

STUDIES ON ANTHRACNOSE – A POSTHARVEST DISEASE OF PAPAYA

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1. INTRODUCTION

Papaya (*Carica papaya* L.) is an important fruit crop, belongs to family *Caricaceae*. *Carica* is the largest of the four genera with 48 species, among which *Carica papaya* L. is most important and cultivated all over the world (Badillo, 1971 and Waller, 1992). The popularity of papaya fruit has made it ubiquitous in tropical and subtropical regions of the world. Papaya is the native of tropical America (Singh, 1990).

It has made its way from kitchen gardens to that of commercial orchards in many tropical and subtropical countries because of its highest production of fruits (75 to 100 tons ha⁻¹) and contributed an income next to banana.

Papaya is a good source of vitamin A, vitamin C and calcium (Arriola *et al.*, 1980 and Hayes, 1993). The raw fruits contain an alkaloid or proteolytic enzyme "Papain", which is a commercial product of several tropical American nations and is used in several medicine and food preparations. Economically, *Carica papaya* is the most important species within the *Caricaceae*, being cultivated widely for consumption as a fresh fruit and for use in drinks, jams, candies and as dried and crystallised fruit (Villegas, 1997). Green fruit and the leaves and flowers may also be used as cooked vegetable (Watson, 1997).

Apart from the fruit, papaya leaves also have some medicinal values and the roots are used to cure yams and piles, the stem and bark can be used for making ropes. The other uses include extraction of oils as a source of protein and in medicine to thirst and as a vermifuse. Hence papaya has been called as "common man's fruit".

The global annual production has been estimated at 65,04,369 metric tons (Anonymous, 2005b). The major papaya producing regions are Asia, South America, North Central America and Africa. About 65 per cent of the world's production is from South America. Another 35 per cent is from North Central America and Africa.

In India, the papaya is grown for table purpose, papin and pectin extraction and concentrated in the state of Kerala, Orissa, West Bengal, Karnataka, Assam and Gujarat (Singhal, 1990). Karnataka ranks fifth in area and first in the production. Approximately 3800 ha area is covered under papaya with a production of 2,61,900 tons with an average productivity of 68.921 tons ha⁻¹ and total production and area of India during 2003-2004 was 18,50,000 tons and 18,000 ha with average productivity of 23.130 tons ha⁻¹ (Anonymous, 2005a).

Papaya is prone to many diseases incited by fungi, bacteria, nematodes and viruses leading to heavy loss in yield, of which papaya anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. appear to be more severe causing devastation of papaya fruits during transit and storage. Papaya anthracnose is the most important disease throughout the year in India and a major limiting factor in transit and storage. It is important in many other tropical regions where papaya is grown (Bolkan *et al.*, 1976).

Fruits are living entities and are highly perishable commodities that are affected by number of factors leading to be postharvest spoilage and hence postharvest losses are major ones.

Postharvest diseases of fresh fruits are traditionally being controlled by synthetic chemical fungicides (Eckert and Ogawa, 1985). However, when harvested fruits are treated with fungicide to manage postharvest diseases, there is greater likelihood of direct human exposure to them. Development of resistance in pathogens to fungicides applied for controlling the postharvest diseases (Spotts and Cervantes, 1986 and Spalding, 1982), underlines the necessity to develop new and effective methods of controlling postharvest diseases that are perceived as safe by the public and pose negligible risk to human health and environment.

Biological control of postharvest diseases is one of the options to overcome these problems in the management of postharvest diseases. It includes the use of naturally derived fungitoxicants, induced resistance, and the use of antagonistic microorganisms (Wilson and Wisniewski, 1989).

First symptoms of papaya anthracnose are round, watersoaked, and sunken spots on the body of the ripening fruit. Brown sunken spots develop on the fruit surface. The symptoms appear only upon ripening and may not be apparent at the time of harvest. The flesh beneath the affected portion becomes soft and begins to rot (Baker *et al.*, 1940 and Dickman and Alvarez, 1983).

In view of wide prevalence of this destructive disease in the area of production, transit and markets. Studies were under taken on anthracnose of papaya with the following objectives.

1. To collect the infected fruit samples and study the symptomatology of anthracnose of papaya.
2. To isolate, identify and prove the pathogenecity of anthracnose of papaya.
3. *In vitro* evaluation of different botanicals, fungicides and biocontrol agents against anthracnose of papaya.
4. *In vivo* evaluation of different salt, biocontrol agents, hot water treatment and fungicides against anthracnose of papaya.

2. REVIEW OF LITERATURE

Colletotrichum spp. are the ethyologic agent of anthracnose disease and play an important role on agricultural subsistence economies world wide. These pathogens infect different crops, from monocotyledons plants, such as papaya and turf grass to higher dicotyledons, such as cashew trees. Nevertheless, despite the fact that *Colletotrichum* affect a wide spread number of crops, its pathogenic range increases caused by a rising number of species identified under these Genera that were classified as anthracnose's agent (Peres, 2002).

Like many other fruits, the papaya fruits are also affected by various postharvest diseases like, anthracnose, stem-end rot, chocolate rot, *Fusarium* rot, *Aspergillus* rot and *Rhizopus* rot etc. The present investigation is on the anthracnose of papaya which is caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc, responsible for the postharvest losses during storage, transit and marketing.

2.1 HISTORICAL

The most common pathogen in all the papaya growing area is *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. Prasad and Verma reported its occurrence in 1970 from Bihar (Sharma and Alam, 1998). Srivastava *et al.* (1964) reported *Gloeosporium papayae* and *C. papayae* causing fruit rot of papaya.

2.2 SYMPTOMATOLOGY

Baker *et al.* (1940); Wallance and Wallance, (1948); Snowdon (1990) and Sharma and Rana (1999) reported that anthracnose spots on green fruits are generally dark brown to black with a pale margin and lenticular in shape. The affected areas increased in size and become sunken and coalesce to form large spots. On ripening fruits, the symptoms were as numerous small dark circular spots, which enlarge, coalesce and become sunken.

Dickman and Alvarez (1983) reported that although the disease usually appears on the ripening portions of the fruit, occasionally the green portions of the fruit may become infected. The disease symptoms are in the form of brown to black depressed spots on the fruits.

Burger (1921) reported that the first symptoms of papaya anthracnose were round, watersoaked, and sunken spots on the surface of the ripening fruit. Lesions may become as large as 5 cm in diameter. Pinkish-orange areas are formed by the conidial masses that cover the lesion center and are frequently produced in a concentric ring pattern.

Simmonds (1965) reported that the first symptoms are small well defined dried pink spots on the surface of ripening fruit. Later, these lesions grow to 5 cm diameter, become rounded, sunken with depth of 3 to 5 mm and brown to black in colour. The lesions can be water-soaked or dried and hard. In the centre of the lesions, the fungus produces dark acervuli, frequently in a concentric pattern and orange to pink gelatinous mass of conidia can be observed. The whole lesion can be easily separated from the flesh of the fruit as a corkscrew, using a knife, leaving a well defined hole in the fruit. Symptoms on immature fruits and leaves are uncommon. Post-harvest infection usually produces stem-end rot.

Stanghellini and Aragaki (1966) reported spores caused infection only on unwounded detached, mature fruits. Tsai (1969) observed that it can cause latent infection of the fruits.

Srivastava and Tandon (1971) observed that symptoms first appear as brown superficial discolorations of the skin and then develop into circular, slightly sunken area, 1- 3 cm in diameter; usually they appear watersoaked. Gradually the lesions coalesce and sparse white mycelial growth often appears on the margin of such spots under humid condition an encrustation of salmon pink spores. Often arranged in a concentric pattern may develop on the surface of some of the older spots.

Hunter and Buddenhagen (1972) reported that in senescing papaya petioles the perfect stage of the anthracnose fungus, *Glomerella cingulata* (Ston.) Spauld. and Schrenk, produces many ascospores. These become air borne and lodge on the fruit surface where they form appressoria upon germination. Isolation from petioles and from healthy papaya fruit

surface usually resulted in recovery of colony type different from that resulting from isolation from anthracnose lesion.

Duran and Mora (1988) observed the part of the fruit near the stem was the most severely affected by the disease and *Glomerella cingulata* causes symptoms earlier to other pathogen and incidence was high after 48 hours of harvesting. Inoculations with *Colletotrichum gloeosporioides* resulted in complete rotting within 5 days (Mohan and Lakshmanan, 1987).

Sepiah *et al.* (1991) reported that symptoms caused by *C. gloeosporioides* occur only when the fruit is fully ripened. Pernezny and Litz (1993) and Simone (1999) reported that the fungus attacks primarily the fruit of papaya, with mature fruit being more susceptible. Petioles and leaves may be infected, but this is thought to be important only as a source of the fungus for further fruit infection. The disease signs begin as small, water-soaked spots on ripening fruit. As the spots develop, they become sunken, turn brown or black, and may enlarge to a few inches. The fungus may produce a pink mass of spores in the middle of the older spots. The pathogen grows into the fruit, resulting in softening of the fruit and an off flavor. Growers practice a prophylactic program for this disease.

Sharma and Alam (1998) reported that the affected tissues become dirty brown, soft and finally rot. Infection may also occur when the fruit is immature and mummification and deformation of fruits take place.

Verma and Sharma (1999) reported that as the disease develops, it frequently produces large mass of spores in the central portions of the lesion causing them to turn light orange or pink.

Rana (2001) reported that the fruit rot also be initiated at the pre-harvest stage when inoculum load is very heavy on the stem and petioles.

The initial symptoms are watersoaked, sunken spots one-fourth to one inch in diameter on fruit. The centers of these spots later turn black and then pink when the fungus produces spores. The flesh beneath the spots becomes soft and watery, which spreads to the entire fruit. Small, irregular-shaped watersoaked spots on leaves may also be seen. These spots eventually turn brown (Anonymous, 2003).

2.2.1 Other pathogens associated with the postharvest diseases of papaya

Microorganisms	Diseases/Symptom	References
<i>Botryodiplodia theobromae</i> [= <i>Diplodia natalensis</i>]	Watersoaked spot increases and turns into dark-brown lesion irregular in outline having greenish watersoaked margin. At later stage the centre of the lesion gets depressed, and dark grayish mycelial growth may appear on the surface.	Alvarez <i>et al.</i> 1977; Siradhana and Jain, 1962.
<i>Ascochyta carica papayae</i> [= <i>A. caricae</i>] [= <i>Mycosphaerella</i> sp.]	Ascochyta rot. Stem-end rot. Fruit rot often starts from the stem-end. Watersoaked lesion increases in size and turns dark-brown to blackish in colour. At later stage numerous dark pycnidial bodies may appear on the surface of the lesion.	Chowdhury, 1950; Chau and Alvarez, 1997.
<i>Alternaria alternata</i> [= <i>A. tenuis</i>]	Brown spots. As the lesion increases, the colour changes to grayish-brown. At later stage the surface of lesion may get covered with dark-brown conidiophores and conidia.	Alvarez <i>et al.</i> , 1977; Barkai-Golan, 1981.
<i>Fusarium acuminatum</i>	Soft rot. Watersoaked spot enlarge and turns light-brown in colour. At later stage the centre of the lesion gets depressed. Often a white mycelial growth appears on the surface	Barkai-Golan, 1981; Pathak <i>et al.</i> 1976.

<i>Rhizopus stolonifer</i> [= <i>R. nigricans</i>]	Soft, watery rot. Watersoaked lesions with irregular margin spread. At later stage the lesion turns brown and white mycelial growth sporangiophores appear on the surface. Finally the fruit collapses watery exudate and emits foul odour.	Tandon and Mishra, 1969; Sarwar and Kamal, 1971.
<i>Pythium aphanidermatum</i>	Soft fruit-rot. Watersoaked lesions spread and turn brown. White mycelial growth appears on the lesion.	Trujillo and Hine, 1965.

2.3 MORPHOLOGICAL STUDIES

McRae (1934) studied the morphological characters of *Glomerella cingulata* on artificial media. The acervuli were round or oblong, black or pink with or without setae. The conidiophores measured about 20-30 µm. the conidia were hyaline or pink in mass, straight rounded at the end and measured 11-32 x 4-55 µm.

Sattar and Malik (1939) observed that the acervuli of the *C. gloeosporioides* developed profusely on diseased parts of the plant (petioles, leaves and fruits). They were irregular and appeared as brown to black dots on the leaves and occurred on both the surface of leaf. Setae were common on twigs but not on fruits. The acervuli when mature exuded pink masses of conidia under moist conditions. The acervuli were reported to measure 80-250 µm. The marginal setae were rare when present they were dark fuliginous, cylindrical, continuous and reported to measure 40-90 x 4-6 µm (mean 60 x 5 µm). The conidia were borne on distinct, well-developed hyaline conidiophores. The conidia were straight, cylindrical or oval and the size of the conidia varied from 8-20 x 5-7 µm, hyaline usually with two, rarely one oil globules.

Arx (1957) made a detailed study on the species of the genus *Colletotrichum* and assigned the ascogenous state of *Colletotrichum gloeosporioides* as *Glomerella cingulata*. The fungus *Colletotrichum* was described by Corda (1831-32) under the name *Colletotrichum* with a single species. Saccardo (1884) and Potebnia (1910) placed *Colletotrichum* in Melanconiales and Acervulales, respectively.

There are around 600 synonyms of *C. gloeosporioides* (Arx, 1957), among them are *Gloeosporium papayae* P. Henn., *Colletotrichum. papayae* P. Henn. and *C. papayae* Petrak. are causing anthracnose of papaya.

Colletotrichum gloeosporioides has been associated with 470 genera of plants (Sutton, 1980). Isolates from papaya can infect avocado, banana, eggplant, guava, apple, mango, watermelon, strawberry, orange, rubber tree, coffee and cotton.

Colletotrichum gloeosporioides is a ubiquitous pathogen that has been associated with quiescent infections and post-harvest diseases on several fruits such as avocado, mango, papaya, guava, passion fruit, citrus, apple, grapes and cashew (Simmonds, 1965; Hartill, 1992 and Alahakoon *et al.*, 1994). Morphology of *C. gloeosporioides* was described by several workers (Simmonds, 1965; Mordue, 1971; Irwin and Cameron, 1978; Louis and Cooke, 1985; Davis *et al.*, 1992; Sudhakar, 2000 and Prasanna Kumar, 2001)

Mordue (1971) reported that perithecia aggregated, globose, dark brown to black, 85-300 µm in diameter; the ostioles are periphysate and paraphyses are present. The asci are 8-spored and have short stalks, clavate to cylindrical, thickened at apex, 35-80 x 8-14 µm. The ascospores are hyaline, unicellular and narrowly oval to cylindrical to fusiform. Acervuli are produced on lesions, and usually setae. Conidiophores are cylindrical phialidic. The conidia are cylindrical with obtuse ends, 9-24 x 3-6 µm, unicellular, hyaline or faintly brown. Appressoria are 6-20 x 4-12 µm, ovate to obovate, sometimes lobed. Bose *et al.* (1953) reported that the acervuli measured 115-467 x 15-22 µm and the size of conidia varied from 11-16 x 4-6 µm.

The causal agent was characterized by immersed, branched, septate and hyaline to brown mycelium and separate to confluent acervuli, which may produce brown smooth, septate, tapered setae. Conidiophores were hyaline, determinate conidiogenous cells. Conidia of *C. gloeosporioides* were hyaline, aseptate prior to germination, smooth and thin

walled, cylindrical or oval, straight and size of the conidia varied from 9-24 x 4-12 µm (Sutton, 1980, 1993).

Holiday (1980) and Jefferies *et al.* (1990) reported that the conidia were hyaline, unicellular and highly cylindrical with obtuse ends or ellipsoidal with a round apex and a narrow, truncate base. The conidial size was 7-20 x 2.5-5 µm and formed on hyaline to faintly brown conidiophores in acervuli that are irregular in shape and about 500 µm in diameter. Setae were 4-8 x 200 µm, one to four septate brown and slightly swollen at the base and tapered at the apex.

Naik (1985) recorded that, the conidia of *C. gloeosporioides* isolates of *Colletotrichum* from peach and apple produced rounded ends conidia and fusiform conidia on PDA respectively.

Colletotrichum gloeosporioides was grayish to dark gray on Potato Dextrose Agar (PDA) and produced aerial mycelium ranging from a thick mat to sparse or tufts of mycelium. Conidia were hyaline, unicellular and either cylindrical with obtuse ends or ellipsoidal with a rounded apex and narrow, truncate base. They measured 7-20 µm x 2.50-5.00 µm in diameter. Setae were 4-8 x 200 µm, 1 to 47 septate, brown and slightly swollen at the base and tapered at the apex. Orange slimy conidial masses can be formed as the acervuli matured (Litz, 1997).

Ekbote (1994) reported that conidia of *Colletotrichum gloeosporioides* were oblong or cylindrical, hyaline, non-septate with round ends, thin walled, having oil globules in the centre. Conidia on the culture media were found to be in reddish mass. They were usually found in aggregates. The conidia on potato dextrose agar measured 12.12–20.78 µm x 4.33–9.52 µm and average being 17.09 x 7.74 µm.

The fungus was grayish white to dark grey on potato dextrose agar and produce aerial septate mycelia. Conidia were hyaline, unicellular and either cylindrical with obtuse ends or ellipsoidal with a rounded apex and narrow, truncate base. Orange slimy conidial mass formed as the acervuli matured. Conidia measured 4.98-15.69 x 2.76-5.52 µm (Prasanna Kumar, 2001).

Rana, (2001) reported that the mycelium is immersed, branched and consists of rather narrow, sparsely septate, hyaline hyphae which turn slightly darken with age. Acervuli are formed on dark stroma and conidia are sub-hyaline, pinkish in mass, variable in shape, mostly oblong to cylindrical, straight, with obtuse ends, sometime slightly curved, 1-celled but may become 2-celled at the time of germination, contain 1 or 2 oil globules and measure 10-25 x 3.5-7.0 µm mostly 12-16 x 4-6 µm.

2.4 SINGLE SPORE ISOLATION

Munjal and Gupta (1965) purified the *Colletotrichum gloeosporioides* isolated from cocks comb by single spore isolation. Similarly, Moses and Govind Rao (1969) and Latham and Williams (1983) purified the *Glomerella cingulata* culture collected from infected tissues of coriander and apple respectively, by single spore isolation. Booth (1971) recommended that after isolation of the fungus, it should be reisolated from the isolation plates as a series of single spore culture.

Naik (1986) and Bhat (1987) also purified the culture by single spore isolation after isolating *Colletotrichum gloeosporioides* from infected tissue of betel vine and cashew, respectively.

Colletotrichum gloeosporioides produced white to grayish mycelial growth with abundant sporulation on Czapek's, host leaf extract, two per cent sucrose, oat meal agar and Richard's agar media (Hiremath *et al.*, 1993).

Ekbote *et al.* (1997) reported that maximum radial growth of *Colletotrichum gloeosporioides* was reported in Richard's, Brown (90 mm) and PDA followed by Czapek's agar (89.60 mm). All these media did not differ significantly. Least colony growth was recorded in Asthana's and Hawker's media.

Akthar (2000) reported that the fresh potato extract was the best source for routine isolation and growing *Colletotrichum gloeosporioides* causing mango anthracnose.

Sudhakar (2000) reported that, maximum radial growth of *Colletotrichum gloeosporioides* was recorded in five media (Sabouraud's agar, Richard's agar, Brown's agar, Potato dextrose agar and Oat meal agar). The least colony growth was recorded in Asthana and Hawker's 'A' medium.

Rani and Murthy (2004) reported that, maximum radial growth of *Colletotrichum gloeosporioides* which causes anthracnose of cashew was recorded in Richard's agar medium (8.84 cm), Brown's agar medium (8.76 cm) and Potato sucrose agar medium (8.62 cm). All these media did not differ significantly. Less radial growth was recorded in corn meal medium (3.16 cm). Richard's agar medium was found to be significantly superior to other media recording an abundant sporulation.

2.5 PATHOGENICITY TEST

The method employed for pathogenicity tests were similar to those described by Stanghellini and Aragaki (1966). Circular 3-4 cm diameter were drawn with a felt tip pen on fruits previously surface sterilized by dipping in 1% sodium hypochlorite for 4 minute and rinsing in sterile water. The circled tissue was either left unwounded or was wounded with a sharp sterile scalpel. The inoculum was prepared by growing fungus to be tested in Petriplates (90 mm diameter) containing 15 ml PDA or 10% V-8 juice agar. After 7 days of incubation under continuous light at 25°C, the contents of the plates (mycelium + fruiting bodied + agar) were added to 100 ml sterile distilled water and one ml of this was placed in centre of each marked circle. Fruits were incubated at 22-24°C for 8 days. The fruits were examined daily and as lesions developed the causal organism was reisolated and identified.

Chau and Alvarez (1983) proved the pathogenicity, mature green papaya fruits were disinfected with hot water at 48°C for 20 min, cooled and inoculated with spore suspension from 7 to 10 days old culture of *Colletotrichum gloeosporioides* five microliters of a suspension containing $2-3 \times 10^3$ spores was placed within a circular area (1.0 cm diameter) on the fruit surface. Inoculated fruits were incubated at room temperature (~24°C) in moist chambers. Samples were excised from the inoculated area at 18, 24, 30, 48, 96 and 120 hours after incubation and reisolated the fungus and identified.

In pathogenicity studies, the conidial suspension of *Colletotrichum gloeosporioides* was sprayed on healthy fruits and typical symptoms were noticed, seven days after incubation. Similar results were recorded while proving pathogenicity by Bhat (1991) and Ekbote (1994) on pomegranate and mango respectively. Kota (2003) proved the pathogenicity of *Colletotrichum gloeosporioides* on mango and banana.

2.6 CULTURAL STUDIES

2.6.1 Growth character on different solid media

Miller (1955) reported that V-8 juice agar as a general purpose medium for *Colletotrichum* spp. and also for many other plant pathogenic fungi.

The pathogenic monospore culture of *Colletotrichum gloeosporioides* made good, white to grayish mycelial growth with abundant sporulation on Czapek's, host leaf extract, two per cent sucrose, oat meal agar and Richard's agar media (Hiremath *et al.*, 1993).

Ekbote (1994) observed that maximum radial growth of 90 mm observed in Richard's agar, Brown's agar and Potato dextrose agar next followed by Czapek's agar (89.6). All these four media did not differ significantly among them.

Ashoka (2005) reported that maximum radial growth of *Colletotrichum gloeosporioides* was on potato dextrose agar (90.00 mm) and Richard's agar (90.00 mm) and was par with oat meal agar (89.5 mm). They were superior over all other media. Minimum radial growth of fungus was obtained in Ellitto's agar (40.00 mm) and Asthana's and Hawker's agar (44.50 mm).

2.6.2 Growth phase

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

Hiremath *et al.* (1993) studied the growth phase of *Colletotrichum gloeosporioides* causing Shisham blight. They noticed that the fungus reached the maximum growth after 12 days of inoculation on potato dextrose broth at temperature of 25°C.

Ekbote (1994) reported that the fungus *Colletotrichum gloeosporioides* causing anthracnose of mango reached the maximum growth after 12 days of inoculation and least growth was observed on 2 day after inoculation. Ashoka (2005) reported that dry mycelial weight of the fungus was minimum on second day after inoculation, it significantly increased and finally reached maximum on 12th day (330.33 mg).

