, screening of the genotypes was included.

In view of the above facts, the present investigation was therefore initiated to elucidate some of the aspects of the pathogen, host and relative damage caused by pathogen with the following objectives.

1. Survey and surveillance on incidence of anthracnose of pomegranate in Northern Karnataka.
2. To study physiological, morphological and cultural characters of pathogen.
3. To evaluate fungicides, bioagents, and botanicals against pathogen both *in vitro* and *in vivo.*
4. To screen the genotypes for resistance. .

2. REVIEW OF LITERATURE

Anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. is the major pre and post harvest disease of pomegranate. Presently, very little literature is available in India on *Colletotrichum gloeosporioides* causing anthracnose of pomegranate fruit (Bose *et al*., 1973). Hence, the available literature on anthracnose of pomegranate and other fruit crops is being reviewed here under.

2.1 History and symptomatology

2.1.1 History

Butler (1918) reported *Colletotrichum gloeosporioides* for the first time in India causing leaf spot in coffee. While, McRae (1924) reported it as causal organism of anthracnose of mango. Singh and Chohan (1972) reported fruit rot of pomegranate due to *Glomorella cingulata* and rotting was noticed from fruit end portion. The fungus has been reported on various fruit crops (Rawal, 1990).

2.1.2 Symptomatology

Prakash & Srivastava (1987) reported that fruit was attacked during any stage of development. Young fruits of a week or two weeks old often become severely infected and fall in large numbers. On older fruits black spots were produced. Initially the spots were usually round but often become so numerous that they run together and form irregular blotches on fruit. Then the blackened areas become covered with minute pustules or fruiting bodies and produced large number of minute spores, each of which was capable of causing fresh infection.

Mazzani (1994), described symptoms of *Colletotrichum gloeosporioides* in plantation of pomegranate in Venezuela. Typical spot on fruits of pomegranate, annona and ber have also been described by Rawal and Saxena (1996).

Prashanth (2007) described typical symptoms on inflorescence, leaves and fruits. Black to brown necrotic depressed spots appeared on the leaves with circular margin, in advanced stage, spots coalesced and resulted in bigger patches, lead to defoliation. On fruits, brown spherical depressed spots occurred in scattered form on the pericarp. In advanced stage, spots coalesced to form necrotic rotten patches. However, the anthracnose spots caused by *Colletotrichum gloeosporioides* were also associated with various fungi.

2.2 Survey and surveillance on incidence of anthracnose of pomegranate

Sudarshan Rao (1975) stated that, survey and surveillance form the basis for any successful plant protection strategy. Plant protection to be successful, depends upon early detection of the disease followed by timely adoption and application of preventive measures.

Padule and Kaulgud (1991), reported that, in ambiabahar and hastabahar prominent leaf and fruit spot of pomegranate was anthracnose.

Sonawane *et al.* (1994), mentioned that extent of field loss in pomegranate due to biotic and abiotic factors and they recorded 44-64% fruit disease incidence in Jan-Feb flowering crop compared to other seasons.

Mandhare *et al.* (1996), conducted survey in Nashik district (Maharashtra) during December 1994. Most of the gardens of pomegranate (cv. Ganesh) were severly affected by fruit spot to the extent of 40-60%.

Venkataravanappa (2002) reported that in field survey of Northern and Southern districts of Karnataka showed diverse incidence form of anthracnose on mango, such

variation in disease incidence was usually attributed to environmental variations and variability in pathogenic fungus.

Amarjit Singh *et al*. (2007) reported appearance of disease in severe form on crops in rainy season compared to winter. The disease varied from 0.17 to 68.51 per cent in different guava growing areas and was more prevalent in Ludhiana as compared to Hoshiarpur and Patiala districts.

Prashanth (2007) conducted roving survey during August to September 2006 in major pomegranate growing areas of north Karnataka to know the incidence and severity of anthracnose. He recorded maximum disease incidence in Aug-Sep which was attributed to susceptibility of cultivars and favorable environmental conditions. Anthracnose appeared to be in severe form in the all pomegranate growing areas, which ranged from 18.14-35.84% of severity on a tree

Benagi *et al.* (2009) conducted roving survey during *mrigbahar* and *hastabahar* (August to January) of 2008-09 in major pomegranate growing areas of north Karnataka to know the incidence and severity of diseases. Plantations of atleast three years and above were selected for survey. Bacterial blight appeared to be in severe form in the all pomegranate growing areas, which ranged from 0.67-94.80% of severity on a tree and they also noticed 18% incidence of anthracnose disease.

2.2.1 Single spore isolation

Naik (1985) and Bhat (1987) purified the culture by single spore isolation after isolating *Colletotrichum gloeosporioides* from infected tissues of betelvine and cashew respectively. Similarly, Ekbote (1994) and Sudhakar (2000) purified the *Colletotrichum gloeosporioides* culture isolated from the infected tissues of mango and stylosanthes respectively by single spore isolation.

Venkataravanappa (2002) and Prashanth (2007) purified the culture by single spore isolation after isolating *Colletotrichum gloeosporioides* from infected tissues of mango and pomegranate respectively using two percent water agar media.

2.2.2 Pathogenicity

Sehgal *et al*. (1965) sprayed *Colletotrichum capsici* spore suspension obtained from 15 days old culture on coriander plants before and after flowering. They noticed the development of symptoms 12 days after inoculation only on the inflorescence, which later extended to the adjacent foliage. Similarly, Latham and Williams (1983) inoculated the apple fruit with perithecial culture of *Glomorella cingulata* under laboratory condition and observed the production of lesions with orange conidial mass, which later became grey then, black after 10-12 days incubation.

Amusa and Alabi (1996) isolated *Colletotrichum gloeosporioides* from infected pepper (*Capsicum*) and its pathogenicity was confirmed.

Anthracnose of bell pepper caused by *Colletotrichum capsici* was observed from three to 15 per cent. The most severe disease occurred on ripened fruits and pathogenicity was confirmed by tooth prick inoculation method (Roy *et al*., 1997).

Venkataravanappa (2002) reported that, pathogenicity was proved by spraying spore suspension (10^6 spores/ml) of fungus on five month old mango seedlings and infection occurred twelve days after inoculation.

Prashanth (2007) proved pathogenicity by artificial inoculation (pin prick method) of fungus on pomegranate fruit.

2.3 Cultural studies on different media

Sonada *et al*. (1974) reported that, conidia of *Colletotrichum gloeosporioides* grown either on potato dextrose agar or V-8 juice agar appeared pink in mass. Whereas, Lenne and Sonada (1978) found that *Colletotrichum gloeosporioides* produced bright orange spores mass on oatmeal agar.

Lande and Utikar (1978) reported that abundant mycelium growth and sporulation of *Drecheslera rostrata* causes fruit spot of pomegranate observed on PDA, M-2 agar, Czapek (Dox) agar and Coon's medium.

Yashoda *et al*. (1993) studied the growth phase of *Colletotrichum gloeosporioides* causing anthracnose of arecanut and observed that the fungus reached the maximum growth after 10 days of inoculation, beyond which autolysis occurred.

Ekbote *et al*. (1997) evaluated ten different synthetic and non-synthetic media for sporulation and growth of *Colletotrichum gloeosporioides*. Among the tested media maximum radial growth of *G. cingulata* was recorded on Richards', Browns' and potato dextrose agar, followed by Czapek (Dox) agar.

Sudhakar (2000) reported that maximum radial growth was recorded in five media (Sabouraud dextrose agar, Richards' agar, Browns' agar, potato dextrose agar and oatmeal agar) and they did not differ significantly. The least colony growth was recorded in Asthana and Hawker's 'A' medium. Further, he reported that in liquid media the maximum mycelial weight was recorded in Richards' broth (288.33 mg) and least in Asthana and Hawker's 'A' broth (166.66 mg) after 16 days of incubation.

Prashanth (2007) reported that, among non / semi synthetic media maximum radial growth and good sporulation of the fungus was recorded on PDA followed by oatmeal agar, While least growth and poor sporulation was observed on host leaf extract media. Among the synthetic media, maximum growth and fair sporulation were recorded on Richards' agar and least growth with poor sporulation was recorded on Czapek (Dox) agar. In case of broth, maximum dry mycelial weight was recorded in Richards' medium broth and least dry mycelial weight was recoded in host leaf extract broth after thirteen days of incubation.

Vinod and Benagi (2009) worked on papaya anthracnose caused by *Colletotrichum gloeosporioides* and he reported that among different solid media Richards' agar showed good growth and sporulation. The fungus in liquid media recorded good growth upto 10 days of incubation and decreased after eleventh day.

2.4 Morphological studies

The genus *Colletotrichum* was described by Corda (1831-32) under the name *Colletotrichum* with a single species. Later, he changed the name as *Colletotrichum gloeosporioides.* Saccardo (1884) and Potebinia (1910) placed *Colletotrichum* in Melanconiales and Acervulaes respectively.

Penz (1884) first time described *Colletotrichum gloeosporioides*. Later in 1924, McRae described the morphological characteristics of *Colletotrichum gloeosporioides*.

Earlier reports (Small 1926) revealed that *Colletotrichum gloeosporioides,* which has been considered as conidial stage of *Glomerella cingulata*. This fungus has a wide host range belonging to various families *viz*., anacardiaceae, musaceae, palmaceae, coricaceae, vitaceae sapotaceae, etc (Bilgrami *et al*., 1979). Bhat (1987) reported conidial measurement as 5.5-6.7 × 3-4.2 µm.

Mordue (1971) enumerated the morphological characters of *Colletotrichum gloeosporioides*. The acervuli were usually setose or glabrous, round to elongate or irregular in shape and attained as much as 500 µm in diameter, conidia were hyaline, cylindrical, aseptate, uninucleate, measuring 9-24 × 36 µm formed on hyaline or fairly brown cylindrical

phialidic conidiophores on potato dextrose agar. The conidia varied in size and shape than those one the host.

Sutton (1980) revealed that conidia of *Colletotrichum gloeosporioides* were hyaline, aseptate prior to germination, smooth and thin walled, cylindrical or oval, straight and size of the conidia varied from 9-24× 6-12µm.

Colletotrichum gloeosporioides fungus with grayish white to dark gray on potato dextrose agar and produced aerial mycelium ranging from a thick mat to sprase or tufts of mycelium. Conidia were hyaline, unicellular and either cylindrical with obtuse ends or ellipsoidal with a rounded apex narrow, truncate base. They measured 7.0-20.0 µm × 2.5-5.0 µm and formed hyaline brown conidiophores in acervulli that were irregular in shape and about 500 µm in diameter. Setae were 6-8 × 200 µm, one to four septate, brown and slightly swollen at the base and tapered at the apex. Orange slimy conidial masses can be formed as the acervulli matured (Litz, 1997)

Rajesh (1999) recorded the morphological characters of *Colletotrichum gloeosporioides* isolated from cashew. Acervulus were numerous, dark and measured 5.5-12.0 µm in diameter. Setae were dark brown, irregularly arranged in the acervulus. Conidiophores were single celled, hyaline and non–septate, closely packed together in rows. Conidia are oblong to cylindrical with rounded ends, hyaline, non septate with one or two vacuoles, measured 8-10×3.5-4.0 µm.

Venkataravanappa (2002) reported that conidia of *Colletotrichum gloeosporioides* were oblong or cylindrical, one celled, hyaline straight, one to two oil globules were observed in the conidium. Conidia on the culture media were found to be in reddish mass.

Prashanth (2007) reported that mycelial colour varied from white to light red color. The growth was flat to raise fluffy and sparse. Pigmentation in fungi also varied in different media i.e. brown to black and light pink to orange.

C. capsici obtained from chilli host and culture recorded the acervulli about 53.3-136.4µm in host and 71.0-161.9µm in culture, setae 150µm long in and conidia 16-30×2.5-4 µm in size (Vinaya, 2008).

2.5 Physiological studies

2.5.1 Effect of temperature on *Colletotrichum gloeosporioides*

Sattar and Malik (1939) found the best growth of *Glomerella cingulata* at 25-29^{o} C and the minimum and maximum range of temperatures were 10-15^{o} C and 35^{o} C respectively.

Rajak (1983) reported the temperature of 25^{o} C as the optimum for the growth of *Colletotrichum gloeosporioides*.

Naik (1985) reported that, 20-30^{o} C as optimum temperature range for *Colletotrichum gloeosporioides* and maximum growth was observed at 25^{o} C.

Artificial inoculation with *Colletotrichum fragariae* on strawberry plants, showed higher severity rate of disease at temperature 35 ^{o}C and 100 per cent relative humidity for 48 hrs (Smith and Black, 1988).

Bhat (1991) reported that, growth of *Colletotrichum gloeosporioides* gradually increased as temperature increased from 10-30°C. Red pigmentation of *Colletotrichum acutatum* was observed at 32 ^{o}C on potato dextrose agar (Gunnel and Gubler, 1992).

Yashoda *et al*. (1993) conducted *in vitro* experiment on growth of *Colletotrichum gloeosporioides* [*Glomerella cingulata*] and obtained maximum growth after 10 days incubation on potato dextrose broth, with optimum pH in the range 5.0-6.5 and optimum temp. in the range 25-35 ^{o} C.

Mesta (1996) reported a temperature 30ºC for growth of *Colletotrichum capsici* . Similar results were also reported by Vinaya (2008).

Sudhakar (2000) found that pathogen could grow well at temperature of 20 to 30° C and relative humidity of 95 per cent. Similar results were also reported by Estrada *et al.,* 2000; Prasanna Kumar, 2001 and Prashanth, 2007.

Venkataravanappa (2002) reported that highest spore germination (98.69%) of *Colletotrichum gloeosporioides was recorded at 25* ℃ and the least spore germination was recorded at 10 and 40℃.

Sangeetha and Rawal (2009) reported that there are significant differences between temperature and their interaction, when observed three days after inoculation. Among all temperature, the maximum colony diameter was observed at 28℃ followed by 25℃. Temperature of 15℃ showed least growth.

Vinod *et al*. (2009) reported that 30℃ is required for the good growth of *Colletotrichum gloeosporioides* causal agent of anthracnose of papaya.

2.5.2 Effect of light on anthracnose of pomegranate

Choudhuary (1936) reported that, continuous light or darkness was found to inhibit sporulation of *Colletotrichum graminicola* (Cesati) G. W. Wilson. Where as cultures exposed to alternate light and darkness were found to sporulate earlier and more conspicuous.

Minussi and Kimati (1978) reported that *Colletotrichum graminicola* of sorghum isolated from four localities sporulated abundantely under continuous light.

Growth and sporulation of *Colletotrichum graminicola* at different light exposures *in vivo* and *in vitro* tests showed that diurnal light exposure favoured growth and sporulation of the pathogen. Disease was more when the pathogen was exposed to diurnal light compared to continuous light or darkness. (Mishra and Siradhana, 1980).

Mesta (1996), Sudhakar (2000) Prashanth (2007) and Vinod *et al* (2009) reported that, exposure of *Colletotrichum spp*. to alternate cycles of 12 hr light and 12hr dark , showed maximum growth and sporulation.

Venkataravanappa (2002) reported that exposure of *Colletotrichum gloeosporioides* to alternate cycles of light and darkness recorded maximum radial growth followed by continuous darkness.

2.6 Toxin studies

As early as 1886, de Bary gave the toxin concept to explain the symptom of plant parasite relationships, while working with *Sclerotinia sclerotiarum* (Lib.) de Bary. He succeeded in producing soft rot by applying a sterile from rotten carrots to healthy carrot tissue.

The term vivotoxins, pathotoxins, phytotoxins and specific toxins have been defined by early workers (Dimmond and Waggoner, 1953; Wheeler and Luke, 1963; Pringle and Scheffer, 1964).

Sharma and Sharma (1969) observed that an isolate of *Colletotrichum gloeosporioides* from *citrus limettoides* produced toxin in liquid media culture. Maximum production of the toxin was found in Richard's solution after 22 days of incubation at 27-28 ℃. Further they found that the toxin was non host specific, thermolabile and toxic to the spore germination of *Colletotrichum gloeosporioides* itself. Dilution with water decreased its toxicity.

Several phytotoxins produced by microorganisms were reported to be responsible for the induction of disease in plant (Owens, 1969 and Strobel, 1974). Many plant pathogenic

microorganisms produced toxic metabolites in culture media and in plant tissues, which were involved in the disease syndrome (Wood *et al.,* 1972).

Production of toxin by any fungus *in vitro* has been provided by a number of bioassay methods of plant cutting seed germination bioassay, root and shoot elongation bioassay (Anahosur, 1976).

Naik *et al.* (1991) observed that the pathogen *Colletotrichum gloeosporioides* produced non-specific toxic metabolites in culture filtrate. The maximum amount of toxin was produced on sixteenth day of incubation. Culture filtrate inhibited the seed germination of coriander, tomato and radish drastically. Toxin proved to be thermostable and retain its toxicity even after autoclaving. The dilution of toxin with water reduced the toxicity to a great extent. The dilution of the culture filtrate and the time taken for symptom expression were positively related.

Jayashankar (1999) found that the *Colletotrichum gloeosporioides,* the causal agent of mango anthracnose produced toxin *in vitro.* The partially purified toxin presumed to be a *Colletotrichum* caused anthracnose like symptoms on young mango leaves and toxic to embryonic suspension cultures of mango cultivars hindi and carabao and it also inhibited *in vitro* seed germination of two non hosts, lettuce and tobacco. There were linear relationships between concentration of the partially purified toxin and mortality of mango embryogenic cultivars. Embryogenic cultivars grown in the presence of partially purified toxin showed significant lower growth rates than the control and Czapek dox. Partially purified toxin can be used as in vitro selection agent for to screen for the resistance to the fungus.

Venkataravanappa (2002) observed that 10+0 dilution culture filtrate of *Colletotrichum gloeosporioides* inhibited the 82.18% germination of sorghum seeds followed by 9+1 dilution (73.03%) and uninoculted Richards' broth and control (Dist. water) showed least inhibition of seed germination and maximum vigour index. Further tomato seedlings showed marginal necrosis of leaves with in 24 hrs followed by drooping of leaves finally became brittle, curled and wilted in 48 hrs at 10+0 dilution.

2.7 Management of diseases

2.7.1 *In vitro* evaluation of fungicides

Bernard and Schrader (1984) found chlorothalonil as most effective fungicide against *Colletotrichum gloeosporioides*. Naik and Hiremath (1986) reported the complete inhibition of mycelial growth of *Colletotrichum gloeosporioides* with blitox (0.1, 0.2 and 0.3%) followed by foltaf and cuman-L. (0.3%).

Bhat (1991), reported captofal, mancozeb, copper oxy chloride, and carbendazim inhibited the conidial germination of *Colletotrichum gloeosporioides*.

Lonsdale (1992) recommended the pre flowering application of copper oxy chloride followed by monthly application of copper oxy chloride from fruit set onwards for effective in control of mango anthracnose.

Mesta (1996), and Hegde (1998), reported that carbendazim(0.1%) , chlorothalonil(0.1%), bitertinol(0,1%) were more effective in inhibiting mancozeb(0.1%), *C.capsici* under laboratory conditions.

Ekbote *et al*. (1996) reported that among the six fungicides tested, derosal gave cent per cent inhibition of mycelial growth at 0.05 and 0.10 per cent concentration, while Indofil M-45 gave cent per cent inhibition at 0.3 per cent concentration. The least per cent inhibition of mycelial growth was observed in kavach at all the tested concentration.

Dinesh Kumar (1998) reported that bavistin (500 ppm) reduced the fruit rot and increased TSS (Total soluble Solids), while acidity and ascorbic acid contents were decreased steadily with an increase in duration of storage.

Banik *et al*. (1998) evaluated two systemic (carbendazim and thiophanate methyl) and three non systemic (captan, ziram and chlorothalonil) fungicides against *Colletotrichum gloeosporioides*. They indicated that carbendazim and thiophanate methyl gave the best control followed by ziram and captan.

Jamadar *et al.* (1998) tested the various fungicides for control of pomegranate fruit spots (*Colletotrichum gloeosporioides*). Among the treatments, spraying with mancozeb along with carbendazim was most effective in reducing the incidence of fruit spot recording the disease reduction of 88 .9 per cent.

Srinivasan and Gunasekaran (1998) reported that, contaf (hexaconazole) at all the four (0.10, 0.15, 0.20, and 0.40 per cent) concentrations completely inhibited the mycelial growth, while, Indofil M-45 at 0.50 per cent concentration inhibited only up to 88 per cent.

Elliott and Patterson (2000) observed the efficacy of nine fungicides against *Colletotrichum gloeosporioides* using poison food technique with concentration of 0.1 per cent.

Gaikwad (2000) reported that seven sprays of fungicide, carbendazim (0.1%) and mancozeb (0.2%) was found to be effective for controlling leaf and fruit spot of pomegranate.

Sanders *et al*. (2000) reported the pre-harvest application of copper oxy chloride and benomyl which gave a significant reduction of inoculum level in the field.

Venkataravanappa and Nargund (2002) reported tricyclazole as very effective under laboratory condition against *Colletotrichum gloeosporioides* among all tested fungicides.

Abhishek and Verma (2007) evaluated fungicides against *Colletotrichum gloeosporioides* causing anthracnose of mango. Bavistin and topsin-M proved most effective at the concentration of 100µg/ml.

Jamadar *et al.* (2007) identified score 25 EC at 0.1 per cent and prochloraz 45 EC at 1.25 percent were effective in reducing the anthracnose of pomegranate (7.7% and 15%) with highest total soluble solids and fruit yield of 9831 kg /ha and 9273 kg / ha respectively.

Prashanth *et al.* (2008) reported that among the non-systemic fungicides, combi-product carbendazim + mancozeb recorded highest per cent inhibition of mycelial growth (89.23%) of fungus, which was followed by propineb (87.78%) and the least inhibition of fungus was recorded in chlorothalonil (53.78%) at 0.1 per cent concentration. Among four systemic fungicides maximum per cent inhibition of growth of *Colletotrichum gloeosporioides* was observed in difenconazole (90.78%) and propiconazole (90.78%) which were on par with each other and was followed by carbendazim (88.89%) while least per cent inhibition of fungus was recorded in iprobenfos (75.99%) at 0.1 per cent concentration.

Gud and Raut (2008) reported that, thiophenate-methyl and propiconazole were most effective against *Colletotrichum gloeosporioides* followed by hexaconazole and carbendazim.

Patel (2009) reported that, carbendazim, mancozeb+carbendazim, propiconazole, tricyclazole were on par with each other, which showed 100% effectiveness.

Watve *et al.* (2009) reported that, carbendazim (0.1%), propiconazole (0.1%), difenconazole (0.1%) and copper oxychloride (0.3%) inhibited the growth and sporulation to the extent of 100% followed by bordeaux mixture (1%), tridemefon (0.1%), and mancozeb (0.1%)

Vinod *et al*. (2009) reported that carbendazim found to be effective among the over all the tested chemicals and gave cent per cent mycelial inhibition of *Colletotrichum gloeosporioides*

2.7.2 *In vitro* evaluation bioagents

Jeffries and Koomen (1992) reviewed the biological control of mango anthracnose. Patil (1992) reported that, *Trichoderma* sp. can be used as an effective antagonistic microorganism against mango fruit rots incited by *Lasiodiplodia theobromae* and *Rhizopus.*

Koomen and Jeffries (1993) identified that out of total of 648 microorganisms, which included bacteria, yeasts and fungi, which were isolated from blossom, leaves and fruits of mango, 558 isolates were found to reduce anthracnose development significantly.

Korsten *et al*. (1993) found that fruit dip of *Bacillus licheniformis* (isolate B 250 and B 251) gave good control of anthracnose and stem end rot on variety, 'Sensation' and 'Keitt' fruit when used at 10^7 cells / ml. Villiers *et al*. (1994) reported that the hot water + *Bacillus licheniformis* followed by wax + ¼ prochloraz treatment was most effective in reducing soft brown rot of mango fruits.

Vyas and Pathak (1995) reported that culture wash of *Trichoderma viride* was proved to be effective against the rots. Villiers and Korsten (1996) reported that dipping the fruits in ethanol (3 min) + *Bacillus licheniformis* (B 251 for 7 min) significantly reduced the incidence levels of soft brown rot and anthracnose compared to the control. Mandhare *et al.* (1996) reported that *Trichoderma* sp. was found to be effective antagonistic, which inhibited the mycelial growth of *Colletotrichum gloeosporioides* cause the anthracnose of pomegranate (72.90%). Chuang and Ann (1997) investigated the antagonistic bacteria *Bacillus subtilis* (isolate Tp-Tu 311), *Pseudomonas fluorescens* (isolate Tn-S 221) and *Pichia ohmeri* (isolate Y 24-8) as post harvest treatment, which tend to decrease anthracnose development in mango fruits. The isolate Tp-TU 311 gave the best control of the disease.

Mandhare *et al*. (1996) mentioned that *Trichoderma sp*. was found to be effective antagonistic which inhibited the mycelial growth of *Colletotrichum gloeosporioides* (72.9%) which is the causal agent of anthracnose of pomegranate.

Bhuvaneswari and Rao (2001) reported that mango fruits inoculated with *T. viride* remained free from *Pestalotia* sp., *Colletotrichum gloeosporioides*, *A. niger*, *A. flavus*, *L. theobromae*, *R. stolonifer* and *M. phaseolina* infection indicating their suppression by the antagonistic nature of *T. viride*. In addition, *T. viride* suppressed the growth of *Penicillium purpurogenum*, *Penicillium* sp. and *Phoma* sp. even as the inoculated fruits were rotten.

Mandhare and Suryawanshi (2003) revealed that toxic properties of metabolic was produced by antagonistic *Bacillus thermophillus* when sprayed on to control pomegranate leaf spot and purple blotch of onion which led to changes in germ tube or mycelium of these pathogens to restrict the fungal growth and invasion in host plant.

Prashanth *et al.* (2008*)* reported that *Trichoderma viridae, T. harazianum, Pseudomonas fluorescence, Bacillus subtillis were* found to be effective antagonistic which inhibited the mycelial growth of *Colletotrichum gloeosporioides. .*

Babu *et al*. (2008) reported that *Trichoderma viride* and plant growth promoting rhizobacteria inhibited the growth of pathogen effectively.

The combination of *Trichoderma viride, T. harzianum* and *Gliocladium virens* were found to be potential antaglnists aginst *Colletotrichum gloeosporioides causing* mango anthracnose. Gud and Raut (2008).

Jadav *et al*. (2008) reported that *T. viride* and *T. harzianum* were found to be more effective antagonist which inhibited the mycelial growth of *Colletotrichum gloeosporioides* causing leaf spot of Kokum.

Vinaya (2008) reported that *Trichoderma viridae, T. koningii T. harzianum Pseudomonas fluorescens, Bacillus subtillis* were found to be effective bioagent which inhibited the mycelial growth of *C. capsici.*

Mallesh *et al.* (2009) reported that RB50 (Rhizobacterial strain) was most effective, showed highest per cent mycelial inhibition against *Colletotrichum gloeosporioides.*

Vinod *et al.* (2009) reported that *Trichoderma viridae* was found to be effective which inhibited the mycelial growth of *Colletotrichum gloeosporioides* causing anthracnose of papaya

Watve *et al.* (2009) reported that *Trichoderma harazianum* (83.33%) followed by *T. viridae* (77.78%) and *Bacillus subtillis* (77.78%) were found to be effective which inhibited the mycelial growth of *Colletotrichum gloeosporioides* causing leaf spot of jatropha.

2.7.3 *In vitro* evaluation plant extracts

In recent years, the increasing use of potentially hazardous fungicides in agriculture has been the subject of growing concern of both environmental and public health authorities. Integration of chemicals, plant extracts, biotic agents along with resistance for managing the plant diseases has been considered as novel approach, as it requires low amount of chemicals, by reducing the cost of control as well as pollution hazards with minimum interference of biological equilibrium (Papavizas, 1973)

Ark and Thompson (1959) demonstrated the aqueous and organic solvent extracts of garlic had potent fungicidal and bactericidal activity against several plant pathogens. Pathak and Jain (1970) reported the antifungal activity of leaf extracts of tulsi.

Jetti *et al*. (1987) observed that, leaf extracts of *Polyalthia longifolia* inhibited the growth of *Colletotrichum gloeosporioides in vitro*.

Chauhan and Joshi (1991) tested the efficacy and persistence of 14 plant extracts and carbendazim in controlling anthracnose of mango fruit caused by *Colletotrichum gloeosporioides*. Carbendazim (0.05%) was the most effective control treatment. Eucalyptus oil (2%) and castor oil (10%) solutions inhibited infection for more than two weeks when fruits were inoculated and were significantly better than the other plant extracts tested. Similarly castor oil (5%), eucalyptus oil (1%), garlic bulb, *Zingiber officinale*, mango, turmeric and lantana leaves also significantly controlled the disease. Persistence was maximum with carbendazim (0.05%) even in the pulp, followed by castor oil (10%), garlic bulb and leaves.

Tulsi (*Ocimum* spp.) leaf extract was found to check spore germination, growth, total proteins and pectolytic and cellulolytic enzymes of various rot pathogens (Saini and Pathak, 1991; Patel, 1991; Patil *et al.,* 1992; Vyas, 1993; Godara and Pathak, 1995).

Escopalao and Silvestre (1996) tested fifteen medicinal plant extracts *in vitro* against *Colletotrichum gloeosporioides* the cause of anthracnose of mango fruits. Only the extracts of komoneigue (fruit) and garlic vine (leaves) crude extracts limited the growth of the fungus. Both extracts were effective against at 1:10 and 1:100 dilution and were comparable to the benomyl chemical check.

Ashashivapuri *et al.* (1997) observed the ethanol extract of *Allium cepa* L, *Allium sativum* L., *Azardirachta indica* Jess, *Calotropis procera* L. *Datura stromonium* L *Ocimum sanctum* L, *Polyalthia longifolia* Benth. and Hook, *Tegetus erecta* L. *Vinca rosea* L. and *Withamia somnifera* L. showed fungi toxic properties against *C. capsici*.

