ECOFRIENDLY MANAGEMENT FOR FRUIT ROT OF CHILLI (*CAPSICUM ANNUUM* L.) CAUSED BY *COLLETOTRICHUM* SPP.

GOLDA. S. B.

2010

Department of Plant Pathology

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM – 695 522

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GOLDA. S. B.

Thesis submitted in partial fulfillment of the requirement for the degree of

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DECLARATION

I hereby declare that this thesis entitled **' Ecofriendly management for fruit rot of Chilli (*Capsicum annuum* L.) caused by *Colletotrichum* spp.'** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title of any other university or society.

Vellayani
31 - 08 – 2010.

Golda. S. B.
(2008 – 11 - 106)

CERTIFICATE

Certified that this thesis entitled **‘ Ecofriendly management for fruit rot of Chilli (*Capsicum annuum* L.) caused by *Colletotrichum* spp.’** is a record of research work done independently by Ms. Golda, S. B. (2008 – 11- 106) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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Dedicated to

The Farming Community

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LIST OF ABBREVIATIONS

%	-	Per cent
˚C	-	Degree celcius
µm	-	Micrometre
@	-	At the rate of
CD	-	Critical difference
cfu	-	Colony forming units
et al.	-	And others
Fig.	-	Figure
g	-	Gram
h	-	hour
ha	-	Hectare
i.e.	-	That is
Kg	-	Kilogram
L	-	Litre
min.	-	Minute
ml	-	Millilitre
mm	-	Millimeter
ppm	-	Parts per million
rpm	-	Rotations per minute
sp.	-	Species (singular)
spp.	-	Species (Plural)
t	-	Tonnes
viz.	-	Namely
w/v	-	Weight/volume

INTRODUCTION

1. INTRODUCTION

Chilli, *Capsicum annuum* L. is an annual herbaceous spice cum vegetable crop coming under the family Solanaceae grown for its pungent fruits used either as dry or raw. It is a rich source of vitamin A, C, and E and assists in digestion. The pungency in chilli is due to an alkaloid capsaicin which has high medicinal value, especially as an anti cancerous agent and prevents heart diseases. The chief constituent of chilli pericarp is a crystalline colourless pungent principle known as Capsaicin or capsicutin, a condensation product of 3-hydroxy-4-methoxy benzylamine and decylenic acid which produces a highly irritating vapour.

The native home of chilli is considered to be the Mexican centre of origin with secondary origin of Gautemala. Chilli was introduced in India by the Portuguese in Goa in the middle of 16^{th} century and since then it spread rapidly throughout the country. The major chilli producing countries are India, China, Peru, Bangladesh and Hungary. World trade in chillies is around 4 lakh tonnes. The Indian share in global production range from 50 – 60 % (Devender Reddy, 2010). The area under cultivation in India was 7.57 lakh ha in 2006 – 2007. During 2009, the production of chillies was 11.67 lakh tonnes and the productivity 1,550 Kg/ha (Devender Reddy, 2010). In Kerala, the area under cultivation was 14,000 ha in 2006 – 2007, the production was 14,000 tonnes and the productivity was about 1000 Kg/ha.

Fruit rot of chilli incited by *Colletotrichum spp*. is reported to cause an yield loss up to 30.7 % in Tamil Nadu (Sujathabai, 1992), 8 – 27% in Maharashtra (Dattar, 1995), 10 – 30 % in Punjab (Rai and Chohan, 1996), and 25 – 48 % in Karnataka (Ekbote, 2001). Anthracnose occurs both as pre harvest or post harvest decay of mature fruits and account for more than 50 % of the yield losses (Poulos, 1992; Bosland and Votava, 2003; Pakdeevaraporn et al., 2005; Ramachandran et al., 2007).

Since 1940, chemical fungicides were applied to control chilli diseases. The indiscriminate usage of a wide range of fungicides has invited many undesirable problems such as development of fungal resistance, food poisoning, toxic residues in the produce, environmental pollution and escalating costs in vegetable production. This necessitates the search for cheaper means of control and environment friendly methods of management of diseases.