2.6.3 Growth on different liquid media

Hiremath *et al.* (1993) reported that, maximum dry mycelial weight of *Colletotrichum gloeosporioides* was recorded in Richard's and potato dextrose broth. Ekbote (1994) reported that maximum growth of the fungus was observed in Richard's broth (443 mg) followed by potato dextrose broth (385.66 mg) and Czapek's broth (317.00 mg).

Ekbote *et al.* (1997) reported that in liquid media the maximum dry mycelial weight of *Colletotrichum gloeosporioides* was recorded in Richard's broth (433 mg) and least weight in Asthana's and Hawker's broth (123 mg).

Sudhakar (2000) observed that in liquid media the maximum dry mycelial weight of *Colletotrichum gloeosporioides* was recorded in Richard's broth (288.33 mg) and least weight was recorded in Asthana's and Hawker's 'A' medium (166.66 mg) after 16 days of incubation.

In liquid media the maximum dry mycelial weight of *Colletotrichum gloeosporioides* causing anthracnose of cashew was supported by Richard's broth (310.00 mg) followed by Brown's broth (297.00 mg) (Rani and Murthy, 2004).

Ashoka (2005) reported that maximum dry mycelial weight of fungus was obtained in Richard's broth (394.00 mg) followed by potato dextrose broth (378.00 mg) and least was obtained in carrot broth (62.66 mg).

2.7 NUTRITIONAL STUDIES

2.7.1 Utilization of carbon sources

Durairaj (1956) observed that among the various carbon sources tested, sucrose was most efficient utilized by *Colletotrichum capsici* followed by glucose and mannose.

Thind and Randhawa (1957) found that *Colletotrichum capsici* grew best on media having dextrose, sucrose, maltose, starch and tartaric acid. Similarly, glucose as a best carbon source for *Colletotrichum gloeosporioides* was reported by many worker (Chandra and Tandon, 1962 and Lal and Tandon, 1968). Result of investigations of Verma (1979) indicated that the total absence of sucrose checked the growth of all species of *Colletotrichum*. The mycelial growth was positively correlated with increased concentration of glucose. In general, higher amount of sucrose supported better growth but only little amount of sucrose was needed for better sporulation, especially for *Colletotrichum gloeosporioides* and *Colletotrichum atramentarium* (Brkk and Br) Taub.

Naik (1985) reported that sucrose was best carbon source for the growth of *Colletotrichum gloeosporioides* of betelvine followed by glucose, dextrose, citric acid and mannitol.

Ekbote (1994) observed maximum growth of the fungus obtained in sucrose (443.00 mg) as a carbon sources, followed by glucose (414.66 mg) and least growth was observed on lactose (131.00 mg).

2.7.2 Utilization of nitrogen source

Durairaj (1956) found that among the nitrogen source tried on *Colletotrichum capsici*, ammonium phosphate supported maximum mycelial growth followed by organic nitrogen sources (urea and asparagines), ammonium oxalate and potassium. Mishra and Mahmood (1960) reported that asparagine at 0.2 per cent nitrogen was found to be good source. Further they observed that for sporulation, peptone appeared to be the best followed by potassium nitrate and sodium nitrate.

Singh and Shankar (1971) in their studies on growth and sporulation of *Colletotrichum capsici* from betelvine found that L-leucine was the best among the different nitrogen sources.

Rajak (1983) reported that potassium nitrate was best inorganic nitrogen source and asparagine was best organic nitrogen source for the growth of *Colletotrichum gloeosporioides*.

Naik (1985) reported that potassium nitrate supported the maximum growth of *Colletotrichum gloeosporioides* on betelvine followed by Di-methioine, L-leucine, glycine and histidine.

Ekbote (1994) reported that potassium nitrate (387.66 mg) supported the maximum growth followed by sodium nitrate (376.00 mg), L-asparagine (341.66 mg) and ammonium nitrate showing least growth (175.33).

2.7.3 Utilization of sulphur sources

Chaturvedi (1965) and Singh and Shankar (1971) reported that magnesium sulphate was utilized more efficiently by *Glomerella cingulata*. Sodium thiosulphate, zinc sulphate, potassium sulphate and sodium bisulphate were also utilized in the decreasing order by *Glomerella cingulata*, next to magnesium sulphate. Hedge (1986) reported that magnesium sulphate was utilized more efficiently by *Colletotrichum gloeosporioides*.

Ekbote (1994) used five sulphur sources for study the utilization of sulphur and maximum growth of the fungus was observed when magnesium sulphate (426.66 mg) was used as a sulphur source, followed by sodium sulphate a (362.66 mg) and least fungus growth was observed on ferrous sulphate (185 mg).

2.8 PHYSIOLOGICAL STUDIES

2.8.1 Effect of hydrogen ion concentration

Satter and Malik (1939) observed fairly good growth of *Glomerella cingulata* in pH range from 4 to 9 and optimum pH was 6.9. Durairaj (1956) found that the growth of *Colletotrichum capsici* increased with increased in pH of the medium and the optimum pH was 7.0. Sitterly (1958) and Chaturvedi (1965) found out an increase in the growth and sporulation of *G. cingulata* at pH 5.5.

The role of pH on the growth of phytopathogenic fungi has been reviewed by Tandon (1961). The optimum pH *in vitro* favoring the growth of fungi pathogenic to the papaya fruit, ranges between 5.5 and 7.0. These pathogens can however, grow at a wider range of pH, especially the acidic side (4.0).

Verma (1969) reported that maximum growth of *Colletotrichum gloeosporioides* was found at an optimum pH of 6.0. Singh and Shankar (1971) obtained the highest growth of *G. cingulata* at pH 5 followed by 4, 6, 7, 8, 9 and 10. Thind and Madan (1979) observed its growth on a neutral alkaline pH range.

Rajak (1983) found that pH of 7.0 was optimum for *Colletotrichum gloeosporioides*. Whereas, Naik (1986) and Hegde (1986) observed that maximum growth of *Colletotrichum gloeosporioides* at a pH 6.0 and 6.5 respectively.

Ekbote (1994) observed maximum growth was at a pH level of 6.5 followed by 6.0. The least growth was recorded at a pH level of 4.0. Whereas, Ashoka (2005) found that maximum dry weight at a pH 6.0 (414.33 mg).

Kamanna (1996) reported that maximum growth of *Colletotrichum gloeosporioides* was found at an optimum pH of 6.0.

2.8.2 Effect of temperature

Sattar and Malik (1939) found the growth of *Colletotrichum gloeosporioides* at 25 to 30°C and the minimum and maximum range of temperature were 10 to 15°C and 35 to 40°C respectively.

Abe and Kono (1956) observed the optimum temperature range for *Glomerella cingulata* as 24- 28°C and thermal death point at 55°C for five minutes (moist) and 10 minutes (dry). Similarly Chowdhuary (1957) and Quimio (1973) obtained maximum mycelial growth of *Colletotrichum gloeosporioides* of papaya was at 30°C.

Verma (1969) and Mancini *et al.* (1973) noted the temperature of 25°C and 27°C as optimum for *Glomerella cingulata* respectively. Rajak (1983) reported a temperature of 25°C as the optimum for the growth of *Colletotrichum gloeosporioides*. Naik (1985) reported that 20-30°C as optimum temperature range for *Colletotrichum gloeosporioides*. Hegde and Hegde (1986) observed maximum growth of the *Colletotrichum gloeosporioides* at 30°C and temperature range for the good growth was 20-35°C.

The fungus *Colletotrichum gloeosporioides* isolated from preivet (*Lingustrum vulgare* L.) showed good growth at temperature range between 20 to 28°C (Orlikowski and Wojdyla, 1991). Agostini *et al.* (1992) reported that the fast growing grey isolate (FGG) from citrus post bloom fruit drop disease, caused by *Colletotrichum gloeosporioides* grew better at 31°C.

Ekbote (1994) reported that maximum growth of fungus was noticed at 26°C. This was followed by 29°C, 23°C, 20°C, 35°C and 15°C which were decreasing order and differed significantly. The least growth recorded at 15°C.

Chakraborty (1997) reported that temperature between 20 to 30°C was necessary for infection in *Stylosanthes* anthracnose caused by *Colletotrichum gloeosporioides*. Sudhakar (2000) observed that the maximum growth of the *Colletotrichum gloeosporioides* was noticed at 30°C followed by 25°C, 20°C and 35°C.

Ashoka (2005) reported that maximum dry mycelial weight of fungus was observed at a temperature of 30°C (454.00 mg) which was significantly over 25 °C (354.00 mg).

2.8.3 Light requirement

Chowdhuary (1936) found that continuous light or darkness was found to inhibit sporulation of *Colletotrichum graminicola* (Ces) G. Wilson, but cultures exposed to alternate light and darkness were found to sporulate earlier and more conspicuous. Minussia and Kimati (1978) reported that *C. graminicola* of sorghum isolate from four localities sporulated abundantly under continuous light.

Growth and sporulation of *C. graminicola* at different light exposures *in vivo* and *in vitro* tests showed that diurnal light exposure favored 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).

Kamanna (1996) showed that exposure of *Colletotrichum gloeosporioides* to alternate cycles of 12 hr light and 12 hr darkness yield maximum growth and sporulation.

Ashoka (2005) reported that the exposure of the *C. gloeosporioides* to alternate cycles of 12 hr light and 12 hr darkness for twelve days resulted in maximum dry mycelial weight (452.85) and maximum radial growth (89.35 mm).

2.9 MANAGEMENT STUDIES

2.9.1 *In vitro* evaluation of 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. The possibilities of controlling plant disease by the integration of several methods have been the subject of extensive research. An integrated control which denotes the rational use of available control measures will have to be considered especially with crops which are infected simultaneously by various types of pathogen, it does offer the possibilities of making up for the deficiencies of any single method. Integration of chemicals, plant extract, biotic agents along with resistance for managing plant disease has been considered as a novel approach, as it requires low amount of chemical, by reducing the cost of control as well as pollution hazardous, with minimum interference of biological equilibrium (Papavizas, 1973).

Ark and Thompson (1959) demonstrated that aqueous and organic extracts of garlic had potent fungicidal and bactericidal activity against several plant pathogens.

Pathak and Jain (1970) reported antifungal activity in leaf extracts of tulsi. Murthy and Amonkar (1974) studied antifungal effects of natural garlic oil and its synthetic form (diallyl-disulphide) on ten air borne and soil borne plant pathogenic fungi. *Glomerella cingulata* required 500 ppm of natural garlic oil and synthetic garlic oil for complete inhibition of mycelial growth. Ahmed and Sultana (1984) found that, whole garlic bulb extract inhibited the mycelial growth of *Colletotrichum corchori* Itaka and Sunaka.

Shekhawat and Prasad (1971) reported that, extracts of *Allium cepa* L., *Allium sativum* L., *Ocimum sanctum* Land Mont., *Metha piperata* L. and *Beta vulgaris* L. showed strong inhibitory action on *Alternaria tenuis* Nees.

According to Jeti *et al.* (1987) leaf extracts of *Polyalthia longifolia* L. inhibited growth of *Colletotrichum gloeosporioides* under *in vitro* condition. Chauhan and Joshi (1990) observed that the eucalyptus oil, castor oil, garlic bulb, *Zinziber officinale*, turmeric and lantana leave significantly controlled the mango anthracnose disease.

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

Chavan (1996) reported that water extracts of ten per cent of concentration of *Allium sativum*, *Azadirachta indica*, *Ocimum sanctum*, *Pongamia pinnata* and *vitex negundo* suppressed the mycelial growth of *Colletotrichum gloeosporioides* causing anthracnose by 84.06, 76.22, 71.19, 64.22 and 61.25 per cent respectively. Escopalao and Silvestre (1996) tested fifteen medicinal plant extracts *in vitro* against *Colletotrichum gloeosporioides* causing anthracnose of mango fruits. Only the extracts of Komontigue (fruit) and garlic crude extracts inhibited the growth of the fungus. Both the extracts were effective at 1:10 and 1:100 dilutions and were comparable to the benomyl fungicide.

Ray and Punithalingam (1996) showed carburin and quercetin isolated from roots of *Clerodendron* inhibited spore germination of *Alternaria carthamii*, *Helminthosporium oryzae* and *Alternaria alternata* (Fries) Keissler and *Fusarium lini*.

Shivapuri *et al.* (1997) noticed that among ten plant extracts tested against *C. capsici*, five plants extracts *viz.*, *Azadirachta indica*, *Datura stramonium*, *Ocimum sanctum*, *Potyalthis longifolia* and *Vinca rosea* were found more fungitoxic.

Thoppil *et al.* (2000) demonstrated that the oil extracted from *Ocimum adscendns* L. showed antimicrobial properties on many bacterial pathogens and also fungal pathogen *Colletotrichum musae*.

Phytoextracts from nineteen species were assayed under *in vitro* condition by poisoned food technique for their antifungal activity against *C. gloeosporioides* causing leaf spot of turmeric. All the phytoextracts inhibited the growth of fungus significantly as compared to control except cactus, tulsi, sankal chain, bodio kalar and mehendi which rather stimulated the growth of fungus. The extract of gando baval produced maximum inhibition (83.58%) followed by bhoy ringni (81.10%) and ginger (74.16%) (Patel and Joshi, 2001).

Shirshikar (2002) found that *Allium sativum* has shown best fungicide activity with its bulb extract totally inhibiting fungal growth and spore germination of *Colletotrichum gloeosporioides* and *Botryodiplodia theobromae*. Leaf extract of *Ocimum sanctum*, *Glyricidia maculate*, *Pongamia pinnata* L. were also effective in reducing per cent spore germination of *B. theobromae* where as, leaf extract of *Pongamia pinnata* and *Catharanthus roseus* were effective in reducing spore germination of *Colletotrichum gloeosporioides*.

Raheja and Thakore (2002) reported that extracts from medicinal plants like *Allium sativum* (cloves), *Azadirachta indica* (leaves), *Mentha arvensis* (leaves) and *Psoralea corylifolia* (seeds) were found most effective to check the mycelial growth of *C. gloeosporioides* followed by *Curcuma longa* (Rhizomes), *Coriander sativum* (leaves) and *Lantana camara* (leaves and flowers). Ashoka (2005) reported that neem was found effective in inhibiting mycelial growth (50.45%).

2.9.2 *In vitro* evaluation of fungicides

Anthracnose was effectively controlled by spraying with carbendazim (0.1%) or Topsin-M (0.1%) or chlorothalonil (0.2%) at 14 days intervals until harvest. Benlate (0.2%)

and Dithane Z-78 (0.2%) are extremely toxic to fungus in culture. However, these have not been tested in the field (Bose *et al.*, 1953).

Postharvest fungicides, applied as a spray or dip, with a food-grade wax have also shown to be effective in reducing anthracnose. This is a common practice especially for fruits shipped to overseas markets (Akamine and Arisumi, 1953).

The fruit dipping in benomyl solution (500 ppm a.i.) or thiobendazole (1000 ppm) immediately before storage reduces the disease to 5 per cent from 29 per cent and Pre-harvest spraying with bavistin (0.1%) or Topsin-M(0.1%) or prochloraz (0.1%) followed by dipping in the same after harvesting have also been found to be effective (Sohi *et al.*, 1973).

Under *in vitro* studies, zineb, thiram, captan, benomyl, phenyl mercuric chlorides, DDB, prochloraz 2 and chlorothalonil were found effective in inhibiting the mycelial growth of *Colletotrichum gloeosporioides* (Eikelenboom, 1964; Saraswathy *et al.*, 1975 and Martinelli and Reis, 1984).

Naik (1986) reported that cent per cent inhibition of mycelial growth was obtained with blitox, foltaf, cumin-L at 0.3 per cent and RH 2161 at 0.025, 0.05 and 0.1 per cent concentration.

Orlikowski and Wajdyla (1991) in their *in vitro* studies found that benlate and carbendazim inhibited the mycelial growth of *Colletotrichum gloeosporioides* isolated from *Lingustrum vulgare*. *In vitro* evaluation of fungicide like prochloraz and chlorothalonil against *Colletotrichum gloeosporioides* of sour cherry exhibited inhibition of colony growth (Ivanovic and Ivanovic, 1992).

Ali *et al.* (1993) reported that complete inhibition of mycelial growth of *Colletotrichum gloeosporioides* of tea was found in case of bavistin and tilt at the concentration of 100 and 200 ppm respectively followed by foliar 400 ppm and calixin 1500 ppm. Koelsch *et al.* (1995) tested fungicides against *Colletotrichum gloeosporioides* of periwinkle, propiconazole inhibited maximum growth (96%) and thiophenate methyl with mancozeb partially growth (50%).

Tomyphilip (1997) reported that among six fungicides tested against *Colletotrichum gloeosporioides* causing black leaf spot in mulberry mancozeb, carbendazim and copper oxychloride proved to be effective in inhibiting radial mycelial growth of the pathogen.

Tomy (1997) reported that among six fungicides tested against *Colletotrichum gloeosporioides* causing black leaf spot in mulberry. Mancozeb, carbendazim and copper oxychloride proved effective in inhibiting radial growth of the pathogen.

Srinivasan and Gunasekaran (1998) reported that contaf (Hexaconazole) at 0.1, 0.15, 0.2 and 0.4 per cent concentration completely inhibited mycelial growth, Indofil M-45 inhibited only upto 88 per cent at 0.5 per cent. Simone, 1999 reported that benomyl is used to manage anthracnose and powdery mildew.

Deshmukh *et al.* (1999) conducted *in vitro* studies with ten fungicides and reported that Bordeaux mixture (1 and 2 per cent) copper oxychloride (0.2 and 0.3 per cent), carbendazim (0.1 and 2%), thiophenate methyl (0.1 and 0.2 %) and fosetyl-AL (0.1 and 0.2%) were very effective in inhibiting the growth and sporulation of *Colletotrichum gloeosporioides* causing anthracnose on anthurium.

Washathi and Bhargava (2000) reported fungicides *viz.*, carbendazim (0.05-0.1%), mancozeb (0.2-0.25%), tetramethyl thiuram disulphate (0.25%) and benomyl (0.15%) showed complete inhibition of growth of *Colletotrichum dematium*.

Patel and Joshi (2002) observed that carbendazim (Bavistin 50% WP), thiophanate methyl (Topsin-M 75% WP), propiconazole (Tilt 25% EC) at 250, 500 and 1000 ppm, hexaconazole (Contaf 5% EC) at 750, 1000 and 1500 ppm and tricyclazole (beam 75% EC) at 500 and 1000 ppm observed cent per cent inhibition of *Colletotrichum gloeosporioides* causing leaf spot of turmeric.

In vitro studies using different fungicides against *Colletotrichum* spp. responsible for premature yellowing and bean shedding in vanilla showed that thiophanate methyl even at

very low concentration i.e. 100 ppm is highly inhibitory to the fungus followed by carbendazim (250 ppm) or carbendazim + mancozeb mixture 2000 ppm (Suseela Bhai *et al.*, 2003).

Ashoka (2005) tested seven systemic and four non-systemic fungicides at three concentrations under *in vitro* condition against *Colletotrichum gloeosporioides*. Systemic fungicide viz. bayleton, benomyl, prochloraz and Saaf were successful in completely (100%) in inhibiting the growth of *Colletotrichum gloeosporioides* at all three concentrations (0.025, 0.005 and 0.1%) whereas, non-systemic fungicide mancozeb (77.65%) found to be effective in inhibiting the growth of fungus.

2.9.3 *In vitro* evaluation of biocontrol agents

Deshmukh and Raut (1992) reported that *Trichoderma harzianum* Rifai and *T. viride* Pers. overgrew colonies of *Colletotrichum gloeosporioides* and *T. harzianum* was more aggressive than *T. viride*. Narendra Singh (1992) revealed that *T. harzianum* was a strong inhibitor of *C. falcatum* under *in vitro* condition.

Arras (1993) reported that, *Bacillus subtilis* strains isolates from cold stored citrus fruits significantly inhibited (60-92%) the growth of *Colletotrichum gloeosporioides*. Whereas, Ashoka (2005) tested six biocontrol agents and noticed that maximum reduction in colony growth was observed in *T. harzianum* (64.65%) followed by *T. viride* (55.38%) and *T. virens* (54.50%) which were on par with each other. These were followed by *Pseudomonas fluorescens* (50.41%), *T. koningii* (46.72%) and *B. subtilis* (42.48%).

Koomen and Jeffries (1993) tested 648 microorganisms, which includes bacteria, yeasts and filamentous fungi against *Colletotrichum gloeosporioides* under *in vitro* conditions and found that 121 organisms inhibited the fungal growth and they were further tested for their ability to affect conidial germination. 45 bacterial and yeasts inhibited germination and finally obtained two potential antagonists identified as *Bacillus cereus* and *Pseudomonas fluorescens*. Medeiros and Menezes (1994) reported that a paired culture method in Petriplates *C. gloeosporioides* showed a high degree of sensitivity to *Trichoderma harzianum*, *Trichoderma polysporum* (Linkex Pers.) Rifai and *Trichoderma pseudokoningii* Rifai.

Mishra and Narain (1994) reported that the four isolate of *Gliocladium virens* and single isolate of *Streptovercillium* inhibited radial growth of *C. gloeosporioides* by the cell free culture filtrates of the bioagents.

Trichoderma spp. were reported to be effective against guava fruit rot pathogens like *Lasiodiplodia theobromae*, *C. gloeosporioides*, *Pestalotiopsis versicolor*, *Phomopsis psidii* and *Rhizopus arrhizus* (Majumdar and Pathak, 1995).

Rocha *et al.* (1998) reported that *Trichoderma koningii* Oudemans and *T. harzianum* with antagonistic potential against *C. gloeosporioides*. Jeyalakshmi *et al.* (1998) observed that the maximum growth inhibition of the pathogen (*C. capsici*) was exerted by *Sccharomyces cerevisiae* followed by *T. viride*, *T. harzianum*, *T. pileatus* and *T. harzianum*.

Bhuvanewari (1999) evaluated *Trichoderma viride* under *in vitro* condition against postharvest pathogens of mango and revealed that the growth of *Pestalotia* spp., *A. flavus* and *C. gloeosporioides* were inhibited 72.88, 70.74, 62.41 and 56.83 per cent respectively.

Gud (2001) found that, among seven antagonists evaluated *Trichoderma viride* (66.4%) proved highly antagonistic against *C. gloeosporioides* followed by *Gliocladium virens* (58.67%). The antagonistic ability of *T. viride*, *T. harzianum*, *T. logidrachytm*, *Gliocladium virens*, *Aspergillus niger*, *Pseudomonas floescence* and *Bacillus subtilis* were tried *in vitro* against *Colletotrichum gloeosporioides* causing leaf spot in turmeric by dual culture technique. All the bioagents proved inhibitory to the growth of pathogen. In dual culture technique, significantly maximum inhibition was recorded in *T. viride* (66.40%) (Patel and Joshi, 2001).

Shirshikar (2002) reported that, culture and culture filtrate of *Trichoderma viride* was more effective than *T. harzianum* in inhibiting the mycelial growth of *Botryodiplodia theobromae* and *Colletotrichum gloeosporioides*.

Santha Kumari (2002) observed that the isolates of T1 and T2 of *T. harzianum* and the isolates of A1 and A2 of *Aspergillus niger* were found effective in inhibiting the growth of *Colletotrichum gloeosporioides* causing anthracnose of black pepper under *in vitro* condition.