Prasanna Kumar *et al.* (2006) evaluated three plant extracts against *Colletotrichum gloeosporioides*, among these Ocimum leaf extracts was found to be best in inhibiting the fungus.

Prabhakar *et al.* (2003) evaluated 26 plant extracts against mango anthracnose, among these *Adenocalyma alleaceum* and *Bougainvillea spectabilis* were most effective in inhibiting the mycelial growth of fungus.

Rangarajulu *et al.* (2003) reported that ethanolic root extracts of *Abrus precatorius* and *Rauvolfia tetraphylla* showed inhibitory effects on both conidial germination and radial growth of *C.capsici*.

Roico *et al.* (2007) evaluated crude extracts of leaves and stems of 40 different plant species against *C.gloeosporoides* causing anthracnose of papaya. The better antifungal effect observed with crude plant extract of night-blooming jasmine and cherimoya after 14 or 18 hr incubation, determind by optical microscopy method and spectrophotometry method.

Babu *et al.* (2008) reported that extraction of plant parts with ethanol and acetone were more inhibitory than water extractions to fruit rot pathogen *Colletotrichum gloeosporioides*. Extracts of *Azadirachta indica, Tagtes erecta* and *Annona squamosa* were effective in inhibiting the growth of the fungus.

Jadav *et al.* (2008) reported that garlic bulb (10%) extract was effective in inhibiting the growth of *Colletotrichum gloeosporioides.*

Prashanth *et al* (2008) evaluated the plant extracts of eucalyptus, garlic extract, datura leaf extract, ocimum leaf extract, *Polyalthia longifolia* leaf extract inhibited the growth of *C.gloeosporoides* the causal organism of pomegranate anthracnose.

Sreelatha and Bhagynarajan (2008) reported that *Ocimum sanctum* was effective at all the tested concentrations inhibiting the growth of *Colletotrichum gloeosporioides.*

Vinod *et al.* (2009) reported that *Lantana camera* at 7.5% was found to be superior (45.54%) followed by turmeric at 7.5% (40.73%) against *Colletotrichum gloeosporioides* on papaya.

Watve *et al.* (2009) reported that maximum inhibition was achieved due to neem leaf extract(78.15%) followed by garlic (58.89%), sadafuli (57.04%) and tulsi (55.93%) and the least colony diameter was observed in glyricidia (25.93%) against jatropha leaf spot caused by *Colletotrichum gloeosporioides*.

2.7.4 *In vivo* evaluation of fungicides, bioagents botanicals

Tandon and Singh (1968) reported that, two sprays of zineb or bordeaux mixture at the time of flowering and continued upto harvest at 14 days intervals for control of anthracnose of mango.

Thompson (1987) reported that five spays of benomyl per season just before flowering and continuing right upto harvest for adequate control of anthracnose of pomegranate

Desai (1998) conducted field studies in Karnataka and reported that by spray of kitazin at 0.05% to 0.2% gave effective control (97.78-99.5%) of anthracnose caused by *Colletotrichum gloeosporioides* on pomegranate.

Hegde (1998), *in vivo* evaluation of fungitoxicants revealed that propiconazole(0.05%), mancozeb(0.2%) , hexaconazole (0.05%) and triademefon (0.05%) were more effective in inhibiting the growth of *C.capsici.*

Jamadar *et al.* (1998) reported that combi product like mancozeb 0.2% + carbendazim 0.05% was more effective in controlling fruit spot incidence over control followed by bordeaux mixture, reduced the disease by more than 88% against control.

Navale *et al.* (1998) evaluated the efficacy of fungicides against the fruit spot of pomegranate in mrig bahar. Ziram at the rate of 0.25 per cent was found to be cheaper than the remaining fungicides tested, which recorded least per cent disease index (0.17%).

Charigkapakorn (2000) reported that, crude extract from rhizome, leaves and creeping branches of sweetflag (*Acorus calamus* L.), palmorosa (*Cymbopogon martinii*) oil, *Ocimum sanctum* leaf extract, and neem (*Azadirachia indica*) oil could restrict growth of the anthracnose fungus. Among the biofungicides used against the fungus *Colletotrichum* spp. on chilli fruit, found that the most effective control was sweetflag crude extract when applied in two intervals when the majority of the plants were at the first bloom stage and at the mature bloom stage.

Freeman *et al*. (2001) reported that, *Trichoderma* species have been applied to control anthracnose of strawberry.

Gaikwad (2000) reported that, seven sprays of fungicides carbendazim (0.1%) and mancozeb (0.2%) was found to be effective for controlling leaf and fruit spot of pomegranate caused by *Colletotrichum gloeosporioides.*

Jahagirdar *et al.* (2000) found that, three sprays with kitazin at the rate of 0.2 per cent was found to be effective in checking the incidence of anthracnose of pomegranate (5.5%) compared to farmers method (16.3%).

Chhata and Kumawat (2001) studied the management of bacterial and fungal fruit spot of pomegranate two years on farmer field and reported that four sprays of bavistin (0.1%) + streptocycline (0.04%) at an interval of 15 days reduced the PDI to 10.42 where as untreated control recorded 71.21 PDI.

Strobilurin fungicides azoxystrobin (Quadris), trifloxystrobin (Flint), and pyraclostrobin (Cabrio) have recently been labeled for the control of anthracnose of chilli (Alexander and Waldenmaier, 2002; Lewis and Miller, 2003).

Gaikwad *et al.* (2002) tested efficacy of mancozeb (0.25%), copper oxychloride (0.25%), carbendazim (0.10 per cent), chlorothalonil (0.25%) mancozeb + carbendazim (0.3 per cent) and bordeaux mixture (1.00%) in controlling fruit rot of custard apple *Annona squamosa* caused by *Colletotrichum gloeosporioides* [*Glomerella cingulata*] and recorded better control of the pathogen compared to the control. Spraying with bordeux mixture resulted in the highest disease control (88.28%) and consequently, in the highest fruit yield (11.49 t/ha), fruit weight (181.42 g), returns (Rs. 94,587 ha^{-1}), additional income over the control (Rs. 82,224 ha^{-1}) and benefit: cost ratio (1: 5.9).

Raghuwanshi *et al.* (2004) tested eight fungicides along with check (unsprayed) against leaf and fruit spot of pomegranate caused by *Colletotrichum gloeosporioides* and found that carbendazim at 0.1 per cent was found to be effective reducing the per cent disease index (4.83) as compared to other fungicides tested.

Boonratkwang *et al*. (2007) reported that *Trichoderma* species have been applied to control *Colletotrichum* species in chilli.

Patel *et al.* (2007) evaluated the efficacy of fungicides on the leaf and fruit spots in Pomegranate. Among different fungicides thiophenate methyl and copper oxychloride showed highest leaf and fruit intensity and difenconazole and propiconazole showing lowest fruit and leaf spot intensity

Babu *et al.* (2008) reported that, *Trichoderma viride* and plant growth promoting rhizobacteria reduced the growth of *Colletotrichum gloeosporioides* of banana fruit rot effectively.

Prasanna Kumar *et al.* (2006) reported that, carbendazim (0.1%) treatment showed lowest per cent disease index followed by tricyclazole, benomyl and copper oxychloride against *Colletotrichum gloeosporioides* of mango anthracnose.

Prashanth *et al.* (2008) reported the efficacy of difenconazole, propiconazole and iprobenfos against *Colletotrichum gloeosporioides* in managing the anthracnose of pomegranate.

Navale *et al*. (2009) reported that, spraying of 0.1% difenconazole aginst *Colletotrichum gloeosporioides* causing anthracnose of pomegranate showed least per cent disease intensity, maximum percent disease control and highest fruit yield followed by 0.1 % propiconazole.

Patel *et al.* (2009) reported that, carbendazim sprayed fruits showed highest per cent disease control over unsprayed fruits and propineb showed lowest percent disease control against *Colletotrichum gloeosporioides* of pomegranate.

2.8 Screening of genotypes

Verma *et.al* (1982) reported that Jelicote local was susceptible to fruit rot of pomegranate caused by *Colletotrichum gloeosporioides* and Muscat white is most susceptible under Northern Indian condition.

Bhat (1991) reported that RCR-1, Jots, Dholka genotypes were resistant and Ganesh variety was more susceptible to fruit rot of pomegranate caused by *Colletotrichum gloeosporioides* and *Aspergillus niger.*

Ekbote (1994) evaluated ten mango cultivars against *Colletotrichum gloeosporioides* through detached leaf method. Out of ten cultivars Dashehari and Amarpalli showed resistant reaction.

Venkataravanappa (2002) evaluated ten mango cultivars against *Colletotrichum gloeosporioides* by detached leaf method. Among ten genotypes evaluated Mallika and Alampur beneshan recorded moderately susceptible reaction.

Pin prick method was commonly used against screening of genotypes in many fruit crops (Anil *et al*., 2008 and Amarjit *et al*., 2009).

3. MATERIAL AND METHODS

The present studies on anthracnose of pomegranate and its management was carried out during 2009-10 in the Department of Plant Pathology, College of Agriculture, and University of Agricultural Sciences Dharwad. Field experiment was carried out at Bandi (Koppal District) in the *ambiabahar*. Dharwad is located in Northern transition zone (Zone-8) of Karnataka state at 15^{o} 15' N latitude, 75^{o} 7' E longitude with an altitude of 389.37 m above mean sea level. The details of materials used and the methodology followed during the course of investigation are described here under.

3.1 General laboratory procedure

3.1.1 Glassware cleaning

For all laboratory experimental studies Corning and Borosil glasswares were used. The glasswares were kept for 24 hrs in cleaning solution containing 60.0 g of potassium dichromate ($K_2Cr_2O_7$), 60.0 ml of concentrated sulphuric acid (H_2SO_4) in 1000 ml of water and were washed with soap powder followed by washing in running tap water and then finally rinsed with distilled water.

3.1.2 Sterilization

All the glasswares were sterilized in an autoclave at 1.1 kg/cm^2 pressure for 20 minutes and further sterilized in hot air oven at 160^{o} C for two hrs. Sterilization of both solid and liquid media was achieved by autoclaving at 1.1 kg/cm^2 (121.6^{o} C) pressure for 20 minutes for all the laboratory studies.

3.2 Disease survey and surveillance

The intensive roving survey was conducted during 2009 and 2010 to know the incidence and severity of anthracnose in the farmers' fields in Bagalkot, Bijapur, Gadag, Koppal and Raichur districts. In each district one to two taluks were selected, in each taluk three villages were selected and in each village one to three fields were surveyed. In field plants were selected in zigzag manner and the severity of anthracnose disease of pomegranate on leaf and fruit were recorded by following 0 to 5 scale.

Grade	Percent area of infection		Reaction
	On fruit	On leaf	
0	No infection	No infection	Immune
1	1-10	Up to 5	Resistant
2	11-25	6-10	Moderately resistant
3	26-50	11-20	Moderately susceptible
4	51-75	21-50	Susceptible
5	>75	50	Highly susceptible

Per cent disease index (PDI) was calculated by using following formula proposed by Wheeler (1969).

$$\text{Per cent disease index (PDI)} = \frac{\text{Sum of the individual disease ratings}}{\text{Number of fruits/leaves observed}} \times \frac{100}{\text{Maximum disease grade}}$$

3.2.1 Collection and isolation of the pathogen

Pomegranate fruits and leaves infected with anthracnose were collected from different places *viz.,* Bagalkot, Bijapur, Gadag, Koppal and Raichur during survey and used for isolation of the fungus *in vitro*. The isolation of the fungus was made by following standard tissue isolation technique as described below.

The infected portions along with some healthy parts were cut and surface sterilized using 1 per cent sodium hypochlorite solution for 60 seconds. These bits were thoroughly washed in sterile distilled water to remove the traces of sodium hypochlorite if any and then aseptically transferred to sterile potato dextrose agar (PDA) slants and incubated at room temperature (27±1^{o}C) and observed periodically for fungal growth and sporulation. Colonies, which developed from the bits, were identified by microscopic observation by taking mycelial and spore character as means for identifying the pathogen. After identification they were transferred to new PDA slants and incubated at 27±1^{o}C for further use.

3.2.2 Single spore isolation

Ten ml of clear filtered two per cent water agar was poured into the sterile Petriplates and allowed to solidify. Diluted spore suspension was prepared in sterilized distilled water from 12 days old culture. One ml of such suspension was spread uniformly on agar plate. These plates were incubated at 27 ± 1^{o} C for 12 hrs. Then such plates were examined under microscope so as to locate germination of conidia. Single isolated germinated conidium was marked with ink on the lower surface of the plates.

The growing hyphal tip portion was transferred to fresh PDA slants with the help of cork borer under aseptic conditions and incubated at 27 ± 1^{o} C. Such obtained pure culture tubes were used for further studies.

3.2.3 Maintenance of the culture

The fungus was sub cultured on PDA slant and allowed to grow at 27 ± 1^{o} C for 12 days. Such slants were preserved in refrigerator at 5^{o} C and renewed once in a month. This pure culture was used for future study.

3.2.4 Identification of the fungus

The morphological characters of the fungus such as mycelial and cultural characters, length and breadth of conidia, fruiting body were studied by using microscope.

3.2.5 Proving the pathogenicity

Detached leaf technique

The healthy young leaves were selected from the pomegranate plant, washed thoroughly with tap water, swabbed with 1per cent sodium hypochlorite and washed with distilled sterile water. Spore suspension of fungal culture was prepared (10^{6} cfu / ml). This suspension was used for inoculating the healthy pomegranate leaves. Control was maintained by spraying with sterile water only. Moist cotton swab was placed at the base of petiole. The inoculated leaves were kept in Petriplates, lined with moist blotting paper to maintain humidity. Further they were placed in humid chamber and incubated at 27 ± 1^{o} C. Observations were made at two days intervals for symptom development. The organism was re-isolated from these artificially inoculated leaves and the culture so obtained was compared with original culture.

3.3 Cultural and morphological studies

3.3.1 Growth phase of *C. gloeosporioides* on potato dextrose broth (PDB)

Thirty ml of potato dextrose broth (PDB) was added into each of 100 ml conical flask and sterilized at 1.1 kg/cm^2 pressure for 20 minutes at 121°C. After strelization these flasks were allowed to cool and then inoculated with 5 mm disc from 12 days old culture and incubated at room temperature. Each treatment was replicated three times. The culture was filtered through Whatman No. 42 filter paper of 9 cm diameter, which were dried to a constant temperature at 60°C in an electric oven prior to filtration. The mycelial mat on the filter paper was thoroughly washed with sterile distilled water to remove traces of salts likely to be associated with it. Three flasks were harvested at 48 hrs after incubation and subsequent harvesting was done at an interval of two days up to 20 days.

The filter paper along with the mycelial mat were dried to a constant weight at 60°C and weighed immediately on an analytical balance. The difference between final and initial weight of filter disc was taken as the weight of the mycelial mat. The data were analyzed statistically.

Dry mycelial weight (mg) = Total weight of filter paper along with mycelia – Initial weight of filter paper

3.3.2 Growth characters of *C. gloeosporioides* on different solid media

The cultural characters of single spore Bagalkot isolate of *C. gloeosporioides* was studied on five non synthetic/semi-synthetic and two synthetic solid and liquid media.

1) Non synthetic or semi synthetic media
    I. Potato dextrose agar
    II. Oatmeal agar
    III. Malt extract agar
    IV. Host leaf extract agar
    V. Sabouraud dextrose agar
2) Synthetic media
    I. Richards' agar
    II. Czapek (Dox) agar

The composition and preparation of the above mentioned synthetic and semi- synthetic media were obtained from Ainsworth and Bisby's 'Dictionary of the Fungi' by Ainsworth (1971) and plant pathological methods, fungi and bacteria by Tuite (1969). The composition of the media is given below.

I. Potato dextrose agar (PDA)

In most of the experimental studies the Potato dextrose agar (PDA) was used. The composition of PDA is as follows

Potato (peeled)	200.00 g
Dextrose	20.00 g
Agar-agar	20.00 g
Distilled water	1000.00ml (to make up)

Two hundred grams of peeled and cleaned potato was made into small pieces. Later these pieces were boiled in distilled water and then extract was collected by filtering through

muslin cloth. Dextrose 20.0 g and agar 20.0 g each were dissolved in the potato extract and the final volume was made up to 1000 ml with distilled water. Known quantity of such medium was dispensed into a number of conical flasks and plugged with non-absorbent cotton and finally wrapped with brown paper. The flask containing dispensed medium were sterilized at 1.1 kg/cm^2 pressure for 20 minutes.

II. Oatmeal agar

Oat flakes	30.00 g
Agar agar	20.00 g
Distilled water	1000.00 ml (to make up)

First oat flakes were boiled in 500 ml distilled water for thirty minutes and filtered through muslin cloth. Agar agar was melted in 500 ml of water separately and then both the solutions were mixed thoroughly and the volume was made up to one liter and sterilized.

III. Malt extract agar

Malt extract	20.00 g
Agar agar	20.00 g
Distilled water	1000.00 ml (to make up)

First malt flakes were boiled in 500 ml distilled water for thirty minutes and filtered through muslin cloth. Agar agar was melted in 500 ml of water separately and then both the solutions were mixed thoroughly and the volume was made up to one liter and sterilized

IV. Host leaf agar (HLA)

Host leaf	200.00 g
Dextrose	20.00 g
Agar-agar	20.00 g
Distilled water	1000.00 ml

Two hundred grams of pomegranate leaves were cut into small bits, boiled in 500 ml distilled water for 30 minutes and extract was collected by filtering through muslin cloth. Dextrose 20 g and Agar agar 20 g each were dissolved in the leaf extract and the final volume was made up to 1000 ml with distilled water and sterilized.

V. Sabouraud dextrose agar

Dextrose ($C_6H_{12}O_6$)	40.00 g
Peptone	10.00 g
Agar-agar	20.00 g
Distilled water	1000.00 ml

All the ingredients were dissolved one by one in 400 ml distilled water and agar was dissolved separately in 500 ml distilled water and mixed with the above solution and the volume was made up to one litre and sterilized as described earlier.

VI. Richards' agar

Sucrose ($C_{12}H_{22}O_{11}$)	20.00 g
Potassium nitrate (KNO_3)	10.00 g
Potassium dihydrogen phosphate (KH_2PO_4)	5.00 g

Magnesium sulphate ($MgSO_4$. $7H_2O$)	2.50 g
Ferric chloride ($FeCl_3$. $6H_2O$)	0.02 g
Agar-agar	20.00 g
Distilled water	1000.00 ml

All the ingredients except Potassium dihydrogen phosphate was dissolved separately in 450 ml of distilled water. Agar melted in 500 ml distilled water was mixed with the above solution. Potassium dihydrogen phosphate was dissolved separately in 50 ml water and mixed together to get and final volume of 1000 ml and sterilized.

VII. Czapek (Dox) agar

Sucrose ($C_6H_{12}O_6$)	30.00 g
Sodium nitrate ($NaNO_3$)	2.00 g
Potassium dihydrogen phosphate (KH_2PO_4)	1.00 g
Magnesium sulphate ($MgSO_4$. $2H_2O$)	0.50 g
Potassium chloride (KCl)	0.50 g
Ferric chloride ($FeCl_3$. $6H_2O$)	0.01 g
Agar-agar	20.00 g
Distilled water	1000.00 ml

Agar-agar was melted in 500 ml distilled water. Ingredients were dissolved in remaining 500 ml distilled water. Two solutions were mixed thoroughly and the volume was made up to 1000 ml and sterilized.

Twenty ml of each medium listed above was poured aseptically into 90 mm diameter Petriplates. After solidification, five mm discs of the *C. gloeosporioides* were selected from actively growing culture using a cork borer and a single disc placed at the center of Petridish. Each set of experiment replicated five and they were incubated at 27 ± 1^o C for 12 days. Cultural characters such as the colony diameter, colony colour, and sporulation were recorded. The sporulation was graded as follows.

Sl. No.	Score	Grade	Conidia/microscopic field (400x)
1.	++++	Excellent	> 75
2.	+++	Good	50-75
3.	++	Moderate	25-50
4.	+	Poor	1-25
5.	-	No sporulation	---

3.3.3 Growth studies of *C. gloeosporioides* on different liquid media

The composition and preparation of different liquid media used, were the same as that of solid media except that the agar was not added. All the liquid media were sterilized and the flasks were inoculated under asceptic condition and were incubated at 27 ± 1℃ for 12 days. The mycelia growth harvested, dried and weighed. The best non-synthetic/ semi-synthetic and synthetic media was found and used as a basal medium for further studies.

Dry mycelial weight (mg) = Total weight of filter paper along with mycelia – Initial weight of filter paper

3.3.4 Spore Morphology

A loopful of culture of *C. gloeosporioides* obtained from twelve days old culture was placed on the slide and mixed thoroughly with lactophenol to obtain uniform spread and cover slip was placed. Length and breadth of one hundred spores were measured using motic images in computer (400 X). The average size of the spore was calculated. Similarly, the spores produced in the host leaf tissue were also measured and the average size was calculated.

3.4 Physiological studies

3.4.1 Effect of temperature on the growth of *C. gloeosporioides*

Effect of temperature on the growth of fungus was tested at 15, 20, 25, 30 and 35^{o} C. Twenty five ml of potato dextrose broth (PDB) was added into each of 100 ml conical flask and sterilized at 1.1 kg/cm^{2} pressure for 20 minutes at 121℃. After sterilization these flasks were allowed to cool and then inoculated with 5 mm disc from 12 days old culture and incubated at 27 ± 1^{o} C for 12 days. For each temperature level five replications were maintained. After incubation period the culture was filtered through Whatman No. 42 filter paper of 9 cm diameter. Dry mycelial weight was recorded for each flask as described earlier.

3.4.2 Effect of light on mycelial growth of *C. gloeosporioides*

The effect of light on the growth of *C. gloeosporioides* was studied by exposing it on potato dextrose agar to alternate cycle of 12 hr light followed by dark, 12 hr dark and light, continuous light and continuous darkness. Petriplates were inoculated with 5 mm disc taken from the periphery of 12 days old pure culture, each treatment was replicated five times and incubated for 12 days. Colony diameter and colony characters were analyzed statistically.

3.5 Toxin studies

3.5.1 Production of toxic metabolites by *C. gloeosporioides*

Culture filtrates of *C. gloeosporioides* obtained by using Potato dextrose broth as basal medium. Aliquots of thirty ml of basal medium was dispensed in 100 ml conical flask and sterilized. Each flask was inoculated with 5 mm disc from 12 days old culture. The flasks were incubated for 20 days at room temperature. Each treatment replicated three times. One set of flasks were harvested on second day after incubation and subsequent harvesting was done at an interval of two days up to 20 days. The culture was filtered through Whatman No. 42 filter paper. The culture filtrate obtained was used for further studies.

3.5.2 Effect of culture filtrate on germination, shoot and root length of sorghum seeds

This experiment was conducted as per the procedure followed by Anahousr (1976). One hundred healthy sorghum seeds were surface sterilized in 1% sodium hypochlorite solution and washed with sterile distilled water to remove the traces of sodium hypochlorite. The sorghum seeds were soaked in culture filtrates for 30 minutes. They were spread on moistened germination paper. Equal numbers of healthy seeds were soaked in the sterile distilled water and Potato dextrose broth, which served as control.

Observations on germination of sorghum seeds were recorded after seven days. Per cent inhibition of seed germination was calculated by the formula given by Vincent (1947). Further root and shoot lengths were recorded in each treatment and seedling vigour index was calculated.

Seedling vigour = (Shoot length + Root length) × Germination percentage

3.5.3 Effect of culture filtrate on tomato seedlings

This experiment was carried out to know production of toxic metabolites by pathogen. The culture filtrates of C*. gloeosporioides* obtained from two days to 20 days at an interval of two days was subjected to find effect on tomato seedlings. Then 25 days old seedlings of same size were selected, and placed in test tubes containing 15 ml of different days old culture filtrates. Each treatment was replicated three times and control was maintained with sterile distilled water and un-inoculated Potato dextrose broth. Effects of culture filtrate on tomato seedlings were recorded at 12 hr and 24 hr, 36 hr and 48 hr after incubation.

Observations on drooping of leaves, curling, marginal necrosis and wilting symptoms were recorded.

3.6 Disease management

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

The efficacy of six non-systemic (one combi- product) and six systemic fungicides were tested against *C. gloeosporioides* for radial growth inhibition on the potato dextrose agar media using poisoned food technique under *in vitro* condition. The non-systemic fungicides were tried at 0.1, 0.2 and 0.3 per cent concentration, whereas systemic fungicides were tried at 0.05, 0.1, 0.15 per cent concentrations. The list of fungicides used along with their chemical and trade names are given below.

List of non-systemic / combi fungicides

Sl. No.	Common name	Chemical name	Trade name
1.	Captan	N-(trichloro methyl thio) – 4 – cyclohexene 1,2, dicarboximide	Captaf 50% WP
2.	Carbendazim 12%+ Mancozeb 63%	Methyl 1-1-2 benzimidazole carbonate +zinc ion and manganese ethylene bis dithiocarbamate.	SAAF 75 %WP
3.	Copper oxychloride	Copper oxychloride	Blue copper-50%WP
4.	Chlorothalonil	Tetrachloroisophthalonitrate	Kavach 75% WP
5.	Mancozeb	Manganese ethylene bis dithiocarbomate plus zinc	Dithane M- 45 75 %WP
6.	Propineb	Zinc propylenebisdithiocarbamate	Antracol 70 %WP

List of systemic fungicides

Sl. No.	Common name	Chemical name	Trade name
1.	Azoxystrobin	Azoxystrobin	Amistar 25%SC
2.	Difenoconazole	trans, cis-3-chloro-6- [6-methyl-2-(1H-1, 2, 6-triazole-1-group methyl)-1, 3-dioxapentane-2 group] phenyl-6 chlorophenylether	Score 25 %EC
3.	Carbendazim	2-(methoxy-carbomyl) benzimidazole	Bavistin 50% WP
4.	Hexaconazole	RS)-2-(2,6-dichlorophenyl-4prophyl,3-dioxolany 2-yl)methyl)-1H-1,2,6-triazole	Contaf 5% EC
5.	Iprobenfos	P-(5-amino-3-phenyl-1h-1,2,6-triazole-1-yl)-N,N,N:N,N-tetramethyl phosphonic diamide	Kitazin 48%EC
6.	Propiconazole	1-{2-(2,6-dichlorophenyl)-6-1 propyl-1-1, 3-dioxolan-2-yl} -1 6-1, 2,6-triazole	Tilt 25% EC

3.6.1.1 Poisoned food technique

The poisoned food technique (Shravelle, 1961) was followed to evaluate the efficacy of non-systemic and systemic fungicides in inhibiting the mycelial growth of *C. gloeosporioides*. The fungus was grown on PDA medium for 12 days prior to setting up the experiment. The PDA medium was prepared and melted. The fungicidal suspension was added to the melted medium to obtain the required concentrations on commercial formulation basis of the fungicide. Twenty ml of poisoned medium was poured in each sterilized Petriplates. Suitable check was maintained without addition of fungicide. Mycelial disc of 5 mm was taken from the periphery of 12 days old colony was placed in the center of Petriplates and incubated at 27 $\pm 1^{o}$ C for 12 days and five replications were maintained for each treatment. The diameter of the colony was measured in two directions and average was recorded. Per cent inhibition mycelial growth of the fungus was calculated by using the formula by Vincent (1947).

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

Where,

I = Per cent inhibition

C = Radial growth in control

T = Radial growth in treatment (fungicide/ botanicals/bioagents)

3.6.2 *In vitro* evaluation of bioagents against *C. gloeosporioides*

The efficacy of four bioagents was tested against *C. gloeosporioides* for radial growth inhibition on the potato dextrose agar media using dual culture technique under *in vitro* condition.

List of bioagents used against *C. gloeosporioides* are mentioned below

1. *Trichoderma viridae,*

2. *Trichoderma harzianum*,

3. *Bacillus subtillis,*

4. *Pseudomonas fluorescence*.

3.6.2.1 Dual culture test

Bioagents were evaluated for their efficacy through dual culture technique. The bio agents and the test fungus were inoculated side by side on a single Petridish containing solidified PDA medium. Five replications were maintained for each treatment with one control by maintaining only pathogen separately. They were incubated for 12 days. The diameter of the colony of both bioagents and the pathogen was measured in two directions and average was recorded. Per cent inhibition of growth of the test fungus was calculated by using the formula of Vincent (1947).

3.6.3 *In vitro* evaluation of botanicals against *C. gloeosporioides*

The present investigation was carried out to evaluate the extracts of seven plant species to know the presence of fungitoxicant properties against *C. gloeosporioides*.

3.6.3.1 Preparation of plant based products

Fresh healthy plant parts of 100 g (leaves/bulb/rhizome) as indicated below were collected from field were washed with distilled water and air dried and crushed in 100 ml of sterile water. The crushed product was tied in muslin cloth and collected the filtrate. The prepared solution gave 100 per cent, which was further diluted to required concentrations of 10, 20 and 30 per cent. The extracts were tested against *C. gloeosporioides* on the PDA using poisoned food technique under *in vitro* condition as described earlier. The per cent inhibition of growth of the test fungus was calculated by using the formula of Vincent (1947).

List of plant extracts used under *in vitro* condition.

Sl. No.	Plants (Common name)	Scientific name	Plant part used
1.	Datura	*Datura stromenium* L.	Leaf
2.	Eucalyptus	*Eucalyptus* globules Labil	Leaf
3.	Garlic	*Allium sativum* L.	Bulb
4.	Ginger	*Zingeiber officinale* Rose	Rhizome
5.	Neem	*Azadirachta indica* Juss	Leaf
6.	Onion	*Allium cepa* L.	Bulb
7.	Tulsi	*Ocimum sanctum* L.	Leaf

3.6.4 *In vivo* evaluation of fungicides and bioagent against *C. gloeosporioides*

An experiment was conducted at farmer's field at Bandi village, Taluk Yalburga, Koppal district, during 2010 *ambiabahar* in relation to manage the anthracnose disease of pomegranate. The variety, Kesar was used and sprayed with different fungicides, and plant products. The experiment included nine treatments and one check with three replications.