The present trend is towards organic farming by which the farmers can fetch a premium price for their produce. The growing realization that plant extracts and plant products have fungitoxic potential has promoted a renewed look at these natural biocides. This investigation was carried out with the objective of evolving an ecofriendly management practice for fruit rot of chilli using biocontrol agents, plant extracts and plant products.

The main objectives of the investigation are

- Study on the symptomatology of fruit rot of chilli.
- Identification of the pathogens of the disease based on colony and conidial morphology.
- Growth studies of pathogen on different solid and liquid media, carbon sources, nitrogen sources, temperature, p^H and light intensities.
- Isolation of antagonists of pathogen from phyllosphere and rhizosphere of healthy chilli plants.
- *In vitro* screening of antagonists against the pathogen.
- *In vitro* screening of plant extracts against the pathogen.
- *In vitro* screening of plant products against the pathogen.
- Screening of chilli varieties released from KAU against fruit rot pathogen.
- To evolve an *in vivo* management practice for fruit rot of chilli using biocontrol agents, plant extract and plant products.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Chilli (*Capsicum annuum* L.) is a popular spice cum vegetable crop valued around the world for its colour, flavour and enriching the nutrition of our daily meals. Chillies are free of cholesterol, rich in vitamin A, C, E, folic acid and potassium and low in sodium content. The sustainability of chilli based agriculture is threatened by a number of factors. Diseases like anthracnose, bacterial wilt, viruses and several other insect pests have been reported to impair the chilli productivity (Isaac, 1992). In India, anthracnose disease is a major problem and is one of the significant economic constraints to chilli production (Ramachandran et al., 2007).

2.1. History, distribution and yield loss

Anthracnose of chilli was first reported from New Jersey, USA, by Halsted in 1890 who described the causal agents as *Gloeosporium piperatum* and *Colletotrichum nigrum*. These taxa were then considered as synonyms of *C. gloeosporioides* by Von Arx (1957). Chilli anthracnose was first reported in India by Sydow in the year 1913 from Coimbatore of the erst while Madras Presidency.

C. capsici (Syd.) Butler and Bisby and *C. gloeosporioides* (Penz) Penz. and Sacc. were reported as the pathogens of chilli anthracnose in tropical Asia (Manandhar et al., 1995 a). Apart from these two species, *C. graminicola,* and C. *atramentarium* were reported from India (Verma, 1973) and *C. coccodes* from Florida (Roberts et al., 2001). Anthracnose of chilli has been shown to be caused by more than one *Colletotrichum spp*. including *C. acutatum* (Simmonds), *C. capsici* (Syd.) Butler and Bisby, *C. gloeosporioides* (Penz.) Penz. and Sacc. and *C. coccodes* (Wallr.) S. Hughes (Simmonds, 1965; Johnston and Jones, 1997; Kim et al., 1999; Nirenberg et al., 2002; Voorrips et al., 2004; Sharma et al., 2005; Pakdeevaraporn et al., 2005; Than et al., 2008). Of the different species of *Colletotrichum, viz., C. capsici, C. gloeosporioides* and *C. acutatum* causing anthracnose in chilli,

C. capsici was the most predominant species in the major chilli growing states *viz.,* Karnataka and Andhra Pradesh in India (Ramachandran et al., 2007). Selvakumar (2007) reported that in addition to *C. capsici,* in north eastern India *C. dematium*, *C. gloeosporioides, C. graminicola* and *C. atramentarium* were also found to be the associated pathogens of chilli anthracnose. Mohan Rao et al., (2007) reported that *C .capsici* was the predominant species in Karnataka, Tamil Nadu, and Maharashtra while *C. gloeosporioides* in Andhra Pradesh.