2.10 *IN VIVO* OF SALTS, BIOAGENTS AND HOT WATER TREATMENT AGAINST ANTHRACNOSE OF PAPAYA

The main approaches to reducing the inoculum level have been to add chlorine sodium *O*-phenylphenate to the water dump to inactivate spore brought into the solution on fruits (Bertrand and Saulie-Carter, 1979; Spotts and Peters, 1980 and Sugar and Powers, 1986).

McLaughlin *et al.* (1990) and Wisniewski *et al.* (1995) reported that application of a two per cent solution of calcium chloride was found to be effective to control of gray and blue mold.

In control of avocado, *Bacillus subtilis*, applied as a dip or a wax formulation remove nutrients from the immediately surrounding of the appressorium of *Colletotrichum gloeosporioides* to ensure maintenance of the dormancy, as demonstrated by Korsten and Jefferies (2000).

The effects of hot water treatments and storage conditions on quality of fresh-cut papaya were investigated. A hot water treatment of 48-50°C for 20 minutes was found to delay fungal storage rots in fruits of Tainung-2 and Red Lady cultivars without negatively affecting sensory quality (Allong *et al.*, 2000).

3. MATERIAL AND METHODS

The present investigations were undertaken at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad and in the laboratory of the Department of Plant Pathology, College of Agriculture, Dharwad during 2007-2008. The details of materials used and the methodology followed during the course of the present investigations are described in the subsequent pages.

3.1 GENERAL PROCEDURE

3.1.1 Glassware cleaning

Corning glassware were used for all the experimental studies, wherever required and were kept in the cleaning solution containing 60 g of potassium dichromate, 60 ml of concentrated sulphuric acid in one liter of water for a day. Then they were cleaned by washing with detergent followed by rinsing several times in tap water and finally in distilled water.

3.1.2 Sterilization

All the glassware were sterilized in an autoclave at 1.1 kg/cm² pressure for 20 minutes and all the solid and liquid media were sterilized for 15 minutes at 1.1 kg/cm² pressure.

3.2 COLLECTION AND ISOLATION OF THE PATHOGEN

Fruits having typical symptoms of anthracnose of papaya were collected from the orchard of Saidapur farm, University of Agricultural Sciences, Dharwad and Dharwad Local market. Infected portion was cut into small pieces along with some healthy portion, they were surface sterilized with sodium hypo chlorite (One part sodium hypo chlorite + three part distilled water) solution for 30 seconds. To remove all the traces of sodium hypo chlorite on the surface, the fruit surface bits were washed for two to three times in sterile distilled water and such bits were then transferred to potato dextrose agar (PDA) slants. The slants were incubated at 27 ± 1°C and the growth of the fungus was observed periodically. Pure colonies which developed from the tissue bits were transferred to slants.

3.2.1 Single spore culture

Ten ml of clear two per cent filtered water agar was poured into sterile Petriplates and allowed to solidify. Dilute spore suspension was prepared in sterile distilled water and from this two ml of spore suspension was spread uniformly on the water agar plates and the excess suspension was drained off aseptically from the plates. The plates were incubated at 27 ± 1°C for few hours. They were examined frequently under the microscope for well isolated germinating spore and were then marked with ink on the glass surface of the plate. These marked agar areas were cut and transferred to PDA slants and incubated at 27 ± 1°C.

3.3 PROVING THE PATHOGENICITY

Pathogenicity of the isolated organism was proved in the laboratory by wound inoculation method. Apparently healthy and uninjured fruits were washed in tap water, followed by surface sterilized by using sodium hypochlorite for 30 seconds and rinsed thrice in sterile distilled water. Wound was made on the fruits with the help of a sterile needle. Sterile cotton pad of about 5.0 sq. cm was dipped in spore suspension and swabbed over the wounded surface of the fruits. The inoculated fruits were kept along with cotton plug dipped in water, to maintain the humidity and incubated at 27 ± 1°C. The pathogen was reisolated from the diseased portion and compared with the original cultures to prove the Koch's postulates.

3.4 MAINTENANCE OF THE CULTURE

The fungus was sub-cultured on PDA slants and allowed to grow at 27 ± 1°C for one week. Such slants were preserved in refrigerator at 5°C and renewed once in two months.

3.5 MORPHOLOGICAL STUDIES

A loopful of culture of *Colletotrichum gloeosporioides* obtained from twelve days old culture was placed on the slide and mixed thoroughly with lactophenol to obtain uniform spread. A cover slip was placed over it, length and breadth of 100 spores were measured under high power objective calibrated micrometer. The average size of the spores was calculated. Similarly the spores produced in the fruit was also measured and the average size was calculated.

3.6 CULTURAL STUDIES

3.6.1 Growth characters on solid media

The culture characters of *Colletotrichum gloeosporioides* were studied using the following fifteen solid media.

1. Potato dextrose agar
2. Malt extract agar
3. Oat meal agar
4. Host leaf extract agar
5. Corn meal agar
6. Potato carrot agar
7. Papaya fruit agar
8. V-8 juice agar
9. Richard's agar
10. Czapek's agar
11. Sabouraud's agar
12. Elliott's agar
13. Tochnal's agar
14. Sach's agar
15. Brown's agar

The composition of the above 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). They are as given below.

Composition and preparation of different media

1. Potato dextrose agar

Potato	:	200 g
Dextrose	:	20 g
Agar agar	:	20 g
Distilled water (to make up)	:	1000 ml

The potatoes were boiled in 400 ml of distilled water and the extract was collected by filtering through a muslin cloth. Agar agar was melted separately in 400 ml of distilled water. The potato extract was mixed in the molten agar and 20 g of dextrose was added to the mixture. The volume was made upto 1000 ml with distilled water and sterilized at 1.1 kg/cm² pressure for 15 min.

2. Malt extract agar

Malt extract	:	25 g
Agar agar	:	20 g
Distilled water (to make up)	:	1000 ml

Malt extract was dissolved in 400 ml of distilled water. Agar agar was melted separately in 400 ml of distilled water. Both the solutions were mixed thoroughly and final volume was made upto 1000 ml with distilled water and autoclaved at 1.1 kg/cm² pressure for 15 min.

3. Oat meal agar

Oat flakes	:	30 g
Agar agar	:	20 g
Distilled water (to make up)	:	1000 ml

First oat flakes were boiled in 400 ml distilled water for 20 minutes and filtered through muslin cloth. Agar agar was melted in 400 ml of water separately. Both the solutions were mixed thoroughly and the volume was made upto one liter with distilled water and autoclave at 1.1 kg/cm² pressure for 15 min.

4. Host leaf extract agar

Papaya leaf	:	200 g
Agar agar	:	20 g
Distilled water (to make up)	:	1000 ml

Papaya leaf were crushed and boiled in 400 ml of distilled water for 20 min and extract was collected by filtering through a muslin cloth. 20 gm agar agar was melted separately in 400 ml of distilled water and both solutions were mixed. The volume was made upto one liter with distilled water and autoclaved at 1.1 kg/cm² pressure for 15 min.

5. Corn meal agar

Maize	:	30 g
Agar agar	:	20 g
Distilled water (to make up)	:	1000 ml

Corn meal was boiled in 400 ml of distilled water for 20 min and extract was collected by filtering through a muslin cloth. Agar agar was melted separately in 400 ml of distilled water and both solutions were mixed. The volume was made upto one liter with distilled water and autoclaved at 1.1 kg/cm² pressure for 15 min.

6. Potato carrot agar

Grated potato	:	20 g
Grated carrot	:	20 g
Agar agar	:	20 g
Distilled water (to make up)	:	1000 ml

Grated vegetables were boiled in 400 ml of distilled water. This was strained through fine sieve. Agar agar was melted separately in 400 ml of distilled water and both solutions were mixed. The volume was made upto one liter with distilled water and autoclaved at 1.1 kg/cm² pressure for 15 min.

7. Papaya fruit agar

Papaya fruit piece	:	200 g
Agar agar	:	20 g
Distilled water (to make up)	:	1000 ml

Papaya fruit cut into pieces were boiled in 400 ml of distilled water and extract was collected by filtering through a muslin cloth. 20 gm agar agar was melted separately in 400 ml of distilled water and both solutions were mixed. The volume was made upto one liter with distilled water and autoclaved at 1.1 kg/cm² pressure for 15 min.

1. V-8 juice agar

V-8 juice	:	8.3 g
L-asparagine	:	10 g
Yeast extract	:	2 g
Calcium carbonate	:	2 g
Glucose	:	2 g
Agar agar	:	20 g
Distilled water (to make up)	:	1000 ml

44.3 g of V-8 juice agar powder obtained from highmedia was suspended in 1000 ml distilled water and sterilized at 1.1 kg/cm² pressure for 15 min.

9. Richard's agar media

Sucrose	:	50 g
Potassium dihydrogen phosphate	:	5 g
Potassium nitrate (KNO ₃)	:	10 g
Magnesium sulphate (MgSO ₄ .7H ₂ O)	:	2.5 g
Ferric chloride (FeCl ₂ .6H ₂ O)	:	0.02 g
Agar agar	:	20 g
Distilled water (to make up)	:	1000 ml

All the above ingredients except potassium dihydrogen phosphate and agar agar dissolved in 450 ml of distilled water. Agar agar melted separately in 500 ml of distilled water and was mixed with the above solution. The volume was made upto 950 ml. potassium dihydrogen phosphate was dissolved in 50 of distilled water. The two solutions were sterilized at 1.1 kg/cm² pressure for 15 min and subsequently mixed together.

10. Czapek's agar

Sucrose (C ₁₂ H ₂₂ O ₁₁)	:	30.0 g
Sodium nitrate (NaNO ₃)	:	2.0 g
Potassium dihydrogen phosphate (K ₂ HPO ₄)	:	1 g
Magnesium sulphates (MgSO ₄ .7H ₂ O)	:	0.5 g
Potassium chloride (KCl)	:	0.5 g
Ferrous sulphates (FeSO ₄ .7H ₂ O)	:	0.01 g
Agar agar	:	20 g
Distilled water (to make up)	:	1000 ml

Agar agar was melted in 500 ml of distilled water. All the other ingredients were mixed 400 ml of distilled water. The two solutions were mixed thoroughly. The volume was made upto 1000 ml by adding distilled water and sterilized at 1.1 kg/cm² pressure for 15 min.

11. Sabouraud's agar

Dextrose (C ₆ H ₁₂ O ₆)	:	20 g
Peptone	:	10 g
Agar agar	:	20 g
Distilled water (to make up)	:	1000 ml

Agar agar was melted in 400 ml of distilled water. All above ingredients were dissolved in 400 ml of distilled water. The two solutions were mixed thoroughly and the volume was made upto 1000 ml by adding distilled water. This was sterilized at 1.1 kg/cm² pressure for 15 min.

12. Elliott's agar

Asparagines (C ₄ H ₈ N ₂ O ₃ .H ₂ O)	:	1.0 g
Dextrose (C ₆ H ₁₂ O ₆)	:	5.0 g
Magnesium sulphate (MgSO ₄ .7H ₂ O)	:	0.5 g
Potassium dihydrogen phosphate (KH ₂ PO ₄)	:	1.36 g
Sodium carbonate (Na ₂ CO ₃)	:	1.06 g
Agar agar	:	20 g
Distilled water (to make up)	:	1000 ml

All the ingredients except agar were mixed in 400 ml of water. Agar agar was melted in 400 ml distilled water. The two solutions were mixed thoroughly. The volume was made upto 1000 ml and sterilized at 1.1 kg/cm² pressure for 15 min.

13. Tochnal's agar

Potassium dihydrogen phosphate (KH ₂ PO ₄)	:	0.5 g
Potassium nitrate (KNO ₃)	:	2.0 g
Magnesium sulphate (MgSO ₄ .7H ₂ O)	:	1.0 g
Ferric chloride (FeCl ₂ .6H ₂ O)	:	Trace
Sucrose (C ₁₂ H ₂₂ O ₁₁)	:	30 g
Agar agar	:	15 g
Distilled water (to make up)	:	1000 ml

Agar agar was melted in 400 ml distilled water. The other ingredients were dissolved in 400 ml of distilled water. The two solutions were mixed thoroughly and the volume was made upto 1000 ml by adding distilled water and sterilized at 1.1 kg/cm² pressure for 15 min.

14. Sach's agar

Calcium nitrate (CaNO ₃)	:	1.09 g
Potassium dihydrogen phosphate (KH ₂ PO ₄)	:	0.25 g
Magnesium sulphate (MgSO ₄ .7H ₂ O)	:	0.25 g
Ferric chloride (FeCl ₂ .6H ₂ O)	:	Trace
Calcium carbonate (CaCO ₃)	:	4.0 g
Agar agar	:	20.0 g
Distilled water (to make up)	:	1000 ml

Agar agar was melted in 400 ml distilled water. The other ingredients were dissolved in 400 ml of distilled water. The two solutions were mixed thoroughly and the volume was made upto 1000 ml by adding distilled water and sterilized at 1.1 kg/cm² pressure for 15 min.

15. Brown's agar

Glucose (C ₆ H ₁₂ O ₆)	:	20.0 g
Asparagines (C ₄ H ₈ N ₂ O ₃ .H ₂ O)	:	2.0 g
Potassium dihydrogen phosphate (KH ₂ PO ₄)	:	1.25 g
Magnesium sulphate (MgSO ₄ .7H ₂ O)	:	0.75 g
Trace element mixture	:	1.0 g
Agar agar	:	20.0 g
Distilled water (to make up)	:	1000 ml

Agar agar was melted in 400 ml distilled water. The other ingredients were dissolved in 400 ml of distilled water. The two solutions were mixed thoroughly and the volume was made upto 1000 ml by adding distilled water and sterilized at 1.1 kg/cm² pressure for 15 min.

Twenty ml of each media was poured aseptically into Petriplates of 90 mm diameter. Five mm discs from an actively growing zone of ten days old culture was placed upside down at the centre of the solidified medium and were incubated at 27 ± 1°C. Each treatment was replicated thrice.

The radial measurements of the colony were taken when the maximum growth was attained in any one of the media tested. The various cultural characters like, rate of growth, type of margin, colour and sporulation on different media were recorded. Photographs were taken to show the growth behaviour of the pathogen. The measurements of colony diameter on different media were measured.

3.6.2 Growth Phase

Thirty ml of potato dextrose broth was added into each of the 100 ml conical flasks and sterilized at 1.1 kg/cm² pressure for 15 minutes. These flasks were allowed to cool and then inoculated with 5 mm discs of inoculum as described earlier and incubated at 27 ± 1°C. Each treatment was replicated three times. Culture was filtered through whatman No. 42 filter paper discs of 12.5 cm diameter, which were dried to a constant weight at 60°C in an electrical oven. The mycelial mat on the filter paper was washed thoroughly with distilled water to remove salts if any associated with it. One set of mycelium from the flasks was harvested on second day after inoculation. Subsequent harvesting was done at an interval of two days up to twenty days. The filter papers along with mycelial mat were dried to a constant weight in an electrical oven at 60°C, cooled in a desicator and weighted immediately on an analytical electrical balance. The results were analysed statistically.

3.6.3 Growth studies in liquid media

The composition and preparation of different fifteen liquid media used, were the same as that of solid except that, the agar was not added. All the liquid media were sterilized and the flasks were inoculated under aseptic condition and were incubated at 27 ± 1°C for ten days. The mycelial growth was harvested, dried and weighed. The data were analysed statistically. The best synthetic media was found and used as a basal media for further studies.

3.7 NUTRITIONAL STUDIES

3.7.1 Carbon utilization

Sucrose, glucose, manital, lactose, soluble starch, fructose and dextrose were used. For this study the carbon in the basal medium (Richard's solution) was replaced by equivalent amount of the above mentioned carbon source individually. Each treatment was replicated three times. After ten days of incubation dry mycelial weight of the fungus was recorded as described earlier and the data were statistically analysed. The best carbon source was found out and used for further studies.

3.7.2 Nitrogen utilization

Potassium nitrate, sodium nitrate, L-asparagine, ammonium sulphates, urea and magnesium nitrate were used for the study. The quantity of nitrogen compound used was determined on the basis of their molecular weight so as to provide an equivalent amount of nitrogen as that of potassium nitrate present in the basal medium. Four replications were maintained for each treatment. Flasks were inoculated under aseptic conditions and incubated at $27 \pm 1^\circ\text{C}$ for 10 days. There after mycelial growth was harvested, dry mycelial weights were recorded and the data were analysed statistically.

3.7.3 Sulphur utilization

In this experiment magnesium sulphates, sodium sulphate, ammonium sulphate, ferrous sulphates and copper sulphate were used for the study.

The quantity of the sulphur compound added was determined on the basis of their molecular weights so as to provide an equivalent amount of sulphur as that of magnesium sulphates present in the basal medium. Four replications were maintained for each treatment. The flasks were inoculated under aseptic conditions and incubated at $27 \pm 1^\circ\text{C}$ for 10 days. There after mycelial growth was harvested, dry mycelial weights were recorded and the data were analysed statistically.

3.8 PHYSIOLOGICAL STUDIES

3.8.1 Hydrogen ion concentration

The liquid medium used in this experiment was Richard's solution and its nitrogen, carbon and sulphur sources were kept unchanged, since they were found to be the best.

Adjustment of pH was done by adding 0.1 N NaOH or 0.1 N HCl. Reaction of the medium was adjusted to the desired pH of 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 by using di-sodium hydrogen phosphate citric acid buffer, according to schedule of Vogel (1951). Thirty ml of the medium was poured into each flask and sterilized in each treatment. There was a slight change in pH of 0.1 after sterilization. The fungus was inoculated under aseptic conditions and incubated for 10 days at $27 \pm 1^\circ\text{C}$. The dry mycelial weight was recorded and the data were analysed statistically as described earlier. The pH of the culture filtrate was determined by using precision pH meter.

3.8.2 Temperature Requirement

Richard's liquid medium was used in this experiment with its nitrogen, carbon and sulphur source kept unchanged as they were found to be the best. The pH of the medium was also adjusted to 6.5, since the maximum growth of the fungus was obtained at this pH. The different temperatures maintained for the growth of *Colletotrichum gloeosporioides* were 10, 15, 20, 25, 30 and 35°C . For each temperature level four replications were maintained. The fungus was inoculated under aseptic condition and incubated for ten days. The data were analysed statistically as described.

3.8.3 Light requirement

Richard's broth was used in this experiment. Conical flasks of 100 ml capacity and each contain 30 ml of liquid medium were inoculated and exposed to different lengths of light hour's viz., alternate cycles of twelve hours and twelve hours darkness, continuous light and continuous darkness in an environmental chamber. Petriplates were inoculated with 5 mm discs taken from the periphery of ten days old pure culture.

Each treatment was replicated seven times and incubated for ten days. Dry mycelial weight was obtained as described earlier and results were analysed statistically.

3.9 MANAGEMENT STUDIES

3.9.1 *In vitro* evaluation of botanicals

Plant based pesticides which are relatively economical, safe and non hazardous can be used successfully against the plant pathogenic fungi. The present investigation was aimed to study the antifungal activity of some plant extracts. The following plant extracts were used.

Sl. No.	Botanical name	Common name	Plant part Used	Family
1.	<i>Allium cepa</i> L.	Onion	Bulb	Amaryllidaceae
2.	<i>Allium sativum</i> L.	Garlic	Cloves	Amaryllidaceae
3.	<i>Aloe vera</i> .	Aloevera	Leaf	Liliaceae
4.	<i>Azadirachta indica</i> A. JUSS	Neem	Leaves	Meliaceae
5.	<i>Azadirachta indica</i> A. JUSS	Neem	kernel	Meliaceae
6.	<i>Clerodendron inerme</i> Gaertn.	Kashmir bouquet	Leaves	Verbenaceae
7.	<i>Curcuma longa</i>	Turmeric	Rhizomes	Zingiberaceae
8.	<i>Lantana camara</i> L.	Lantana	Leaves	Verbenaceae
9.	<i>Ocimum sanctum</i> L.	Holybasil/Tulsi	Leaves	Labiataeae

3.9.1.1 Preparation of cold aqueous extract

Fresh plant material were collected and washed first in tap water and then in distilled water. Hundred grams of fresh sample was chopped and then crushed in a surface sterilized pestle and mortar by adding 100 ml sterile water (1:1 w/v). The extract was filtered through two layer of muslin cloth. Finally filtrate thus obtained was used as stock solution.

3.9.1.2 Effect of botanicals on mycelial growth of *C. gloeosporioides*

To study the antifungal mechanism of plant extract, the poisoned food technique was used (Nene and Thapliyal, 1982). Five ml and 7.5 ml of stock solution were mixed with 95 and 92.5 ml of sterilized molten PDA media, respectively so as to get 5 and 7.5 per cent concentration. The medium was thoroughly shaken for uniform mixing of extract.

Twenty ml of poisoned medium was poured into each of the 90 mm sterile Petriplates. Each plate was seeded with mycelium of five mm size discs form periphery of actively growing culture were cut out by cork borer and one such disc was placed at the centre of each agar plate. Controls were also maintained by growing the pathogen on PDA plates. Then such plates were incubated at $27 \pm 1^\circ\text{C}$ temperature for ten days and radial growth was taken when maximum growth was occurred in the control plates.

The efficacy of plant products or botanicals was expressed as per cent of radial growth over the control which was calculated by using the formula suggested 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

Further, angular transformations were made for data and analysed statistically.

3.9.2 *In vitro* evaluation of fungicides

The efficacy of five systemic fungicides and five non-systemic fungicides were assayed at the concentration of 0.05, 0.1, and 0.15 per cent. The fungicides used are given here under.

Sl. No.	Common name	Chemical name	Trade name
Systemic fungicides			
1	Benomyl	Methyl(1-(butyl carbamoyl)-2-benzimidazole carbamate)	Benlate 50% WP
2	Propiconazole	1-(2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-ylmethyl)-1H-1,4-triazole	Tilt 25% EC
3	Carbendazim	2-(Methoxy-Carbonyl)-benzimidazole	Bavistin 50% WP
4	Hexaconazole	(RS)-2-(2,4-dichlorophenyl)-1-(1,2,4-triazole-1-yl)hexan-2-ol	Contaf 5% EC
5	Triadimefon	1-(4-Chlorophenoxy)-3,3-dimethyl-1H-1,2,4-triazole-1-yl-2-butanone	Bayleton 25% WP
Non-systemic fungicides			
6	Captan	N (trichloromethylthio) Cyclohex-4-ene-1,2 dicarboximide	Captaf 50% WP
7	Chorothalonil	Tetrachloro isophthalonitrile	Kavach 75% WP
8	Propineb	Zinc 1,2-Propylenebis dithiocarbamate	Antracol 65% WP
9	Mancozeb	Manganese ethylene bis dithiocarbonate + Zinc	Indofil M-45 75% WP
10	Zineb	Zinc ethylenebis dithiocarbamate	Indofil Z-78 75% WP

Required quantity of individual fungicide was added separately into molten and cool potato dextrose agar so as to get the desired concentration of fungicide. Later 20 ml of the poisoned medium was poured into sterile Petriplates. Mycelial discs of 5 mm size from actively growing culture of the fungus were cut by sterile cork borer and one such disc was placed at the centre of each agar plate. Control was maintained without adding any fungicide to the medium. Each treatment was replicated thrice. Then such plates were incubated at room temperature for ten days and radial colony growth was measured. The efficacy of a fungicide was expressed as per inhibition of mycelial growth over control that was calculated by using the formula suggested by Vincent (1947).