Location : Bandi (Koppal District)

Variety : Kesar

Spacing : 4.25 m × 3.65 m

Treatments : 10

Number of plants / treatment : 4

Replication : 3

Year : 2009-10

Age of the plant : 5 year

Details of treatments

Treatments		Concentration (%)	Quantity/liter
T_1	Carbendazim	0.2	2 g
T_2	Difenoconazole	0.1	1 ml
T_3	Hexaconazole	0.1	2 ml
T_4	Iprobenfos	0.2	1 ml
T_5	Propiconazole	0.1	1 ml
T_6	Captan	0.3	3 g
T_7	Mancozeb	0.3	3 g
T_8	Carbendazim + Mancozeb	0.3	3 g
T_9	*Trichoderma viride*	1.0	10g
T_{10}	Untreated Control	-	-

Observations on disease severity was scored using 0-5 scale .The per cent disease index (PDI), Area under disease progression curve (AUDPC) and per cent disease reduction over control (PDC) was calculated and angular transformed data were analyzed statistically.

$$A = \sum_{i=1}^{k} \frac{1}{2}(S_i + S_{i-1})\,t$$

A= Area under disease progression curve (AUDPC)

S_i = Disease incidence at i th day of evaluation

k = Number of successive evaluation

t= Interval between i and i-1 evaluation of disease

$$PDC = \frac{PDIC-PDIT}{PDIC} \times 100$$

PDC = Per cent disease index over control

PDIC = PDI in control

PDIT = PDI in treatment

3.6.4.1 Yield parameters

Bahar treatments were imposed during *hastabahar* season because of unseasonal rains received during October, November excessive flower drop as observed in the orchard, further the crop was imposed during *ambiabahar*. After successive seven sprays of different chemicals / bioagent, the yield contributing parameters like fruit number and average fruit weight was recorded from the all treatments. Further estimated the fruit yield / tree later it was calculated per hectare by taking the count of 643 trees / ha (4.25 m × 3.65 m).

3.7 Screening for disease resistance

3.7.1 Evaluation by detached leaf technique

Nineteen pomegranate cultivars were selected from glass house of Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences Dharwad and detached leaf inoculation technique was used for screening under artificial inoculation (Tuite, 1969)

Five young leaves from each genotype were selected and detached from the plant, washed thoroughly with distilled sterile water, swabbed with 1 per cent sodium hypochlorite and washed with sterile water and inoculated with inoculum 5mm disc of 12 days old culture. Control was maintained by spraying sterile water only. Moist cotton swab was placed at the base of petiole. The leaves were kept in Petriplates lined with moist blotting paper to maintain humidity.

Further, these plates were placed in humid chamber and incubated at 27±1℃ for 12 days. After 12 days of incubation the genotypes were evaluated for their reaction on 0-5 scale.

The varieties were classified as follows.

Grade	Percent leaf area covered	Reaction
0	No disease	Immune
1	0 – 5.0	Resistant
2	5.1 -10.0	Moderately resistant
3	10.1 – 25.0	Moderately susceptible
4	25.1 – 50.0	Susceptible
5	>50.0	Highly susceptible

List of genotypes used for screening:

1. Araktha
2. Alandi

3. Ganesh
4. GUT-C-Shah rose pink
5. G-137
6. Jalore seedless
7. Jodhpur red
8. Jural anar
9. Jyoti
10. Kabuli
11. Kaladgi local
12. Kandar
13. Kesar
14. Mridula
15. Muskot
16. RCR
17. Ruby
18. Speen dahedar
19. Yeronad

3.8 Statistical analysis

The experimental data collected were analyzed statistically for its significance of difference by the normal statistical procedure adopted for completely randomized design and randomized block design and interpretation of data was carried out in accordance with Walter (1997). The level of significance used in 'F' and 'T' test was P=0.05 and P=0.01. Critical differences were calculated wherever 'F' test was significant. The values percent disease index was subjected to angular transformation according to the table given by Sundarraj *et al*., (1974).

4. EXPERIMENTAL RESULTS

The results of the experiments conducted on various aspects on anthracnose of pomegranate (*Punica granatum* L.) during the period 2009 to 2010 with reference to survey and surveillance of disease; cultural, morphological, physiological aspects of pathogen; management of disease by fungicides, bioagents, botanicals, host plant resistance and results so obtained are presented here under.

4.1 Disease survey and surveillance

Roving survey was undertaken during 2009 – 2010 to assess the severity of anthracnose of pomegranate in major pomegranate growing areas of Bagalkot, Bijapur, Gadag, Koppal, and Raichur districts by taking three gardens in each village, three villages in each taluk and one to two taluk in every disticts as explained in the “Material and Methods” and results are presented in Table 1a, Plate1.

Per cent disease index on leaf ranged from 8.88 to 29.99 with highest per cent disease index (29.99) was recorded in Kustagi of Koppal district followed by 29.00 PDI in Hebbal (Bagalkot) and 27.55 PDI in Hiremanpur. The least per cent disease index (8.88) was recorded in Tumbargudi (Koppal).

Among the different locations, Per cent disease index on fruit ranged from 12.38 to 37.53. Hebbal (Bagalkot) village recorded highest PDI of 37.53, followed by 37.33 PDI in Kushtagi (Koppal) and 32.88 PDI in both Kaladgi and Jerkundi (Bagalkot and Koppal). The least PDI 12.38 was recorded in Kalakaleshwar (Koppal) village.

District-wise sevrity of anthracnose of pomegranate surveyed during 2009-10 exhibited that (Table 1b and Fig. 2), maximum disease severity of 23.21 PDI on leaf was recorded in Bagalkot district followed by Koppal (21.84 PDI). Lowest leaf disease severity of 14.36 PDI was found in Raichur district. Severity on fruit ranged from 19.99 (Raichur) to 28.76 (Bagalkot) PDI. It was moderate in all other districts.

Looking into the varieties acerage (Table 1c and Fig. 3), Kesar occupied the larger cultivated area of pomegranate followed by Ganesh and Sindhoor. Among the varieties Araktha recorded 26.39 PDI on leaves and 33.61 PDI on fruits which is followed by Sindhoor (22.33 and 28.37 PDI).

Looking into the age of trees (Table 1d and Fig. 4) maximum PDI was observed in old age (>5 years) trees compared to young age trees (up to 2 years). On fruits maximum PDI was recorded in >5 year aged Araktha genotype (34.26 PDI) followed by Ganesh (31.10 PDI). The least PDI was recorded in upto 2 years aged trees (13.20 PDI). On leaf maximum PDI was recorded in >5year aged Araktha genotype (29.00 PDI), followed by Sindhoor (24.06 PDI). The least PDI was recorded in Ganesh genotype (8.88 PDI).

Among the three bahar maximum PDI was recorded in Mrigbahar (leaf - 25.15 and fruit – 28.28) and moderate in Hastabahar. The least PDI of 11.03 and 14.63 was recorded in Ambiabahar on leaves and fruits respectively (Table1e and Fig. 5)

4.2 Symptomatology

The typical anthracnose symptoms were observed on leaves and fruits.

4.2.1 Symptoms on leaves

Pinhead size of black to brown water soaked spots appeared on the leaves with circular margin. In advanced stage, these spots enlarged, coalesced and resulted in bigger patches. In severe case, leaves dried up and drooped down (Plate3a).

Table 1a. Survey on the severity of anthracnose of pomegranate caused by *Colletotrichum gloeosporioides* in major areas of northern Karnataka during 2009 – 10

Sl no.	District	Taluk	Village	No. of orchards	Cropping season	Month	Stage of the crop	Range (Grade)	Variety	Percent disease index	
										On Leaf	On Fruit
1	Bagalkot	Bagalkot	Govinkoppa	2	*Mrigbahar*	June	Fruit colour development	0-3	Sindhoor	21.33	25.30
			Kaladgi	3	*Mrigbahar*	June	Fruit colour development	0-3	Sindhoor	22.21	32.88
		Mudhol	Hebbal	4	*Mrigbahar*	June	Fruit colour development	0-4	Ganesh, Kesar, Araktha, Sindhoor	29.00 24.21 29.00 26.00a	37.53 26.33 34.26 25.00
			Mallingpur	3	*Mrigbahar*	June	Fruit colour development	0-3	Sindhoor	20.33	25.33
Mean										23.21	28.76
2	Bijapur	Bjapur	Babbaleshwar	1	*Mrigbahar*	July	Flowering	0-3	Ganesh	18.66	-
			Hitnalli	3	*Mrigbahar*	July	Flowering	0-3	Ganesh	19.99	-
			Jumnal	5	*Mrigbahar*	July	Flowering	0-3	Ganesh	20.33	-
			Utnal	1	*Mrigbahar*	July	Flowering	0-3	Ganesh	20.00	-
		Indi	Bardol	3	*Mrigbahar*	July	Flowering	0-3	Ganesh	24.44	-
									Mean	19.86	-
3	Gadag	Gajendragad	Vajrabandi	3	*Hastabahar*	November	Fruit colour development	0-3	Kesar	19.99	22.21
		Ron	Kotbal	4	*Hastabahar*	November	Fruit colour development	0-3	Kesar	20.00	25.33
			Udegere	3	*Hastabahar*	November	Fruit colour development	0-2	Kesar	17.33	21.33
Mean										19.10	22.95

Table 1a Contd…

Sl no.	District	Taluk	Village	No. of orchards	Cropping season	Month	Stage of the crop	Range (Grade)	Variety	Percent disease index	
										On Leaf	On Fruit
4	Koppal	Kushtagi	Chumnal	1	*Mrigbahar*	July	Fruit colour development	0-3	Araktha	25.33	29.33
			Hiremanpur	3	*Mrigbahar*	July	Fruit colour development	0-3	Kesar	27.55	31.99
			Kalkbandi	3	*Mrigbahar*	July	Fruit colour development	0-3	Kesar, Araktha	24.00 25.86	27.66 28.86
			Kushtagi	2	*Mrigbahar*	July	Fruit colour development	0-3	Kesar	29.99	37.33
			Moudigere	3	*Mrigbahar*	July	Fruit colour development	0-3	Kesar	24.66	29.66
		Yalburga	Jerkundi	3	*Mrigbahar*	July	Fruit colour development	0-3	Kesar, Ruby red	26.33 22.33	32.88 28.33
			Bandi	1	*Hastabahar*	January	Flowering	0-3	Kesar	22.66	-
			Bandi	1	*Ambiabahar*	May	Fruit colour development	0-2	Kesar	18.66	22.33
			KalaKaleshwara	2	*Ambiabahar*	May	Fruit colour development	0-2	Kesar	9.55	12.38
			Tumbarguddi	1	*Ambiabahar*	May	Fruit colour development	0-2	Ganesh	8.88	13.2
Mean										21.84	23.95
5	Raichur	Raichur	Appledinne	5	*Hastabahar*	September	Fruit colour development	0-2	Kesar	14.22	20
			Tuntapura	3	*Hastabahar*	September	Fruit colour development	0-2	Kesar	15.55	21.33
			Chandrabanda	4	*Hastabahar*	September	Fruit colour development	0-2	Kesar	13.33	18.66
Mean										14.36	19.99

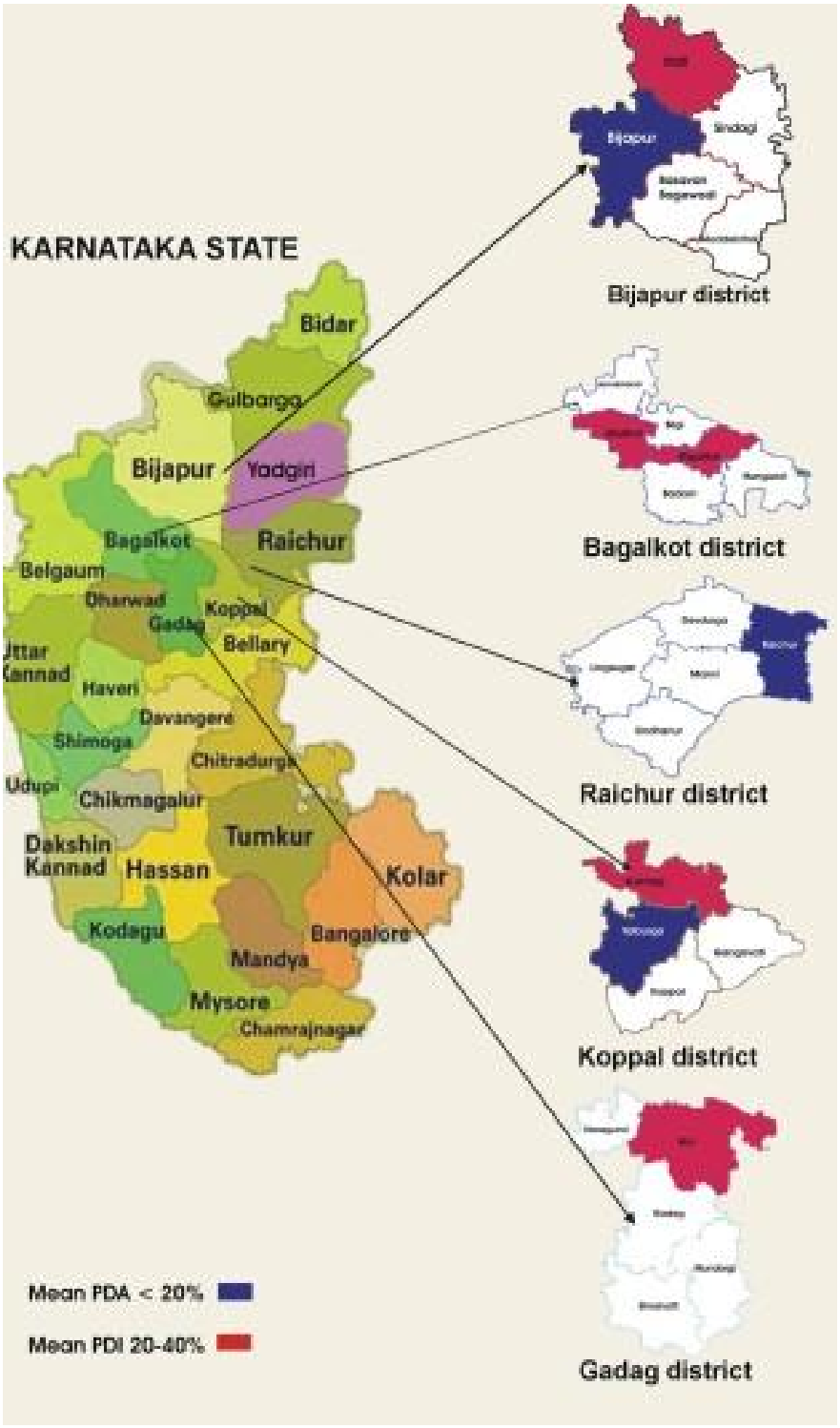


Plate.1. Severity of anthracnose of pomegranate in major growing areas of northern karnataka

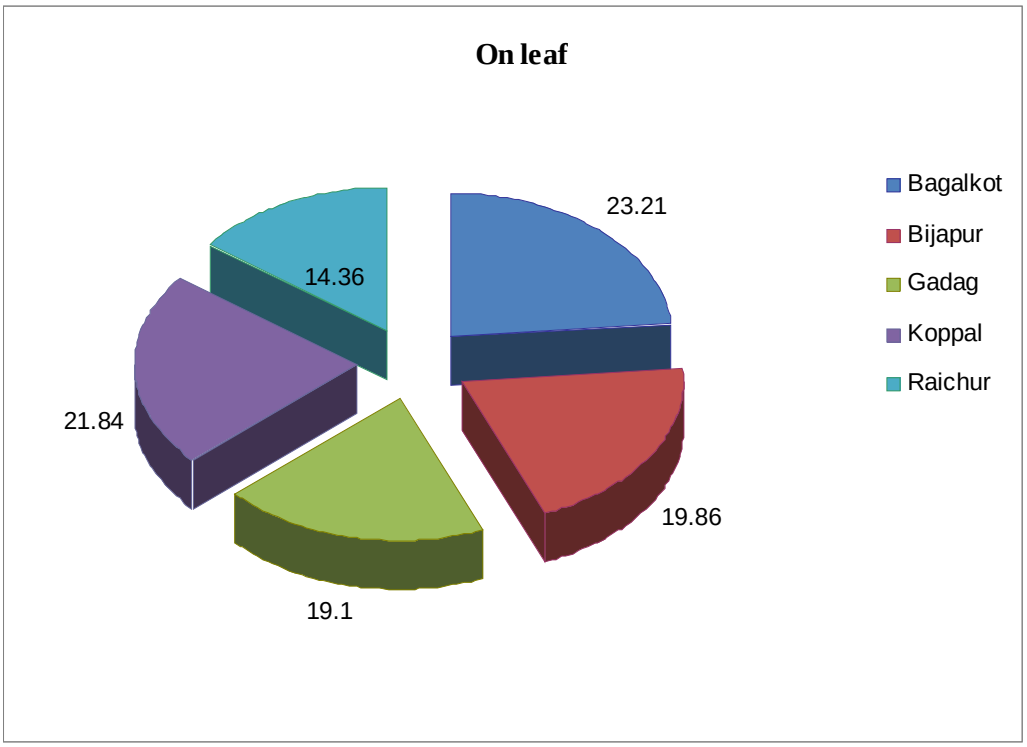
Healthy Orchard

Infected orchard

Plate.2. Severity of anthracnose disease on pomegranate observed during survey

Table 1b. District wise mean severity of anthracnose of pomegranate caused by *Colletotrichum gloeosporioides* in major areas of northern Karnataka during 2009-10

Sl.No.	District	Mean Percent disease index	
		On leaf	On fruit
1.	Bagalkot	23.21	28.76
2.	Bijapur	19.86	-
3.	Gadag	19.10	22.95
4.	Koppal	21.84	23.50
5.	Raichur	14.36	19.99



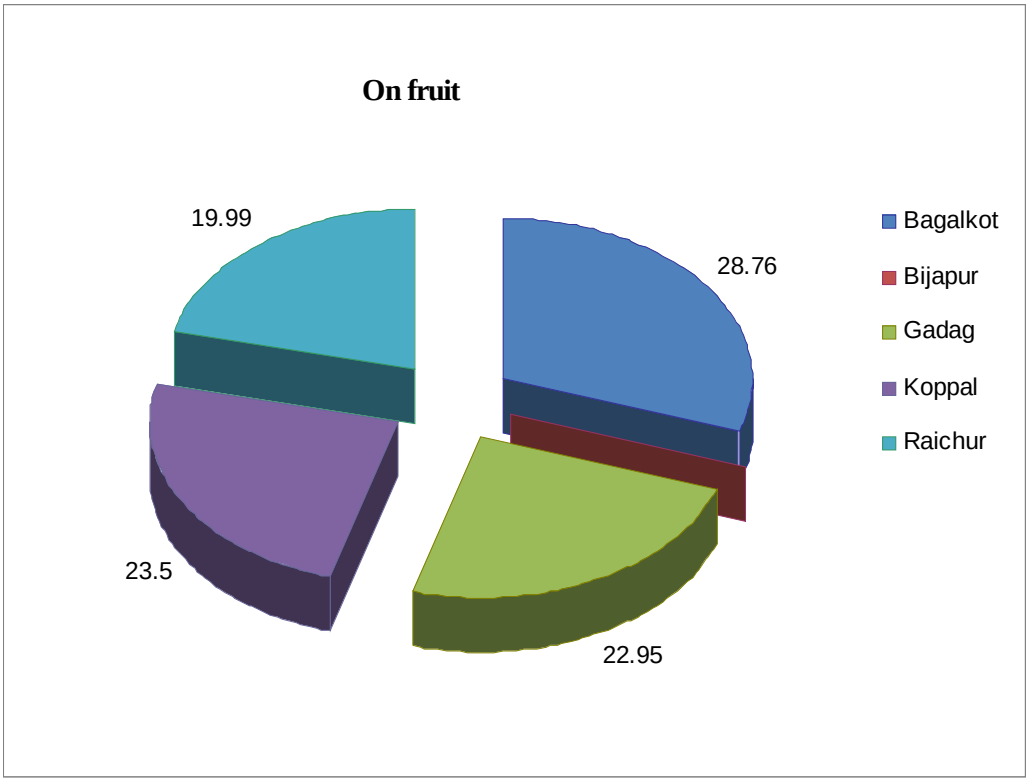
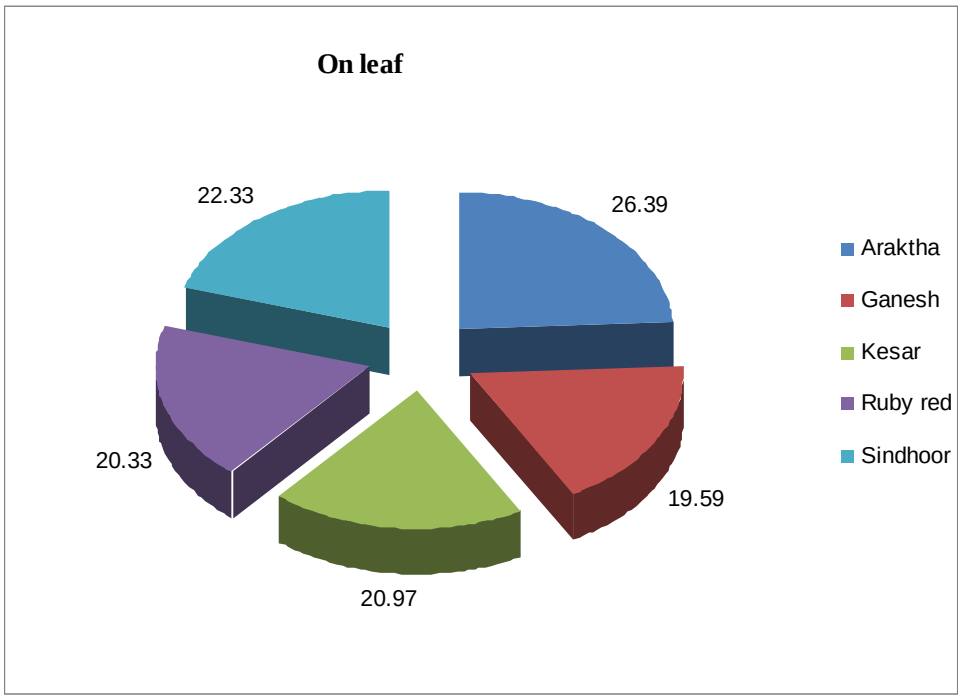


Fig. 2: Mean per cent disease index of pomegranate anthracnose in different districts of northern Karnataka during the year 2009-10

Table 1c. Variety-wise mean severity of anthracnose of pomegranate caused by *Colletotrichum gloeosporioides* in major areas of northern Karnataka during 2009-10

Sl.No.	Variety	Mean Percent disease index	
		On leaf	On fruit
1.	Araktha	26.39	33.61
2.	Ganesh	19.59	27.05
3.	Kesar	20.97	25.90
4.	Ruby red	20.33	26.33
5.	Sindhoor	22.33	28.37



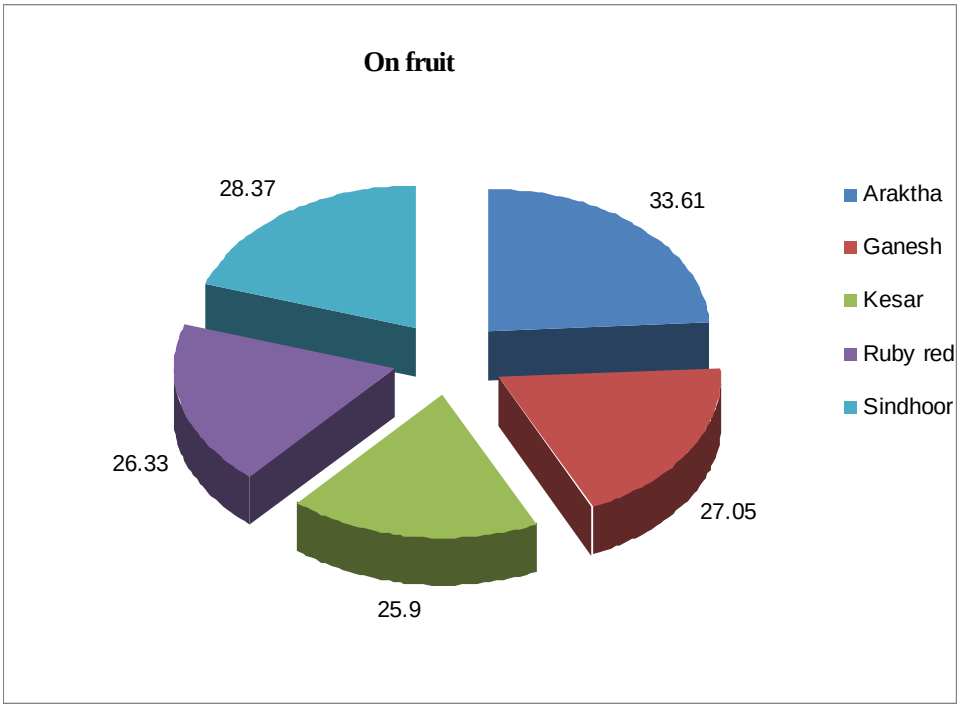


Fig. 3: Mean per cent disease index of pomegranate anthracnose in different varieties during the year 2009-10

Table 1d. Age-wise mean severity of anthracnose of pomegranate caused by *Colletotrichum gloeosporioides* in major areas of northern Karnataka during 2009-10

Sl.No	Age of tree	Mean Percent disease index										
		Araktha		Ganesh		Kesar		Ruby red		Sindhoor		Mean
		Leaf	Fruit	Leaf	Fruit	Leaf	Fruit	Leaf	Fruit	Leaf	Fruit	
1.	Up to 2 year	-	-	8.88	13.20	-	-	-	-	-	-	11.04
2.	2 – 5 year	25.09	29.09	20.33	25.86	19.44	24.47	22.33	28.33	21.77	29.09	24.58
3.	> 5 year	29.00	34.26	23.35	31.10	23.97	28.50	-	-	24.60	30.66	28.18

- No plants observed

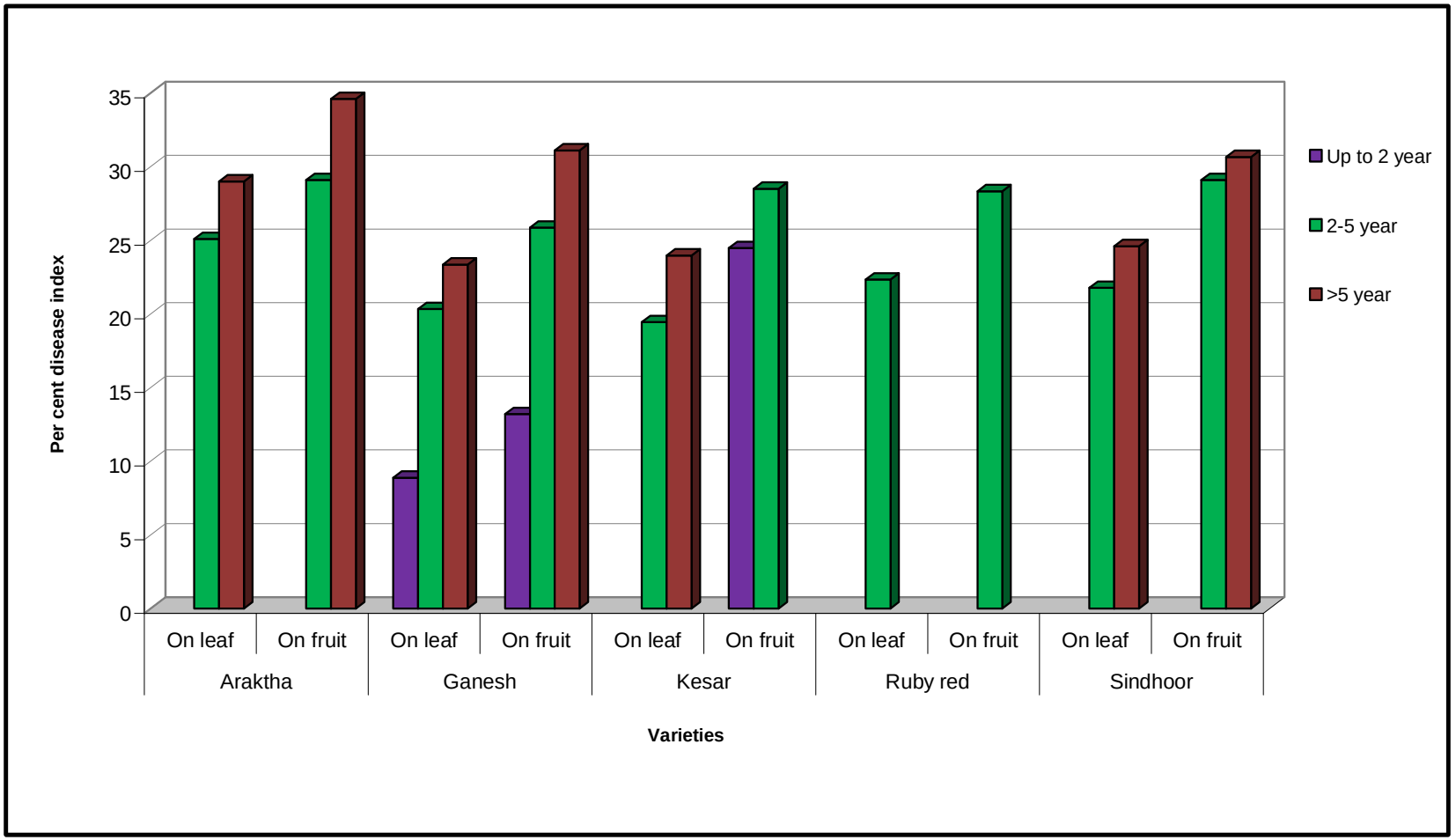
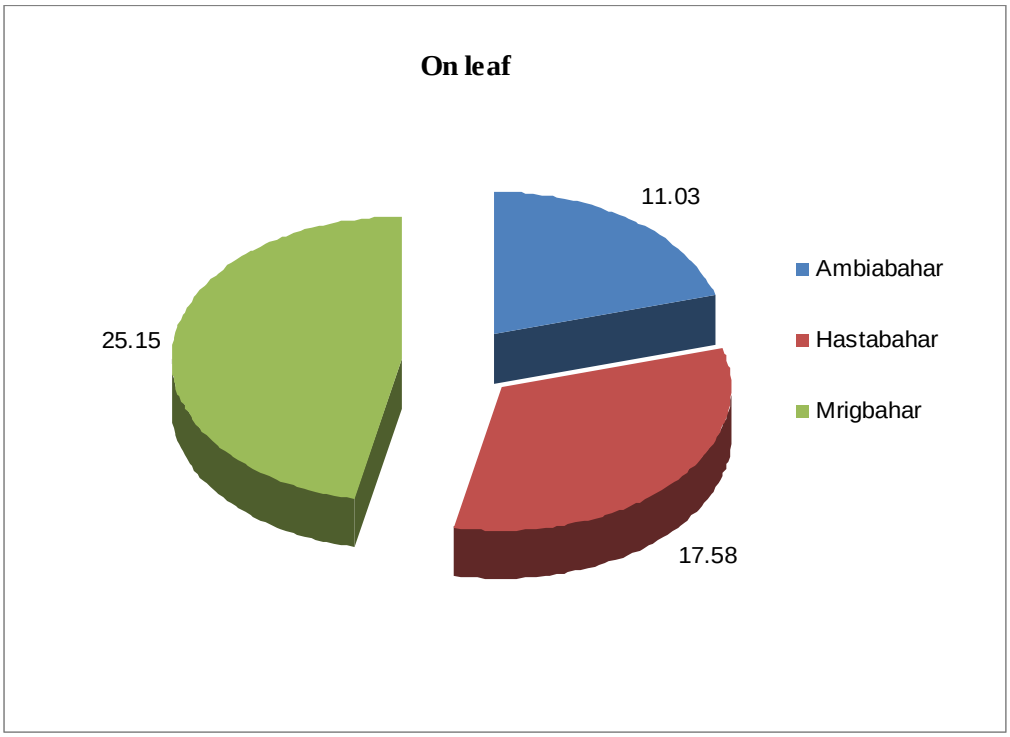