Chilli anthracnose usually develops under high humid conditions when rain occurs after the fruits have started to ripen with reported losses of up to 84 % (Thind and Jhooty, 1985). Fruit rot of chilli incited by *C. capsici* is reported to cause yield losses up to 30.7 % in Tamil Nadu (Sujathabai, 1992), 8 – 27 % in Maharashtra (Datar, 1995), 10 – 30 % in Punjab (Rai and Chohan, 1996)), and 25 – 48 % in Karnataka (Ekbote, 2001). Anthracnose occurs both as pre harvest and post harvest decay of mature fruits which accounts for more than 50 % of the yield losses (Poulos, 1992; Bosland and Votava, 2003; Pakdeevaraporn et al., 2005; Ramachandran et al., 2007). In Thailand, the marketable yield loss due to anthracnose amounted to 10 – 80% of the crop production (Poonpolgul and Kumphai, 2007). Capsicum fruit rot reduces the dry weight, quantities of capsaicin and oleoresin content of the fruits (Mistry et al., 2008).

2.2. Symptomatology

Anthracnose, derived from a Greek word meaning ‘coal’, is the common name for plant diseases characterized by very dark, sunken lesions, containing spores (Isaac, 1992). The disease appears as small circular sunken spots that coalesce to form large elliptical spots on fruits and leaves. Under severe conditions, defoliation of affected plants occurs. Mc Govern (1995) opined that fruit rot began as small, round (1 - 2 mm dia.), brown, and slightly depressed lesions that became surrounded by water soaked areas. Individual lesion enlarged concentrically to about three cm in

dia. and became wrinkled and covered with black acervuli. Total fruit rot often occurred due to the coalescence of multiple lesions. Typical anthracnose symptoms on chilli fruit include sunken necrotic tissues, with concentric rings of acervuli. Fruits showing blemishes have reduced marketability (Manandhar et al., 1995 b). Anthracnose lesions are brown, not orange, and they turn black by the formation of setae and sclerotia. Concentric rings of the acervuli are commonly seen within the lesions (Roberts et al., 2001). Pandey and Pandey (2006) recorded that symptoms include circular, dark brown to black sunken spots surrounded by water soaked areas. Palmateer and Ploetz (2007) noted that lesion centre became white and coalesced to rot. Salmon coloured spores, subepidermal acervuli typically with setae and simple short erect conidiophores were observed in the lesion. According to Gupta et al. (2009) the characteristic symptoms of the disease appeared as small circular or elongated slightly sunken bleached lesions bearing light black, brown or orange coloured acervuli either in rings or in a scattered fashion. On fruits the acervuli were subepidermal with light to dark brown setae disrupting outer epidermal cell walls of the host.

According to Mehrotra and Aneja (1990) acervulus may develop subepidermally or subcuticularly. An acervulus is a saucer – shaped fructification in which the hymenium of conidiogenous cells develops on the floor of the cavity from a pseudoparenchymatous stroma beneath an integument of host tissue which ruptures at maturity. Acervuli form large, brown or black setae among the conidiogenous cells. The setae are external appendages with no cytoplasmic continuity. Heavily colonized chilli seeds had abundant inter and intra cellular mycelia and acervuli in the seed coat, endosperm and embryo, showing disintegration of parenchymatous layers of the seed coat and depletion of food material in endosperm and embryo (Chitkara et al., 1990). Appressoria that formed on immature fruits may remain quiescent until ontogenic changes occur in the fruits (Bailey and Jeger, 1992; Prusky and Plumbley, 1992). Fungi can overwinter on alternative hosts such as other

solanaceous or legume crops, plant debris and rotten fruits in the field (Pring et al., 1995). He also reported that *Colletotrichum* species naturally produce micro sclerotia to allow dormancy in the soil during the winter or when subjected to stressful conditions, and these micro sclerotia can survive for many years. Diseased fruit acts as a source of inoculum, allowing the disease to spread from plant to plant within the field (Roberts et al., 2001).