3.9.3 *In vitro* evaluation of bioagents

Six biocontrol agents such as *Trichoderma harzianum* Rifai, *Trichoderma viride* Pers. ex. Gray., *Trichoderma koningii* Oudern, *Trichoderma virens* Miller, *Pseudomonas fluorescens* Migula and *Bacillus subtilis* Cohn Emend Pras were tested against *Colletotrichum gloeosporioides*. Both biocontrol agents and test fungus were cultured on potato dextrose agar in order to get fresh and active growth of each fungus.

Twenty ml of sterilized and cooled potato dextrose agar was poured into sterile Petriplates and allowed to solidify. For evaluation of fungal biocontrol agent, mycelial discs of

test fungus were inoculated at one end of Petriplate and antagonistic fungus was placed opposite to it on the other end. In case of evaluation of bacterial antagonist the bacterium was streaked at ends of the Petriplates and mycelial discs of the fungus was placed at the centre. four replications were maintained for each treatment. The plates were incubated at $27 \pm 1^\circ\text{C}$ and zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and antagonistic organism. The colony diameter of pathogen in control plate was also recorded. The per cent inhibition of the growth of the pathogen was calculated by using the formula suggested by Vincent (1947).

3.10 *IN VIVO* EVALUATION OF SALT, BIOAGENTS, HOT WATER AND FUNGICIDES AGAINST ANTHRACNOSE OF PAPAYA

3.10.1 *In vivo* evaluation of salt, bioagents and hot water treatment

In vitro study was carried out on papaya fruits against postharvest disease anthracnose caused by *Colletotrichum gloeosporioides* by imposing various salts and bioagents at 2 and 5 per cent concentration. The treatments given were *viz.* sodium chloride, sodium chloride + hot water, calcium chloride, *Trichoderma harzianum*, *Pseudomonas fluorescens*, *Trichoderma harzianum* + *Pseudomonas fluorescens*, hot water treatment and distilled water, which served as control. Fruits were treated with hot water at 49°C for 20 minutes. Disease intensity in different treatments was scored using 0-5 scale (Prasanna kumar, 2001).

Grade	Per cent disease on the fruit surface
0	No. disease
1	0.1 – 5%
2	5.1 -10%
3	10.1 – 25%
4	25.1 – 50%
5	> 50%

Per cent Disease Index (PDI) of postharvest disease was calculated by using the formula given by Wheeler (1969).

$$\text{PDI} = \frac{\text{Sum of individual disease rating}}{\text{No. of samples}} \times \frac{100}{\text{Maximum disease grade}}$$

Papaya fruits were washed through tap water to remove dust and soil particle which were adhere to papaya fruits and surface sterilized in sodium hypochlorite and rinsed thrice with distilled water. Small wound were made by sterilized needle on the fruits surface. These papayas were dipped in postharvest treatment solution as mentioned before for ten minutes. They were allowed to dry and cotton swabs dipped in spore suspension of *C. gloeosporioides* were introduced to surface of papaya. Sufficient humidity was provided by placing the cotton swabs dipped in water along with them. Observations were taken by following 0–5 scale on seven day after inoculation and data were analysed statically.

3.10.2 *In vivo* evaluation of fungicides

In vitro study was carried out on papaya fruits against postharvest disease anthracnose caused by *C. gloeosporioides* by imposing various fungicides at 0.05 and 0.1 per cent concentration. Half gram (0.5 g) and one gram (1 g) of chemical was mixed with 1000 ml of sterilized water, respectively so as to get 0.05 and 0.1 per cent concentration. The medium was thoroughly shaken for uniform mixing of chemical. The treatments given were viz. mancozeb, carbendazim antracol, benomyl, hexaconazole, captan and distilled water, which served as control. Disease intensity in different treatments was scored using 0-5 scale (Prasanna kumar, 2001).

4. EXPERIMENTAL RESULTS

The experiments were conducted in the year 2007–2008 at the Department of Plant Pathology. The results of investigation of anthracnose of papaya caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. are presented here under.

4.1 COLLECTION OF DISEASED SPECIMENS

The anthracnose infected fruits of papaya, showing the typical symptoms were collected from the Saidapur farm (UAS, Dharwad), farmer's gardens and Dharwad fruit market. These infected specimens were used for isolation of *Colletotrichum gloeosporioides*.

4.2 SYMPTOMATOLOGY

4.2.1 *Colletotrichum gloeosporioides*

Colletotrichum gloeosporioides is one of the most important fungal pathogens which cause black spot or anthracnose of papaya.

The symptoms usually appear as small round dark area on the ripening portions of the fruits. As the fruits ripen, these spots enlarge rapidly forming brown superficial discolouration of the skin which develop into circular, slightly sunken and water soaked lesions having dark margins and brown or black central portions. Gradually, the lesions coalesce and sparse mycelial growth often appears on the margins of such spots. An encrustation of salmon pink spores often arranged in a concentric pattern, giving the appearance of "bull's eye" (Plate 1). The fungus also invades fruit tissues underneath producing rot and turning them soft and darker in colour. Finally, the whole fruit turns dirty dark brown and rot. The latex oozes out from the infected fruits soon after the penetration of the pathogen.

4.2.2 *Fusarium oxysporium*

Fusarium oxysporium is another important pathogen of papaya. The symptoms appear in small dark area which enlarge rapidly and become soft and white mycelial growth appears on the surface of the fruit. It produces three types of spores which are micro conidia, macro conidia and chlamydospores. Conidia are sickle shaped, septated and pointed at tip (Plate 3a).

4.2.3 *Rhizopus stolonifer*

Rhizopus stolonifer which is the most destructive of the postharvest pathogens and due to rapid rotting the entire fruit become soft and infected fruit covered by coarse gray mycelium with black sporangia. which appeared in the form of pin head (Plate 3b).

4.3 ISOLATION

Symptoms of anthracnose showing fruits of papaya (*Carica papaya*) were used for the isolation purpose. The standard tissue isolation procedure was followed in the study.

Single spore isolation was done to obtain pure culture of the pathogen as detailed mentioned in "Material and Methods". Thus, obtained pure culture was multiplied for further use in the study. The pathogen involved was sub cultured on potato dextrose agar medium and stored in a refrigerator. For further studies the original culture was sub cultured once in 30 days.

4.3.1 Identification of the pathogen

The pathogen was identified upto species level based on their morphological and cultural characters of the pathogen.



Symptoms on petioles



Shot hole symptoms on leaf



Water-soaked lesion on fruit



Lesion become brown to black in colour
In later stage



Enlargement of lesion and production of
acervuli



Acervuli with concentric pattern conidia
(pinkish colour), bull eye symptom

Plate 1: Different types of anthracnose symptoms on petioles, leaves and papaya fruits

4.4 MORPHOLOGY OF *Colletotrichum gloeosporioides*

4.4.1 Morphology of the *C. gloeosporioides* on host tissue

Microscopic examination of infected tissue revealed that acervuli were circular, measured 126-281.4 μm . The acervulus was covered with a range mucilaginous mass containing numerous conidia. Setae were arising through this mass; they were erect in habit, measuring 37-89 X 1.4- 4.0 μm and were dark brown to black. Conidia were hyaline, single celled and smooth walled. They were oblong or cylindrical and measured 6-11.2 X 2-4 μm .

4.4.2 Morphology of the fungus in culture

The fungal colony from 10 days old culture on potato dextrose agar was white to dull white with smooth margins. The mycelium was hyaline, superficial, septate and branched. The aerial mycelium was white to dull white. Sporulation was abundant with maximum fruiting bodies at the centre of the plate as against mycelium growth towards the periphery. The conidia from the culture measured 9-20 X 3-7.5 μm . Based on the morphology of the fungus the pathogen was identified as *Colletotrichum gloeosporioides* (Table 1 and Plate 2).

4.5 PATHOGENECITY TEST

Fungus was isolated from the infected papaya fruit and pure culture was obtained by single spore isolation method as described in "Material and Methods" and such culture was used for pathogenicity test. Artificial inoculation of the pathogen was carried out as explained in "Material and Methods". Numerous oval or circular water soaked spots were observed on papaya fruit after seven days of inoculation. The fungus was reisolated from such lesion and the culture obtained was found to be the same as that of original culture.

Table 1: Morphological characteristics of *Colletotrichum gloeosporioides*.

Characters	Measurement in μm		
	On culture		On host
	Range	Average	Range
Acervuli	129-281.4	193.19	116.4-264.7
Setae	37-89 X 1.4- 4.0	51.13 X 2.4	-
Conidia	9-20 X 3- 7.5	13.04 X 4.81	6-11.2 X 2-4

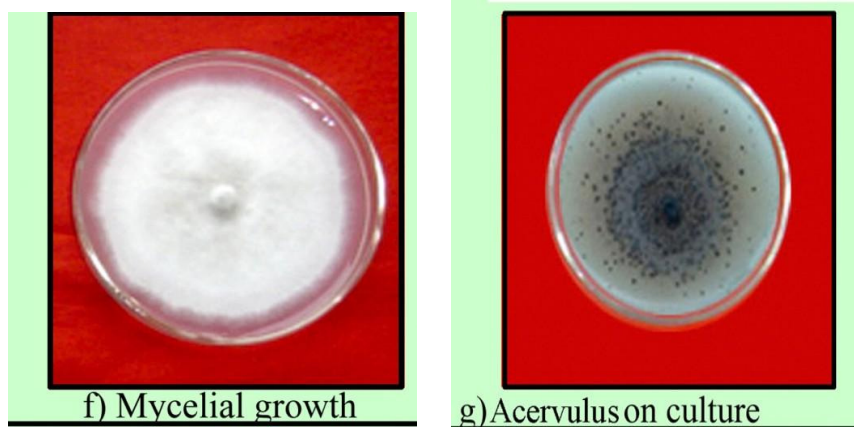
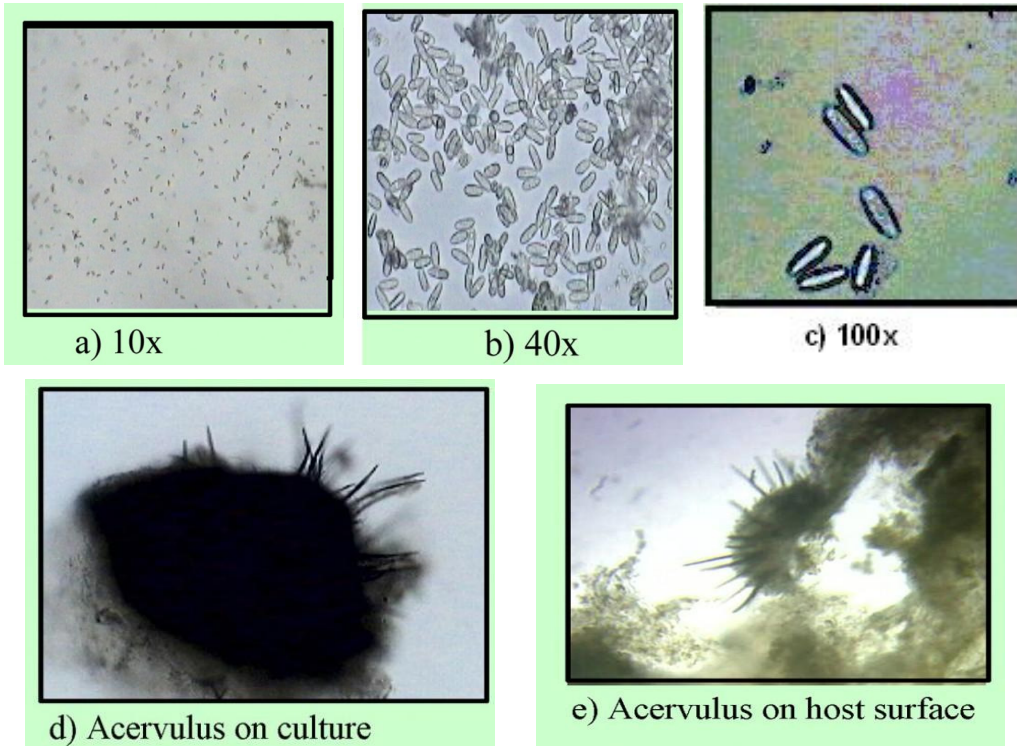


Plate 2: Morphology of *Colletotrichum gloeosporioides*

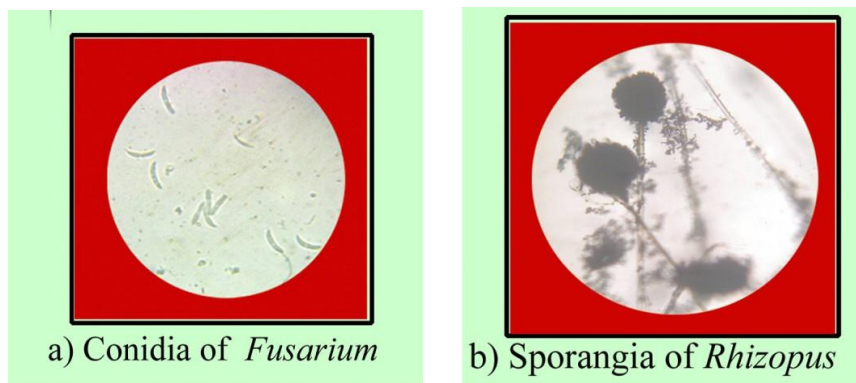


Plate 3: Morphology of *Fusarium oxysporium* (a) and *Rhizopus stolonifer* (b)

4.6 CULTURAL STUDIES

4.6.1 Growth characters on solid media

Cultural characters were studied on fifteen solid media. The radial growth of *Colletotrichum gloeosporioides* was measured. Observations on various cultural characters were recorded. The results are presented in Table 2, Fig. 1 and plate 4a.

Table 2 shows that among the fifteen different solid media, maximum radial growth of the fungus was observed on V-8 juice agar media (87.67 mm) which was significantly superior over all other tested medium followed by oat meal agar (83.63 mm) and Richard's agar (82.65 mm) which were on par with each other. Next best medium were found to be corn meal agar (80.00 mm), malt extract agar (79.20 mm), potato carrot agar (79.00 mm), papaya fruit agar (78.17 mm) which were on par with each other followed by Tochinal's agar (75.50 mm), potato dextrose agar (75.50 mm), Czapek's agar (74.83 mm) and host leaf extract agar (74.27 mm). No growth was observed on Sach's agar.

The growth character on V-8 juice agar and oat meal agar produced good growth, white cottony mycelium with smooth margin. Where as on host leaf extract agar growth was poor with dirty brown mycelium. Observation on sporulation of the fungus indicated that V-8 agar produced excellent sporulation and was significantly superior over other media.

The growth character on Richard's agar was good growth, margin was irregular and mycelium was cottony whitish and Sabouraud's agar shows good growth, less dense mycelium with irregular margin. Where as on Brown's agar and Elliott's agar was poor growth with dirty white mycelium with irregular margin and no growth was observed on Sach's agar media. Sparse sporulation was observed on Richard's agar and Elliott's agar. Sach's agar did not support the sporulation (Table 3)

4.6.2 Growth phase

This experiment was conducted to know the period when the maximum vegetative growth could occur. The fungus was grown in potato dextrose broth and the dry mycelial weight was recorded at two days interval as described in "Material and Methods". The data are presented in Table 4 and Fig 2.

The data from Table 4 indicated that dry mycelium weight of the fungus was minimum on 2nd days after inoculation and on subsequent harvest at 2 days interval (4, 6, 8, 10, 12, 14, 16, 18 and 20 days) it significantly increased and finally reached maximum on 10th day. Later the growth decreased significantly and least dry mycelium weight was observed on the 2nd day after inoculation. As maximum dry mycelium weight was observed on 10th day after inoculation, this period was used as peak period for further studies.

4.6.3 Growth studies in liquid media

The experiment was conducted as explained in "Material and Methods" to find out the best liquid medium for mycelium growth of the fungus. The average mycelium weights of the fungus after 10th days on incubation are given in Table 5, Fig. 3 and Plate 4b.

The data from Table 5 indicated that there was a significant effect of the different liquid media on the growth of the fungus. The maximum growth of fungus was observed in Richard's agar (581.33 mg) followed by malt extract broth (575.00 mg) which were par with each other and significantly superior over all tested medium. Next best were found to be potato dextrose broth (449.17 mg), Brown's broth (425.00 mg), Tochinal's broth (420.33 mg) which par each other followed by oat meal broth (339.00 mg). Medium growth supported media were Czapek's broth (256.13 mg), Sabouraud's broth (253.67 mg), papaya fruit broth (249.03 mg) and V-8 juice broth (217.67 mg). Least mycelial dry weight was observed on potato carrot broth (43.10 mg).

As the maximum growth was recorded in Richard's broth it was selected as a basal liquid media for further studies.

Table 2: Effect of different solid media on mean colony diameter of *Colletotrichum gloeosporioides*.

Sl. No.	Solid media	Mean colony diameter (mm)
1.	Brown's agar	34.83
2.	Corn meal agar	80.00
3.	Czapek's agar	74.83
4.	Elliott's agar	48.45
5.	Host leaf extract agar	74.27
6.	Malt extract agar	79.20
7.	Oat meal agar	83.63
8.	Papaya fruit agar	78.17
9.	Potato carrot agar	79.00
10.	Potato dextrose agar	75.50
11.	Richard's agar	82.65
12.	Sabouraud's agar	68.67
13.	Sach's agar	0.00
14.	Tochinal's agar	75.50
15.	V-8 juice agar	87.67
	Mean	68.15
	SEm \pm	0.857
	CD at 1%	3.33

Table 3: Cultural characters of *Colletotrichum gloeosporioides* on different solid media after ten days on incubation.

Sl. No.	Non synthetic media	Growth characters	Sporulation
1.	Potato dextrose broth	Good growth, whitish raised mycelia with irregular margin	++
2.	Malt extract broth	Good growth, whitish mycelia with smooth margin	++
3.	Oat meal broth	Good growth, margin smooth, whitish mycelium	+++
4.	Host leaf extract broth	Poor growth, dirty brown growth	-
5.	Corn meal broth	Moderately growth, white irregular margin and light brown colour mycelia	+
6.	Potato carrot broth	Moderately growth, white mycelial growth	++
7.	Papaya fruit broth	Good growth, white colony with regular margin	+++
8.	V-8 juice broth	Good growth, white mycelia with regular margin	+++
9.	Richard's agar media	Good growth, white mycelia with regular margin	+++
10.	Czapek agar media	Moderately growth, white mycelia with regular smooth margin	++
11.	Sabouraud's agar media	Moderate growth, margin are irregular , dirty white growth mycelium	++
12.	Elliott's agar media	Poor growth	-
13.	Tochinal's agar media	Moderate growth, white mycelia with regular margin	++
14.	Sach agar media	No growth was observed	-
15.	Brown agar media	Poor growth, dirty white mycelia growth	+

Table 4: Effect of incubation on dry mycelium weight of *Colletotrichum gloeosporioides* on potato dextrose broth.

Sl. No.	Days after seeding	Mean dry mycelial weight (mg)
1	2	97.83
2	4	153.26
3	6	259.03
4	8	327.33
5	10	486.07
6	12	479.19
7	14	454.30
8	16	328.45
9	18	302.11
10	20	288.40
Mean		317.60
SEm \pm \pm		1.05
CD at 1%		4.24

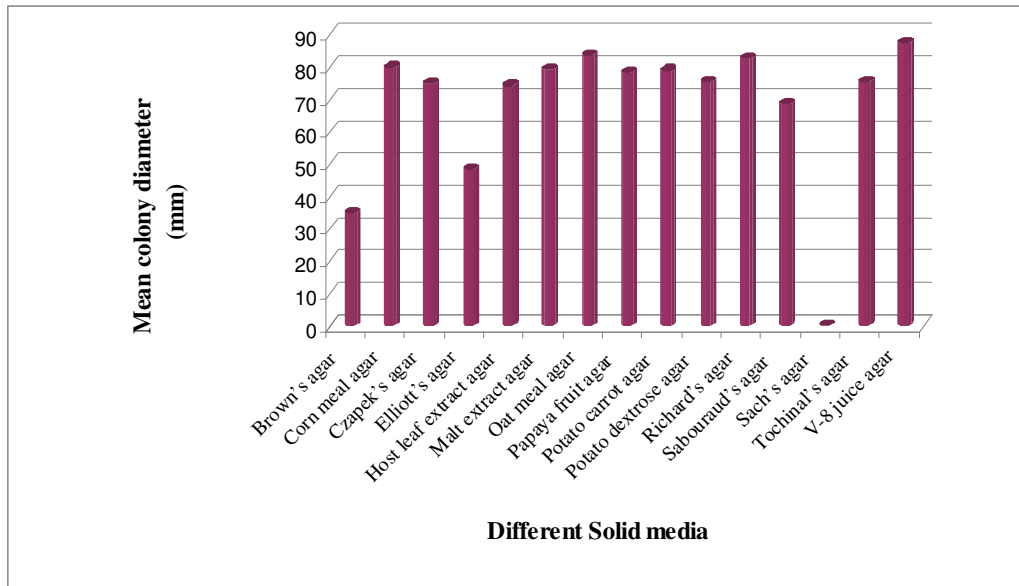


Fig. 1: Effect of different solid media on mean colony diameter of *Colletotrichum gloeosporioides*.

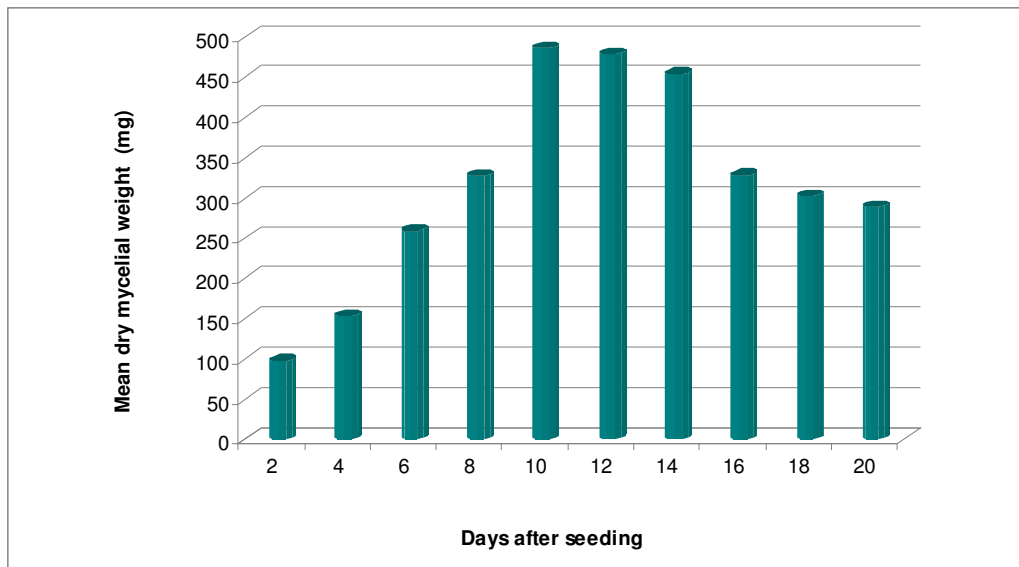


Fig. 2: Effect of incubation on dry mycelium weight of *Colletotrichum gloeosporioides* in potato dextrose broth

4.7 NUTRITIONAL STUDIES

4.7.1 Carbon utilization

The utilization of seven carbon sources by *Colletotrichum gloeosporioides* was tried in the present investigation as described in "Material and Methods". The data are presented in Table 6, Fig. 4 and Plate 5a.