Fig. 4: Mean per cent disease index of pomegranate anthracnose in different age of varieties during the year 2009-10

Table 1e. Bahar-wise mean severity of anthracnose of pomegranate caused by *Colletotrichum gloeosporioides* in major areas of northern Karnataka during 2009-10

Sl.No	Season	Mean Percent disease index	
		On leaf	On fruit
1.	*Ambiabahar*	11.03	14.63
2.	*Hastabahar*	17.58	21.47
3.	*Mrigbahar*	25.15	28.28



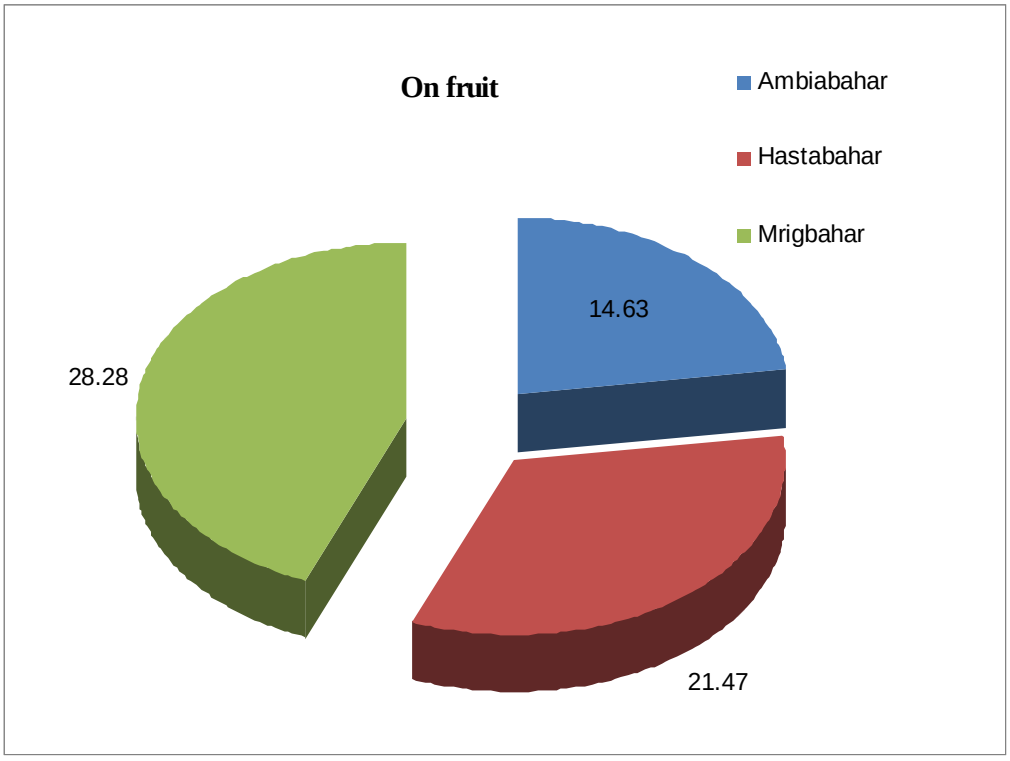


Fig. 5: Mean per cent disease index of pomegranate anthracnose in different bahar during the year 2009-10

4.2.2 Symptoms on fruits

On fruits, brown spherical depressed spots occurred in scattered form on the pericarp. In advanced stage, these spots coalesced to form necrotic patches over the surface of the fruit. When such diseased fruits were cut open the rotting symptoms were observed. Brown to dark brown colored seeds were seen in infected fruits. In advanced stage can observe the minute dark coloured acervulli were observed on depressed spots. However no smell was emitted from affected fruit. Looking to these symptoms, the disease is referred as “anthracnose” (Plate3b).

4.3 Isolation of the pathogen

Standard tissue isolation technique was followed to obtain causal agent from the pomegranate fruits showing typical anthracnose symptoms. Repeated isolation yielded a species of C*olletotrichm*.

4.3.1 Proving pathogenicity

Fungus was isolated from infected pomegranate fruit and pure culture was obtained by single spore isolation method as described in “Material and Methods” and such culture was used for pathogenecity test. Detached leaf method was carried as explained in “Material and Methods” (Plate 4).

On fourth day of inoculation, minute pin head size brown to black coloured water soaked spots were seen on leaves. On sixth day, these spot turned slightly brown surrounded by chlorotic halo, while on eighth day these spots enlarged and showed dark brown colour surrounded by slight chlorotic halo with irregular margins and were not deliminated by veins. On twelth day the central portion of spot showed minute dark coloured acervulli, later these spots coalesced. The fungus was reisolated and pathogenic culture thus obtained was compared with the original culture of *C. gloeosporioides*.

4.2.3 Identification of the pathogen

Identification of the fungus was carried out based on the morphological characters of the isolated fungus. The fungus in the present study produced septate mycelium. Later it produced Conidiophores arising singly or closely packed together in rows. Conidiophores were single celled, hyaline and aseptate with one or several conidial scars. The conidia were oblong or cylindrical or slightly dumbel, hyaline, aseptate with rounded ends, one to two oil globules were observed in the conidium .

4.4 Morphological characters

4.4.1 Spore morphology

In the present study conidia of *C. gloeosporioides* obtained from infected fruit and from culture were measured and compared in respect of their spore morphology (Plate 5)

The conidia were oblong or cylindrical or slightly dumbel, hyaline, aseptate with rounded ends and one to two oil globules. Conidia on the culture media were found to be in reddish mass. They were rarely found in aggregates. The conidia collected from potato dextrose agar measured 11.48-20.01 µm × 4.25-6.62 µm and average being 15.74 × 5.43 µm where as the conidia from host fruit measured 11.24-19.65 µm × 4.35-6.45 µm and average being 16.80 µm × 6.40 µm

4.4.2 Growth phase studies

The experiments was conducted as mentioned in “Material and Methods" to ascertain the period for the maximum growth of the fungus by dry mycelial weight method, starting from the 2^{nd} day to 20^{th} day. The results obtained are presented in the Table 2 and Fig. 6.

Early stage

Later stage

On Leaves

Healthy flowers

Infected flower

Infected flower

Infected flower

On floweres

Plate.3a. Symptoms of anthracnose on leaves and flowers

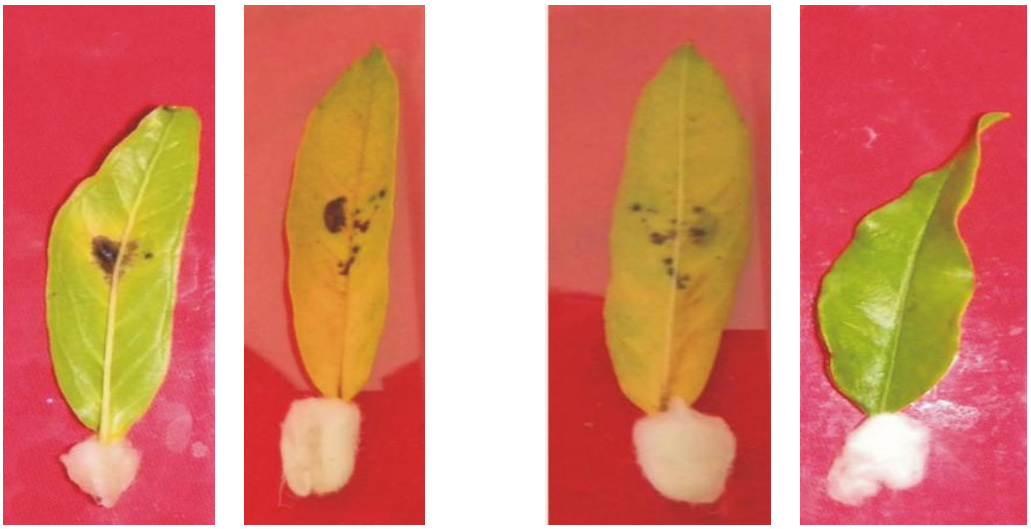





Infected fruits

Healthy fruits

Plate.3b. Symptons of anthracnose on fruits

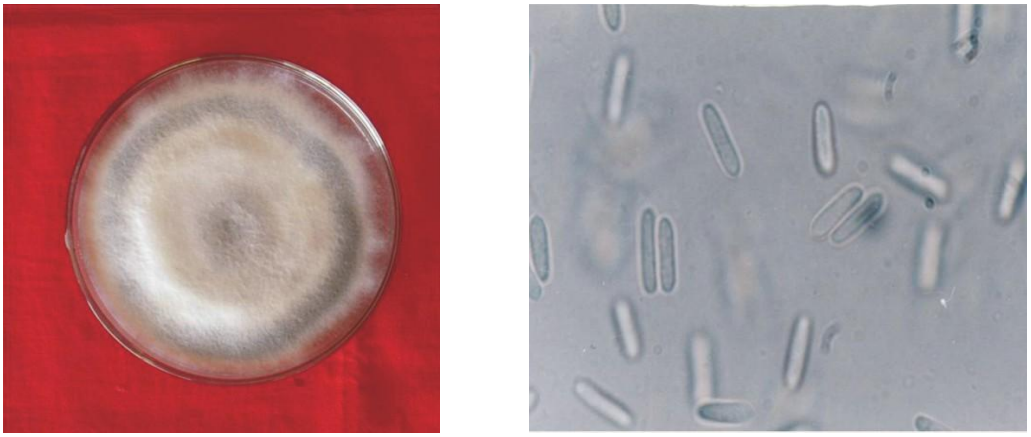


Infection on upper surface of leaf

Lower surface
Of leaf

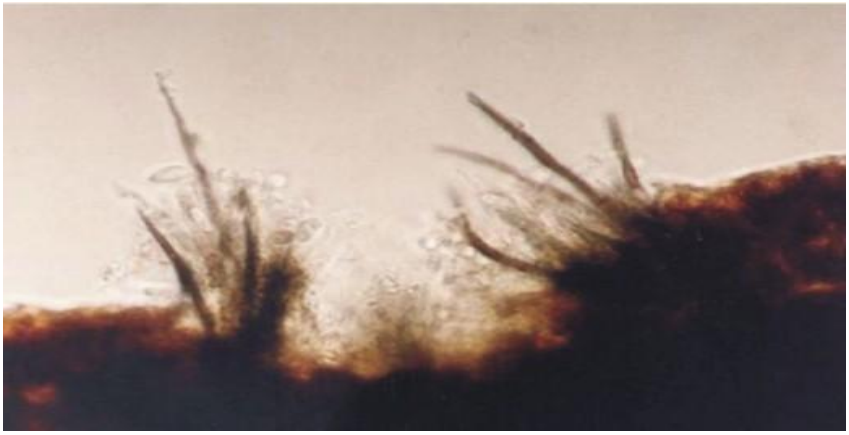
Healthy leaf

Plate.4. Proving pathogenicity



Culture

Spores



Acervulli

Plate.5. Culture and morphology of colletotrichum gloeosporioides

It is evident from the data that there were significant differences in the different incubation periods. Dry mycelial weight of *C. gloeosporioides* recorded gradual increase starting from second day (63.66 mg) and reached peak growth on 12thday (426.77 mg), and remained significantly superior over remaining treatments. Later the dry mycelia weight declined to reach 296.70 mg on 20th day of inocubation. The dry mycelia weight on 10th and 14th day remained on par with each other. Similarly on par results were recorded on 8th and 18th day of incubation.

As highest growth of the fungus was recorded at 12th day it was taken for further studies.

4.4.3 Effect of different liquid media on growth of *C. gloeosporioides*

The results of the experiment pertaining to identification the best liquid media for growth under laboratory conditions are presented in Table 3; Fig.7 and Plate 6. For this two synthetic and five non synthetic / semi synthetic broth were tested as described in "Material and Methods".

The data revealed that, there were significant differences among the different liquid media on growth of *C. gloeosporioides*. Maximum dry mycelial weight of *C. gloeosporioides* was recorded in Potato dextrose broth (416.67mg), which was found to be significantly superior to all the tested broths. Next best basal medium was oatmeal broth (393.33mg) followed by Richards's broth (336.67mg) and Sabourauds dextrose broth (326.67mg). Further Richards's broth and Sabourauds dextrose broth remain on par with each other. Least dry mycelial weight of *C. gloeosporioides* was recorded in host leaf extract broth (150.00mg). Between synthetic and non / semi synthetic media maximum growth was recorded in non / semi synthetic media.

As the maximum growth was recorded in Potato dextrose broth, it was selected as a basal liquid medium for further studies.

4.4.4 Cultural characteristics of *C. gloeosporioides* on different solid media

Diversity in cultural and morphological characters of *C. gloeosporioides* were studied in five non synthetic / semi synthetic and two synthetic media at room temperature 27 ± 1^{o} C as described in "Material and Methods" and the results obtained are presented in Table 4; Fig. 8 and Plate 7.

The radial growth, colony characters and sporulation of the fungi were recorded, when the maximum growth was attained on any one of the tested media. The effect of different culture media on the growth of fungi differed significantly. Maximum radial growth of *C. gloeosporioides* was recorded on potato dextrose agar (90.00 mm), which was found to be significantly superior to all other media followed by oat meal agar (84.90mm), Sabouraud dextrose agar (82.00mm), Richards's agar (81.00 mm), host leaf extract agar (81.00mm), and malt extract agar (80.40mm) were on par with each other. The least radial growth was recorded in Czapek (Dox) agar (72.70 mm). The non synthetic / semi synthetic media recorded maximum growth compared to synthetic media.

Mycelium colour varied from white to black. The growth varied from flat, raised fluffy to sparse. Pigmentation in the media also varied from brown to black and light pink to orange. Sporulation also showed greater variation in different media, ranging from excellent to poor sporulation.

Excellent sporulation was recorded on potato dextrose agar and moderate sporulation in Oatmeal agar and Richards's agar. Poor sporulation was recorded in malt extract agar and Czapek (Dox)agar.

Table 2 . Growth phase of *Colletotrichum gloeosporioides* in potato dextrose broth

Sl .No.	Days after incubation	Dry mycelial weight (mg)[#]
1	2	63.66
2	4	159.33
3	6	253.33
4	8	313.33
5	10	386.77
6	12	426.77
7	14	390.00
8	16	333.33
9	18	306.70
10	20	296.70
S.Em. ±		6.95
CD at 1%		19.88

[#] Mean of three replications

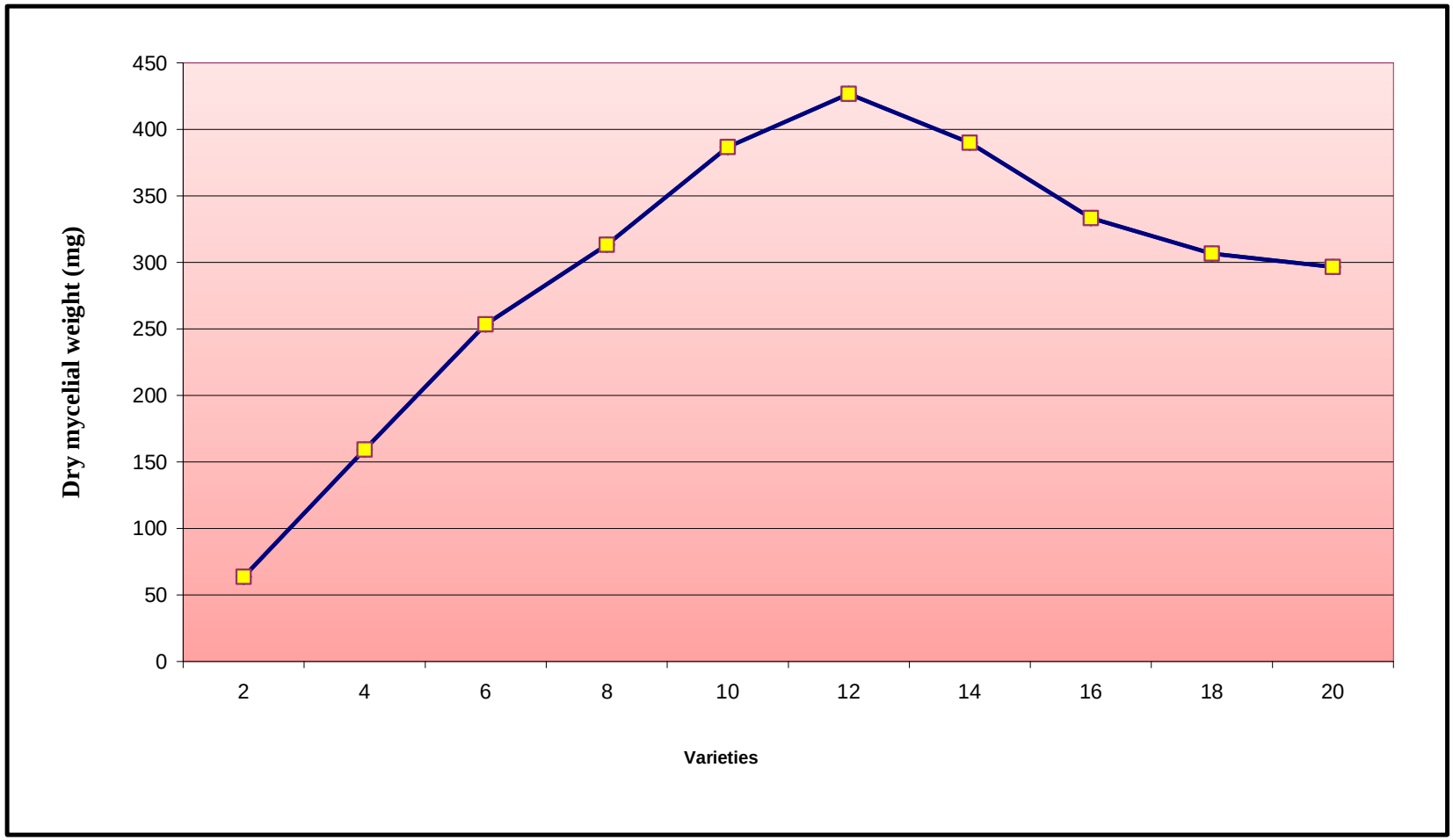


Fig. 6: Mean dry mycelial weight of Colletotrichum gloeosporioides in potato dextrose broth

Table 3. Effect of different liquid media on growth of *Colletotrichum gloeosporioides*

Sl. No.	Broth	Dry mycelial weight (mg)[#]
Non synthetic / semi synthetic media		
1.	Host leaf extract broth	150.00
2.	Malt extract broth	273.33
3.	Oatmeal broth	393.33
4.	Potato dextrose broth	416.67
5.	Sabouraud dextrose broth	326.67
Synthetic media		
6.	Czapek (Dox) broth	210.00
7.	Richards's broth	336.67
S.Em. ±		7.34
CD at 1%		21.87

[#] Mean of three replications

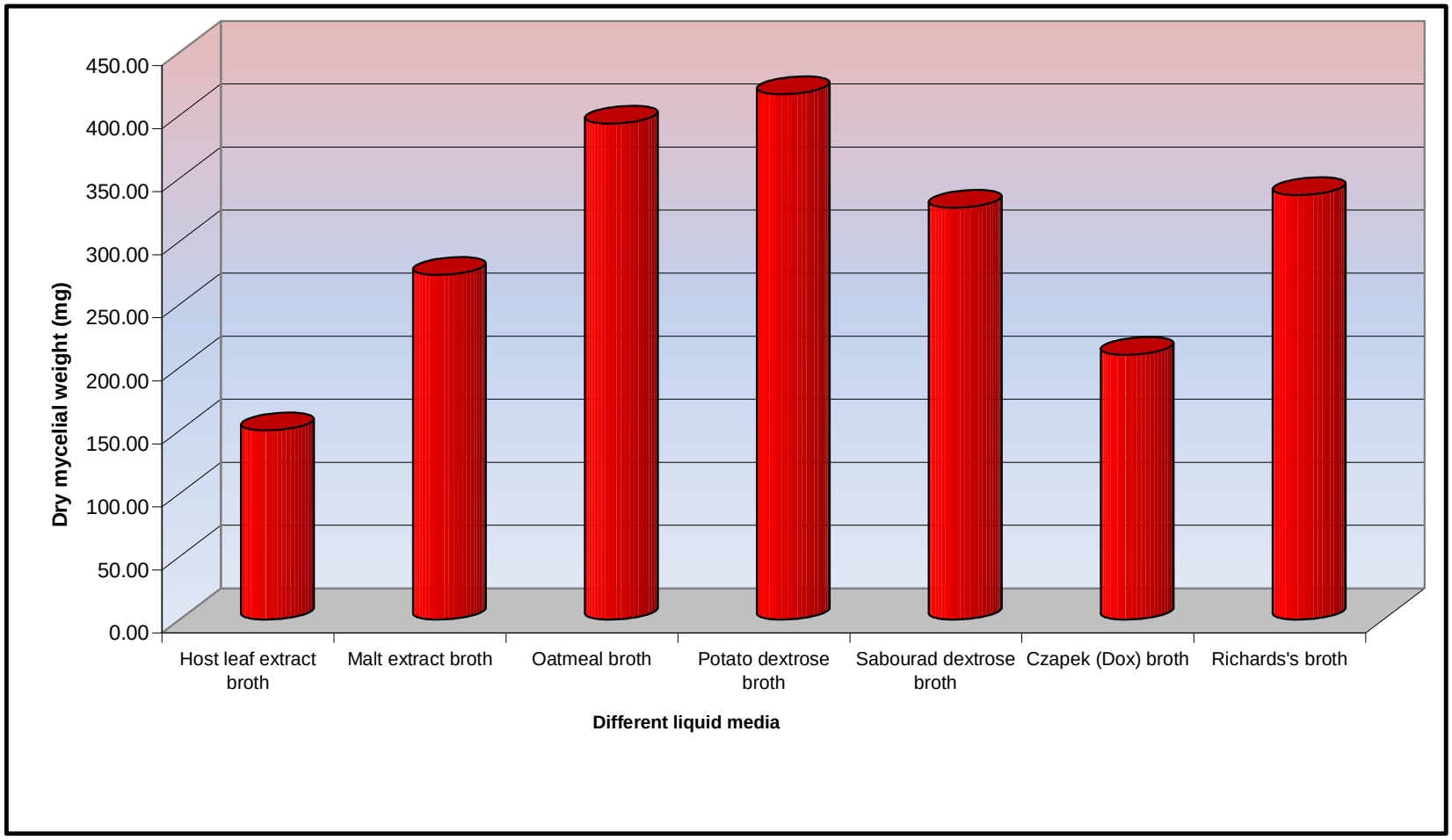


Fig. 7: Dry mycelial weight of Colletotrichum gloeosporioides on different liquid media

4.5 Physiological studies

4.5.1 Temperature studies

4.5.1a Effect of temperature on growth of *C. gloeosporioides* on liquid media

C. gloeosporioides was grown on potato dextrose broth at five temperature levels *viz.,* 15, 20, 25, 30 and 35^{o}C to know the optimum temperature required for maximum dry mycelial weight. The results obtained are presented in Table 5; Fig. 9a and Plate 8

The temperature of 30^{0}C was found to be significantly superior to other temperature levels by recording the maximum dry mycelial weight (416.00 mg) followed by, 25^{o} C (382.00 mg). The least dry mycelial weight was recorded at 15^{o} C (92.00 mg). Further 20 ^{o}C (268.00 mg) and 35^{o} C (260.00 mg) remaind on par with each other.

4.5.1b Effect of temperature on *C. gloeosporioides* on solid media

C. gloeosporioides was grown on potato dextrose agar at five temperature levels *viz.,* 15, 20, 25, 30 and 35^{o}C to know the optimum temperature required for maximum radial growth and sporulation. The results obtained are presented in Table 5; Fig. 9b and Plate 8.

Growth of *C. gloeosporioides* on PDA showed gradual increase as temperature increased from 15 to 30^{o} C and later declined with further increase in temperature. The maximum mycelial radial growth was recorded at 30^{o} C (90.00 mm), which was significantly superior to all other temperatures tested, followed by 25^{o} C (87.80 mm), 35^{o} C (73.80 mm) and 20^{o} C (66.00 mm) but least radial growth was recorded at 15^{o} C temperature (0.60 mm). The growth differences at all the temperatures were statistically significant from each other. The sporulation was excellent at 30^{o} C, good at 25^{o} C and moderate at 35^{o} C. Poor sporulation was recorded at 20^{o} C and no sporulation at 15^{o} C. Light orange mycelial color was observed at 20^{o} C and 35^{o}C.

4.5.2 Effect of light

4.5.2a Effect of light and darkness on growth and sporulation of *C. gloeosporioides*

The experiment was conducted to study the effect of light on growth and sporulation of *C. gloeosporioides* by exposing the culture to alternate cycle of 12 hr dark followed by 12 hr light and 12 hr light followed by 12 hr dark, continuous darkness and continuous light for about twelve days and results are presented in Table 6; Fig.10 and Plate 9.

Alternate cycles of 12 hr light and 12 hr dark recorded maximum radial growth of 88.88 mm which was on par with alternate cycles dark and light 88.44mm, both recorded excellent sporulation. But there was significantly reduced mycelial growth when exposed continuous light and continuous dark. Least radial growth of 57.94 mm was recorded at continuous dark which was on par with continuous light (59.38mm) with poor sporulation. Pigmentation of the fungus also varied from white to pink.

4.6 Toxin studies

4.6.1 Effect of culture filtrate on seed germination, shoot and root length of sorghum

The details of this experiment are given in the “Material and Methods” and results are given in Table 7a and Fig. 11

From the table it is clear that culture filtrate of *C. gloeosporioides* affected the germination of sorghum seeds, seedling growth (shoot and root length) and vigour index. The maximum per cent inhibition of seed germination was recorded in both 18 and 20 days culture filtrate (60.30%) which differed significantly with other treatments. Least per cent seed germination inhibition was recorded in 2 days (20.00%) culture filtrate which was on par with 4 days (23.70%) culture filtrate.