C. capsici generally caused disease on ripe red fruit, while *C. gloeosporioides* and *C. acutatum* produced disease both on young and mature green fruits (Hong and Hwang, 1998; Kim et al., 1999). Hegde et al. (2001) observed maximum per cent disease index (PDI) in red fruits (58 %) which is on par with fruits at the turning stage from green to red (54.5 %). Green fruits recorded the least PDI (22.5 %). Rajapakse and Ranasinghe (2002) reported that conidia of *C. capsici* germinated and differentiated into appressoria on fruit surfaces at all maturity stages, but the highest rates occurred on surface of red ripe fruits. Anthracnose lesions initiated after fruits had ripened. Chilli fruits at red ripe stage were more susceptible to *C. capsici* than green fruits. Kim et al. (2004) reported that different species cause diseases of different organs of the chilli plant; for example, *C. acutatum* and *C. gloeosporioides* infect chilli fruits at all developmental stages, but usually not the leaves or stems, which are mostly damaged by *C. coccodes* and *C. dematium*. Mesta et al. (2007) reported that the stage at which fruits turn from green to red was prone to infection. Resistance in green chillies was attributed to the higher phenol and wax content.

The Pathogen, *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc.

Colletotrichum is an economically important genus of the family Melanconiaceae, order Melanconiales, class Coelomycetes, sub division Deuteromycotina.

Teleomorph : *Glomerella cingulata* (Stonem.) Spauld and Schrenk.

2.3. Morphological and cultural characters of the pathogen

2.3.1. Morphological characters

2.3.1.1. Colony characters

Grey, dark grey or black colonies of *Colletotrichum capsici* produced profuse sporulation while cottony white, white and dirty white colonies with fluffy growth sporulated weakly (Misra and Dutta 1963; Jeyalakshmi and Seetharaman 1999; Chander Mohan et al., 2006). Mc Govern (1995) reported that on Potato Dextrose Agar *C. gloeosporioides* formed greyish white colonies with sparse to wooly mycelium with little aerial growth. The colonies were creamy white at first, turning to grey black with age while *C. capsici* formed whitish cream to blackish grey colonies, with thin mat of mycelium and with out aerial growth. Reverse side of the colonies were brownish grey to black. Rohana Wijesekara and Agarwal (2006) reported that the colonies were having entire or wavy margins. Mohan Rao et al. (2007) reported that *C. capsici* produced fluffy or flat colonies while *C. gloeosporioides* produced either fluffy or slightly fluffy colonies. He also reported that *C. gloeosporioides* produced either pink or orange mycelia and it grew faster than *C. capsici*. Gupta et al. (2009) reported that colonies of *C. gloeosporioides* on PDA were pinkish white to grey with reverse dark brown. The mycelium in culture formed brown to orange acervuli. Acervuli bearing setae were rarely observed in the culture.

2.3.1.2. Morphology of Conidium, Acervulus and Setae

Considerable variation was observed in the size of the conidia of *C. gloeosporioides* and *C. capsici.*

Sutton (1992) reported that the conidial dimensions of *C. gloeosporioides* were 12.0 – 17.0 × 3.5 – 6.0 µm. Yee and Sariah (1993) reported that the conidia

were cylindrical with obtuse ends, hyaline, aseptate, uninucleate and measured 4 – 24 × 2 - 6 µm. They were formed in setose or globose acervuli. The shape of the acervuli ranged from round to elongated to irregular and measured 60 – 250 µm in dia. Setae were sparse to profuse, dark brown to black, straight to slightly curved, 1- 4 septate, swollen at the base and tapering towards the apex, 70 – 165 µm long. Mc Govern (1995) also reported the shape of conidia as cylindrical with obtuse ends having an average size of 12.2 × 3.7 µm. The size of conidia observed by Rohana Wijesekara and Agarwal (2006) was 8.54 – 21.95 × 2.44 – 7.32 µm. Amarjit Singh et al. (2006) noted that Yeast extract agar induced the production of setae of *C. gloeosporioides* to the extent of 20 – 25 per acervulus followed by Guava fruit decoction agar, Potato dextrose agar and Oat meal agar producing 12 – 15, 5- 8 and 2-3 setae, respectively. Palmateer and Ploetz (2007) reported that colonies produced abundant conidia that were hyaline, one celled, straight, cylindrical, and averaged 14.7 × 5.0 µm with ranges of 12.5 to 17.5 × 3.8 to 7.5 µm. Gupta et al. (2009) reported that the conidiophores were closely packed on the surface of the acervulus and were hyaline. The conidia of *C. gloeosporioides* were oblong to cylindrical with obtuse ends having one to three oil globules. The conidia measured 4.12 – 24.72 × 1.03 - 8.24 µm.