The data from Table 6 indicated that the effect of different carbon sources on the growth of the fungus was significant. Maximum growth of the fungus was obtained in dextrose (620.00 mg) followed by fructose (567.67 mg), sucrose (410.33 mg), glucose (374.33 mg), soluble starch (347.00 mg), manital (204.33 mg) and least mycelial growth was observed on lactose (136.67 mg). But the fungus did not show any significant difference in utilization of glucose and soluble starch. Except the above two sugars, the other carbon sources differed significantly among them.

4.7.2 Nitrogen utilization

The utilization of six different nitrogen sources by *Colletotrichum gloeosporioides* was tried in the present experiment as detailed in "Material and Methods". The data are presented in Table 7, Fig. 4 and Plate 5b.

The data indicate that effect of different nitrogen sources on the growth of the fungus was significant. Potassium nitrate (525.00 mg) supported the maximum growth followed by Ammonium sulphate (378.33 mg), Magnesium nitrate (311.67 mg), L-asparagine (267.00 mg) and Sodium nitrate (253.33 mg) and urea (106.00 mg). The above mentioned all nitrogen sources differed significantly among themselves. Urea was found to be the least utilized nitrogen source.

4.7.3 Sulphur utilization

The utilization of five sulphur sources by *Colletotrichum gloeosporioides* was studied as described in the "Material and Methods" and the data are presented in the Table 8, Fig.4 and Plate5c.

The data from Table 8 indicate that the effect of different sulphur sources on the growth of the fungus was significant. Maximum growth of the fungus was observed when magnesium sulphate (588.25 mg) was used as sulphur source, followed by Ammonium sulphate (463.75 mg), sodium sulphate (417.25 mg) and ferrous sulphate (339.25 mg) which were significantly superior over rest of the compounds tested. The least utilized sulphur compound was copper sulphate (190.25 mg). The medium without sulphur source has also supported better mycelial growth.

4.8 PHYSIOLOGICAL STUDIES

4.8.1 Hydrogen ion concentration

The effect of pH on the growth of the *Colletotrichum gloeosporioides* was studied as described in "Material and Methods". The results obtained are presented in Table 9, Fig. 5 and Plate 6a.

The data from Table 9 indicate that the effect of different pH levels on the growth of *Colletotrichum gloeosporioides* was significant. The maximum growth of the fungus was noticed at a pH level of 6.5 (602.67 mg) followed by 6.0 (538.00 mg), 7.0 (487.33 mg), 5.5 (427.00 mg), 5.0 (310.33 mg), 7.5 (474.33 mg), 8.0 (431.00 mg), 8.5 (420.67 mg), 9.0 (408.33 mg), 4.5 (234.67 mg) and least growth was noticed in case on 4 pH (194.67 mg).

4.8.2 Temperature studies

In the present study, effect of different temperature levels on the growth of *Colletotrichum gloeosporioides* was evaluated as explained in "Material and Methods" and the data are present in Table 10, Fig.6 and Plate 6b.

Table 5: Effect of different liquid media on dry mycelium weight of *Colletotrichum gloeosporioides*

Sl. No.	Liquid media	Mean dry mycelial weight* (mg)
1.	Brown's broth	425.00
2.	Corn meal broth	136.33
3.	Czapek's broth	256.13
4.	Elliott's broth	80.33
5.	Host leaf extract broth	93.33
6.	Malt extract broth	575.00
7.	Oat meal broth	339.00
8.	Papaya fruit broth	249.03
9.	Potato carrot broth	43.10
10.	Potato dextrose broth	449.17
11.	Richard's broth	581.33
12.	Sabouraud's broth	253.67
13.	Sach's broth	145.23
14.	Tochinal's broth	420.33
15.	V-8 juice broth	217.67
	Mean	284.31
	SEm \pm	2.32
	CD at 1%	9.02

*Observation of mean dry mycelial weight was recorded ten days after incubation

Table 6: Effect of carbon sources on dry mycelial weight of *Colletotrichum gloeosporioides* after 10 days on incubation.

Sl. No.	Carbon sources	Mean dry mycelial weight (mg)
1.	Sucrose	410.33
2.	Glucose	374.33
3.	Soluble starch	347.00
4.	Manital	204.33
5.	Lactose	136.67
6.	Fructose	567.67
7.	Dextrose	620.00
Mean		379.62
SEm \pm		1.17
CD at 1%		4.91



4a: Growth of *Colletotrichum gloeosporioides* on different solid media.



4b: Growth of *Colletotrichum gloeosporioides* on different liquid media.

- 1= Potato dextrose agar
- 2= Malt extract agar
- 3= Oat meal agar
- 4= Host leaf extract agar
- 5= Corn meal agar
- 6= Potato carrot agar
- 7= Papaya fruit agar
- 8= V-8 juice agar

- 9= Richard's agr
- 10= Czapek' agar
- 11= Sabouraud's agar
- 12= Elliott's agar
- 13= Tochnal's agar
- 14= Sach's agar
- 15= Brown's agr

Plate 4: Cultural Studies of *Colletotrichum gloeosporioides*.

Table 7: Effect of nitrogen sources on dry mycelial weight of *Colletotrichum gloeosporioides* after 10 days of incubation.

Sl. No.	Nitrogen sources	Mean dry mycelial weight (mg)
1.	Potassium nitrate	525.00
2.	Sodium nitrate	253.33
3.	L-asparagine	267.00
4.	Ammonium sulphates	378.33
5.	Urea	106.00
6.	Magnesium nitrate	311.67
7.	Control	96.33
Mean		276.76
SEm \pm \pm		0.72
CD at 1%		3.04

Table 8: Effect of sulphur sources on dry mycelial weight of *Colletotrichum gloeosporioides* after 10 days of incubation.

Sl. No.	Sulphur sources	Mean dry mycelial weight (mg)
1.	Magnesium sulphates	588.25
2.	Sodium sulphate	417.25
3.	Ammonium sulphate	463.75
4.	Ferrous sulphates	339.25
5.	Copper sulphate	190.25
6.	Control	290.50
Mean		382.42
SEm \pm		1.05
CD at 1%		4.26

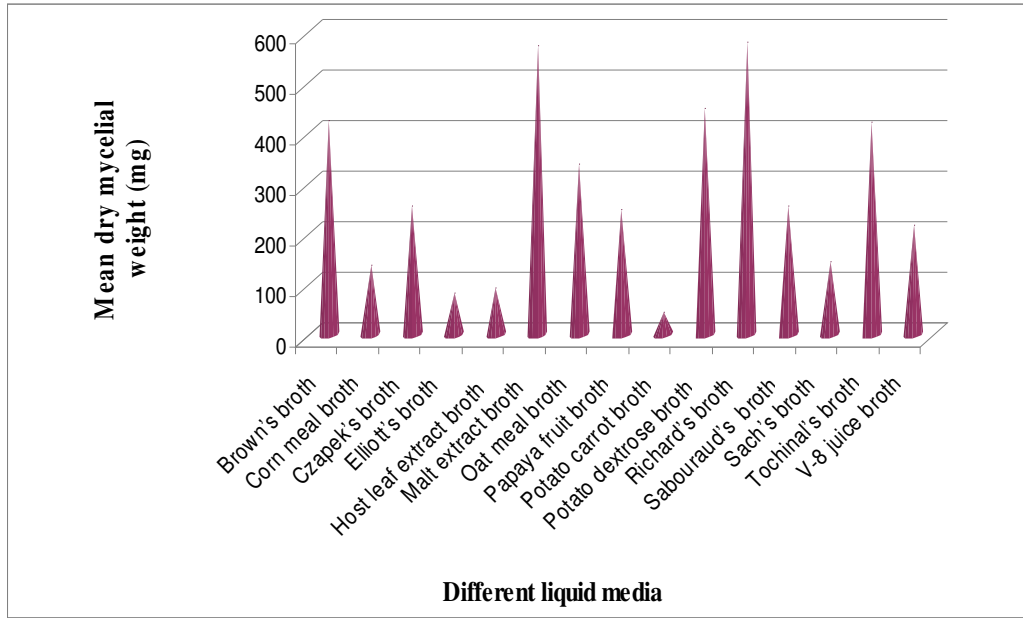


Fig. 3: Effect of different liquid media on dry mycelium weight of *Colletotrichum gloeosporioides*.

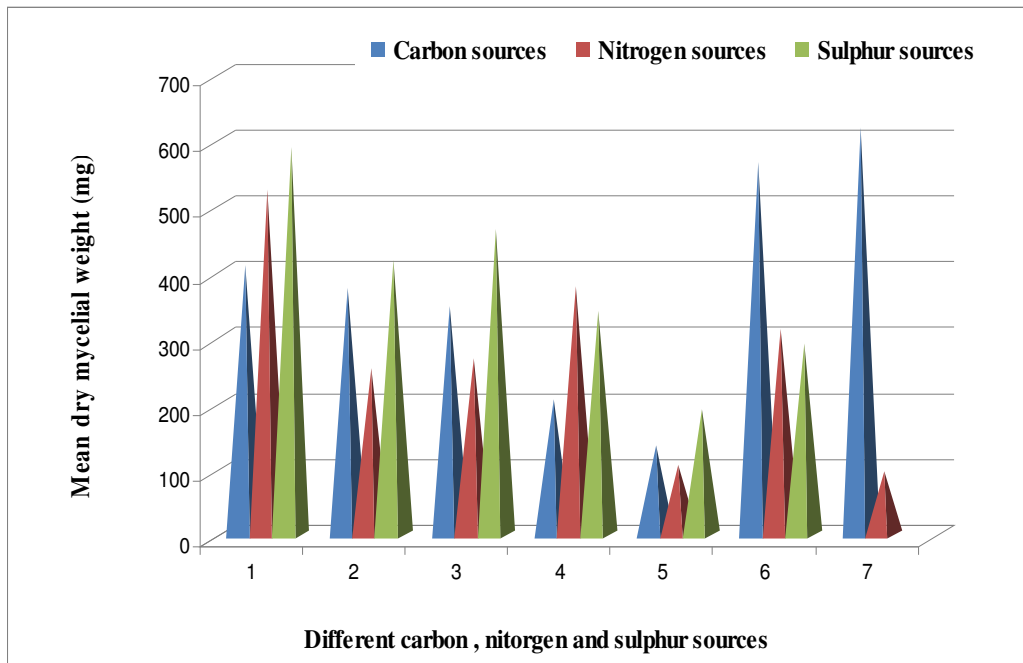
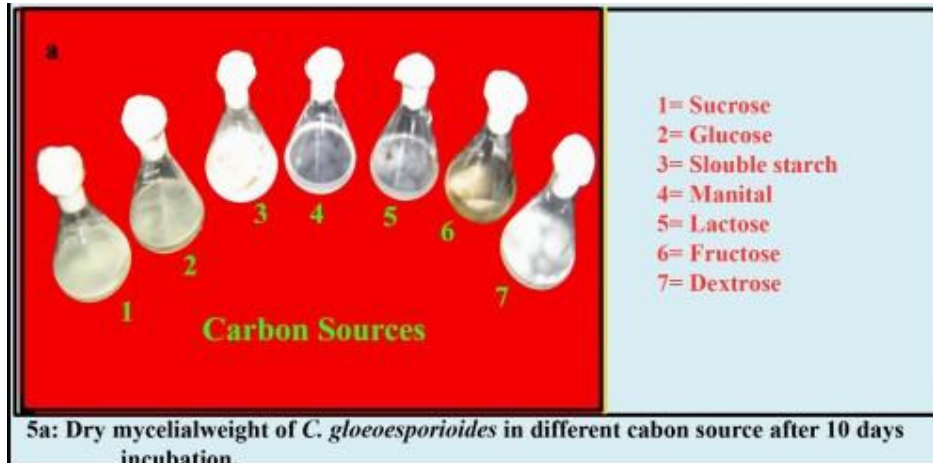


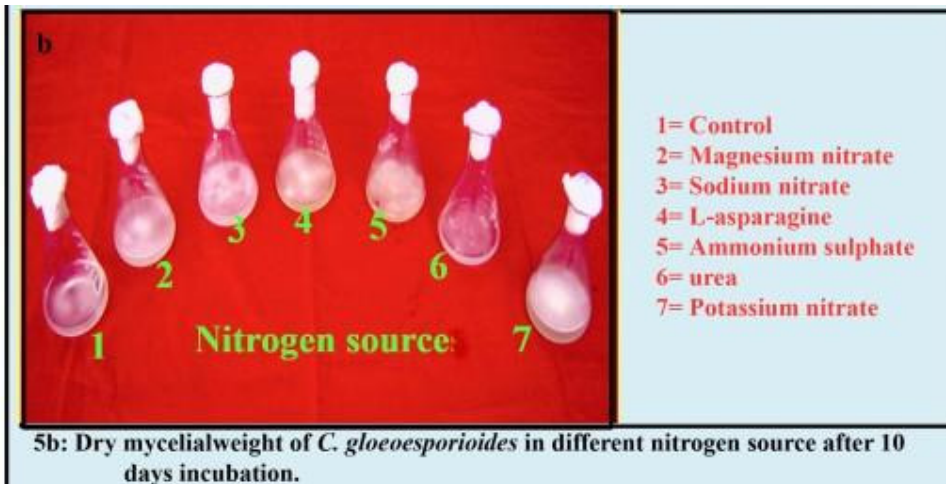
Fig. 4: Effect of carbon, nitrogen and sulphur source media on dry mycelium weight of *Colletotrichum gloeosporioides*.

Table 9: Effect of pH levels on dry mycelial weight of *Colletotrichum gloeosporioides* after 10 days of incubation.

Sl. No.	pH levels	Mean dry mycelial weight (mg)
1	4.0	194.67
2	4.5	234.67
3	5.0	310.33
4	5.5	427.00
5	6.0	538.00
6	6.5	602.67
7	7.0	487.33
8	7.5	474.33
9	8.0	431.00
10	8.5	420.67
11	9.0	408.33
Mean		411.73
SEm \pm		0.59
CD at 1%		2.37



5a. Dry mycelial weight of *C. gloeosporioides* in different carbon source after 10 days incubation



5b: Dry mycelial weight of *C. gloeosporioides* in different nitrogen source after 10 days incubation

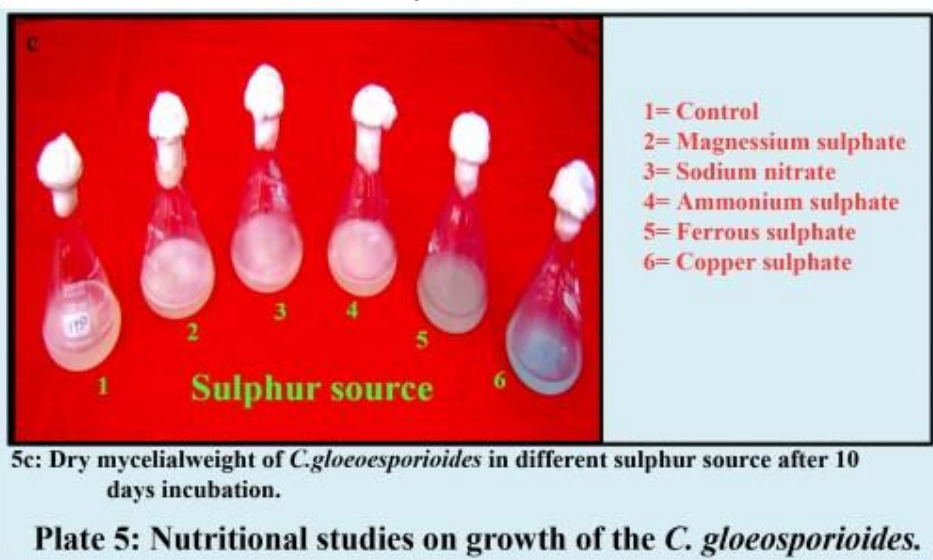


Plate 5: Nutritional studies on growth of the *C. gloeosporioides*.

Table 10: Effect of different temperature levels on dry mycelial weight of *Colletotrichum gloeosporioides* after 10 days of incubation.

Sl. No.	Temperature (°C)	Mean dry mycelial weight (mg)
1	10	179.75
2	15	248.00
3	20	427.50
4	25	505.50
5	30	558.50
6	35	300.50
Mean		369.88
SEm ±		0.70
CD at 1%		2.86

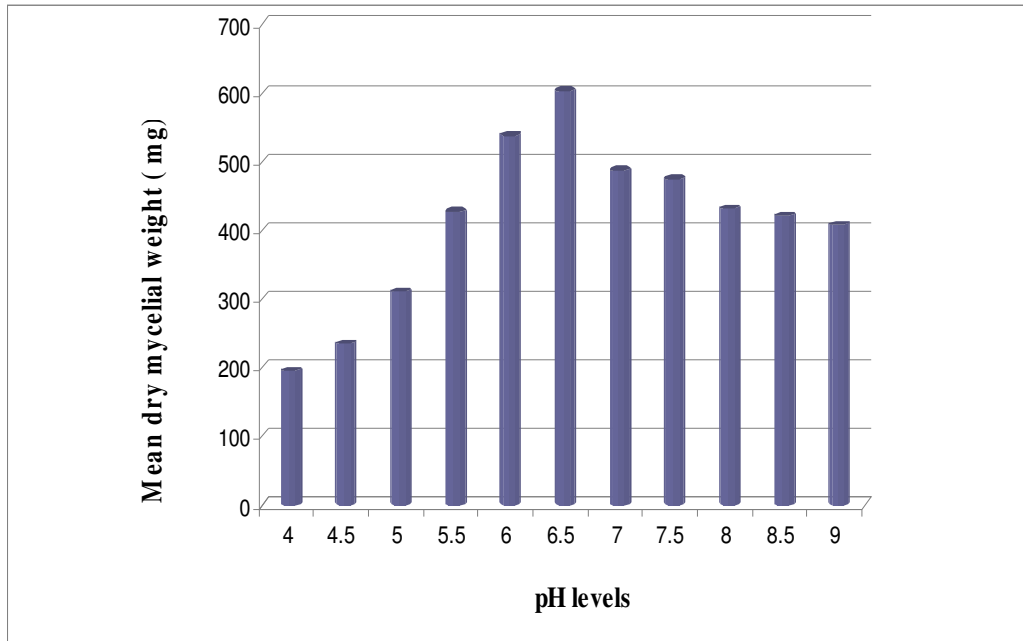


Fig. 5: Effect of pH levels on dry mycelial weight of *Colletotrichum gloeosporioides* after 10 days of incubation

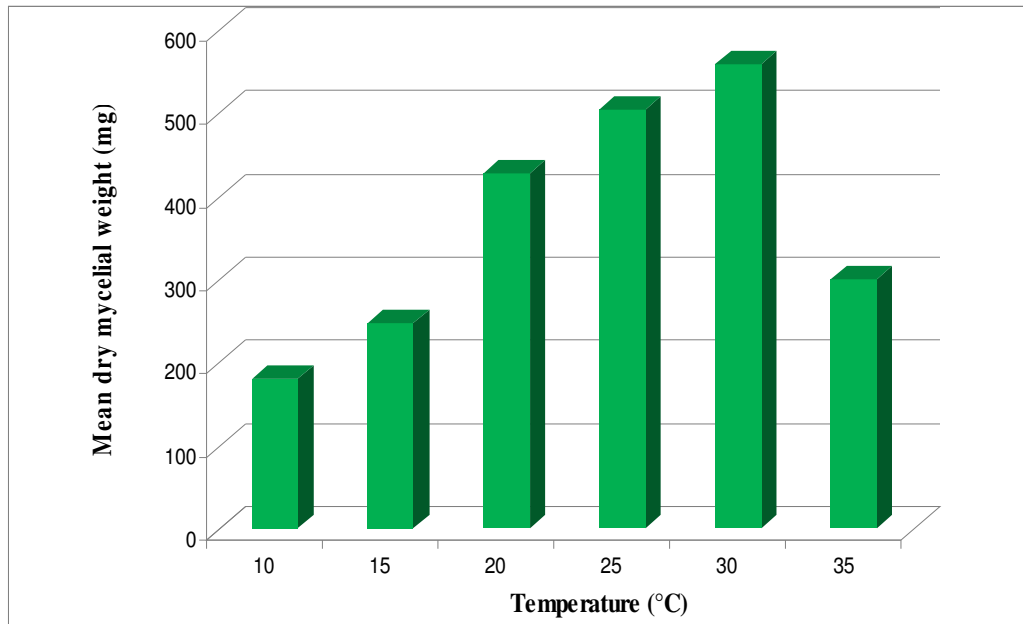


Fig. 6: Effect of different temperature levels on dry mycelial weight of *Colletotrichum gloeosporioides* after 10 days of incubation

Table 11: Effect of light intensity levels on dry mycelial weight and radial growth of *Colletotrichum gloeosporioides* after ten days of incubation

Sl. No.	Treatments	Mean colony dry mycelial weight (mg)	Mean colony diameter (mm)
1	Continuous light	468.29	72.14
2	Continuous dark	359.86	68.86
3	Alternate cycles of 12 hr light and 12 hr darkness	593.71	89.29
Mean		473.95	76.76
SEm \pm		1.34	0.37
CD at 1 %		5.47	1.50

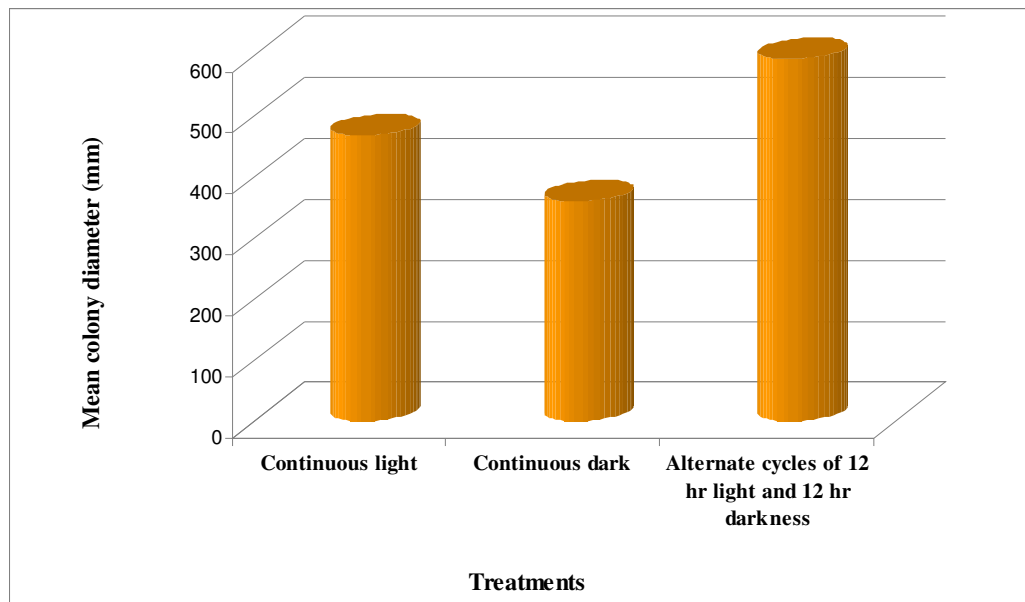
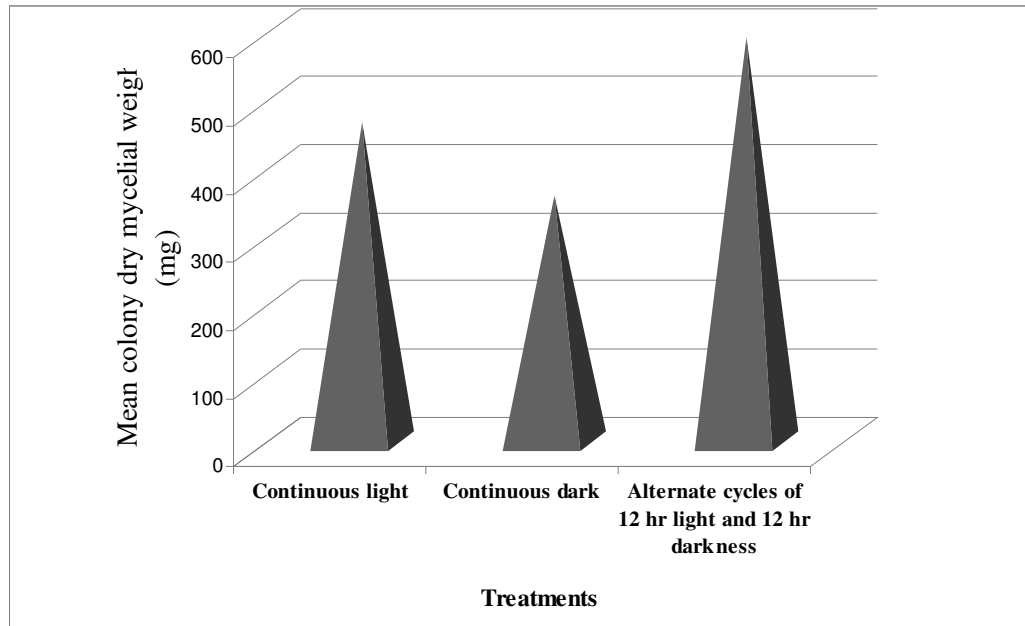