Table 4. Cultural and morphological characters of *Colletotrichum gloeosporioides* on different solid media

Sl. No.	Different Media	Mycelium				Sporulation
		Radial growth (mm)[#]	Colour	Type of growth	Pigmentation	
Non synthetic / semi synthetic media						
1	Host leaf extract agar	81.00	Ash	Flat growth circular	Black	+++
2	Malt extract agar	80.40	Light white and black	Sparse irregular	Brown to black	+
3	Oatmeal agar	84.90	Intermixed black and white	Sparse circular	White	++
4	Potato dextrose agar	90.00	Intermixed black and white	Fluffy raised circular	Light pinkish	++++
5	Sabouraud dextrose agar	82.00	Black and White	Fluffy raised circular	White	+++
Synthetic media						
6	Czapek (Dox) agar	72.70	Intermixed white and black	Fluffy raised irregular	Reddish orange	+
7	Richards's agar	81.00	white	Fluffy raised irregular	Pinkish orange	++
S.Em. ±		1.34				
CD at 1%		3.71				

[#] Mean of five replications

Sporulation	Conidia /microscopic field (400 X)
++++	>75
+++	50 - 75
++	25 – 50
+	1-25
-	No conidia

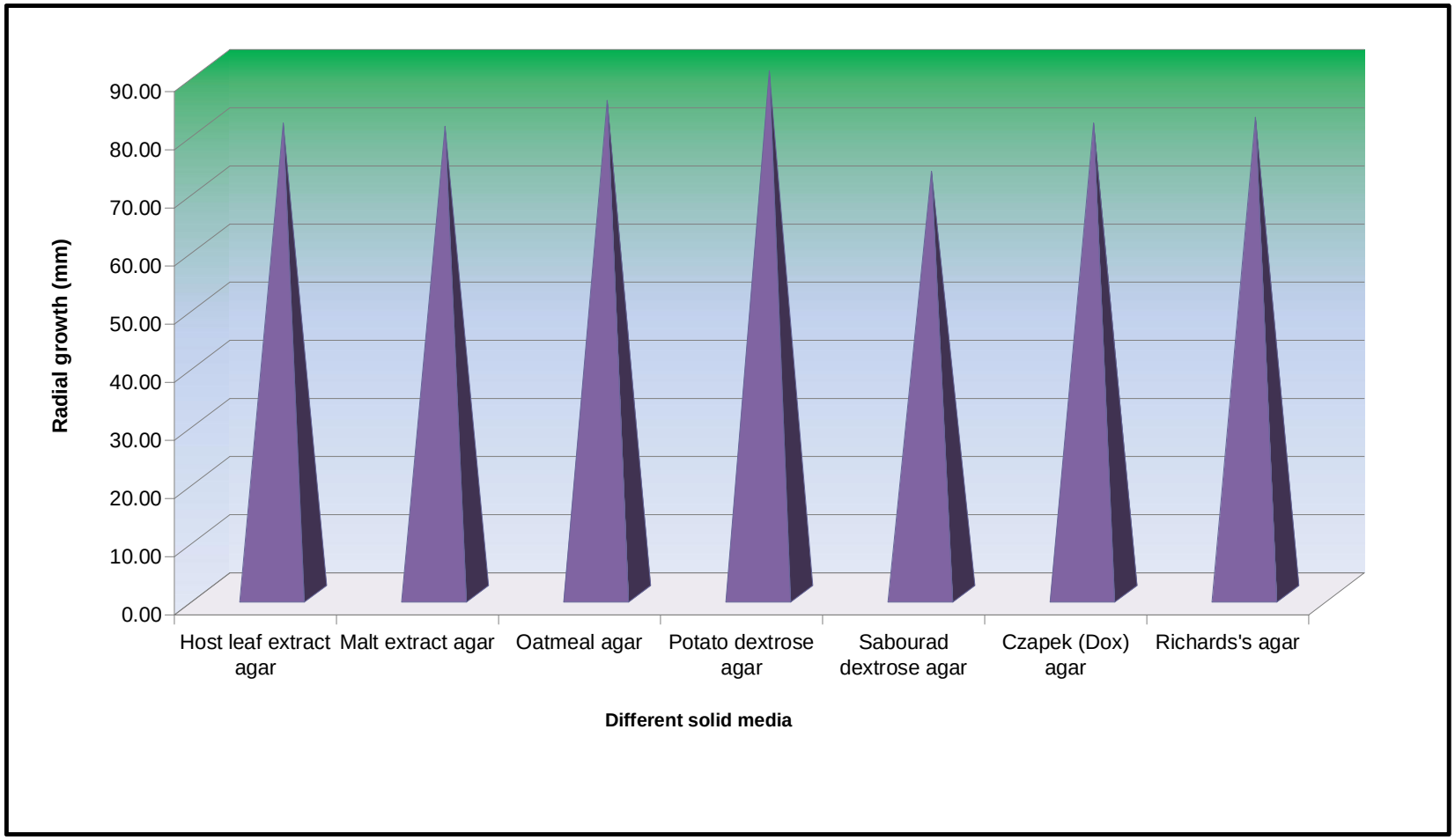
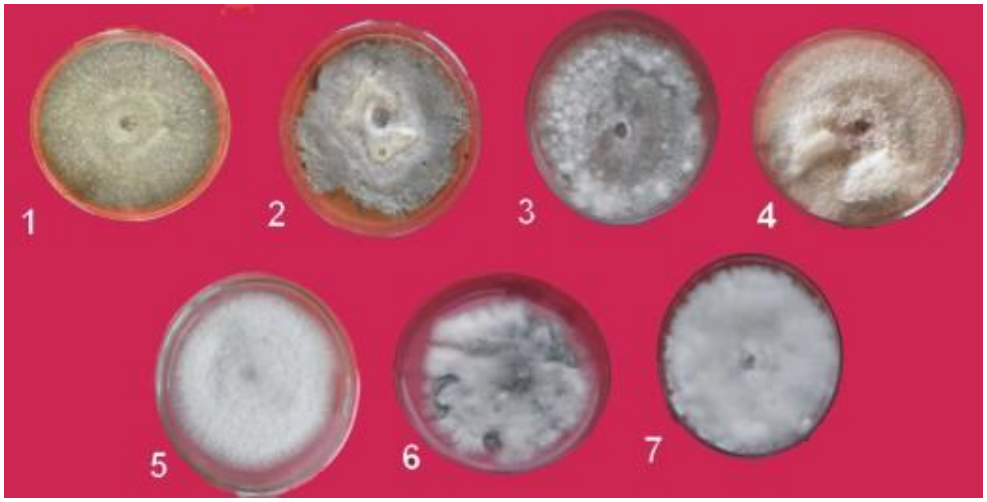


Fig. 8: Radial growth of colletotrichum gloeosporioides on different solid media

1) Czapek (Dox) broth
2) Malt extract broth
3) Host leaf extract broth
4) Richard's broth
5) Oatmeal
6) Sabouraud dextrose broth
7) Potato dextrose broth

Plate.6. Growth of Colletotrichum gloeosporioides on different liquid media



1)Host leaf extract agar
2) Malt extract agar
3) Oatmeal agar
4) Potato dextrose agar
5) Sabouraud dextrose agar
6) Czapek (Dox) agar
7) Richards's agar

Plate.7. Growth of colletotrichum gloeosporioides on different solid media

Table 5. Effect of temperature on growth and sporulation of *Colletotrichum gloeosporioides* in liquid and on solid media

Sl. No.	Temperature (^{o}C)	On Potato dextrose broth	On Potato dextrose agar		
		Dry mycelia weight (mg)[#]	Radial growth (mm)[#]	Mycelial colour	Sporulation
1.	15	92.00	0.60	-	-
2.	20	268.00	66.00	Suppressed, orange	+
3.	25	382.00	87.80	Raised, white	+++
4.	30	416.00	90.00	Raised, intermixed black with white circular	++++
5.	35	260.00	73.80	Suppressed, light orange	++
S.Em. ±		5.93	0.62		
CD at 1%		16.88	1.78		

[#] Mean of five replications

Sporulation	Conidia /microscopic field (400 X)
++++	>75
+++	50 - 75
++	25 – 50
+	1-25
-	No conidia

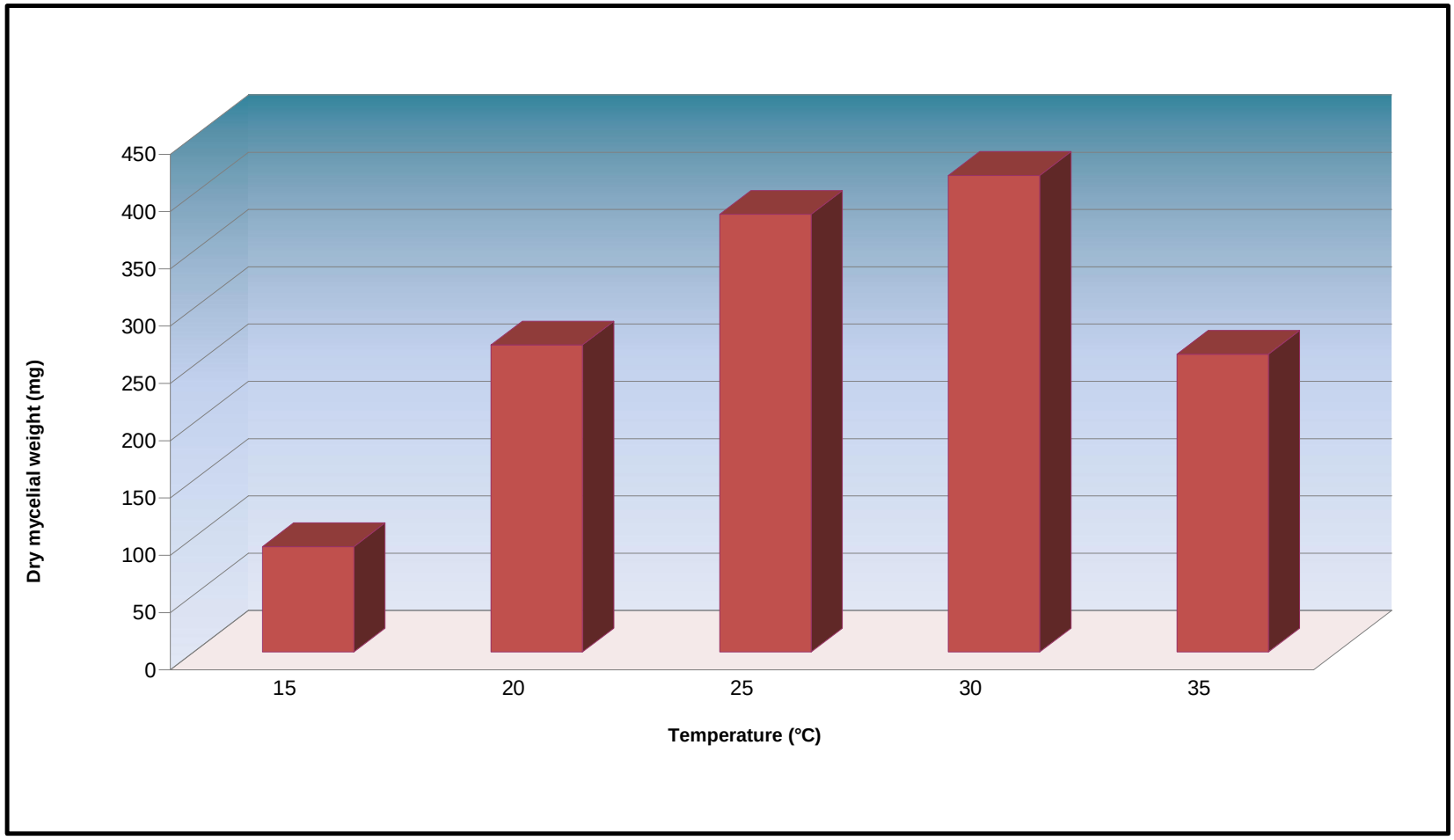


Fig. 9a: Effect of different temperature levels on the growth of Colletotrichum gloeosporioides on potato dextrose broth

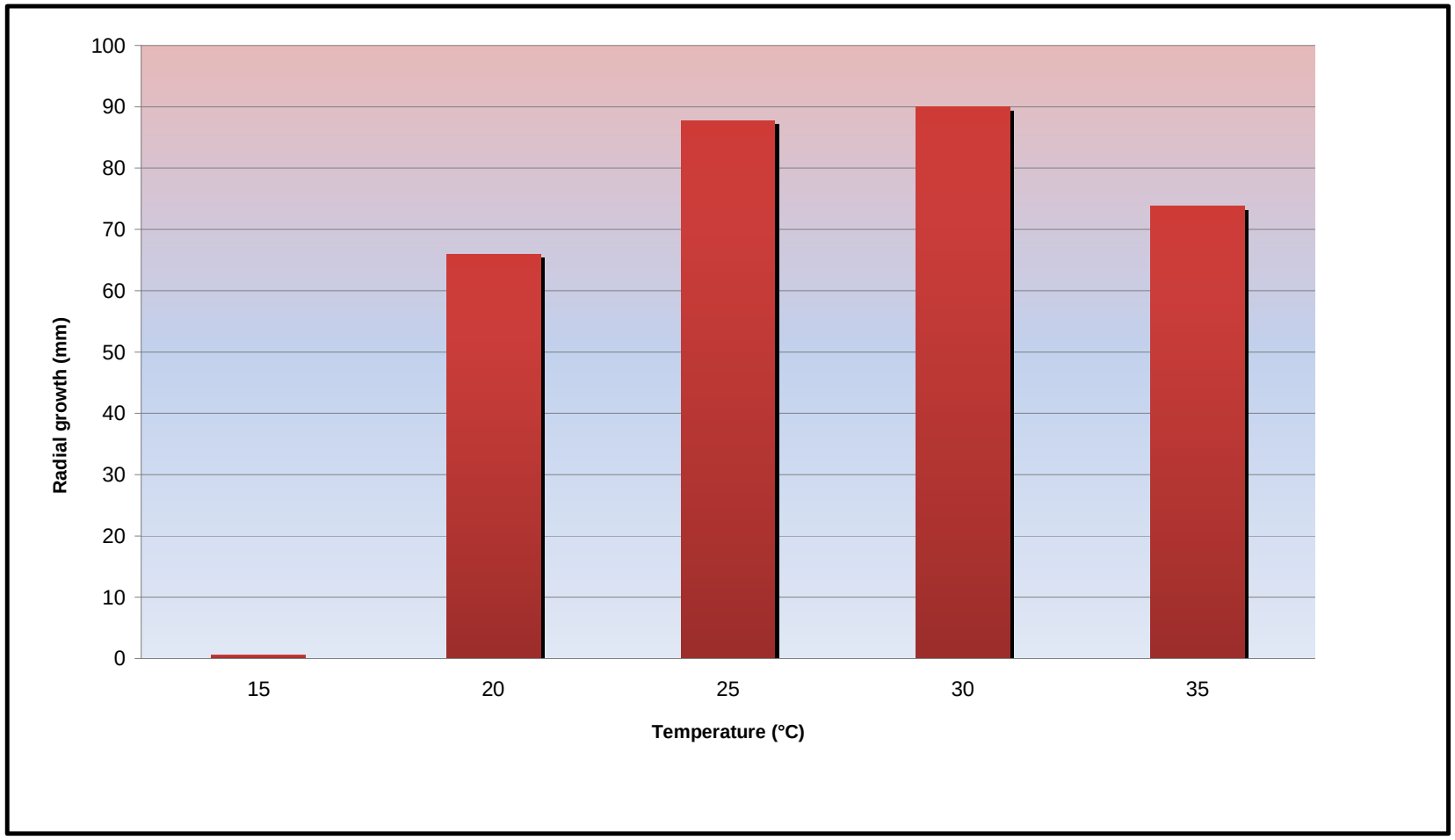


Fig. 9b: Effect of different temperature levels on the growth of Colletotrichum gloeosporioides on potato dextrose agar

Table 6. Effect of light on growth and sporulation of *Colletotrichum gloeosporioides*

Sl. No	Treatment	Radial growth (mm)[#]	Pigmentation	Sporulation
1.	Continuous 24 hours light	59.38	White	+
2.	Continuous 24 hours dark	57.94	White	+
3.	12 hours alternate dark and light	88.44	White	++++
4.	12 hours alternate light and dark	88.88	Slight pink	++++
S.Em. ±		2.07		
C.D.at 1%.		6.33		

[#] Mean of five replications

Sporulation	conidia /microscopic field (400 X)
++++	>75
+++	50 - 75
++	25 – 50
+	1-25
-	No conidia

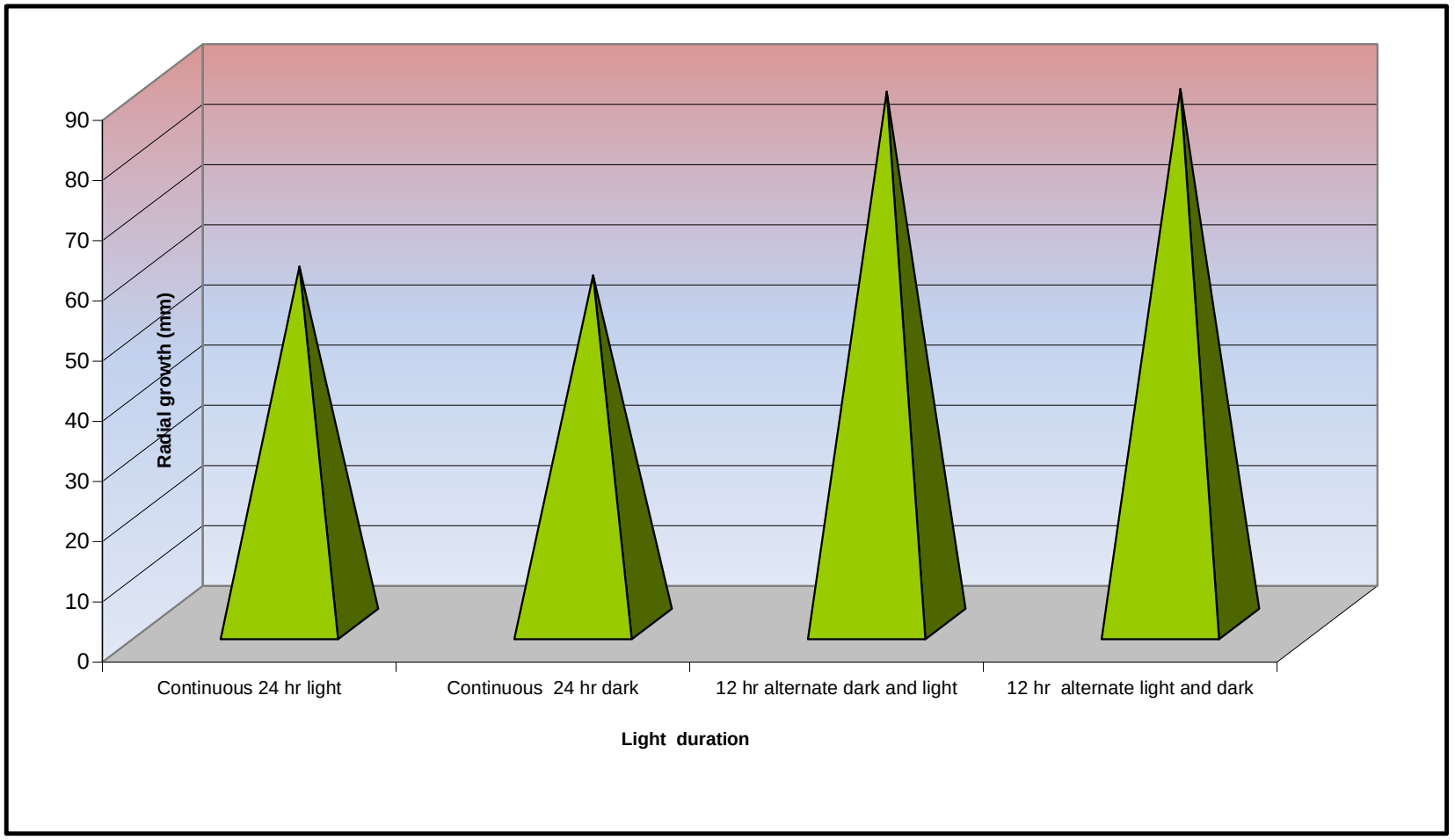
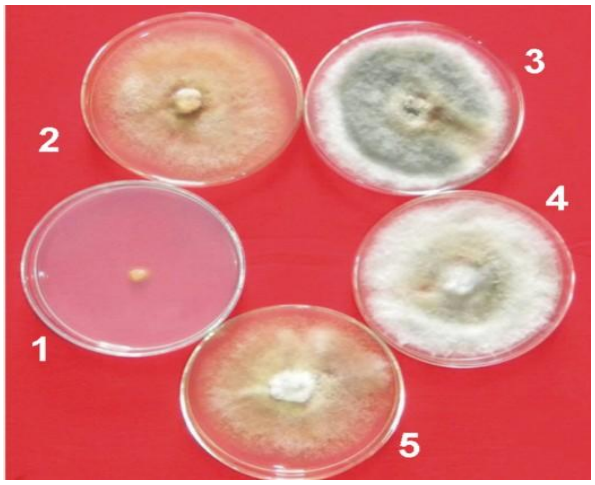


Fig. 10. Effect of light on the growth of Colletotrichum gloeosporioides on potato dextrose agar



Growth of colletotrichum gloeosporoides on potato dextrose agar

1. 15^0 C
2. 20^0 C
3. 25^0 C
4. 30^0 C
5. 35^0 C

Growth of colletotrichum gloeosporoides on potato dextrose broth

Plate.8. Effect of different temperature leve on growth of colletotrichum gloeosporoides on potato dextrose agar and potato dextrose broth

Continuous 24 hrs light	Continuous 24 hrs dark	Alternate 12 hrs dark& Light	Alternate 12 hrs light & dark

Plate.9. Effect of light on growth of Colletotrichum gloeosporoides

Maximum inhibition of sorghum shoot length (56.80%) and root length (74.10%) were recorded in 20 days old culture filtrate followed by days 18 and 16 days and were on par with each other. Least per cent shoot length and root length inhibition was recorded in 2 days culture filtrate (11.00 and 11.40%)

Further, the seedling vigour index was calculated and presented in the table 7a.It was noticed that higher seedling vigour index was noticed in sterile distilled water (2700) followed by 2 days old culture filtrate (2048). While, the least vigour index was noticed in 20 days old culture filtrate (379.80) followed by 18 days culture filtrare (468.30).

4.6.1 Effect of culture filtrates of *C. gloeosporioides* on tomato seedling

Effect of culture filtrate of *C. gloeosporioides* of different days of incubation (*viz*., 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 days) on tomato seedlings was tested as explained in "Material and Methods". The results on the effect of these culture filtrates on tomato seedling recorded at 12 hr, 24 hr, 36 hr, 48 hr, 60 hr, 72 hr, and 96 hrs of incubation are presented in Table 7b.

The tomato seedlings were placed in different days culture filtrates of *C. gloeosporioides.* No symptoms were found upto 12 hr in all treatments. Among the different culture filtrates, 2 days and 6 days culture filtrates showed symptom after 24 hr. Slight drooping of bottom leaves, marginal necrosis was the initial symptoms observed in all treatments at 24 hr. The tomato seedlings showed partial (with in 60 – 72 hr) and complete wilting (with in 72 – 96 hr). Whereas 20 days culture filtrate showed complete wilting within 72 hr.

4.7 Management studies

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

Screening of fungicides was done against *C. gloeosporioides* under laboratory condition by following poisoned food technique as described in "Material and Methods".

Six each of systemic and non-systemic fungicides (one combi product) were evaluated against *C. gloeosporioides* in laboratory at three concentrations by poisoned food technique.

4.7.1a *In vitro* evaluation of non systemic and combi fungicides against *C. gloeosporioides*

Data with respect to inhibition of mycelial growth of *C. gloeosporioides* at three concentrations of five non-systemic fungicides and one combi product was recorded and presented in Table 8; Fig. 12 and Plate10

Data from table revealed that, the efficacy of different non-systemic fungicides, concentrations and their interaction on per cent inhibition of mycelial growth of *C. gloeosporioides* differed significantly.

Maximum percent inhibition (75.10%) of *C. gloeosporioides* was recorded in combi product carbendazim + mancozeb which was significantly superior over all other fungicides followed by captan (60.77%), propineb (21.33%), mancozeb (16.39%) and chlorothalonil (11.69%). Least per cent inhibition was noticed in copper oxychloride (0.9%). However the maximum per cent inhibition of mycelial growth was at 0.3 per cent concentration irrespective of fungicides.

At 0.3 per cent concentration combi product carbendazim + mancozeb recorded highest per cent inhibition of mycelial growth (81.88%) of fungus which was significantly superior over other fungicides followed by captan (73.88%) . The least inhibition of fungus was recorded in copper oxychloride (1.55%).

At 0.1 per cent concentration maximum per cent inhibition of mycelial growth (68.99%) of the fungus was recorded in carbendazim + mancozeb. Further 0.1 per cent

Table 7a: Effect of age of culture filtrate of *Colletotrichum gloeosporioides* on percent inhibition of seed germination, shoot and root length of sorghum

Treatments	Percent inhibition			Vigour index
	Seed germination	Shoot length	Root length	
2days	20.00 (26.55)*	11.00 (19.3)	11.40 (19.76)	2048.00
4days	23.70 (29.09)	18.00 (25.10)	17.90 (25.03)	1776.75
6days	33.00 (35.02)	25.00 (30.00)	34.30 (35.86)	1293.39
8days	39.00 (38.63)	27.70 (31.70)	37.40 (37.66)	1193.77
10days	41.00 (39.77)	34.10 (35.70)	39.10 (38.66)	1064.30
12days	42.30 (40.57)	39.10 (38.80)	43.30 (41.13)	957.60
14days	50.70 (45.36)	43.50 (41.30)	50.20 (43.56)	751.20
16days	52.30 (46.31)	48.30 (44.00)	60.20 (49.33)	623.30
18days	60.30 (50.94)	48.40 (44.20)	61.00 (50.96)	468.30
20days	60.30 (5.94)	56.80 (48.90)	74.10 (59.36)	379.80
Control (Sterile distilled water)	0.00	0.00	0.00	2700.00
S.Em. ±	0.93	0.43	1.05	
CD @ 1%	2.66	1.23	3.00	

*Arcsine transformed values

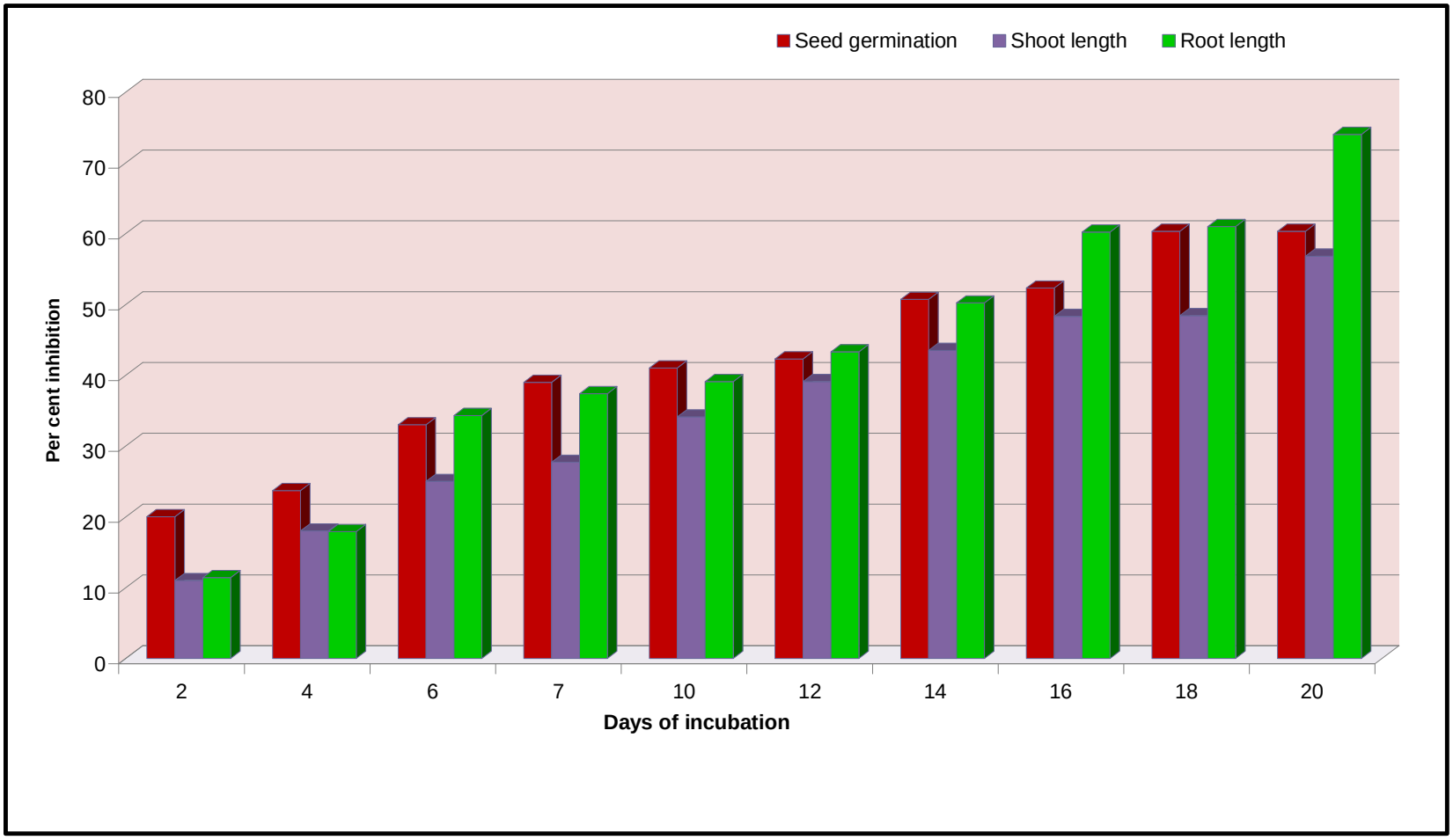


Fig.11: Effect of age of culture filtrates of Colletotrichum gloeosporioides on per cent inhibition of seed germination, shoot and root length of sorghum

Table 7b: Effect of age culture filtrate of *Colletotrichum gloeosporioides* on tomato seedlings upto 96 hours of incubation

Treatments	Incubation period in hours							
	12	24	36	48	60	72	84	96
2days	NS	NS	D	CL	MN	PW	PW	CW
4days	NS	NS	D	CL	MN	PW	PW	CW
6days	NS	D	CL,	B	MN	PW	PW	CW
8days	NS	D	D,CL	MN	PW	PW	CD	CW
10days	NS	D	B, CL	B, CL	PW	PW	CD	CW
12days	NS	D, MN	B, CL	B, CL	PW	PW	CW	-
14days	NS	D,MN	B,CL	PW	PW	PW	CW	-
16days	NS	D, MN, CL	B,	B, CL	PW	CD	CW	-
18days	NS	D, MN, CL	B	B	PW	CD	CW	-
20days	NS	D, MN, CL	B	PW	CD	CW	CW	-
Control (Sterile distilled water)	NS	NS	NS	NS	NS	NS	NS	NS

D: Drooping CD : Complete drooping PW :Partial wilting
B : Browning CL : Curling of leaves MN : Marginal necrosis
CW: Complete wilting BL: Brittling of leaves NS : No symptoms

proineb (14.83%) and 0.2 per cent of mancozeb (16.19%) remain on par with each other. The least per cent inhibition (0.27%) of fungus was in copper oxychloride.