Fig. 7: Effect of light intensity levels on dry mycelial weight (A) and radial growth (b) of *Colletotrichum gloeosporioides* after ten days of incubation

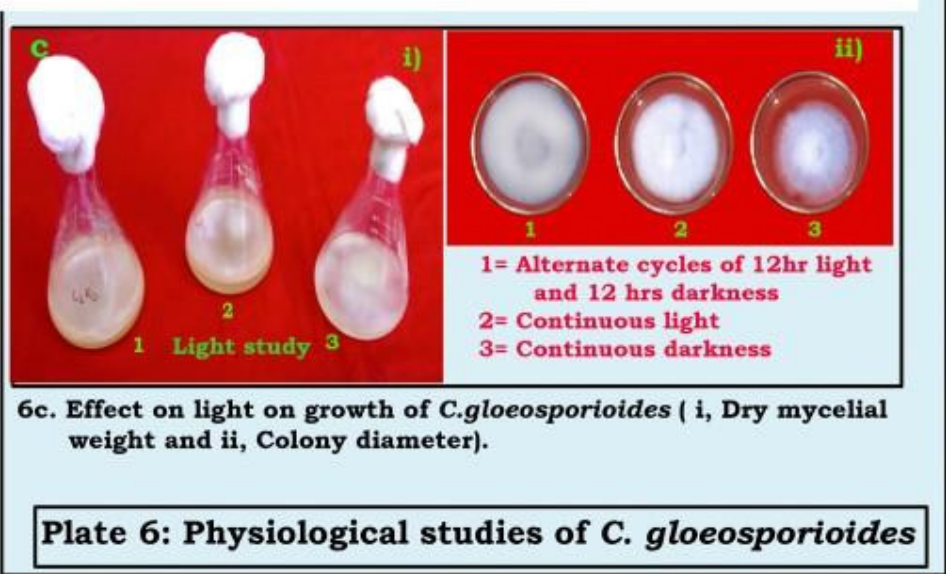
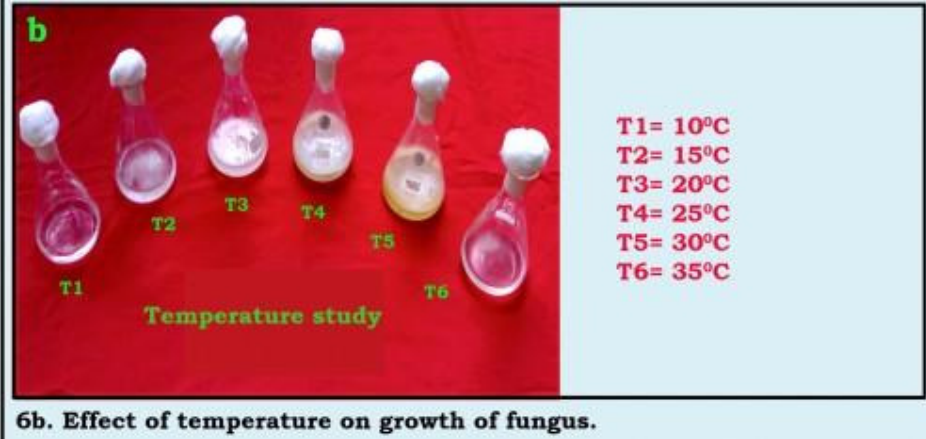
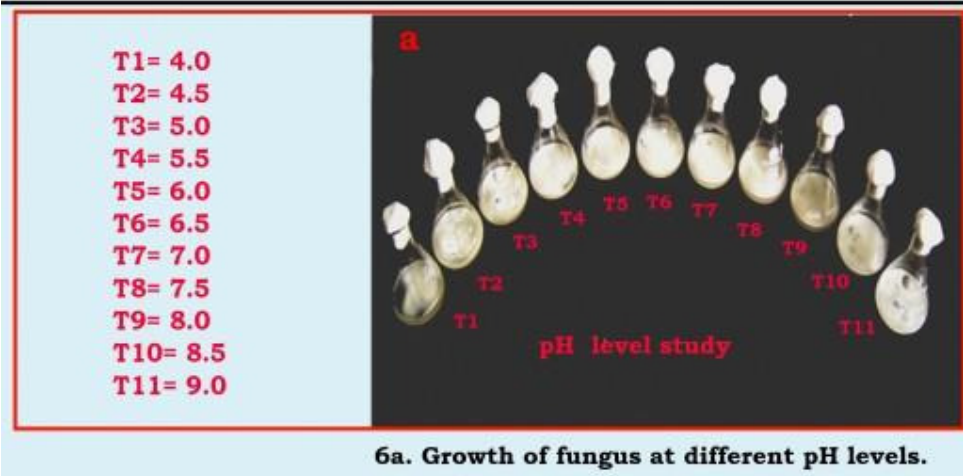


Plate 6: Physiological studies of *C. gloeosporioides*

Table 12: *In vitro* evaluation of different plant extracts against mycelial growth of *Colletotrichum gloeosporioides*.

Sl. No.	Plant extracts		Per cent inhibition of mycelial growth		
			Concentrations (%)		Mean
	Common name and part used	Botanical name	5	7.5	
1.	Onion (Bulb)	<i>Allium cepa</i> L.	35.16 (36.35)*	38.23 (38.17)	36.70 (37.26)
2.	Lantana (Leaves)	<i>Lantana camara</i>	42.66 (40.76)	45.54 (42.42)	44.10 (41.59)
3.	Neem (leaf)	<i>Azadirachta indica</i> A. JUSS	18.427 (25.40)	23.703 (29.05)	21.06 (27.23)
4.	Neem (kernel)	<i>Azadirachta indica</i> A. JUSS	30.03 (33.21)	32.33 (34.62)	31.18 (33.92)
5.	Kashmir bouquet (leaves)	<i>Clerodendron inerme</i> Gaertn.	15.31 (23.01)	22.33 (28.09)	18.77 (25.55)
6.	Tulsi /Holybasil (Leaves)	<i>Ocimum sanctum</i> L.	10.33 (18.73)	12.52 (20.68)	11.42 (19.70)
7.	Garlic (Cloves)	<i>Allium sativum</i> L.	15.50 (23.146)	20.16 (26.64)	17.83 (24.89)
8.	Turmeric (Rhizome)	<i>Curcuma longa</i>	37.46 (37.72)	40.73 (39.64)	39.10 (38.98)
9.	Aloevera (Leaf)	<i>Aloe vera</i> .	3.66 (10.99)	12.00 (20.22)	7.83 (31.06)
Mean			23.17 (28.77)	27.50 (31.63)	25.33 (29.38)
Source			SEm ±		CD at 1 %
Plant extract (P)			0.58		2.24
Concentration (C)			0.27		1.06
P X C			0.82		3.17

* Figures in parentheses indicate angular transformed values.

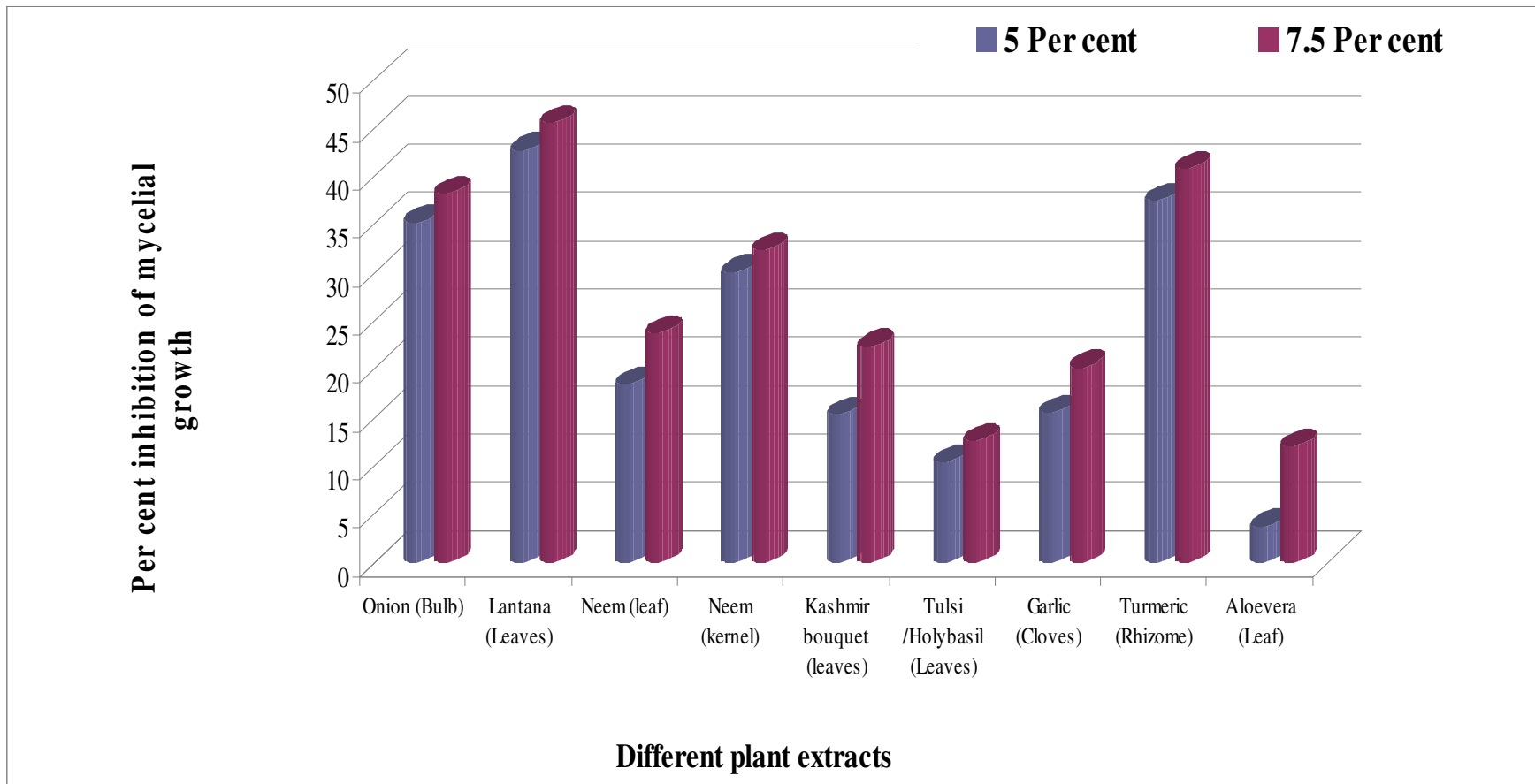


Fig. 8: In vitro evaluation of different plant extracts against mycelial growth of *Colletotrichum gloeosporioides*.



Plate7a: Per cent inhibition of mycelial growth of *C. gloeosporioides* at 5 per cent.



Plate7b: Per cent inhibition of mycelial growth of *C. gloeosporioides* at 7.5 per cent.

- | | |
|--------------------------|----------------------|
| 1= Onion bulb | 6= Tulsi leaves |
| 2= Lantana camara leaves | 7= Garlic cloves |
| 3= Neem leaves | 8= Turmeric rhizomes |
| 4= Neem kernals | 9= Aloevra leaf |
| 5= Clerodendron leaves | |

Plate 7: *In vitro* evaluation of botanicals against *C. gloeosporioides*

Plate 7: In vitro evaluation of botanicals against *C. gloeosporioides*

It is clear from the Table that, there was a significantly difference in the growth of the fungi at different temperature levels. Maximum growth of *Colletotrichum gloeosporioides* was noticed at 30°C (558.50 mg), which was followed by the growth at 25°C (505.50 mg). Next best temperature levels were 20°C (427.50 mg), 35°C (300.50 mg) and 15°C (248.00 mg). The least growth was observed at 10°C (179.75 mg).

4.8.3 Light requirement

i) On dry mycelial weight

The exposure of the fungus to alternate cycles of 12 hr light and 12 hr darkness for ten days resulted in maximum dry mycelial weight (593.71 mg), which was significantly superior over other two treatments tested. The dry mycelial weight of fungus exposed to continuous light resulted in 468.29 mg and continuous darkness resulted in 359.86 mg (Table 11, Fig. 7(A) and Plate 6c(i)).

ii) On radial growth

The exposure of the fungus to alternate cycles of 12 hr light and 12 hr darkness for ten days resulted in maximum radial growth of *C. gloeosporioides* (89.29 mm), which was significantly superior over other two treatments tested. The radial growth of fungus exposed to continuous light resulted in 72.14 mm and continuous darkness resulted in 68.86 mm (Table 11, Fig. 7(B) and Plate 6c(ii)).

4.9 MANAGEMENT STUDIES

4.9.1 *In vitro* evaluation of botanicals

The antifungal activity of nine plant extracts, viz., onion bulb, *lantana camara* leaf, neem leaf, neem kernel, clerodendron leaf, tulsi leaf, garlic cloves, turmeric rhizomes and aloe vera leaf were tested against *Colletotrichum gloeosporioides* by following method described in "Material and Methods". Inhibition of mycelial growth of the postharvest pathogen was assayed and presented in Table 12, Fig. 8 and Plate 7.

The results revealed that, effect of plant extracts on the fungal growth was significant. *Lantana camara* (44.10%) was found effective in inhibiting mycelial growth which was significantly superior over all other plant extracts evaluated. Turmeric (39.10%) and onion (36.70%) were the next best followed by neem kernel (31.18%). Neem leaf (21.06%), clerodendron (18.77%) and garlic (17.83%) followed by tulsi (11.42%) which were on par with each other and next in order. Least growth inhibition of *C. gloeosporioides* was obtained from aloe vera (7.83%).

The leaf extract at 7.5 per cent were significantly superior over 5 per cent. *Lantana camara* (45.54%) at 7.5 per cent was the best and significantly superior over all other plant extracts. Next best was turmeric (40.73%) followed by onion (38.23%), neem kernel (32.33%), neem leaf (23.33%), garlic (20.16%), tulsi (12.52%) and aloe vera (12.00%). Tulsi at 5 per cent (10.33%) and aloe vera at 5 per cent (3.66%) were least effective in inhibiting the mycelial growth of *C. gloeosporioides*.

4.9.2 *In vitro* evaluation of fungicides

Each five systemic and non-systemic fungicides were screened at three concentrations in the laboratory for their efficacy against *C. gloeosporioides*. Poisoned food technique was followed as detailed in "Material and Methods" (Table 13, Fig. 9 and Plate 8).

Table 13 revealed that, the effect of different fungicides on growth of *C. gloeosporioides* was significant. Among systemic fungicides carbendazim was found to be most effective which inhibited cent per cent growth of the fungus at all three concentrations (0.05, 0.1 and 0.15 per cent). Similarly 100 per cent inhibition was observed in case of propiconazole at 0.15 per cent concentration, followed by hexaconazole (94.33%) at 0.15 per cent concentration. The per cent inhibition was least in benomyl at 0.05 per cent (58%).

Table 13: *In vitro* evaluation of fungicides against mycelial growth of *Colletotrichum gloeosporioides*.

Sl. No.	Fungicides	Per cent inhibition of mycelial growth			
		Concentrations (%)			Mean
		0.05	0.1	0.15	
1.	Benomyl	58.00 (49.58)*	63.33 (52.75)	83.33 (65.90)	68.22 (56.08)
2.	Propiconazole	68.14 (55.64)	75.48 (60.31)	100.00 (89.96)	81.21 (68.64)
3.	Carbendazim	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)
4.	Hexaconazole	92.11 (73.73)	94.07 (76.42)	94.33 (76.34)	93.50 (75.50)
5.	Triadimefon	71.48 (57.71)	81.10 (64.27)	92.14 (73.88)	81.57 (65.29)
6.	Captan	62.66 (52.38)	89.66 (71.35)	100 (89.96)	84.09 (71.23)
7.	Chorothalonil	55.18 (47.96)	55.18 (47.96)	56.40 (48.66)	55.59 (48.19)
8.	Propineb	62.77 (52.39)	68.93 (56.18)	69.26 (56.33)	66.98 (54.97)
9.	Mancozeb	58.88 (50.14)	65.55 (54.05)	88.61 (70.35)	71.01 (58.18)
10.	Zineb	71.45 (57.72)	71.48 (57.71)	84.85 (67.09)	75.93 (60.84)
Mean		70.72 (59.11)	76.60 (63.16)	86.12 (72.37)	77.81 (64.88)
Source		SEm ±		CD at 1 %	
Fungicide (F)		0.843		3.17	
Concentration (C)		0.462		1.73	
F X C		1.46		5.49	

* Figures in parentheses indicate angular transformed values.

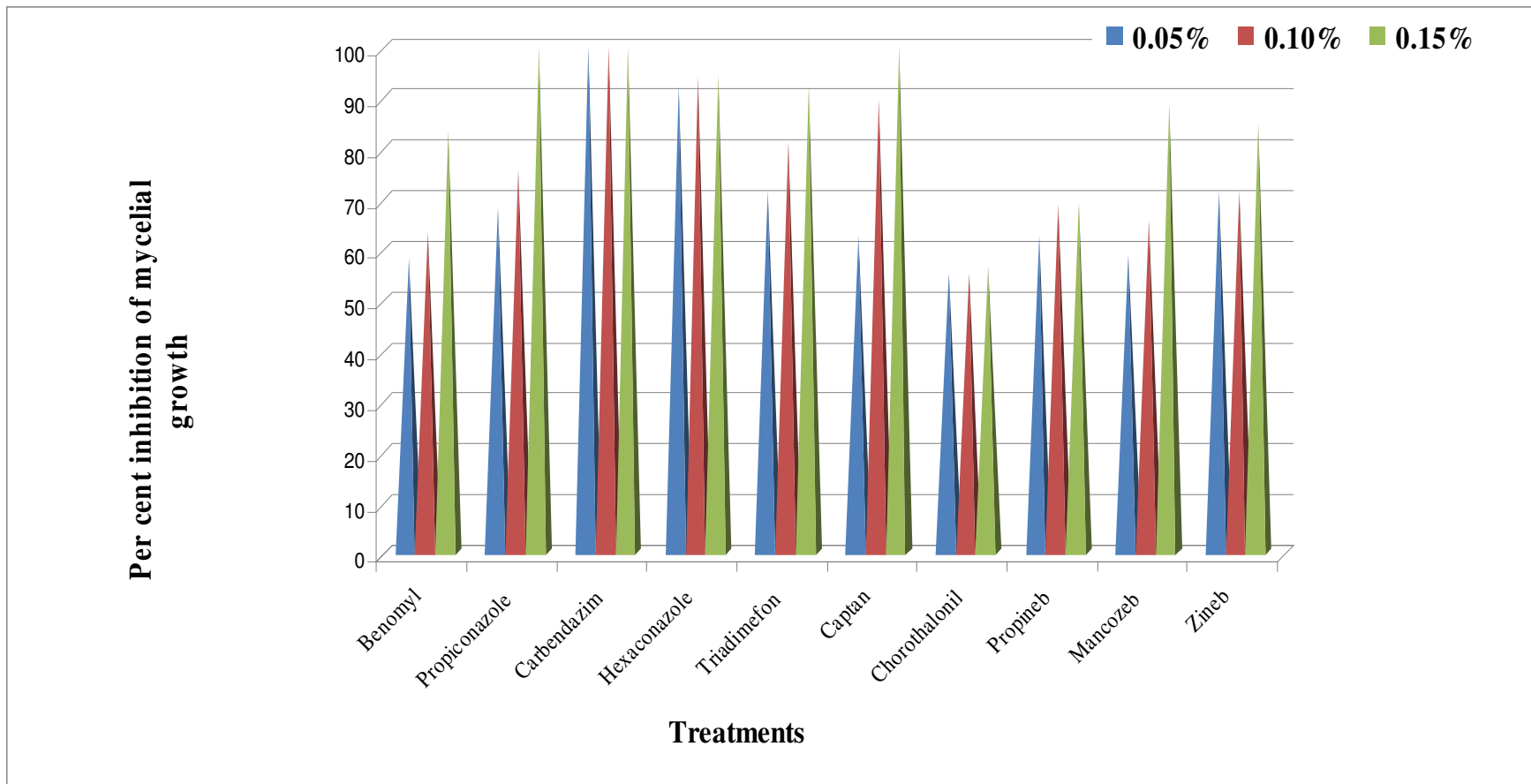
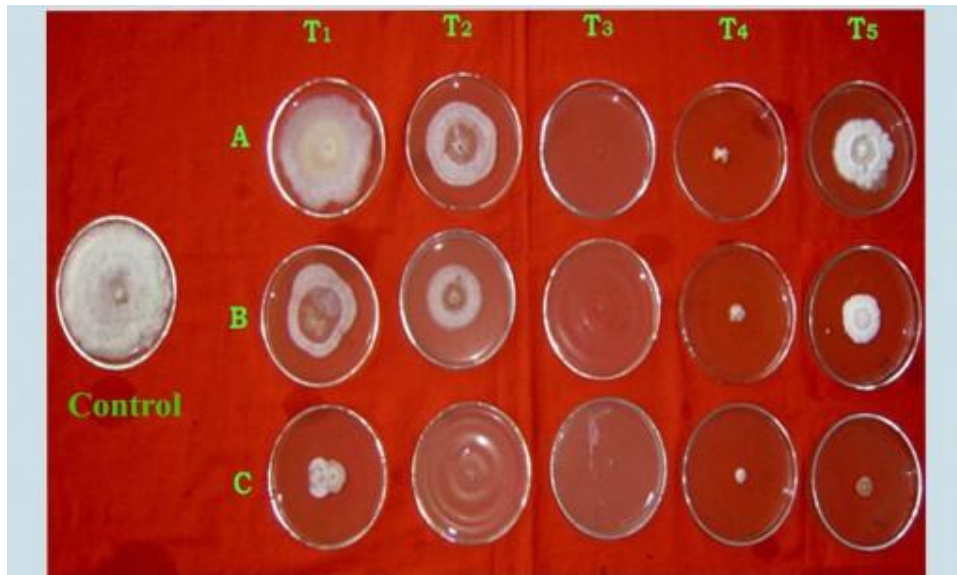
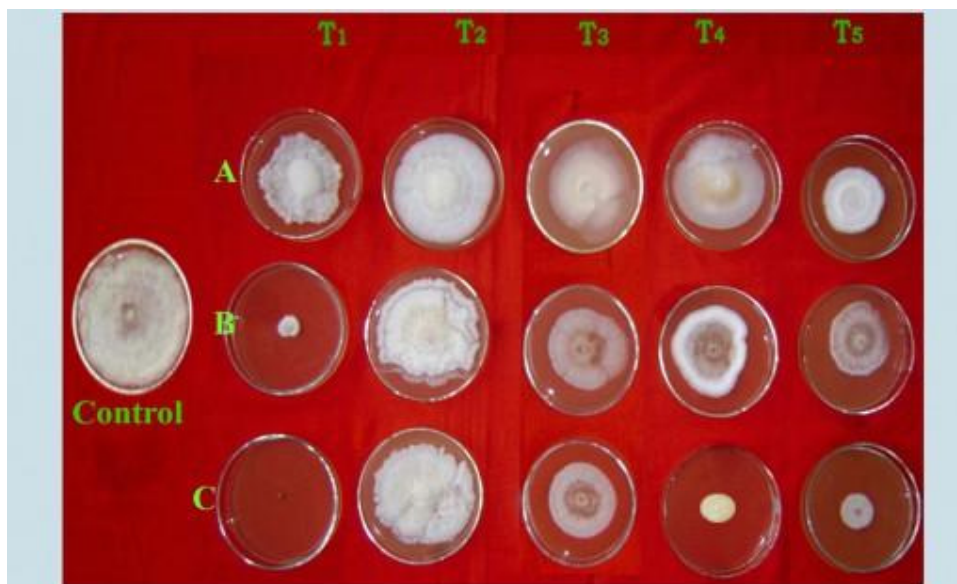


Fig. 9: In vitro evaluation of fungicides against mycelial growth of *Colletotrichum gloeosporioides*.



8a . Per cent inhibition of mycelial growth of *C.gloeosporioides* by systemic fungicides



8b . Per cent inhibition of mycelial growth of *C.gloeosporioides* by non systemic fungicides.

A= 0.05, B= 0.1 and C= 0.15%

Plate 8 : *In vitro* evaluation of fungicides against *C. gloeosporioides*

Plate 8: In vitro evaluation of fungicides against *C. gloeosporioides*

Table 14: *In vitro* evaluation of biocontrol agents against mycelial growth of *Colletotrichum gloeosporioides*.

Sl. No.	Biocontrol agents	Per cent inhibition of mycelial growth
1.	<i>Trichoderma harzianum</i>	51.89 (46.06)*
2.	<i>Trichoderma viride</i>	50.11 (45.04)
3.	<i>Trichoderma koningii</i>	53.32 (46.88)
4.	<i>Trichoderma virens</i>	60.87 (51.26)
5.	<i>Pseudomonas fluorescens</i>	42.87 (40.88)
6.	<i>Bacillus subtilis</i>	50.97 (45.54)
Mean		51.67 (45.94)
SEm ±		0.64
CD at 1%		2.60

* Figures in parentheses indicate angular transformed values.

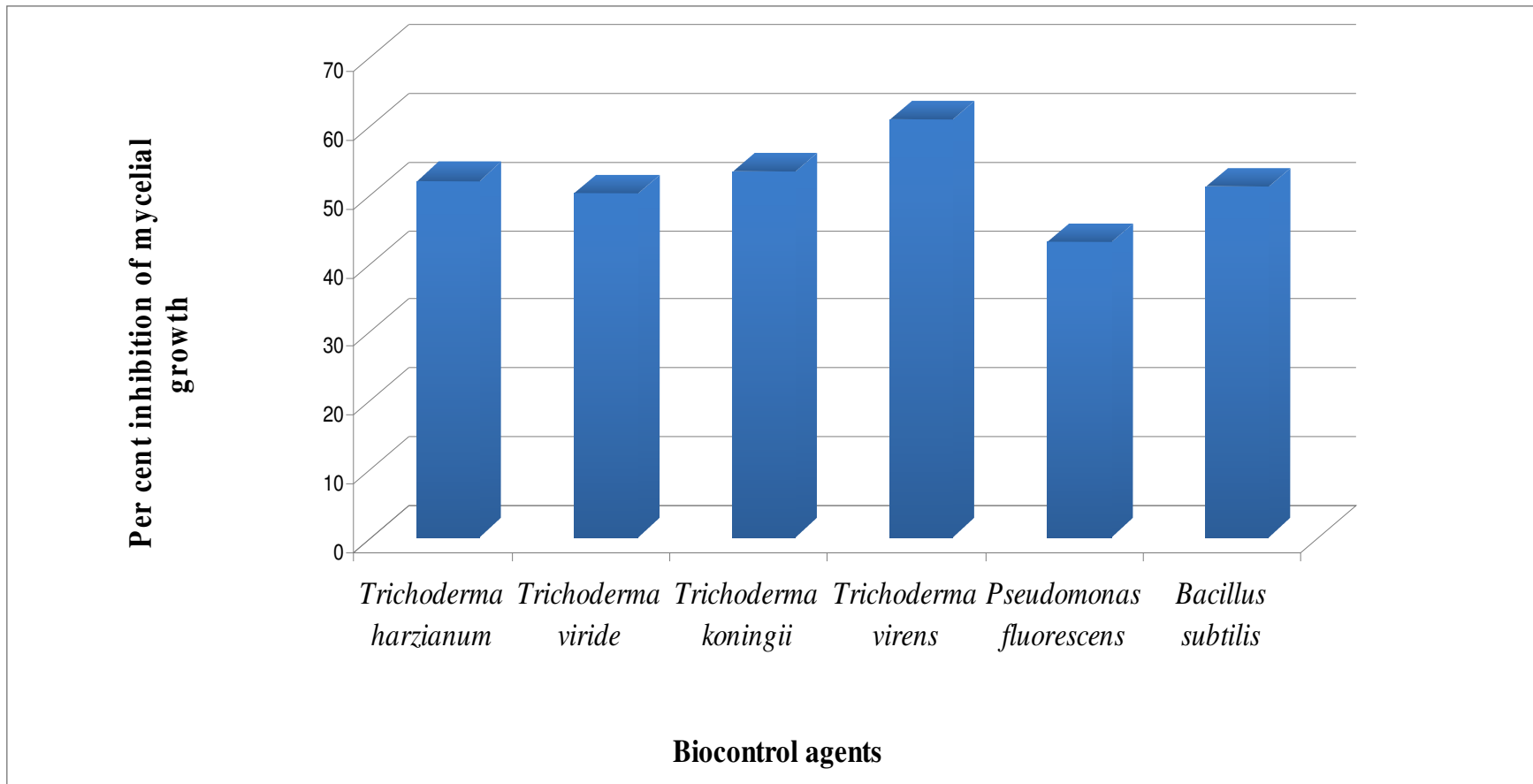


Fig. 10: In vitro evaluation of different biocontrol agents against mycelial growth of *Colletotrichum gloeosporioides*.

Table 15: *In vivo* evaluation of salt, bioagents and hot water treatment against anthracnose of papaya.

Sl. No.	Treatment	Per cent Disease Index (PDI) at 6 DAI		
		2 %	5%	Mean
1.	Sodium chloride	53.33 (46.91)*	33.33 (35.26)	43.33 (41.07)
2.	Sodium chloride + Hot water (49°C)	36.67 (37.27)	26.67 (31.09)	31.66 (34.16)
3.	Calcium chloride	60.00 (50.77)	46.67 (43.09)	53.33 (46.91)
4.	<i>Trichoderma harzianum</i>	76.67 (61.12)	56.67 (48.83)	66.67 (54.95)
5.	<i>Pseudomonas fluorescens</i>	60.00 (50.77)	53.33 (46.91)	56.67 (48.82)
6.	<i>Trichoderma harzianum</i> + <i>Pseudomonas florescence</i>	56.67 (48.83)	43.33 (41.17)	50.00 (44.98)
7.	Hot water (49°C)	46.67 (43.09)	46.67 (43.09)	46.67 (43.09)
8.	Control	90.00 (71.57)	90.00 (71.57)	90.00 (71.57)
	Source	SEm ±		CD at 1 %
	Treatments (T)	0.21		0.81
	Concentration (C)	0.10		0.40
	T X C	0.30		1.14

* Figures in parentheses indicate angular transformed values.

Table 16: *In vivo* evaluation of fungicides against anthracnose of papaya.

Sr. No.	Treatment	Per cent Disease Index (PDI) at 8 DAI		
		0.05 %	0.1%	Mean
1.	Antracol	63.33 (52.73)	53.33 (46.91)	58.33 (49.80)
2.	Benomyl	83.33 (65.91)*	46.67 (43.09)	65.50 (54.77)
3.	Captan	80.00 (63.43)	43.33 (41.17)	61.83 (52.38)
4.	Carbendazim	40.00 (39.23)	26.67 (31.09)	33.33 (35.14)
5.	Hexaconazole	70.00 (56.79)	56.67 (48.83)	63.33 (52.79)
6.	Mancozeb	53.33 (46.91)	40.00 (39.23)	46.67 (43.05)
7.	Control	90.00 (71.57)	90.00 (71.57)	90.00 (71.57)
	Source	SEm ±	CD at 1 %	
	Treatments (T)	0.31	1.24	
	Concentration (C)	0.17	0.66	
	T X C	0.45	1.75	

* Figures in parentheses indicate angular transformed values.

Plate 10a: Effect of salts, biocontrol agents and hot water against *C. gloeosporioides*.

T1 = Sodium chloride + hot water (49°C)

T2 = Sodium chloride

T3 = Hot water (49°C)

T4 = Calcium chloride

T5 = *Trichoderma harzianum*

T6 = *Pseudomonas fluorescens*

T7 = *Trichoderma harzianum* + *Pseudomonas fluorescens*

C = Control

Plate 10b: Effect of salts, biocontrol agents and hot water against *C. gloeosporioides*.

T1 = Sodium chloride + hot water (49°C)

T2 = Sodium chloride

T3 = Calcium chloride

T4 = *Trichoderma harzianum*

T5 = *Pseudomonas fluorescens*

T6 = *Trichoderma harzianum* + *Pseudomonas fluorescens*

C = Control

Plate 11: Effect of fungicides against *C. gloeosporioides*.

T1 = Mancozeb

T2 = Carbendazim

T3 = Antracol

T4 = Captan

T5 = Hexaconazole

T6 = Benomyl

C = Control

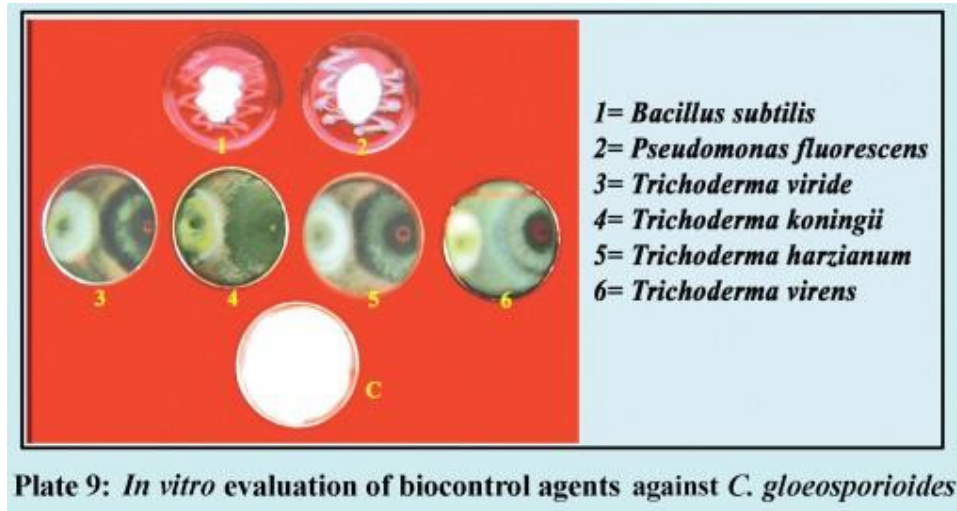


Plate 9: In vitro evaluation of biocontrol agents against *C. gloeosporioides*

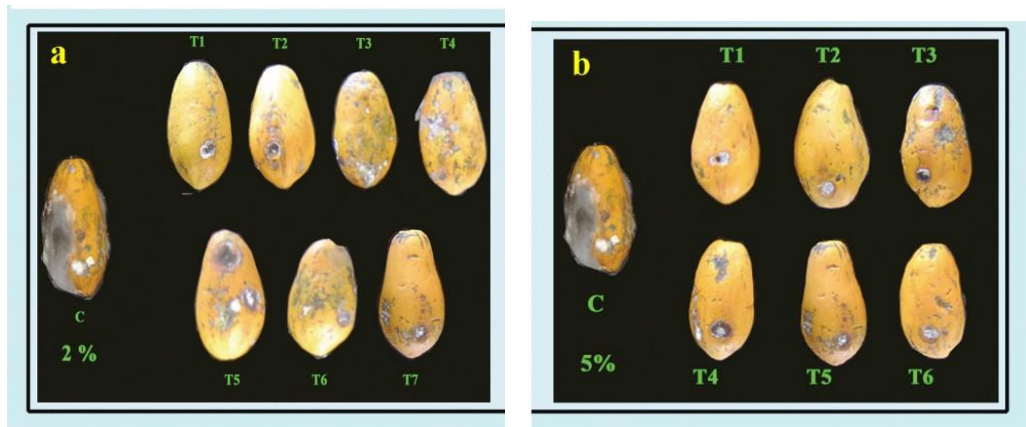


Plate 10: Effect of salts, biocontrol agents and hot water against *C. gloeosporioides*.

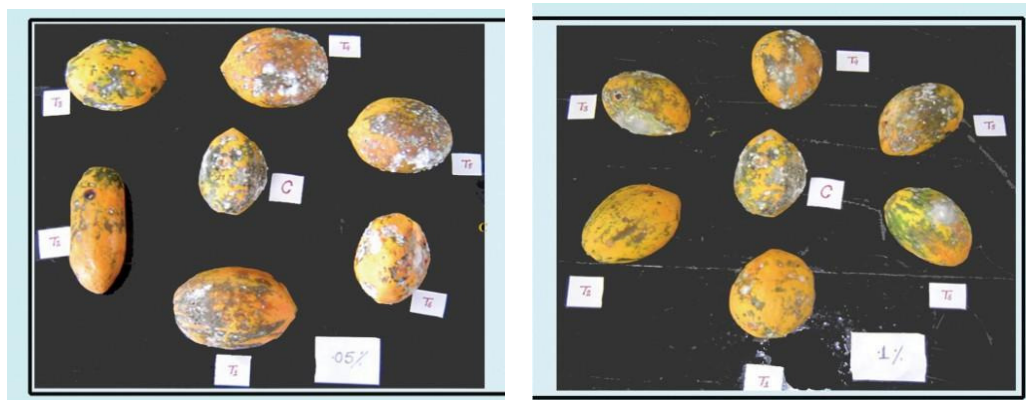


Plate 11: Effect of fungicides against *C. gloeosporioides*.

Among the non-systemic fungicides captan showed highest inhibition of mycelial growth (100%) followed by mancozeb (88.61%) at 0.15 per cent and zineb (84.85%) at 0.15 per cent. The least mycelial growth was noticed in case of chlorothalonil (55.18%) at 0.05 and 0.1 per cent concentration. Non-systemic fungicides at 0.15 per cent were significantly superior over 0.05 and 0.10 per cent concentrations in inhibiting growth of fungi.

4.9.3 *In vitro* evaluation of biocontrol agents

Six biocontrol agents viz. *Trichoderma harzianum*, *Trichoderma viride*, *Trichoderma koningii*, *Trichoderma virens*, *Pseudomonas fluorescens* and *Bacillus subtilis* were tested against *Colletotrichum gloeosporioides*. The results are presented in Table 14, Fig. 10 and Plate 9.

The results revealed that all the antagonists significantly reduced the growth of *C. gloeosporioides* either by over growing or by exhibiting inhibition zones. Most of antagonists inhibited colony growth of *C. gloeosporioides* by fast and over growing nature as observed in antagonists.

After measuring the colony diameter of *C. gloeosporioides*, it was noticed that maximum reduction in colony growth was observed in *Trichoderma virens* (60.87%) which was significantly superior over all the bioagents tested. Next best in inhibiting the mycelial growth of fungus was *Trichoderma koningii* (53.32%), *Trichoderma harzianum* (51.89%), *Bacillus subtilis* (50.97%) and *Trichoderma viride* (50.11%) which were on par with each other. Least mycelial reduction was noticed in case of *Pseudomonas fluorescens* (42.87%).

4.10 *IN VIVO* EVALUATION OF SALTS, BIOAGENTS, HOT WATER TREATMENT AND FUNGICIDES AGAINST ANTHRACNOSE OF PAPAYA

4.10.1 *In vivo* evaluation of salt, bioagents and hot water treatment

The data pertaining to the *in vivo* evaluation of salt, bioagents and hot water treatment on *Colletotrichum gloeosporioides* of papaya are presented in Table 15 and Plate 10.

Data are the significantly differ over control, the minimum per cent disease index was observed in sodium chloride + hot water (49°C) (31.66%) followed by sodium chloride (43.33%) and hot water (46.67%). Next best treatment was *Trichoderma harzianum* + *Pseudomonas florescence* (50.00%) followed by calcium chloride (53.33%) and + *Pseudomonas florescence* (56.67%). Maximum PDI was observed in *Trichoderma harzianum* (66.67%).

Among the tested two concentrations, 5 per cent concentration was showing the significant differed over 2 per cent concentration. Minimum PDI was observed in sodium chloride + hot water (49°C) (26.67%) followed by sodium chloride (33.33%) and *Trichoderma harzianum* + *Pseudomonas florescence* (43.33%). Maximum PDI was observed in *Trichoderma harzianum* (76.67%) followed by calcium chloride and *Pseudomonas florescence* (60.00%) which were on par with each other.

4.10.2 *In vivo* evaluation of fungicides

Per cent disease index (PDI) at 8 days after postharvest treatment at two different concentrations (0.05 and 0.1 per cent) is presented in Table 16 and Plate 11.

Data are the significantly differ over control, among the different fungicides, carbendazim (33.33%) was found to be most effective followed by mancozeb (46.67%), and antracol (58.33%). Captan (61.33%) and hexaconazole (63.33%) were found to be next best effective which were on par with each other.

Among the two concentrations tested 0.1 per cent was found to be significant over 0.05 per cent. Minimum PDI was observed in carbendazim (26.67%) followed by mancozeb (40.00%) and antracol (53.33%) at 0.10 per cent concentration. Maximum PDI was observed in benomyl (83.33%) at 0.05 per cent.

5. DISCUSSION

Papaya (*Carica papaya* L.) is considered as the “common man’s fruit” crops. The fruits are immensely important as they are delicious, have high food value, rich in vitamin A and C and also fats and occupy a very prominent place in the diet of human being. Papaya suffers from several diseases and anthracnose is one of them and this disease is one of the limiting factors for papaya marketing because it caused postharvest losses.

The present investigation on anthracnose of papaya caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. included symptomatology, isolation of the fungus, morphology of the fungus, pathogenicity test, cultural studies, physiological studies, *in vitro* evaluation of botanicals, fungicides and biocontrol agents against pathogen, effect of salts, biocontrol agents and hot water on fungus and effect of fungicides of papaya fruit against pathogen. The results of the present investigation are discussed below.

Anthracnose of papaya, exhibited as small round dark area on ripening portion which enlarge rapidly forming circular, slightly sunken and watersoaked lesions having dark margins with black centre and under humid condition pinkish spore masses were formed on the affected fruit and frequently produced in a concentric ring. Similar symptoms were reported by various workers (Simone, 1999; Pernezny and Litz, 1993; Burger, 1921 and Snowdon, 1990)

The causal organism, *C. gloeosporioides* was isolated from diseased papaya fruits by following standard tissue isolation method. Further, pure culture was obtained by following single spore isolation. Pure culture of fungi thus obtained was used in rest of the studies. Similar work had been done by Naik (1985) and Bhat (1987).

Identified as *Colletotrichum gloeosporioides* based on colony and spore morphology which revealed that acervuli were circular to elliptical, measuring 129-281.4 μm , setae were erect in habit, measuring 37-89 X 1.4-4.0 μm . conidia were hyaline, single celled and smooth walled. They were cylindrical and measuring 9-20 X 3-7.5 μm . These characters are in agreement with those of Bose *et al.* (1953) where the morphology of the pathogen reported that the acervuli measured 115-467 μm and size of conidia varied from 11-16 X 4-6 μm . Similarly Sutton (1993) reported that the conidia of *Colletotrichum gloeosporioides* were hyaline, smooth and thin walled, cylindrical or oval, straight and size of the conidia varied from 9-24 X 4-12 μm . Holiday (1980) and Jeffries *et al.* (1990), reported that conidial size were 7-20 X 2.5 -5 μm and setae were 4-8 X 200 μm .

In pathogenicity studies, the conidial suspension of *Colletotrichum gloeosporioides* was sprayed on healthy fruits and typical symptoms were noticed, seven days after incubation. Similar results were recorded while proving pathogenicity by Bhat (1991) and Ekbote (1994) on pomegranate and mango respectively and Kota (2003) proved the pathogenicity of *C. gloeosporioides* on mango and banana. The method employed for pathogenicity test was similar to those described by Stanghellini and Aragaki (1966).

Every living being required food for its growth and reproduction and fungi are not exception to it. 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. All media are not equally good for fungi, nor there can be a universal substrates or artificial medium, upon which all fungi grow. So, different media including both synthetic and non-synthetic were tried for *Colletotrichum gloeosporioides* in the present experiment. The amount of vegetative growth can be estimated by measuring either the diameter of the colony or by weighting the dry mycelial mass. The former method was made use of while studying the growth of the fungus on different solid media and the latter in different liquid media.

Among the fifteen solid media evaluated, maximum radial growth of *Colletotrichum gloeosporioides* was observed on V-8 juice agar (87.67 mm) and followed by oat meal agar (83.63 mm), Richard’s agar (82.65 mm), corn meal agar (80.00 mm), malt extract agar (79.20 mm), potato carrot agar (79.00 mm), papaya fruit agar (78.17 mm), potato dextrose agar (75.50 mm), Tochinal’s agar (75.50 mm), Czapek’s agar (74.83 mm) and host leaf extract agar (74.27 mm) and potato dextrose agar and Tochinal’s agar were found on par with each other. Elliott’s agar (48.45 mm) and Brown’s agar (34.83 mm) were found to be next best solid media for radial growth. No growth was noticed in case of Sach’s agar media (0.00 mm). The

results are in confirmation with that of Durairaj (1956) in case of *C. capsici*, Ekbote *et al.* (1997); Sudhakar (2000) and Rani and Murthy (2004) in case *C. gloeosporioides*. Sporulation of fungus was found to be abundant on Richard's agar, V-8 juice agar, oat meal agar and malt extract agar. They were in agreement with observation of Ekbote *et al.* (1997); Akthar (2000); Sudhakar (2000) and Rani and Murthy (2004) in case of *C. gloeosporioides*.