At all the tested concentrations among nonsystemic and combi fungicides, carbendazim + mancozeb recorded highest inhibition at 0.2 and 0.3 per cent concentrations.

4.7.1b *In vitro* evaluation of systemic fungicides against *C. gloeosporioides*

Data with respect to inhibition of mycelial growth of *C. gloeosporioides* at three concentrations of six systemic fungicides were recorded and per cent inhibition is presented in Table 9; Fig. 12 and Plate11.

It was observed that, fungicides, concentrations and their interaction differed significantly with respect to inhibition of the mycelial growth of *C. gloeosporioides.*

Among six systemic fungicides, maximum per cent inhibition of growth of *C. gloeosporioides* was observed in propiconazole (85.84%) which was significantly superior to all other fungicides followed by iprobenfos (84.14%), difenoconazole (65.13%), hexaconazole (61.62%) and azoxystrobin (58.55%). The least per cent inhibition of fungus was recorded in carbendazim (44.15%) at 0.1 per cent concentration.

Among the tested three concentrations, 0.15 per cent concentration of all fungicides was significantly found superior to 0.1 and 0.05 per cent. Maximum per cent inhibition of mycelial growth (87.99%) of the fungus was recorded in iprobenfos followed by propiconazole (87.10%) which remained on par with each other.

Further difenconazole at 0.15 per cent (67.21%) remained significantly superior to hexaconazole (64.55%) and carbendazim (62.09%).

At 0.1 per cent concentration, maximum per cent inhibition of mycelial growth (86.37%) of the fungus was recorded in propiconazole. Further 0.1 per cent hexaconazole (65.77%) and 0.15per cent of difenconazole (64.43%) remained on par with each other. The least per cent inhibition of mycelia growth was recorded in carbendazim (50.55%).

At 0.05 per cent concentration, maximum per cent inhibition of mycelial growth (84.06%) of the fungus was recorded in propiconazole. The least per cent inhibition of mycelial growth was recorded in carbendazim (19.81%). Further azoxystrobin and hexaconazole were on par with each other. Similarly difenconazole at 0.05 and 0.1 per cent and hexaconazole at 0.15 per cent remained on par with each other and also 0.15% of carbendazim at 0.15 percent was difenconazole at 0.05 and 0.1 per cent.

4.7.2 *In vitro* evaluation of bioagents against *C. gloeosporioides*

Efficacy of bacterial and fungal bioagents was studied under *in vitro* condition by following dual culture method as described in “Material and Methods” and the results are presented in Table 10; Fig. 13 and Plate12

There were significant differences among all the tested bioagents. *Trichoderma viride* (86.82%) was found to be significantly superior in inhibiting the mycelial growth of *C. gloeosporioides* followed by *Trichoderma harzianum* (72.47%) and *Pseudomonas fluorescens* (67.00%). Furthe *Trichoderma viride* and *Pseudomonas fluorescens* remained on par with each other. The least inhibition of mycelial growth of *C. gloeosporioides* was recorded in *Bacillus subtilis* (53.88%).

4.7.3 *In vitro* evaluation of botanicals against *C. gloeosporioides*

An experiment was conducted to assess the antifungal activity of seven plant extracts as described under “Material and Methods” and the results are presented in Table 11; Fig. 14 and Plate13.

The effect of plant extracts on the per cent inhibition of mycelial growth of *C. gloeosporioides* at three concentrations differed significantly.

Table 8. *In vitro* evaluation of non systemic and combi fungicides against *Colletotrichum gloeosporioides*

Sl. No.	Fungicides		Percent inhibition			
			Concentration (%)			
	Common name	Trade name	0.1	0.2	0.3	Mean
1	Captan	Captaf 50% WP	48.55 (44.15)*	59.88 (50.67)	73.88 (59.49)	60.77 (51.36)
2	Carbendazim + mancozeb	SAAF 75% WP	68.99 (56.14)	74.44 (59.69)	81.88 (64.79)	75.10 (60.20)
3	Copper Oxychloride	Blue copper 50% WP	0.27 (2.81)	0.88 (5.28)	1.55 (7.26)	0.90 (5.11)
4	Chlorothalonil	Kavach 75% WP	5.65 (13.68)	9.10 (17.51)	20.32 (26.33)	11.69 (19.17)
5	Mancozeb	Indofil M- 45 75% WP	9.99 (18.37)	16.19 (23.71)	22.99 (28.63)	16.39 (23.57)
6.	Propineb	Antracol 70% WP	14.83 (22.63)	20.22 (26.70)	29.10 (32.35)	21.33 (27.22)
Mean			24.71 (14.34)	30.12 (43.72)	38.29 (35.31)	31.04 (31.12)
			Fungicides (F)	Concentration (C)	F × C	
S.Em. ±			0.31	0.22	0.55	
C.D @ 1 %			0.84	0.67	1.46	

[#] Mean of five replications
*Arcsine transformed values

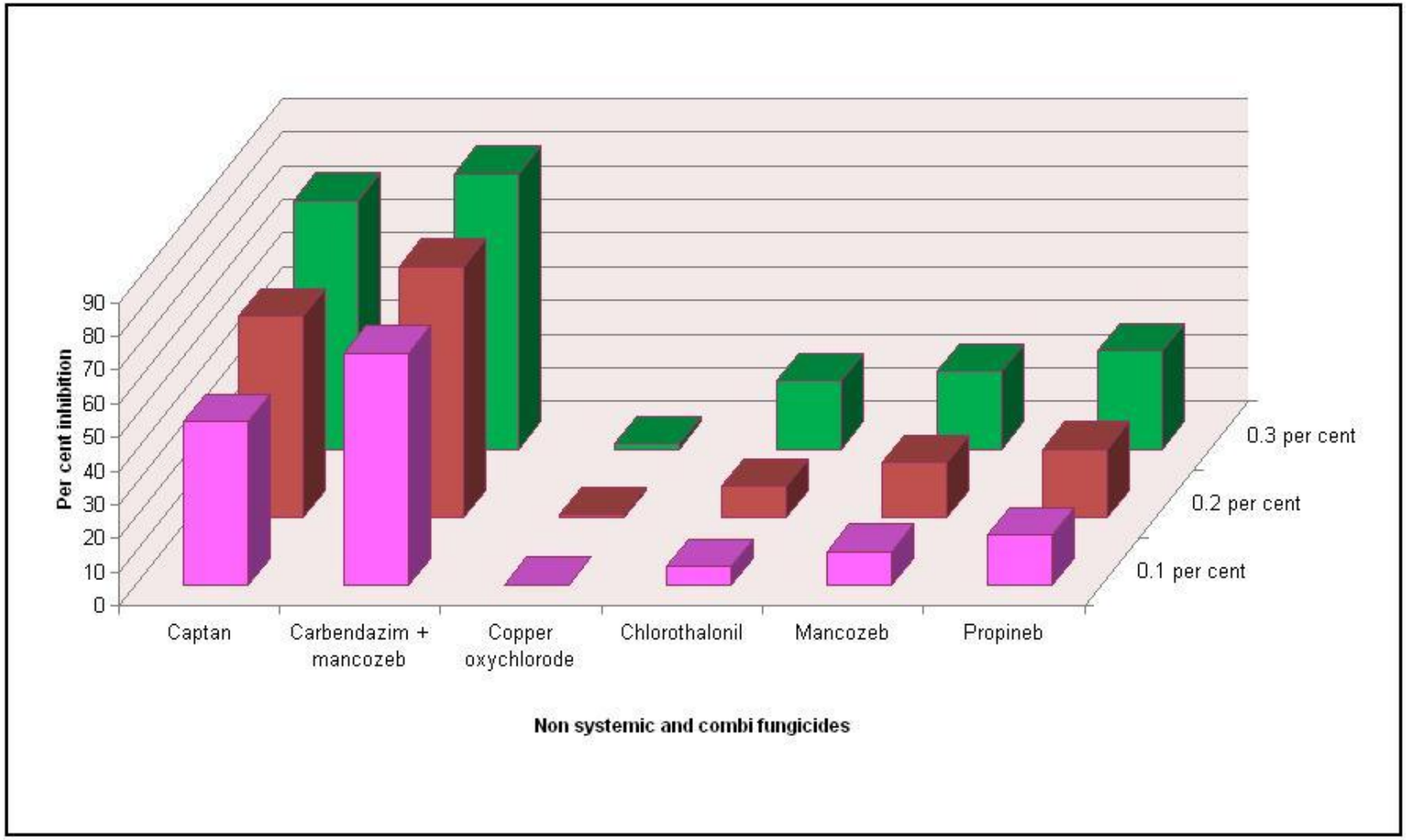
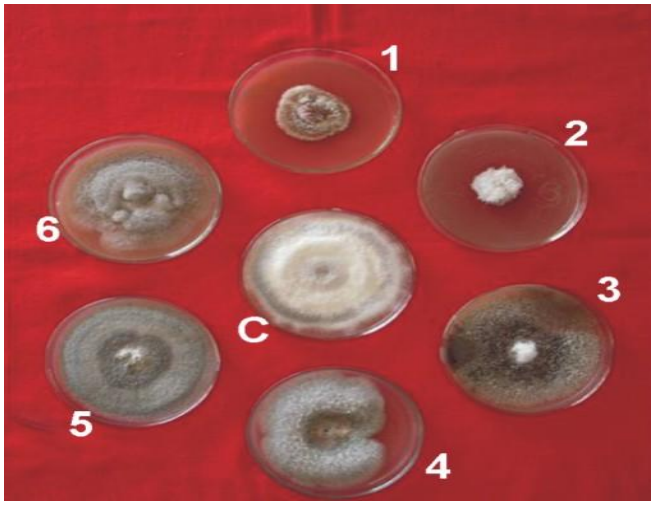
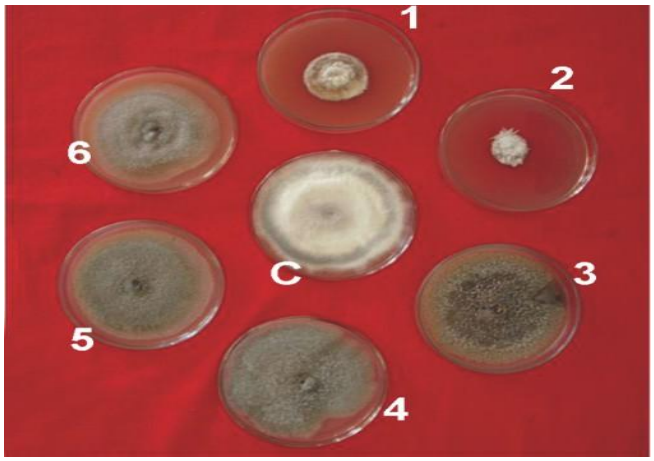


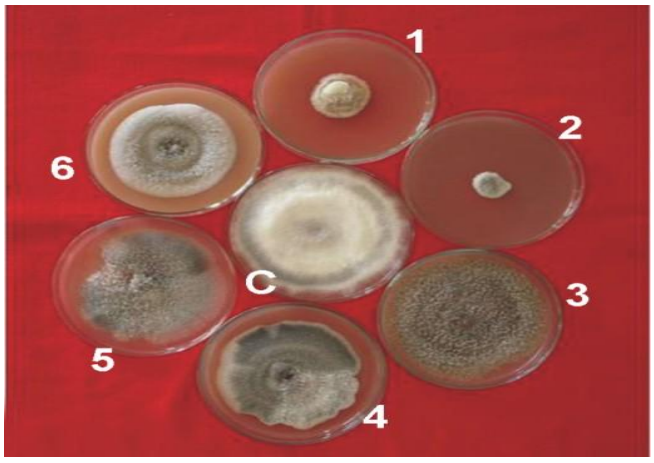
Fig.12: In vitro evaluation of non systemic and combi fungicides on per cent inhibition of Colletotrichum gloeosporioides



0.1 percent



0.2 percent



0.3 percent

1. Captan
2. Carbendazim+Mancozeb
3. Copperoxychloride
4. Chlorothalonil
5. Mancozeb
6. Propineb

C- Control

Plate.10. In vitro evaluation of non systematic and combi fungicides against Colletotrichum gloeosporoides

Table 9. *In vitro* evaluation of systemic fungicides against *Colletotrichum gloeosporioides*

Sl. No	Fungicides		Percent inhibition			
			Concentration (%)			
	Common name	Trade name	0.05	0.1	0.15	Mean
1.	Azoxystrobin	Amistar 25%SC	55.01 (47.85)*	57.88 (49.51)	62.77 (52.43)	58.55 (49.93)
2.	Carbendazim	Bavistin 50% WP	19.81 (26.53)	50.55 (45.29)	62.09 (51.98)	44.15 (41.27)
3.	Difenconazole	Score 25 %EC	63.75 (52.84)	64.43 (53.37)	67.21 (55.05)	65.13 (53.72)
4.	Hexaconazole	Contaf 5% EC	54.55 (47.58)	65.77 (54.15)	64.55 (53.42)	61.62 (51.72)
5.	Iprobenfos	Kitazin 48%EC	80.33 (63.63)	84.12 (66.50)	87.99 (69.67)	84.14 (66.60)
6.	Propiconazole	Tilt 25% EC	84.06 (66.39)	86.37 (68.28)	87.10 (69.47)	85.84 (68.05)
Mean			59.58 (50.80)	68.18 (56.18)	71.95 (58.67)	66.57 (55.21)

	Fungicides (F)	Concentration (C)	F × C
S.Em±	0.22	0.15	0.38
C.D. at 1 %	0.59	0.41	1.01

[#] Mean five replications
*Arcsine transformed values

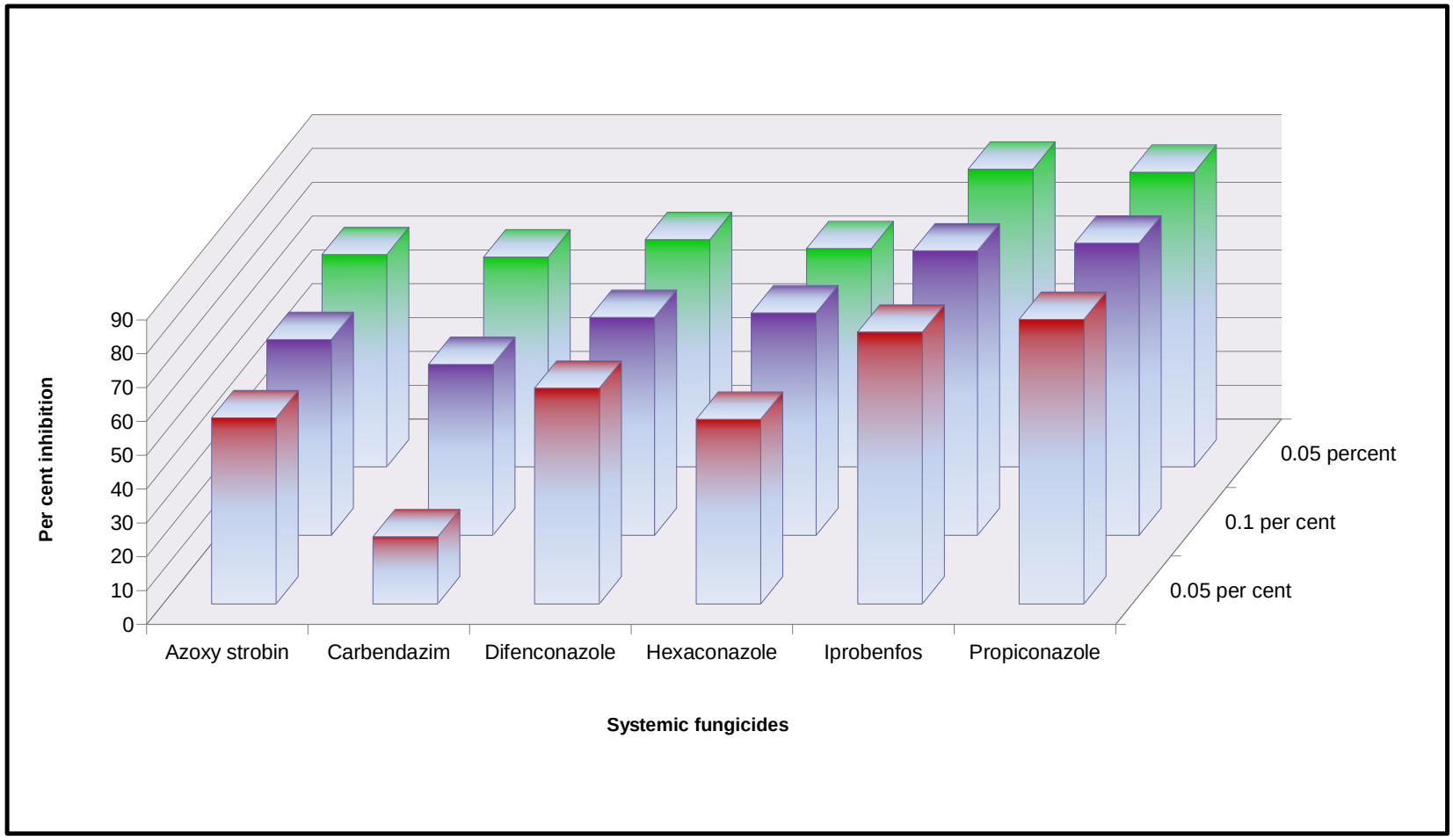
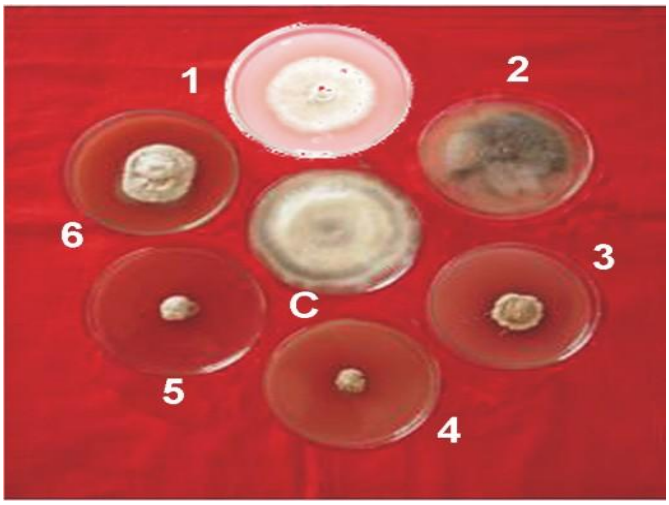
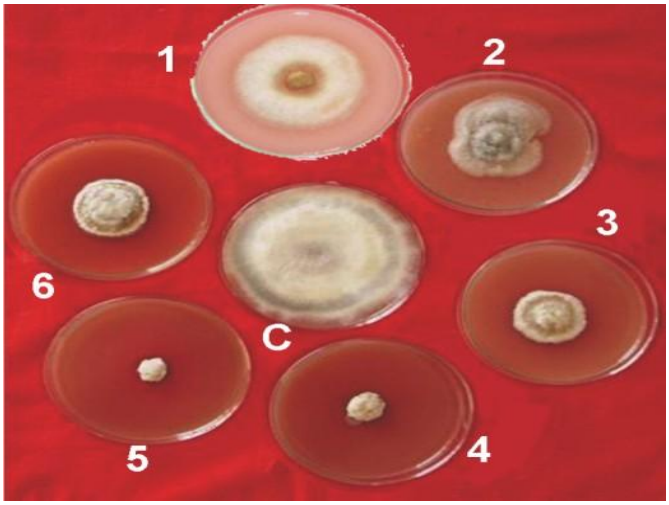


Fig. 13: In vitro evaluation of systemic fungicides on per cent inhibition of Colletotrichum gloeosporioides

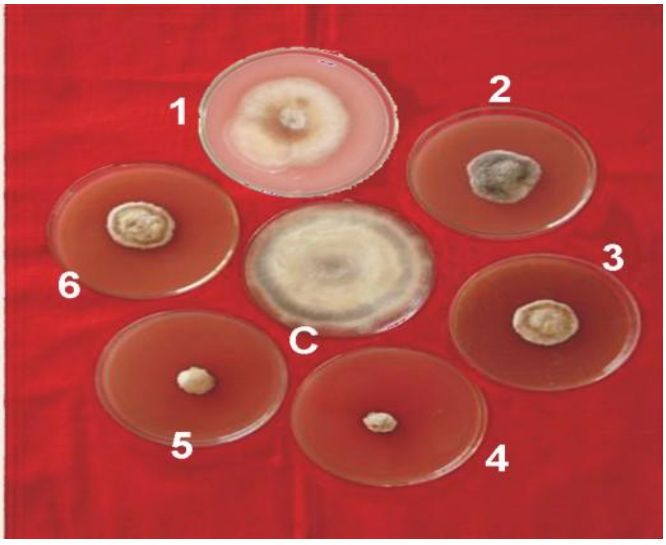


0.05 percent



0.1 percent

1. Azoxystrobin
2. Carbendazim
3. Difenconazole
4. Iprobenfos
5. Propiconazole
6. Hexaconazole



0.15 percent

Plate.11. In vitro evaluation of systemic fungicdes against Colletotrichum gleosprioides

Table 10. *In vitro* evaluation of bioagents against *Colletotrichum gloeosporioides*

Sl. No.	Bioagents	Per cent inhibition[#]
1.	*Bacillus subtilis*	53.88 (46.63)*
2.	*Pseudomonas fluorescens*	67.0 (54.64)
3.	*Trichoderma harzianum*	72.47 (57.67)
4.	*Trichoderma viride*	86.82 (67.85)
S.Em±		1.32
C.D at 1%		4.04

[#] Mean of five replications
*Arcsine transformed values

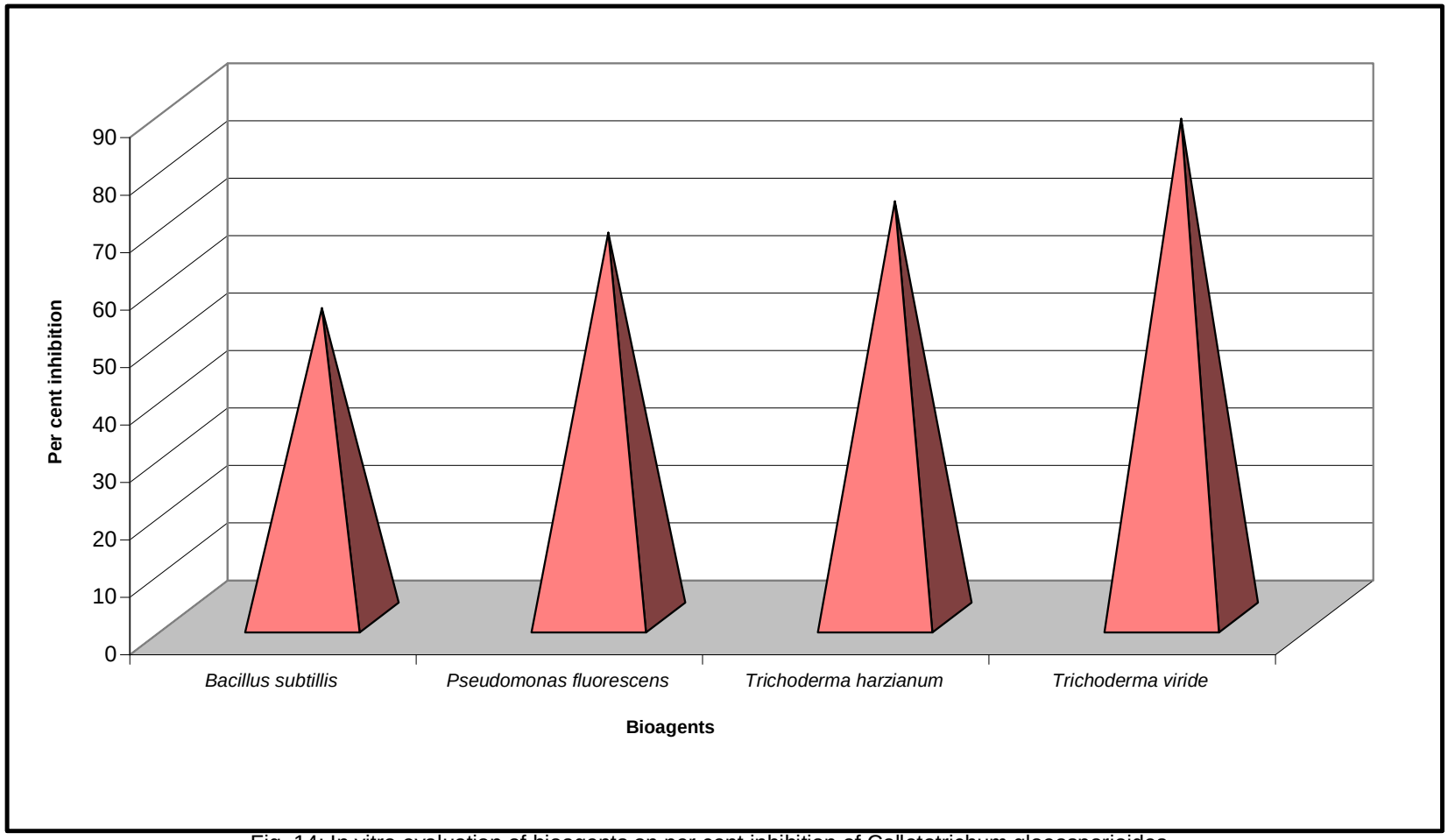


Fig. 14: In vitro evaluation of bioagents on per cent inhibition of Colletotrichum gloeosporioides

Among the seven plant extracts, maximum of per cent inhibition of mycelial growth (58.42%) was recorded in datura leaf extract which was significantly superior to other all treatments tested, followed by Onion bulb extract (36.58%), ginger rhizome extract (31.23%), garlic bulb extract (20.92%), neem leaf extract (5.46%) and eucalyptus leaf extract (2.37%). Least inhibition was recorded in tulasi leaf extract (0.56%).

At 30 per cent concentration of plant extracts, maximum of 61.70 per cent inhibition of mycelial growth was recorded in datura leaf extract followed by garlic bulb extract (50.00%) and onion bulb extract (43.32%). Further tulasi leaf extract, eucalyptus leaf extract and neem leaf extract showed less mycelial inhibition of 0.7, 5.27 and 10.83per cent respectively.

Similar trend was observed at 10 and 20 percent concentration. Mycelial inhibition of onion bulb extract at 10 per cent (28.18%) remained on par with ginger rhizome extract of 20per cent (27.8%).

4.7.4 *In vivo* evaluation of fungicides and bioagent against pomegranate anthracnose during *ambiabahar* 2010

This study was undertaken to evaluate the relative efficacy of different fungicides and bioagent for management of anthracnose of pomegranate during *ambiabahar* 2010 (Plate14)

The experiment was conducted during *ambiabahar* cropping season 2010, (Jan-May) with nine treatments and one untreated control as described in “Material and Methods”. Totally 7 sprays were given at an interval of 15 days. The observation on pomegranate anthracnose was recorded at 15 days interval. Further these observations were converted into per cent disease index (PDI) using formula given by Wheeler (1969). The per cent disease reduction over control was worked. Statisticaly analyzed data are presented in the Table 12a and 12b; Fig. 15 and 16

From the table maximum PDI on leaves and fruit was noticed in untreated control (16.00 and 22.66 PDI) after seventh spray (Plate16).

Results of the experiment revealed that (Table 12a), severity of the disease on leaves before the treatment application was non-significant and almost uniform in the plots and significant differences among the treatments were observed after second spray.

Before first spray the disease was relatively consistent and all the treatments remained on par with each other. On he contrary after 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th}, and 7^{th} spray treatment differed significantly.

Before 2^{nd}, spray minimum PDI (11.83) was recorded in T_2 treatment which was on par with T_1, T_6 and T_9.Maximum PDI was recorded in untreated control.

Before 4^{th} spray T_5 recorded the lowest PDI (10.33) and remained on par with 0.2, 0.3, 0.2 per cent concentration of T_1 (10.66), T_4 (10.66) and T_8 (11.00). Where as T_9 was least (12.83) effective.

Before 6^{th} spray, lowest PDI of 7.33 was recorded at 0.3 per cent concentration of T_8 which was on par with other fungicides viz., T_5, T_1, T_2 . Where as T_9 was found to be least (13.33 PDI) effective.

After 7^{th} spray T_5 at 0.1per cent and T_8 at 0.3per cent remained on par with each other and differed significantly with other treatments. Minimum disease severity of 5.00 PDI was recorded in T_5 and T_8 at 0.1 per cent and 0.3 per cent concentration followed by T_4 (6.50 PDI), T_1 (6.60) and T_2 (6.60) at 0.2, 0.2 and 0.1 per cent concentration. *T. viride* was found to be less effective in the management of anthracnose in field condition.