Vegetative growth of the fungus reached maximum on 10th day of incubation (486.07 mg dry mycelial weight). This was indicative of optimum growth period beyond, which the autolysis occurred. Lilly and Barnett (1951) have discussed the onset of autolysis after maximum growth during which the cellular enzymes begin to digest the various cell constituents. This was similar finding of Ekbote (1994) in case of *C. gloeosporioides* and Hiremath *et al.* (1993) in case of *C. gloeosporioides* causing Shisham blight.

Fungi possess an ability to utilize a wide range of nutrients as a source of energy. Among the liquid media used for growth of *C. gloeosporioides*, Richard's broth (581.33 mg) and malt extract broth (575.00mg) were supported maximum growth both were on par with each other followed by potato dextrose broth (449.17 mg), Brown broth (425.00 mg), Tochnal's broth (420.33) and oat meal broth (339.00 mg). Czapek's broth (256.13 mg), Sabouraud's broth (253.67 mg), papaya fruit broth (249.03 mg), V-8 juice broth (217.67 mg), Sach's broth (145.23 mg), corn broth (136.33 mg) and host leaf extract broth (93.33 mg) were found to be next best media. Least mycelial growth was observed in potato carrot broth (43.10 mg). Hiremath *et al.* (1993) and Ekbote *et al.* (1997) reported that, maximum dry mycelial growth of *C. gloeosporioides* was recorded in Richard's broth.

In the radial measurement, it is not possible to consider the amount of submerged mycelium. Hence, Cochrane (1958) has opined the determination of dry mycelial weight as the best method for precise work. The ability of the fungus to grow in Richard's medium indicated the requirement of certain nutrients and vitamin which may be present in the medium.

Carbon occupies a unique position among the essential elements required by fungi. Almost half of the dry mycelial weight of fungi cells consists of carbon which is a main structural element and which also plays an important functional role (Lilly and Barnett, 1951; Bilgrami and Verma, 1978).

The utilization of various carbon compounds may depend either on the activity of the fungus to utilize certain simpler forms or on its power to convert the complex carbon compound into simpler forms, which may be easily utilized. In present study dextrose supported maximum growth of fungi (620.00 mg) followed by fructose (567.67 mg), sucrose (410.33 mg), glucose (374.33 mg) and soluble starch (347.00 mg). Least growth was reported in case of lactose (136.67 mg). Dextrose and fructose are the best source of energy and comes under complex sugar. Sucrose being major component of photosynthetic plants is generally utilized as good source by most of plant pathogenic fungi (Lilly and Barnett, 1951) and similar observation were also made by Naik (1986). Glucose also promoted growth in fungi since, it is a simple sugar (Chandra and Tandon, 1962). Fairly good growth was noticed in soluble starch also indicating the presence of enzyme having power to break down starch into simple sugar, which can be utilized easily. Among the seven carbon sources, lactose supported least growth of the fungus, since it is an uncommon sugar for plant pathogenic fungi. Over and above, galactocyclase a enzyme required for breaking down lactose to galactose and glucose had not been commonly reported in fungi, but adoptive enzyme system which enables fungi to grow on such a common sugars have been discussed (Cochrane, 1958).

Nitrogen, a compound of protein is an essential element and like carbon, it is also used by fungi for functional as well as structural purposes. But all the source of nitrogen are not equally good for the growth of fungi. The fungus *Colletotrichum gloeosporioides* showed variation in its ability to utilize different nitrogen sources. It utilized potassium nitrate (525 mg) more efficiently and was a better nitrogen source than any other nitrogen sources tested. Similar observation has been made by Rajak (1983); Naik (1985) and Ekbote (1994) in case of *C. gloeosporioides*. The nitrate compounds are excellent nitrogen sources for imperfect fungi and also ascomycetes (Bilgrami and Verma, 1978). The next best nitrogen sources were Ammonium sulphate (378.33 mg), magnesium nitrate (311.67 mg), L-asparagine (267 mg) and sodium nitrate (253.33 mg). Mishra and Mahmood (1960) noticed the maximum

growth of *C. gloeosporioides* in L-asparagine. The fact that good growth in these amino acids indicated the direct utilization of these compounds in protein synthesis (Lilly and Barnett, 1951). The poor growth was observed in urea. Cochrane (1958) has opined that urea breaks down to ammonia during autoclaving and ammonia in high concentration is toxic to fungi.

Sulphur being a compound of the sulphahydril or thiol group of many enzymes, co-enzymes and vitamins affect various other vital processes of fungi (Bilgrami and Verma, 1978). In the present study, maximum mycelial growth of *C. gloeosporioides* was obtained when magnesium sulphate was used as sulphur source and least growth was noticed in copper sulphate (190.25 mg) over the control (290.50 mg) so it can be help full to mangle the disease. Similar results were obtained by Hegde, 1986 who found *C. gloeosporioides* infecting arecanut made the best growth when magnesium sulphate was supplied as sulphur source. Chaturvedi (1965) also obtained the best growth of *C. gloeosporioides* by supplementing magnesium sulphate in the medium.

The fungi generally utilize substrate in the form of solution, only if the reaction of the solution is conductive to fungal growth and metabolism. This brings the importance of hydrogen ion concentration for a better fungal growth. In the present study maximum growth of the fungus was obtained at 6.5 (602.67 mg) followed by 6.0 (538 mg) and 7.0 (487.33 mg), where as optimum pH range was found to be 5.5 to 7.5 and least growth was observed at pH 4.0 (194.67 mg). Abe and Kono (1956) found that a pH range 5.2 to 5.8 is the best for *Glomerella cingulata*. Verma (1969) and Hegde (1986) observed maximum growth of *C. gloeosporioides* at pH of 6.0. Rajak (1983) claimed a pH of 7.0 as optimum for *C. gloeosporioides*. Katti (1981) found the maximum growth of *Glomerella cingulata* at 5.8 pH and optimum pH range was 5.2 to 6.4. A pH of 5.5 to 6.5 was found to be optimum for growth and sporulation of *C. falcatum*, (Ahmed and Divingaraciam, 1974). Ekbote (1994) reported that the maximum growth of fungus at 6.5 pH and optimum pH range was found to be between 5.0 to 7.5. At reduced pH, the cell membrane becomes saturated with H⁺ ions which limit the passage of cations. The reverse condition could be obtained when the medium is alkaline and accumulated OH⁻ ions present the passage of essential anions. Enzyme activity is also conditioned by the reaction of the medium, as a result, the reduced growth of fungi is observed at extremities (Bilgrami and Verma, 1978). The role of pH on the growth of phytopathogenic fungi has been reviewed by Tandon (1961).

Among the external abiotic factors which influence the growth of fungi, temperature plays an important role. Temperature affects almost every function of fungi, including growth, spore germination and reproduction. The fungus under study grew best at temperature of 30°C (505.50 mg dry mycelial weight), whereas optimum range is between 20 to 30°C. Similarly, Naik (1985) reported, 20-30°C as an optimum temperature range for a *Colletotrichum gloeosporioides* and also Hegde and Hegde (1986) achieved maximum growth of *Colletotrichum gloeosporioides* at 30°C and temperature range for the good growth was 20 to 35°C. Sattar and Malik (1939) have reported the optimum temperature for the sporulation of *C. gloeosporioides* to be 30°C. Quimio (1973) reported that the optimum temperature for growth, sporulation and spore germination is 30°C.

Light has a profuse effect on growth of fungi. The preliminary studies carried out in present investigation with *Colletotrichum gloeosporioides* indicated a maximum radial growth (89.29 mm colony diameter and 593.71 mg dry mycelial weight) was observed when expose to alternate cycles of light and darkness. This was followed by continuous light (72.14 mm diameter and 468.29 mg dry mycelial weight) and continuous darkness resulted in 68.86 mm colony diameter and 359.86 mg dry mycelial weight of fungi. Kamanna (1996); Sudhakar (2000); Ashoka (2005) and Narendra Kumar (2006) observed that exposure of *C. gloeosporioides* to alternate cycles of 12 hours light and 12 hours darkness resulted in maximum growth. Similarly these studies are in agreement with the Chowdhuary (1936); Mishra and Siradhana (1980) and Saifulla and Ranganathaiah (1990).

Continuous use of chemical fungicides in the management of disease also brought new problems with them. More alarming amongst them are pollution of air, water, soil, residual toxicity, development of resistance in pathogen against chemicals and harmful effects on non-targeting organisms. Consequently, there has been alarming development of harmful environment for human beings. Contrary to the problems associated with the use of synthetic chemicals, botanicals are environmentally non pollutive, renewable, inexhaustible, indigenously available, easily accessible, largely non phytotoxic, systemic ephemeral, thus

readily biodegradable, relatively cost effective and hence constitute as 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 to consider as one of the component in the integrated disease management (Khadar, 1999 and Nagesh, 2000).

In present investigation nine plant extracts were evaluated under *in vitro* condition against *C. gloeosporioides* to know the fungitoxic nature of their extracts. Though complete inhibition of the pathogen was not observed in any of the plant extracts tested but considerable amount of inhibition was noticed in some of them. Among the nine plant extracts tested against *C. gloeosporioides*, *lantana camara* at both the concentration 5 (42.66%) and 7.5 per cent (45.54%) was significantly superior over all other plant extracts. Next best was turmeric at 7.5 per cent (40.73%) followed by onion at 7.5 per cent (38.23%), neem (kernel) at 7.5 per cent (32.33%). Least growth was observed in case of aloe vera (3.66%) at 5 per cent concentration. In present investigation, the mycelial growth of fungus was inhibited to greater extent by *lantana camara* leaf extract, which is said to have pesticides property followed by turmeric. The fungicidal spectrum of neem, *Azadirachta indica* has been attributed to Azadirachtin which belong to C₂₅ terpenoides (Subramanian, 1993). The effectiveness of neem is well supported by Rahejha and Thakore (2002). In effectiveness of tulsi leaf extract against *C. gloeosporioides* is supported by the work of Patel and Joshi (2001), where in they reported that tulsi leaf extract was ineffective in inhibiting the mycelial growth of fungus.

In vitro evaluations of fungicides provide useful preliminary information regarding its efficacy against a pathogen within a shortest period of time and therefore, serve as guide for further field testing in future. In the present study five systemic and five non systemic fungicides were tested at three concentrations.

Among systemic fungicides carbendazim was successful in completely (100%) inhibiting the growth of *C. gloeosporioides* at all three concentrations (0.05%, 0.10 and 0.15%). Propiconazole was successful in completely (100%) inhibiting growth only at concentration at 0.15 per cent, similar results were obtained by Biradar (2002) carbendazim was found to be very effective in the investigations conducted by Orlikowski and Wajdyla (1991); Tomy (1997) and Kumbhar and Caudhary (1979). Carbendazim and Triadimefon are the best fungicides for inhibiting the growth of *C. gloeosporioides*. Carbendazim and Benomyl being a Benzimidazole group fungicide, they interfere with energy production and cell wall synthesis of fungi (Nene and Thapliyal, 1982). Further, they also reported the effectiveness of triazole, which inhibit sterol biosynthesis pathway in fungi. According to Davidse (1986) carbendazim induced nuclear instability by disturbing the mitosis and meiosis. Among the five non-systemic fungicides tested against *C. gloeosporioides* Captan only at 0.15 per cent concentration was found highly effective in inhibiting the growth by 100 percent, which was followed by mancozeb (88.61%) at 0.15 per cent. Result was confirmed by Biradar (2002).

Biological control is an effective, ecofriendly and alternative approach for any disease management practice. In the present investigation, four fungal and two bacterial bio-control agents were tested against *C. gloeosporioides*.

The results of dual culture technique on *C. gloeosporioides* revealed that all the antagonists significantly reduced the growth of *C. gloeosporioides* either by over growing or by exhibiting inhibition zones. Most of antagonists inhibited colony growth of *C. gloeosporioides* by their fast and over growing nature as observed in antagonists.

It was noticed that maximum reduction in colony growth was observed in *T. virens* (60.87 mm) which was significantly superior over all the bioagents tested. Next best was *T. koningii* (53.32 mm), *T. harzianum* (51.89 mm), *B. subtilis* (50.97), *T. viride* (50.11 mm) and *P. fluorescens* (42.87 mm). Least mycelial growth reduction was observed in *P. fluroncenc*. Similar results were obtained by Deshmukh and Raut (1992); Santha kumari (2002); Patel and Joshi (2001) and Raheja and Thakore (2002) in case of *C. gloeosporioides*.

T. virens and *T. koningii* showed more mycelial inhibition at organism compared to bacterial antagonists. This can be attributed to higher competitive ability of these *Trichoderma* spp.

The antagonism of *Trichoderma* spp. against many fungi is mainly due to production of acetaldehyde compound (Robinson and Park, 1966 and Dennis and Webster, 1971). This

may also be the reason for its antagonistic effect on *C. gloeosporioides*. Godtfredsen and Vagedal (1965) reported trichodermin, Pyke and Dictz (1960) found dermadin as major volatile antibiotic produced by *Trichoderma* spp., which suppress several plant pathogens.

Effect of salt, biocontrol agents and hot water treatment 49°C for 20 minutes were tested against the postharvest disease of papaya. In this investigation least per cent disease index was noticed in sodium chloride + hot water treatment (31.66%) followed by sodium chloride (43.33%) and hot water treatment (46.67%). Sodium chloride + hot water at 49°C (26.67%) found to be effective over the all other tested treatment at 5 per cent concentration followed by sodium chloride (33.33%) at 5 per cent and *Trichoderma harzianum* + *Pseudomonas fluorescens* (43.33%) at 5 per cent concentration. Hot water treatment and calcium chloride at 5 per cent found to be equal effect to reduce the infection of pathogen (46.67%). Allong *et al.* (2000) reported that a hot water treatment of 48-50°C for 20 minutes was found to delay fungal storage rots in fruits of papaya cultivars without negatively affecting sensory quality. The postharvest phase of anthracnose can be controlled by submerging fruits in water at 46 to 49°C for 20 minutes shortly after harvest (Akamine, 1953; Akamine and Arisumi, 1953 and Tsai, 1969). But this treatment leads to the uneven degreening of fruits (Patel *et al.*, 1973). Khare and Dhingra (1974) reported that different salt can be completely checked the growth of the fungus in culture.

Many chemical compounds have been used as part of postharvest treatment of tropical fruits for the prevention or retardation of microbial infection. In this present investigation, fruits dipped in different fungicides solution at two different concentration (0.5% and 0.1%), among that carbendazim (33.33%) found to be a best over all the other tested fungicides followed by mancozeb (46.67%) and captan (61.83%). Among the two concentrations tested, 0.1 per cent concentration found to be effective over 0.05 per cent concentration. Carbendazim (26.67%) was found least PDI followed by mancozeb (40.00%) at 0.1 and carbendazim (40.00%) at 0.05 per cent found to be equal effective against the growth of pathogens. Maximum growth of pathogens was noticed in benomyl (83.33%) at 0.05 per cent.

Among the two test concentrations, 0.1 per cent was found to be superior over the 0.05 per cent concentration fungicides. Similar studied were conducted by many workers, Spalding and Reeder (1972); Quimio and Quimio (1974) and Singh and Bhargava (1977) reported that fruit dipped in benomyl give a better control of disease. Bolkan *et al.* (1976) and Tsai (1969) have found that when papaya fruits are dipped for 3 minutes in either benomyl or thiabendazole solution (with a sticker) reduce the postharvest decay caused by *C. gloeosporioides*.

Future line of work

There is no full stop to gain inside scientific knowledge. Any amount of work does not satisfy the hunger of scientists as problems. Circumvent and idea will continue to flow and this work is not an exception. The present investigation has given rise to new idea on fungal diseases of papaya anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. Hence, the following future lines of work are being suggested.

- 1) To conduct systematic and comprehensive survey of all the papaya growing area of Karnataka for anthracnose of papaya.
- 2) Systematic crop loss assessment to anthracnose disease in papaya.
- 3) Detailed studies on influence of various ecological factors on the distribution and survival of the inoculum.
- 4) Detailed investigation on the interaction between the fungal and viral diseases is essential.
- 5) Detailed investigation on epidemiology of fungal diseases needs immediate attention.
- 6) Molecular aspects of host pathogen interaction and variability in pathogenesis.
- 7) Effect of other non chemical methods like e treatment or irradiation on postharvest diseases.
- 8) Characterization of principle chemical compound involved in the biofungicides.
- 9) Use of higher concentration of plant extracts to manage the plant diseases.

6. SUMMARY AND CONCLUSION

Papaya constitutes the bulk of fruit production of India and world. It is mainly known as a "common man's fruit" and is distributed throughout tropical and subtropical countries. They are affected by various postharvest pathogens, which take heavy toll of these fruit crops. The present investigation was directed towards developing the effective control methods for managing the postharvest diseases of these crops. Investigations were carried out at Department of Plant Pathology, UAS, Dharwad on the symptomatology of postharvest disease anthracnose of papaya, their causal organism, cultural studies, nutritional studies, physiological of the organism, evaluation of botanicals, fungicides and biocontrol agents against pathogen and effect of different fungicides, salts, biocontrol agents to reduce the infection of pathogen. The major findings of the investigations are presented in brief, as follows.

In papaya, anthracnose disease was observed very commonly after the harvesting of the fruits or under storage conditions. Anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. appeared as either as small circular, watersoaked spots on ripening fruit. As the spots develop, they become sunken, turn brown or black pinkish orange area are formed by conidial masses that cover lesion. The conidia were cylindrical, hyaline, single celled with oil globules in the centre and measured 9-20 x 3-7.5 µm. Acervulus measured 129-281.4 µm.

Pure culture of these fungi was obtained by following single spore isolation technique from the disease fruits. The fungal culture thus obtained was found to be pathogenic to papaya fruit.

Maximum radial growth was on solid medium was on V-8 Juice agar followed to be significant over all tested media followed by Oat meal agar, Richard's agar, corn meal agar and no growth was observed in case of Sach's agar. Maximum dry mycelial weight was observed on 10th day of incubation. This was taken as maximum peak period for further experiment studies, while least growth was observed after two days of incubation.

Among the different liquid media maximum growth was observed on Richard's broth followed by malt extract broth which was par with each other. Next best media was potato dextrose broth followed by Brown's broth, Tochnal's broth and least growth was observed in potato carrot broth.

Among the carbon sources tested, dextrose was found to be the best for the growth of *C. gloeosporioides* than other carbon sources and lactose was least utilized. Among the nitrogen sources tested, potassium nitrate was found to be the best for growth of *C. gloeosporioides* followed by Ammonium sulphate. Also among the sulphur utilization, magnesium sulphate yielded maximum mycelial weight of pathogen among the other sulphur compounds tested, and least growth was observed in case of copper sulphate over the control (190.25 mg) so copper is useful to manage the disease incidence.

C. gloeosporioides grew at all pH levels tested, however, the maximum growth of fungus was obtained at pH 6.5 while optimum range of pH was found to be 5.5 to 7.5.

The temperature studies revealed that maximum growth of *C. gloeosporioides* was observed at 30^oC and range to 20- 30^oC least growth of was recorded at 10^oC.

C. gloeosporioides exposure to continuous light, continuous darkness and alternate cycles of 12 hours light and 12 hours darkness for ten days resulted maximum dry mycelial weight was recorded on alternate cycles of light and darkness.

In vitro evaluation of the nine different botanicals against the pathogen revealed that *Lantana camara* was effective at the concentration of 7.5 per cent followed by turmeric at 7.5 per cent concentration and least effect was noticed in case of aloe vera at the concentration of 5 per cent.

Out of the ten fungicides tested, *in vitro* against *C. gloeosporioides*, carbendazim at all three concentrations 0.05, 0.1 and 0.15 per cent inhibited the mycelial growth upto cent per cent. Among the non systemic fungicides, captan (84.09%) was found to be effective at the concentration of 0.15 per cent, it was inhibited the mycelial growth 100 per cent followed by mancozeb (88.61%) at the concentration of 0.15 per cent.

Under *in vitro* condition, per cent inhibition of mycelial growth of *C. gloeosporioides* by different biocontrol agents, *Trichoderma virens* was found to be effective (60.87%) over all the biocontrol agents followed by *T. koningii* (53.32%). Least growth was observed in case of *Pseudomonas fluorescens* (42.87%).

Under *In vivo* evaluation of effect of salts, biocontrol agents solution at the two concentrations (2 and 5 per cent) and hot water treatment at 49°C for 20 minutes, among the different treatment, the least PDI was found in case of sodium chloride + hot water at 49°C for 20 minutes (26.67%) followed by sodium chloride (33.33%), hot water (46.67%) and *T. harzianum* + *Pseudomonas floescence* (43.33%) at 5 per cent concentration and more growth of fungus was noticed in *T. harzianum* (76.67%). Similarly *in vivo* evaluation of fruit treatment with different fungicides at the two concentration (0.05 and 0.10%), among the different fungicides treatment tested, carbendazim was found to be effective and it showed least PDI (26.67%) followed by mancozeb (40.00%) at 0.1 per cent concentration and carbendazim (40.00%) at 0.05 per cent concentration. Less inhibition was noticed in benomyl (83.33%) at 0.05 per cent concentration.

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* Original not seen.

STUDIES ON ANTHRACNOSE - A POSTHARVEST DISEASE OF PAPAYA

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ABSTRACT

Papaya (*Carica papaya* L.) an important tropical and subtropical fruit crop, is being affected by several post-harvest diseases among which anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. Studies on *C. gloeosporioides* include isolation, identification and proving pathogenicity test. The conidia were cylindrical or oblong, hyaline and single celled with oil globules. Cultural studies revealed that among solid media, V-8 juice agar and oat meal agar were found to be good for radial growth and sporulation of *C. gloeosporioides* and among the liquid media, Richard's and Malt extract broth supported maximum dry mycelial weight of *C. gloeosporioides* on 10th day of incubation.

Nutritional studies revealed that dextrose, potassium nitrate and magnesium sulphate were better among the carbon, nitrogen and sulphur sources, respectively for growth, sporulation and yielded maximum dry mycelial weight of the pathogen.

Physiological studies revealed that optimum pH of 6.5 was favourable for growth and sporulation of pathogen. Maximum dry mycelial weight was obtained at optimum temperature of 30°C. Alternate cycles of 12hours light and 12 hours darkness favoured the maximum radial growth and dry mycelial weight of *C. gloeosporioides* followed by continuous light.

Under *in vitro* evaluation of botanicals, that *Lantana camara* and turmeric (5 and 7.5%) were found to be effective against *C. gloeosporioides*. Among the fungicides tested, carbendazim was found to be cent per cent effective at all three concentration (0.05, 0.1 and 0.15%) and among the biocontrol agent *Trichoderma virens* was found to be effective followed by *T. koningii*.

Under *in vivo* evaluation of salt, bioagents and hot water, the minimum per cent disease index was observed in sodium chloride (5%) + hot water at 49° at 15 minutes. Among the *in vivo* evaluation of fungicides, carbendazim was a superior at 0.1% and found to be a least per cent disease index.