Similarly, disease severity was also recorded on flowers after first spray and on fruits after third spray. Before spray the maximum PDI was noticed in untreated control (7.33). Results of respective treatments revealed that, all did not differed significantly in their performance over unprotected control. Minimum disease severity of 5.00 PDI was recorded in

Table 11. *In vitro* evaluation of botanicals against *Colletotrichum gloeosporioides*

Sl. No.	Botanicals	Percent inhibition			
		Concentration (%)			
		10	20	30	Mean
1.	Datura leaf extract	54.58 (47.25)*	59.02 (50.17)	61.70 (51.72)	58.42 (49.84)
2.	Eucalyptus leaf extract	0.45 (3.83)	1.38 (6.67)	5.27 (13.17)	2.37 (7.89)
3.	Garlic bulb extract	7.80 (16.08)	21.66 (27.72)	50.00 (44.98)	20.92 (29.59)
4.	Ginger rhizome extract	15.94 (19.27)	27.8 (31.78)	33.33 (35.23)	31.23 (31.35)
5.	Neem leaf extract	0.83 (5.14)	4.70 (12.41)	10.83 (19.19)	5.46 (12.27)
6.	Onion bulb extract	28.18 (32.05)	38.23 (38.88)	43.32 (41.14)	36.58 (37.36)
7.	Tulsi leaf extract	0.35 (3.09)	0.65 (4.61)	0.70 (4.78)	0.56 (4.16)
Mean		15.44 (19.27)	21.92 (24.62)	29.30 (30.04)	22.22 (24.64)
		Botanicals(B)	Concentrations (C)	B×C	
SEm.±		0.32	0.21	0.55	
CD at1%		0.84	0.55	1.46	

[#] Mean of five replications
*Arcsine transformed values

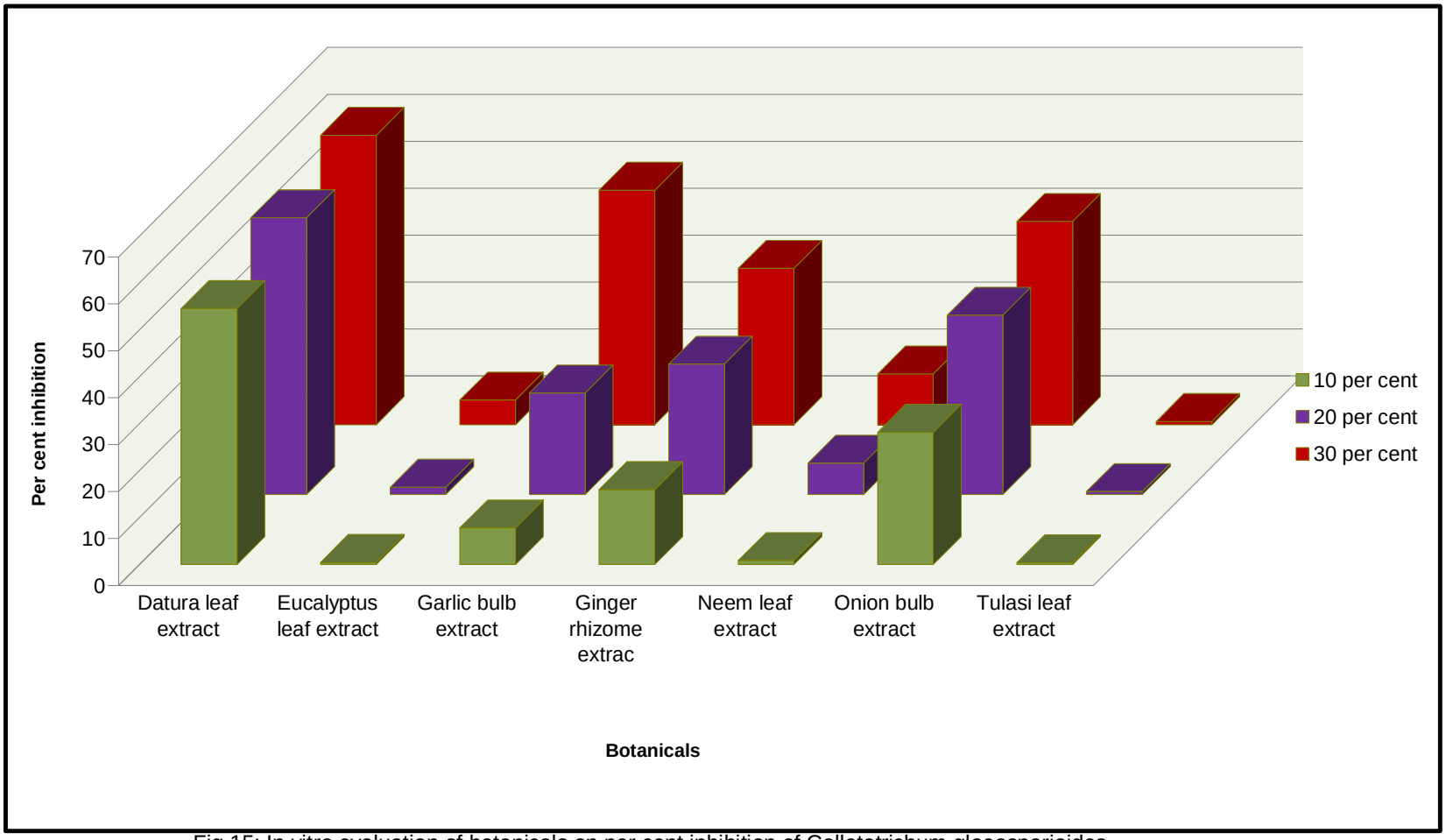
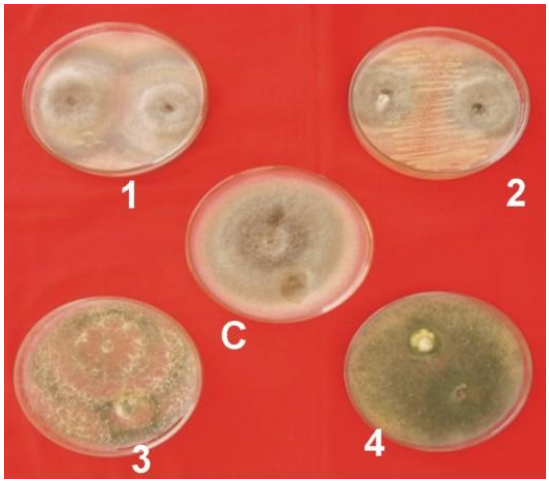


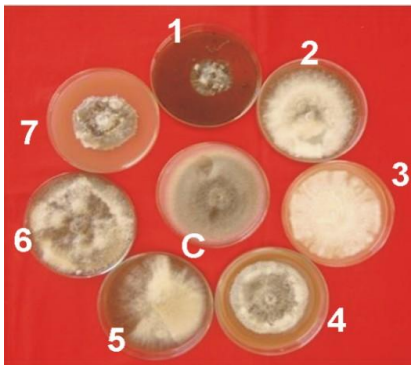
Fig.15: In vitro evaluation of botanicals on per cent inhibition of Colletotrichum gloeosporioides



1. Bacillus subtilis
2. Pseudomonas fluorescens
3. Trichoderma harzianum
4. Trichoderma viride

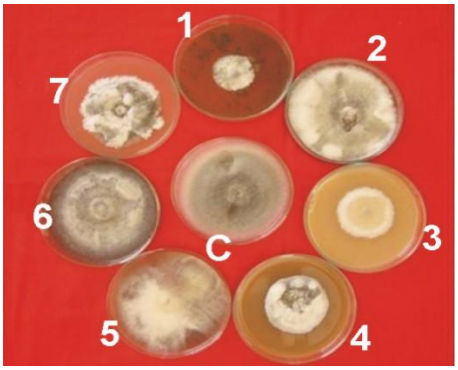
C- Control

Plate.12. In vitro evaluation of bioagents against colletotrichum gloeosporioides



10%

20%



30%

1. Datura leaf extract
2. Eucalyptus leaf extract
3. Garlic bulb extract
4. Ginger rhizome extract
5. Neem leaf extract
6. Tulsi leaf extract
7. Onion bulb extract

C- Control

Plate.13: In vitro evaluation of plant extracts against colletotrichum gloeosporioides

Plate.14 :Field experiment plot

Table 12a: *In vivo* evaluation of fungicides and bioagent against anthracnose of pomegranate in orchard during *ambiabahar* 2010

Treatments		Conc. (%)	PDI on leaves								Per cent disease control	AUDPC values
			Before spray							After VII spray		
			I	II	III	IV	V	VI	VII			
T_1	Carbendazim	0.2	14.00 (21.96)*	12.33 (20.54)	11.83 (20.10)	10.66 (19.05)	9.66 (18.10)	7.8 3 (16.24)	7.16 (15.15)	6.66 (14.95)	58.35	1007.57
T_2	Difenconazole	0.1	13.58 (21.66)	11.83 (20.06)	11.33 (19.66)	10.62 (19.04)	10.33 (18.74)	8.50 (16.93)	7.50 (15.80)	6.66 (14.95)	58.35	1052.98
T_3	Hexaconazole	0.1	13.66 (21.67)	13.58 (21.66)	12.00 (20.25)	11.16 (19.51)	12.16 (20.40)	10.50 (18.89)	9.50 (17.94)	9.00 (17.43)	43.75	1203.45
T_6	Iprobenfos	0.2	13.83 (21.81)	13.33 (21.40)	11.66 (19.96)	10.66 (19.05)	10.66 (19.05)	9.33 (17.78)	8.00 (16.42)	6.50 (14.75)	59.37	1107.06
T_5	Propiconzole	0.1	13.83 (21.81)	13.16 (21.25)	11.33 (19.66)	10.33 (18.74)	9.50 (17.94)	7.50 (15.88)	6.16 (14.37)	5.00 (12.90)	68.75	1025.80
T_6	Captan	0.3	14.33 (22.22)	13.16 (21.26)	12.00 (20.25)	11.50 (19.81)	10.66 (19.05)	10.00 (18.43)	9.66 (18.10)	9.60 (18.10)	40.00	1184.17
T_7	Mancozeb	0.3	14.00 (21.94)	13.33 (21.40)	13.16 (21.26)	12.16 (20.40)	11.16 (19.51)	10.33 (18.74)	9.83 (18.26)	9.50 (17.90)	40.60	1225.52
T_9-	Carbendazim + mancozeb	0.3	14.33 (22.23)	14.00 (21.96)	12.00 (20.25)	11.00 (19.36)	9.33 (17.78)	7.33 (15.68)	6.33 (14.56)	5.00 (12.90)	68.75	1197.22
T_9	*Trichoderma viride*	1.0	13.66 (21.66)	13.16 (21.25)	13.00 (21.11)	12.83 (20.98)	13.00 (21.12)	13.33 (21.39)	13.33 (21.39)	13.00 (21.11)	18.75	1396.60
T_{10}	Control	-	14.16 (22.09)	14.16 (22.09)	14.50 (22.37)	15.83 (23.40)	15.66 (23.30)	15.66 (23.30)	15.00 (22.77)	16.00 (23.56)	-	1588.3
S. Em. ±			NS	0.70	0.33	0.35	0.31	0.51	0.35	0.47	-	
CD at 5%				1.47	0.69	0.75	0.67	1.08	0.74	0.99		

*Arcsine transformed values

Table 12b: *In vivo* evaluation of fungicides and bioagent against anthracnose of pomegranate in orchard during *ambiabahar* 2010

Treatments		Conc. (%)	PDI on flowers	PDI on fruits				Per cent disease control	AUDPC values for fruits
			Before spray	Before spray			After VII spray		
			III	IV	V	VI	VII		
T_1	Carbendazim	0.2	6.67 (14.79)*	6.33 (14.56)	10.67 (19.05)	8.33 (16.73)	4.33 (11.99)	4.00 (11.53)	82.30	299.92
T_2	Difenconazole	0.1	6.00 (14.14)	6.00 (14.14)	11.00 (19.36)	10.33 (18.72)	7.67 (16.06)	4.00 (11.53)	82.30	382.49
T_3	Hexaconazole	0.1	6.50 (14.76)	6.50 (14.76)	12.00 (20.26)	11.33 (19.66)	5.33 (13.33)	6.50 (14.70)	71.23	388.64
T_6	Iprobenfos	0.2	6.00 (14.07)	5.67 (13.68)	10.67 (19.05)	10.67 (19.05)	5.00 (12.92)	2.41 (8.89)	89.23	333.14
T_5	Propiconzole	0.1	7.00 (15.31)	5.33 (13.33)	8.33 (16.77)	6.00 (14.17)	4.00 (11.53)	1.20 (6.31)	94.58	221.47
T_6	Captan	0.3	6.67 (14.92)	6.67 (14.92)	10.67 (19.05)	9.33 (17.78)	7.00 (15.31)	4.83 (12.69)	78.54	361.19
T_7	Mancozeb	0.3	6.67 (14.95)	6.67 (14.95)	12.00 (20.26)	10.33 (18.74)	7.67 (16.06)	6.33 (14.56)	71.91	407.24
T_9	Carbendazim + mancozeb	0.3	6.00 (14.14)	5.00 (12.92)	8.00 (16.42)	4.00 (11.53)	4.00 (11.53)	0.83 (5.18)	96.22	186.22
T_9	*Trichoderma viride*	1.0	6.67 (14.95)	6.67 (14.95)	13.33 (21.41)	15.00 (22.77)	18.00 (25.07)	17.00 (24.33)	24.97	722.25
T_{10}	Control	-	7.33 (15.70)	7.33 (15.70)	13.33 (21.41)	17.33 (24.58)	20.66 (27.02)	22.66 (28.41)	-	839.54
S. Em. ±				0.81	0.31	0.61	0.53	0.72	-	
CD at 5%			2.48	1.71	0.65	1.30	1.13	1.53	-	

*Arcsine transformed values

Table 13. Effect of chemicals/bioagent on severity of anthracnose of pomegranate caused by *Colletotrichum gloeosporioides* and fruit yield

Treatments	Conc.	Severity (After 7th spray)	Fruit yield parameters			Fruit yield/tree (kg)	Estimated fruit yield per ha (tons)
			Total number of fruits/plant	Total number of healthy fruits/plant	Average weight of fruit (g)		
T_1- Carbendazim	0.2%	4.00 (11.53)*	43.50	39.83	229.91	9.16	5.89
T_2- Difenconazole	0.1%	4.00 (11.53)	44.25	40.25	226.02	9.10	5.85
T_3- Hexaconazole	0.1%	6.50 (14.70)	39.67	33.17	252.16	8.36	5.37
T_4- Iprobenfos	0.2%	2.41 (8.89)	43.83	40.72	228.32	9.29	5.97
T_5- Propiconzole	0.1%	1.20 (6.31)	51.00	49.79	196.09	9.76	6.28
T_6- Captan	0.3%	4.83 (12.69)	41.00	36.17	243.93	8.82	5.67
T_7- Mancozeb	0.3%	6.33 (14.56)	36.83	30.50	271.80	8.28	5.33
T_9 - Carbendazim + mancozeb	0.3%	0.83 (5.18)	53.00	52.33	188.69	9.87	6.35
T_9- *Trichoderma viride*	10g/lt	17.00 (24.33)	32.17	23.17	310.90	7.20	4.63
T_{10}- Control	-	22.66 (28.41)	28.42	11.41	352.07	4.01	2.57
S. Em. ±		0.72	0.72	1.05	4.96	0.20	0.12
CD at 5%		1.53	1.52	2.20	10.42	0.42	0.27

*Arcsine transformed values

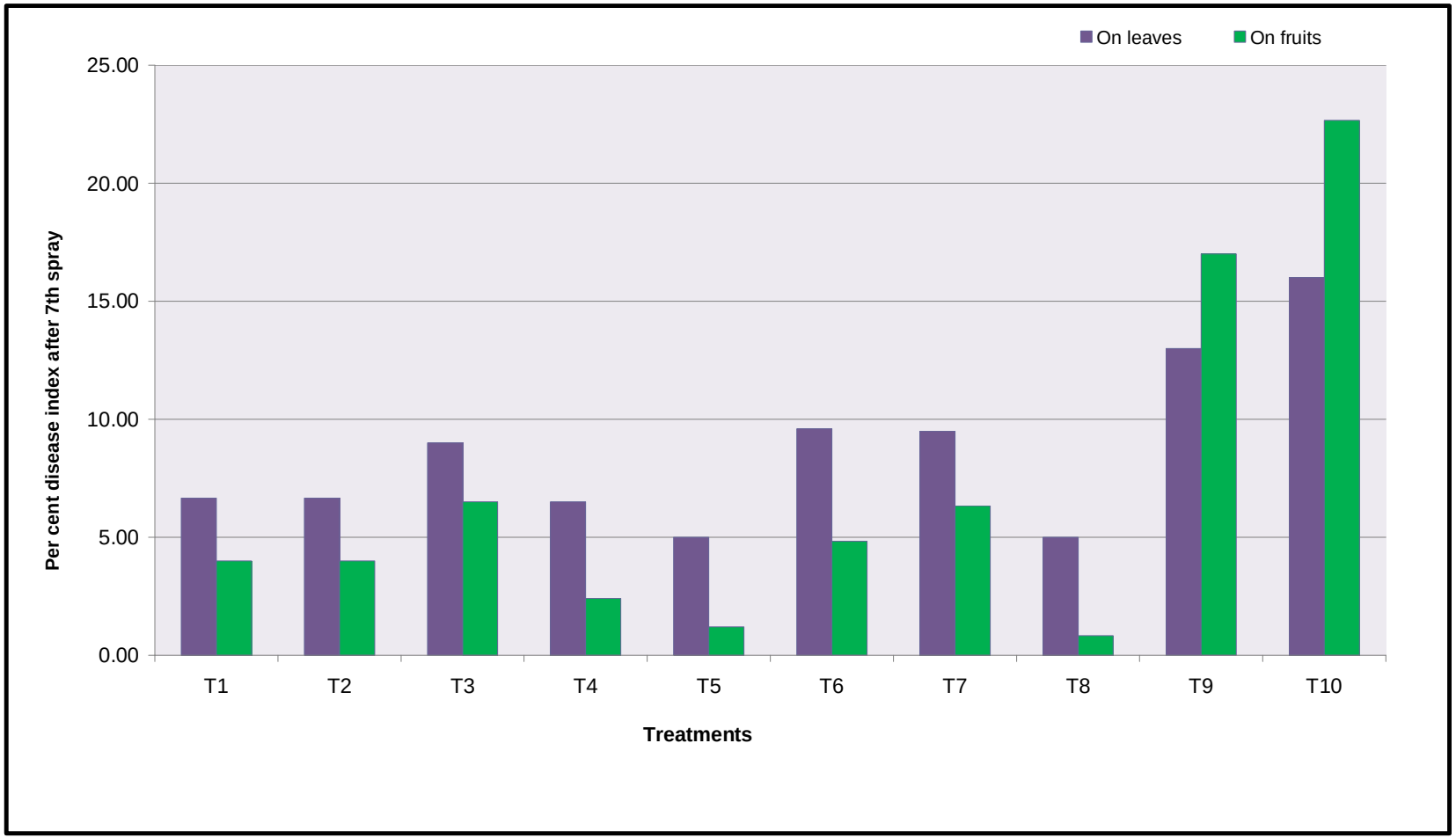


Fig.16: In vivo evaluation of fungicides and bioagents against severity of anthracnose of pomegranate

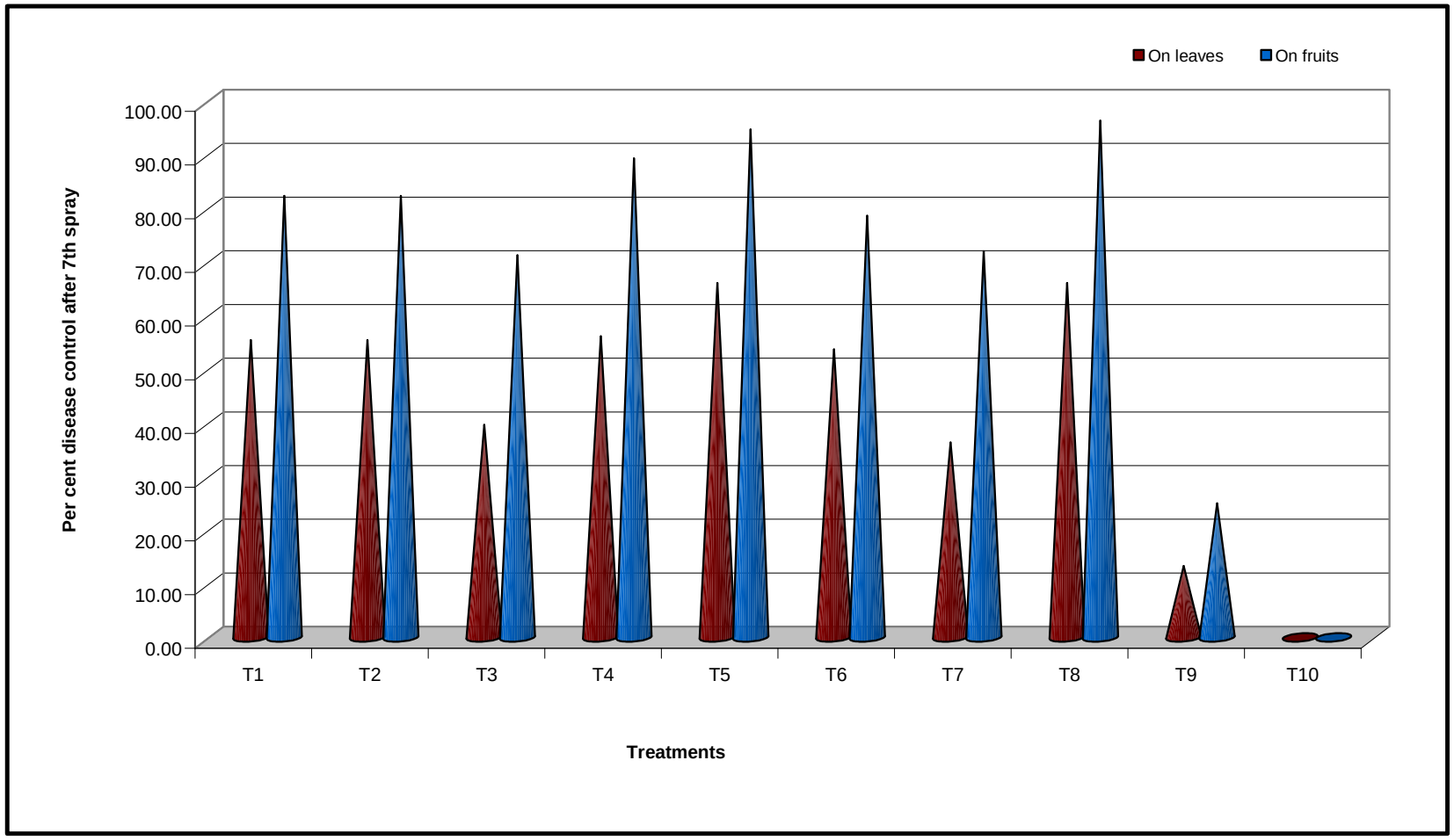
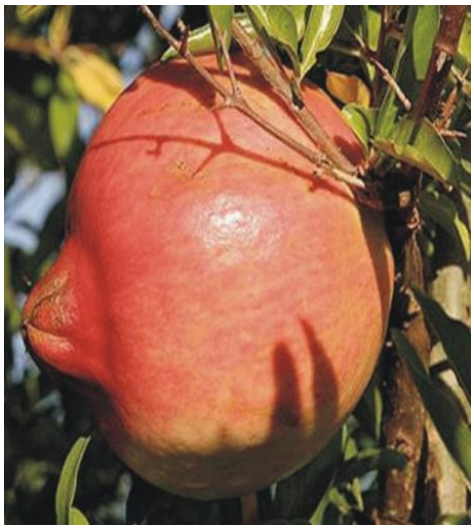


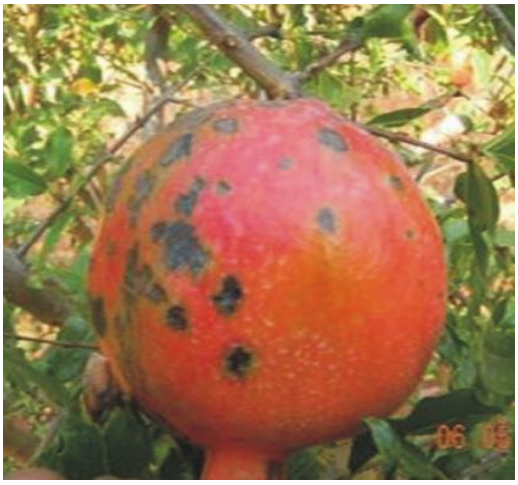
Fig.17 In vivo evaluation of fungicides and bioagent against anthracnose of pomegranate



0 grade

1 grade

2 grade



3 grade

4 grade

5 grade

Plate.15. Disease scoring using 0-5 scale in field

Carbendazim + Mancozeb (0.3%)

Unsprayed control

Plate.16. Management of anthracnose

T_8 at 0.3 per cent concentration followed by T_5 and T_4 at 0.1 and 0.2 per cent after third spray and *T. viride* was found to be less effective.

Looking into the fruits disease severity recorded, it was observed that, before 5th spray minimum disease severity of 8.00 PDI was observed in T_8 (carbendazim + mancozeb) at 0.3 per cent which was significantly superior over other treatments followed by T_5 (propiconazole) with a PDI of 8.33. Further T_8 and T_5 were statistically on par with each other.Maximum PDI was recorded in untreated control.

Before 6th spray minimum disease severity of 4.00 was observed in T_8 (carbendazim + mancozeb) at 0.3 per cent and T_5 (propiconazole) at 0.1 per cent followed by T_1 with a PDI of 8.33. Further T_5 andT_8 were on par with each other and were significantly superior over remaining treatments except T_2 which was also on par with T_5 and T_8. The highest PDI was recorded in T_9.

After 7th spray lowest disease severity of 0.83 PDI was observed in T_8 (carbendazim + mancozeb) at 0.3 per cent which was significantly superior over other treatments followed by T_5 (propiconazole) with a PDI of 1.20 at 0.1 per cent. Further T_8 and T_5 were statistically on par with each other. The other fungicides *viz.*, iprobenfos (0.2%), carbendazim (0.2%), difenconazole (0.1%), captan (0.3%) were found effective. The bioagent like *T. viride* was found to be less effective than fungicides. Maximum disease severity *i.e.,* 22.60 PDI was recorded in untreated control.

Further percent disease reduction over control (PDC) was calculated for all treatments. Among nine treatments highest PDC of 68 .75 on leaves was calculated in T_8 (carbendazim + mancozeb) at 0.3 per cent and T_5 (propiconazole) at 0.1 per cent. Similarly on fruits maximum PDC of 96.22 and 94.58 was recorded in T_8 (carbendazim + mancozeb) at 0.3 per cent and T_5 (propiconazole) at 0.1 per cent.

4.7.5 Yield as influenced by fungicides/bioagent

Yield obtained in fungicides/bioagent treated plots indicated that, highest and significant yield of 6.35 tonns per ha was recorded in carbendizim + mancozeb at 0.3 per cent concentration treated plot (Table 13) followed by propiconazole (0.1%) with a yield of 6.28 tonns/ha. Further carbendazim + mancozeb and propiconazole remain on par with each other.

The next on par yield levels were obtained in iprobenfos (0.2%), carbendazim (0.2%), difenconazole (0.1%), captan (0.3%) and mancozeb (0.3%) treated plots. The lowest yield of 2.57 tonns per ha, obtained in untreated control plot.

4.8 Screening of genotypes by detached leaf technique

Nineteen genotypes were subjected to screening by detached leaf inoculation technique in laboratory as described in “Material and Methods” and results are presented in Table 13.

No variety was found to be immune, resistant, and moderately resistant. Ganesh, Araktha and Kesar showed susceptible reaction. Where as other sixteen cultivars showed moderately susceptible reaction.

Table14: Screening of pomegranate genotypes against *Colletotrichum gloeosporioides* by detached leaf technique

Sl. No.	Genotypes	Disease reaction
1	Araktha	S
2	Alandi	MS
3	Ganesh	S
6	GUT-C-Shah rose pink	MS
5	G-137	MS
6	Jalore seedless	MS
7	Jodhpur red	MS
8	Jural anar	MS
9	Jyoti	MS
10	Kabuli	MS
11	Kaladgi local	MS
12	Kandar	MS
13	Kesar	S
14	Mridula	MS
15	Muskot	MS
16	RCR	MS
17	Ruby	MS
18	Speen dahedar	MS
19	Yeronad	MS

S=Susceptible
MS=Moderately Susceptible

5. DISCUSSION

Pomegranate (*Punica granatum* L.), regarded as a fruit of paradise, is one among the major fruit crops of arid zone. The fruits are immensely important as they are delicious, have high food value and medicinal properties, rich in many nutrients and vitamins. Hence, they are consumed by many people of the world. The crop is becoming more popular among the growers and attained a status of commercial crop. The diseases are also spreading faster and becoming a major limiting factor in attaining high yield.

Among the various fungal diseases, anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. has become potentially destructive disease both under field and post-harvest storage conditions. In severely affected orchards, it leads to defoliation, drooping of flowers and fruit dropping from plant resulting in drastic reduction of fruit yield while, after harvest it reduces the market values of pomegranate fruits. In India McRae (1924) reported this disease and observed it as one of the limiting factors for pomegranate production.

In recent years the disease has assumed to be serious problem in northern Karnataka. Therefore, investigations were carried out on various aspects of the pathogen with respect to symptomatology, severity of disease in different locations, morphological, cultural, physiological aspects of pathogen, evaluation of fungicides, bioagents and botanicals against the disease in both laboratory and in orchards, and screening of varieties for host plant resistance for developing an effective disease management approach. The results so obtained are discussed here under.

5.1 Disease survey and surveillance

Survey on the incidence and severity of anthracnose of pomegranate reveals the magnitude of the problem on hand and serves as a precursor for evolving the management strategies. It also gives clue about survival of pathogen during off season as weed or collateral hosts.

The work was initiated on survey to know the severity and distribution of the disease in major pomegranate growing areas of Bagalkot, Bijapur Koppal, Gadag and Raichur districts of northern Karnataka.

The survey also supplements the information about intensity and existence of biotypes in particular geographical locations. The severity of anthracnose of pomegranate expressed as per cent disease index (PDI). The survey also revealed that the severity of anthracnose varied from location to location, season to season and also varietal performance differed from place to place, obviously due to various factors like temperature, relative humidity, pattern of rainfall and even it could also be attributed to existence of variability in the pathogen.

Among all the districts the maximum disease severity was recorded in Bagalkot, which may be due to susceptibility of cultivars and favourable environmental condition viz., optimum temperature and relative humidity, moisture conditions that must have favoured to build up of inoculums and subsequently showing increase in disease severity. Where as in Raichur district the disease was recorded less compared to other districts. This may be due to unfavourable environmental condition which reduced the build up of inoculums thus reduced the severity.

Sudarshanrao (1975) stated that, survey and surveillance form the basis for any successful plant protection that depends on early detection of the disease followed by timely adoption of control measures. Hence, in the present investigation, roving survey was undertaken for two years in major pomegranate growing areas of northern Karnataka to assess the incidence and severity of anthracnose. During the survey, it was generally observed that, disease incidence on fruit was more than other parts of plants in most of the areas surveyed.

From the results of survey, it was observed that, fruits were more vulnerable to the attack by anthracnose than leaf as evidenced by more disease severity on fruits, irrespective of season, location and variety. Among the different districts under survey, maximum fruit infection of 37.53 PDI was recorded in Hebbal (Bagalkot) village followed by 32.88 PDI of Kushtagi (Koppal) districts. Correspondingly, average severity of the disease on fruits was observed as maximum in Bagalkot district (28.76 PDI). Where as minimum severity of 19.99 PDI was recorded in Raichur district.

Similarly, intensity of the disease on leaf ranged from 8.88 to 29.99 PDI with average highest leaf severity of 23.21 PDI in Bagalkot district. On the contrary, lowest disease index on leaf was recorded in Raichur (14.36) district. In general, the disease incidence and severity vary from season to season in different agro-climatic zones and varieties, which may be due to variation in pathogen, host varieties or climatic condition. Mandhre *et al*. (1996) in their survey recorded the highest severity of anthracnose of pomegranate to an extent of 40 to 60 per cent in the Nashik district (Maharashtra).

Prashanth (2007) recorded the highest severity of anthracnose of pomegranate to an extent of 38.88 per cent in the villages of Bagalkot. On the contrary, in the present survey work, minimum fruit and leaf severity of 19.99 and 14.36 PDI was recorded in Raichur district, because most of the farmers in both the years had grown their crop during Hastbahar (September to March) during which weather, conditions such as no rainfall from third week of November till the harvest of the crop (in both the years) and high temperature which were found unfavourable for the disease development and spread during growth period of the crop. However, the minimum disease severity on leaf and fruit was observed due to favourable environment for a shorter period (intermittent rainfall and low temperature) from September to October. Similar reasons for low disease incidence and severity (anthracnose of pomegranate) in hastbahar were attributed by Prashanth (2007). Contrary to this maximum disease severity was recorded in Bagalkot district. The reason being that the crop by most of the farmers was grown during *mrigbahar* followed by *ambiabahar*, which were the most vulnerable seasons for the attack of anthracnose due to the existence of favourable environment such as continuous intermittent rainfall, maximum temperature ranges between 30.80 to 33.62^{0}C and minimum ranges between 21.50 to 23.84^{0}C and presence of variability in pathogenic fungus.

The present findings are also in conformity with the work of Padule and Kaulgud (1991), who reported the leaf and fruit spot of pomegranate caused by *Colletotrichum gloeosporioides* in *ambiabahar* and *hastabahar*. Among the varieties, Araktha was found more susceptible with more average disease severity on leaf (26.39 PDI), fruits (33.61PDI). The disease severity on leaf was comparatively less on Ruby red and Ganesh (20.33 and 19.59 PDI). Among the seasons, mrigbahar was found more vulnerable than *hastbahar* and *ambiabahar*.

Contrary to the above observations, heavy infection of Araktha has been reported in *mrigbahar* at the time of fruits maturity because of continuous rainfall. Thus observation recorded in present investigation, It clearly point out to the severity of disease in cultivars

in the stage of the crop growth when moist, high humid, rainy period that prevails during July to September period confirm the various earlier reports.

5.2 Symptomatology

Anthracnose caused by *Colletotrichum gloeosporioides* is the major and threatening disease of pomegranate. Temperature and relative humidity requirement is going to decide the development of disease in orchards. The disease is manifested on leaves, flowers and fruits of pomegranate in all stage of crop growth.

The first evidence of disease appeared on the young leaves as minute dark brown water soaked lesion later become necrotic depressed spots of one to two millimeter diameter. Often these spots enlarged, coalesce and produced bigger patches. Later downward progression of the disease was observed and leaves dried up and dropped. The disease affects the tender part of fruit. Symptoms varied according to the plant part infected. Normally

black necrotic sunken patches appeared on the fruits. Similar descriptions of the symptoms of anthracnose of pomegranate were given by previous workers in mango (Ekbote,1994, Venkataravanappa, 2002), and in pomegranate (Prashnth, 2007). The disease in pomegranate exhibits two types of damages. Firstly, reductions in fruit yield due to immature fruit dropping, secondly reduction in the market value of partially affected fruits. This disease is more serious in rainy season and is a limiting factor in successful cultivation of pomegranate. Mazzani (1994) also described similar symptoms caused by *Colletotrichum gloeosporioides* in commercial plantation of pomegranate in Venezuela.

5.2.1 Collection and isolation

The field survey of disease conducted during 2009-10 in northern districts of Karnataka showed diverse incidence of anthracnose on pomegranate in different locations. Affected disease samples, showing typical anthracnose symptoms were collected from different locations during survey. Upon tissue isolation the pathogen from the locality was brought into pure culture and identified as *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. based on morphological, cultural characters and pathogenicity in accordance with the description given by Ekbote (1994); Sudhakar (2000); Prasanna Kumar (2001); Venkataravanappa (2002) and Prashanth (2007).

5.2.2 Pathogenicity

Pathogenicity test was proved employing detached leaf technique. The description given agreed with earlier worker (Jamadar, 2007).

5.2.3 Identification

The study on morphological characteristics of *Colletotricum gloeosporioides* indicated, septate hyphe, conidiomata acervulli and hyaline conidiophore, conidia single celled, hyaline, straight, cylindrical, rounded at both the ends, with one to two oil globules in the conidium. The conidia measured 11.48-20.01 µm in length and 4.25-6.62µm in width. The findings are in agreement with the findings of earlier workers (Simmondes, 1965; Mordue, 1971, Irwin and Camerson, 1978; Holiday, 1980; Jeffries *et al*., 1990; Ekbote., 1994; Sudhakar 2000; Prasanna 2001; Venkataravanappa 2002 and Prashanth, 2007). Based on the mycelial and spore morphology, asexual fruiting body of the fungus under study was identified as *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc.

5.3 Growth phase

Maximum dry mycelial weight of *Colletotrichum gloeosporioides* was attained on 12th day (426.70 mg) of incubation in potato dextrose broth and was considered as optimum period for growth of fungus for future studies. The study indicated that further increase in the incubation period resulted in decrease in the dry mycelia weight of the fungus, which may have been due to autolysis of the mycelium, accumulation of toxins and exhaustion of nutrients in the medium after incubation for optimum number of days. The results are in conformity with the findings of Lilly and Barnett (1951) who have discussed the onset of autolysis after maximum growth, during which cellular enzyme begin to digest the various cell constituents .The present studies are also in conformity with Ekbote *et al*. (1997), but slightly differ with Sudhakar (2000) who found that the maximum growth of fungus was attained on 14th day of incubation. and also prashanth (2007) found that the maximum growth of fungus was attained on 13th day of incubation. Which may be attributed to variability of the fungus in relation to geographical and host adoptability.

5.3.1 Growth and sporulation of *Colletotrichum gloeosporioides* on different solid and liquid media

Fungi secure food and energy from the substrate upon which they live in nature. In order to culture the fungus in the laboratory it is necessary to furnish those essential elements and compounds in the medium for their growth and other life processes. Not all media are not equally good for all fungi, nor there is a universal substrate or artificial medium upon which all

fungi can grow. So, different media including both non or semi -synthetic and synthetic were tried for *Colletotrichum gloeosporioides*.

Among the non or semi-synthetic and synthetic media used for growth and sporulation of *Colletotrichum gloeosporioides*, maximum growth (90.00 mm) and good sporulation of the fungus was recorded on PDA followed by oatmeal agar, which recorded growth of 84.90 mm. While least growth (72.70 mm) and poor sporulation was observed on Czapek (Dox) agar media. Present studies are in accordance to the better performance of *Colletotrichum gloeosporioides* on PDA which may be attributed to inherent complex nature of material supporting good fungal growth owing to provision of some additional nutrients as reported by Ekbote *et al. (*1997), Sudhakar (2000) Akhtar (2000) and Prashanth (2007).

However, in case of broth, maximum dry mycelial weight (416.67 mg) was recorded in potato dextrose broth and least (150.00 mg) in host leaf extract broth after twelve days of incubation. The present studies are in accordance to Prashanth (2007) who reported that maximum radial growth was recorded in potato dextrose agar medium. Further, he reported that in liquid media, the maximum mycelial weight was recorded in Richards' broth and least in host leaf extract after 12days of incubation. The present studies are in accordance to Sudhakar (2000).

5.4 Physiological characters of *Colletotrichum gloeosporioides*

Among the external factors, temperature is an important factor governing distribution, growth, reproduction and survival of the fungus. Temperature affects almost every function of fungi including the growth and sporulation. Temperature has profound effect on the vegetative and reproductive activity of the fungi. Effect of temperature on mycelial radial growth and dry mycelial weight revealed that maximum growth (90.00 mm and 416.00mg) was at 30^{o} C. Further increase in temperature level to 35^{o} C decreased mycelial growth and least mycelial growth (0.60 mm and 92.00 mg) was observed at 15^{o} C. However the temperature at 20-30^{o} C was found to be optimum for the growth and sporulation of *Colletotrichum gloeosporioides*. This is in agreement with the observation made on *Glomeralla cingulata*, *C. capsici* and *Colletotrichum gloeosporioides* by various workers who noticed best growth at 25-29^{o} C. (Sattar and Mallik, 1939; Abe and Kono 1956; Choudhuary, 1957; Verma, 1969; Mancini *et al*., 1973; Ekbote *et al*., 1996; Prasanna Kumar, 2001; Venkataravanappa, 2002; Prashanth2007 and Vinaya, 2008). Excellent sporulation of fungus was at 30^{o} C and least sporulation was found at temperature 15^{o} C and 35^{o} C, which is an important pathogenic character. The present results are also in accordance with Estrada *et al*. (1993 and 2000).

Light has a profound effect on growth and sporulation of fungus. The preliminary studies carried out in the present investigation with *Colletotrichum gloeosporioides* indicated a maximum growth (88.88mm) and excellent sporulation when it was exposed to alternate light and dark condition which was on par with 12 hours dark and 12 hours light (88.44mm). Least radial growth (59.38 mm and 57.94mm) and poor sporulation were recorded when *Colletotrichum gloeosporioides* was exposed to continuous light and continuous dark and both were on par with each other. When exposed to alternate light and darkness it attained maximum radial growth which might be due to induction of certain metabolic process necessary for growth and sporulation of the fungus, which usually doesn't occur in continuous light. Similarly Venkataravanappa (2002) and Prashanth (2007) observed in their studies that the exposure of alternate light and darkness favoured good growth and sporulation compared to continuous light and continuous dark.

5.5 Toxin studies

A preliminary indication of toxin production by any fungus *in vitro* has been provided by a number of bioassay methods of plant cutting (Sharma and Sharma, 1969; Naik *et al*., 1991)) seed germination bioassay , root and shoot elongation bioassay (Anahosur, 1976, Venkataravanappa, 2002) and spore germination inhibition method (Sharma and Sharma, 1969).

Tomato seedlings of same age and size were kept in culture filtrates of 2 to 20 days Culture filtrate showed complete wilting of seedlings within 72 hr but 2 days old culture filtrate showed complete wilting after 96 hr.Symptom expression increased with increases in days of incubation from 2 to 20 days. The results are in agreement with observations of Sharma and Sharma (1969) and Venkataravanappa (2002) who reported toxicity of culture filtrate of *Colletotrichum gloeosporioides* on sorghum seeds, tomato seedlings.

Further the effect of culture filtrates were studied on seed germination, shoot and root length on sorghum seedlings. Maximum inhibition of sorghum seed germination and also root and shoot length was noticed. There was 60.30 percent inhibition of sorghum seed germination in 20 days and 18 days culture filtrates. Increase in the days of culture filtrate the seed germination, shoot and root length and tomato seedling growth inhibition decreased showing in linear manner.

The present findings clearly indicated that, active metabolite released by the fungus in culture was toxic to seed germination of sorghum and growth of tomato seedlings.

The present findings are in agreement with Venkataravanappa (2002) who reported that *Colletotrichum gloeosporioides* produced nonspecific toxic metabolites in culture filtrate, which inhibited the seed germination of sorghum. Jayashankar *et al.* (1999) found that the *Colletotrichum gloeosporioides* produced phytotoxin *in vitro,* which inhibited the seed germination of lettuce and tobacco seeds.

5.6 Disease management

5.6.1 *In vitro* evaluation of fungicides

In the absence of resistant cultivars, use of fungicides to manage the disease is an old-age practice. When there is outbreak of epidemic for any reason perhaps use of fungicides is one of the best options available. These fungicides have to be used judiciously according to the need and kind of organism involved. Availability of new fungicides necessitates evaluation of fungicides under *in vitro* conditions to know their efficacy, and initiate spray schedule in field conditions.

In vitro evaluation of new synthetic molecules of fungicides is very much necessary before they are tried under field condition. Among the non-systemic fungicides at 0.3 per cent concentration carbendazim + mancozeb showed 75.10 per cent inhibition of mycelial growth of fungus followed by captan with 60.77 per cent and least inhibition of mycelial growth was recorded in copper oxychloride with 0.9 per cent. Ekbote *et al*. (1996) reported that among the four fungicides tested, carbendazim + mancozeb gave cent per cent inhibition of mycelial growth at 0.1 per cent concentration. The least per cent inhibition of mycelial growth was observed in chlorothalonil at all the tested concentration.

Among the systemic fungicides, iprobenfos showed 87.99 per cent inhibition of mycelial growth of fungus and was followed by propiconazole (87.10%) at 0.15 per cent concentration while, least per cent inhibition of mycelial growth was recorded in carbendazim (62.09). The effectiveness of the triazole fungicides like propiconazole may be attributed to their interfeance with the biosynthesis of fungal sterols and inhibit the ergosterol biosynthesis. In many fungi, ergosterol is essential to the structure of cell all and its absence cause irreparable damage to cell wall leading to death of fungal cell.

A similar study was reported for the effectiveness of triazoles, which inhibit the sterol biosynthesis pathway in fungi (Nene and Thapliyal, 1973).

At higher concentration most of the fungicides inhibited maximum mycelial growth but decreased with reduced concentration. These results are in agreement with that of Sudhakar (2000) and Prashanth (2007).

5.6.2 *In vitro* evaluation of bioagents

Regular use of synthetic chemicals/ fungicides in pomegranate has been a norm in many orchards that brings with it many hazards such as pesticide residue, development of resistant strains among the pathogen, ecological consideration, etc.

Use of bioagents, now a days, is best and has been most emphasized and widely accepted practice as it is environmentally safe and can overcome the residual problems associated with heavy use of fungicides for management disease Hence, the present investigation was taken up to screen the bioagents for effective management of pomegranate anthracnose.

Among the bioagent tried during present investigation *Trichoderma viride* was found to be best in inhibiting mycelial growth of *Colletotrichum gloeosporioides* (86.82%) followed by *Trichoderma harzianum* (72.47%) and *Pseudomonas fluorescens* (67.00%) and least per cent inhibition of mycelial growth was observed in *Bacillus subtillis* (53.88). Present studies recorded significant mycoparasitism of *Trichoderma viride* and *Trichoderma harzianum* on anthracnose fungus that caused lysis of the hyphae and the spores *in vitro*.

Mandhare *et al (*1996) and Prashanth (2007), who reported that *Trichoderma* sp. most effective antagonist, to inhibit the anthracnose of pomegranate.

5.6.3 *In vitro* evaluation of botanicals

Extensive use of fungicides has led to various environmental problems, human health and their persistence in the fruits. To sort out these problems botanicals were tested in laboratory against *Colletotrichum gloeosporioides*.

Continuous use of chemical fungicides in the management of disease also brought new problems with them. Amongst them are pollution of air, water, soil, residual toxicity, development of resistance in the pathogen against chemicals there by the need to apply them more with their escalating prices and harmful effects on non target organisms. Consequently, fungicides have been alarming development of harmful environment for human beings. Contrary to the problems associated with use of synthetic chemicals, botanicals are environmentally non-pollutive, renewable, in-exhaustible, indigenously available, thus readily biodegradable relatively cost effective and hence constitute as a suitable plant protection in the strategy of integrated disease management. Hence, screening of plant products for its effective antifungal activity against the pathogen is essentially required to minimize the use of fungicides and considered as one of the components in the integrated disease management (Khadar, 1999 and Nagesh, 2000).

It revealed that, most of the plant extracts showed fungistatic nature at higher concentration (30%). Two plant extracts *viz*. Datura leaf extract (61.70%), garlic extract (50.00%) showed ≥50 per cent inhibition of mycelial growth, while least inhibition of myceliall growth was noticed in tulasi leaf extract (0.70%).

At 20 per cent concentration, three plant extracts namely garlic leaf extract, onion bulb extract and garlic bulb extract showed more than 30 per cent inhibition of mycelial growth. The extracts of datura leaf shown maximum inhibition of mycelial growth of *Colletotrichum gloeosporioides* even at 10 per cent concentration. The effectiveness of onion and garlic as a pesticide due to an acrid volatile oil which contains diallyl disulphide, diallyl trisulphide and sulphoxides derived from allicin has been well established. (Venkataravanappa, 2002)

Effectiveness of eucalyptus leaf, garlic bulb and ocimum leaf extract against *Colletotrichum gloeosporioides* is supported by the work of Prashanth (2007). The toxicity of *Allium cepa*, *A. sativum, Ocimum sanctum*, *Datura stromonium*, *Polyalthia longifolia, Tagetas errecta* and *Vinca rosea* on has been reported by Ashashivapuri *et al*. (1997). Plants are resourceful ≥ biopesticides and need to be evaluated to find out the chemicals involved / synthesized in them. Fungicidal spectrum of neem (*Azadirachta indica), Tridax procumbense*

and *Datura stramonium* has already been investigated by Ameresh *et al*. (1998) in oil seed disease management.

5.6.4 *In vivo* evaluation of fungicides and bioagent against *Colletotrichum gloeosporioides* during ambiabahar 2010

By utilizing the in vitro information generated on bioassay of fungicides and bioagent, a field experiment was planned and executed during *ambiabahar* 2010 (Jan - may) at Bandi farmer village Koppal district. Eight different fungicides (five non-systemic, one combi product and two sysemic) one bioagent (*Trichoderma viride*) with untreated control were evaluated for their efficacy disease control on pomegranate diseased leaves, flowers and fruits.

The results after seven sprays reavealed that, among systemic fungicides, combi product (carbendazim + mancozeb) and propiconazole at 0.1 per cent concentration was significantly superior over other fungicides, where as iprobenfos (0.2%), carbendazim (0.2%) and difenconazole (0.1%) remained statistically on par with each other. The results are in agreement with findings of several workers (Abhishek and Verma, 2007; Desai, 1998; Gaikwad, 2000; Hegde, 1998; Jahagirdar *et al.,* 2000; Jamadar,et al., 2007; Navale *et al*., 2009; Patel *et al*., 2007 Prasanna Kumar *et al*., 2001; Prashanth *et al*., 2008; Raghuwamshi *et al*., 2004 Venkataravanappa, 2002). The effectiveness of triazole, which inhibit the sterol biosynthetic pathway in fungi.

Among non-systemic and combi fungicides, combi product like carbendazim + mancozeb at 0.3 percent concentration was was significantly superior where as captan and mancozeb were less effective. The results are in agreement with findings Prashanth *et al*., 2008.

5.7 Screening of genotypes

The management of disease through host plant resistance has been an important choice in all crop improvement programme. Utilization of resistance is most simple, effective and economical method in the management of biotic stress. Besides, the resistant cultivars conserve natural resources and reduce the cost, time and energy when compared to other methods of disease management. Increase in use of fungicides to control the anthracnose has led to consciousness of their persistence and development of new strains of pathogen. To avoid this situation, identifying the resistant cultivars against anthracnose is most significant one.

Nineteen pomegranate cultivars were screened for their reaction under artificial condition. None of them were resistant to anthracnose fungus. Three cultivars *viz.,* Ganesh, Araktha and Kesar showed susceptible reaction where as sixteen cultivars showed moderately susceptible reaction. The results are in agreement with findings of Bhat (1991).

Future line of work

1. Needs to be tested in tow or three locations preferably in Mrigbahar
2. Variability in relation to molecular characteristics
3. Need to develop the forecast models based on 3-4 years data
4. Reduces the fungicide and develop alternate methods

6. SUMMARY AND CONCLUSIONS

An investigation on anthracnose of pomegranate caused by *C. gloeosporioides* was carried out with reference to survey on the incidence and severity of anthracnose in orchards, cultural, physiological and morphological characters of the *C. gloeosporioides*, evaluation of fungicides, botanicals and bioagents against the disease both under laboratory and field conditions and varietal reaction to the pathogen. The results obtained are summarized here under,

An extensive roving survey was carried is northern parts of Karnataka to assess the severity of anthracnose on pomegranate. This study revealed that severity of disease on leaves was recorded in Koppal district and on fruits highest disease severity was recorded in Bagalkot district. Correspondingly, the lowest disease severity on leaves and fruits was recorded in Raichur district. Among the varieties, Araktha was found more susceptible and recorded highest PDI on both leaves and fruits. The maximum disease severity was recorded in >5 year old plants and severe in mrigbahar cropping season.

The colony morphology in general indicated that fungus on potato dextrose agar produced greyish white mycelium, conidiophores hyaline, with conidia single celled hyaline, straight cylindrical, round at both the ends, with one to two oil globules present in conidium. On the basis of cultural and morphological characters, the fungus was identified as *C. gloeosporioides*. Pathogenicity of the fungus on pomegranate leaf was proved by detached leaf technique.

The maximum dry mycelial weight of *C. gloeosporioides* was observed on 12 days of incubation on potato dextrose broth (426.70 mg) and this period was considered as optimum period for fungal growth.

The fungus *C. gloeosporioides* exhibited diversified cultural characters, on both synthetic and semi / non-synthetic liquid and solid media. Among the liquid media maximum dry mycelial weight (416.67 mg) was recorded on potato dextrose broth and least weight (150.00 mg) on host leaf extract. However, in case of solid media maximum radial growth of the fungus (90.00 mm) was recorded on potato dextrose agar where as least on Czapek (Dox) agar.

Temperature of 30^{o} C was found to favour the growth of the fungus in both solid and liquid media, which recorded the highest radial growth (90.00 mm), dry mycelial wight (416.00 mg) with excellent sporulation where as least radial growth (0.60 mm) and dry mycelial weight (92.00 mg) with poor sporulation was recorded at 15^{o} C. The light also plays an important role in growth of *C. gloeosporioides*. The maximum radial growth (88.88 and 80.44mm) was recorded when it was exposed to both 12 hr alternate light followed by 12 hr dark and 12 hr dark followed by 12hr light and least radial growth of the fungus (57.94 mm) was recorded when exposed to continuous dark.

The different days of incubation of culture filtrates of *C. gloeosporioides* differed in their action to inhibit the sorghum seed germination, root and shoot length and induction of phytotoxic symptoms on tomato seedlings. The maximum inhibition of seed germination, root and shoot length, least vigour index of sorghum and complete wilting of tomato seedlings was noticed within 72 hr in 20 days old culture filtrate and least inhibition and highest vigour index and complete wilting of tomato seedlings after 96 hr in 2 days culture filtrate.

Among the nonsystemic (one combi-product) and systemic fungicides evaluated *in vitro*, carbendazim+mancozeb recorded highest per cent inhibition of mycelial growth (81.88). However least per cent inhibition of mycelium (1.55) was recorded in coper oxychloride at 0.3 per cent concentration. In case of systemic fungicides, iprobenfos at 0.15 per cent recorded highest per cent inhibition of mycelium growth (87.99) and it was on par with propiconazole at 0.15 and at 0.1 per cent concentration (87.10 and 86.34) while least per cent inhibition of mycelial growth (62.09) was recorded in carbendazim at 0.15 per cent concentration. Propicinazole and iprobenfos were very effective in inhibiting the mycelial growth of the fungus at 0.1 and 0.15 per cent concentrations.

Four bioagents were tested against *C. gloeosporioides* by following dual culture technique. Among maximum inhibition (86.82%) of mycelial growth of the fungus was recorded in *T. viride*. The least inhibition of mycelial growth (53.88%) of the fungus was noticed in *Bacillus subtilis*.

Among plant extracts, datura leaf extract and garlic bulb extract showed higher inhibition (≥50%) of mycelial growth of *C. gloeosporioides* and onion bulb extract and ginger rhizome extract showed less than 45 per cent inhibition at 30 per cent concentration and least inhibition of mycelium growth (0.70%) was recorded in tulasi leaf extract.

The management of anthracnose of pomegranate using different fungicides and bioagent *in vivo* indicated that the spraying of carbendazim + mancozeb at 0.3 per cent was significantly most effective and recorded higher yield and on par with propiconazole at 0.2 per cent. Captan (0.3 per cent) and mancozeb (0.3 per cent) were found to be least effective. The bioagent *T. viride* found to be less effective in the management of anthracnose in field condition

Of the 19 pomegranate genotypes screened for their reaction to anthracnose under artificial conditions, the genotypes *viz*., Ganesh, Araktha and Kesar showed susceptible reaction where as, other 16 genotypes showed moderately susceptible reaction. None of the cultivar was resistant to *C. gloeosporioides* infection.

Conclusions

* Symptoms of anthracnose of pomegranate appeared as black to brown necrotic depressed spots on the leaves with circular margin, which coalesced and to form bigger patches in advanced stage.

* Maximum mean disease severity on fruit was recorded in Bagalkot district followed by Koppal, Gadag and Raichur district.

* Maximum mean disease severity on leaves was recorded in Bagalkot district followed by Bijapur, Koppal, Gadag and Raichur district.

* Maximum mean disease severity on leaves and fruit was recorded Araktha variety.

* Maximum disease severity was recorded in >5 year old age trees and the disease was more severe in mrigbahar.

* The fungus was grayish white on potato dextrose agar and produced septate mycelium. Conidia were hyaline, unicellular with cylindrical base. One to two oil globules were present in the conidium.

* Pathogenecity of pomegranate anthracnose was proved by detached leaf technique in artificial condition.

* Maximum growth of *C. gloeosporioides* was attained on 12 days after incubation (426.70 mg).

* On non-synthetic media maximum radial growth of *C. gloeosporioides* was recorded on potato dextrose agar (90.00 mm) with excellent sporulation and having pinkish white mycelial colony.

* Maximum dry mycelial weight of *C. gloeosporioides* was recorded in potato dextrose broth.

* Maximum dry mycelia weight and radial growth with excellent sporulation was recorded at 30^{o} C.

* Alternate cycle of first 12 hr light followed by 12 hr dark recorded maximum radial growth of 88.88 mm with good sporulation followed by alternate cycles of 12 hr dark and 12 hr light.

* Among the culture filtrates tested maximum inhibition of seed germination, root and shoot length, least vigour index of sorghum and complete wilting of tomato seedlings as recorded within 72 hr in 20 days old culture filtrate. The least inhibition of seed germination, root and shoot length and highest vigour index and complete wilting of tomato seedlings was recorded after 96 hr in 2 days old culture filtrate.

* Among the non-systemic fungicides, carbendazim + mancozeb (combi–product) recorded highest inhibition of mycelial growth (81.88%) at 0.3 per cent concentration under laboratory condition.

* Among four systemic fungicides, maximum inhibition of growth of *C. gloeosporioides* was recorded in iprobenfos and propiconazole at 0.15 per cent concentration. Further they remain on par with each other at 0.15 per cent concentration.

* Among four bioagents tested, *T. viride* was found to be best in inhibiting the mycelial growth of *C. gloeosporioides* under laboratory condition.

* Among seven plant extracts, a maximum per cent inhibition of mycelial growth of fungus was recorded in datura leaf extract followed by garlic extract at 30 per cent concentration under laboratory condition.

* Combi product (carbendazim + mancozeb) at 0.3 per cent and propiconazole at 0.1 per cent managed the anthracnose very effectively in the orchard and yielding more number of healthy fruits.

* Among nineteen genotypes tested for their reaction, Ganesh, Araaktha and Kesar found to be susceptible, and none of them was resistant.

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STUDIES ON ANTHRACNOSE OF POMEGRANATE CAUSED BY *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc.

JAYALAKSHMI K. 2010 NARGUND V. B.
(MAJOR ADVISOR)

ABSTRACT

Pomegranate anthracnose is an important disease which affects leaves and fruits. The severity of pomegranate anthracnose was more in Bagalkot district followed by Koppal, Bijapur Gadag and Raichur districts. The identity of the fungus was confirmed as *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. The fungus showed maximum growth on Potato dextrose broth on 12 th day after incubation at 27±1^{o}C. Culture of *C. gloeosporioides* which exhibited diversity with respect to cultural characters like type of the growth, mycelial colour, pigmentation and sporulation with maximum growth on Potato dextrose agar. The highest radial growth and sporulation of the fungus was recorded at 30^{o}C, and light condition having 12 hours light alternated with 12 hours dark. The different days of incubation of culture filtrates of *C. gloeosporioids* differed in their action to inhibit the seed germination, root and shoot elongation of sorghum seeds and induction of phytotoxic symptoms on tomato seedlings. Among the fungicides, bioagents and botanicals tested against the *C. gloeosporioides,* carbendazim + mancozeb, propiconazole, *T. viride*, and datura leaf extract, were superior, in inhibiting the mycelial growth of the fungus under *in vitro* condition. The bioefficacy of fungicides and bioagent which performed well *in vitro* condition were tested *in vivo* condition as well. Among them, carbendazim + mancozeb at 0.3 per cent and propiconazole at 0.1 per cent concentration were effective in reducing the per cent disease index of anthracnose disease. Ganesh, Araktha and Kesar showed susceptible reaction while other 16 genotypes showed moderately susceptible reaction under detached leaf technique.