Sutton (1992) has recorded the conidial and appressorial dimensions of *C. capsici* as 18 – 23 × 3.5 – 4.0 µm and 9.0 – 14.0 × 6.5 – 11.5 µm. Rohana Wijesekara and Agarwal (2006) described the size of conidia of *C. capsici* were 13.41 – 31.71 × 1.22 – 6.1 µm while Ruchi Garg et al. (2007) reported as 22.5 × 3.3 µm. Jameel Akhtar and Singh (2007) reported that conidial size of *C. capsici* measured 25.27 – 26.15 × 3.14 – 3.67 µm. Conidiophores were aseptate and unbranched. The packed conidia in mass or group were pinkish but individually they appeared hyaline. The conidia were produced singly at the tip of unbranched conidiophore, single celled, curved with narrow ends and fusoid. Jameel Akhtar et al. (2008) noted that conidia of *C. capsici* were falcate, fusiform with acute apices, and

narrow truncated. They were one celled, hyaline and uninucleate. The conidia measured as 16 – 30 × 2.5 – 4 µm in size and acervuli were rounded, elongated, approximately 350 µm in dia. Setae were abundant, brown, 1 – 5 septate, rigid, hardy swollen at the base, slightly tapered towards the paler acute apex. They were up to 250 µm long and 5 – 8 µm wide.

2.3.1.3. Conidial germination

Maximum conidial germination of *C. capsici* was recorded in one per cent sucrose solution (Chung and Lee, 1986) and 84 % germination of *C. gloeosporioides* in one per cent glucose solution (Sunil Kumar and Yadav, 2007)

2.3.2. Cultural characters

2.3.2.1. Growth and sporulation of *Colletotrichum* in different solid and liquid media

Radziah (1985) and Amarjit Singh et al. (2006) revealed that maximum growth and sporulation of *C. gloeosporioides* was obtained on Potato dextrose Agar (PDA). Hegde (1986) reported that the maximum radial growth of *C. gloeosporioides* was observed on Sabouraud's agar and potato dextrose agar. He obtained the highest dry mycelial weight in Sabouraud's broth. But according to Hiremath et al., 1993; Ekbote, 1994; Vinod Tasiwal and Benagi, 2009; Ekbote et al., 1997 maximum dry mycelial weight of *C. gloeosporioides* was recorded in Richards' broth. Ekbote (1994) observed maximum radial growth of *C. gloeosporioides* on Richards' agar, Potato dextrose agar, and Brown's agar. Vinod Tasiwal and Benagi (2009) opined that best solid medium for growth and sporulation of *C. gloeosporioides* was on V – 8 juice agar and Richards' agar respectively.

Mesta (1996) found that PDA was the best solid medium for growth and sporulation of *C. capsici*. Among different liquid media tested highest dry mycelial

weight was observed in Richards' broth. Ruchi Garg et al. (2007) reported that among ten different nutrient media tested Richards' Synthetic agar was best for maximum radial growth of *C. capsici* and Martin's Rose Bengal agar gave the minimum radial growth, while PDA was best for sporulation after 6th DAI.

2.3.2.2. Toxin production by the fungus

Goodman (1960) reported colletotin, a toxin produced by *Colletotrichum fuscum.* Sharma and Sharma (1969) reported that Richards' liquid medium as the best medium for the toxic metabolite production by *C. gloeosporioides* causing citrus die back in India. They found that the toxin production started at eight days growth and the toxicity increased up to a maximum of 22 days growth, after which it declined slowly up to 30 days. Twigs kept in the filtrates showed mild symptoms in 8, 10 and 12 days old culture, moderate reaction in 16, 20, 26, 28, and 30 days old cultures and severe symptoms in 22 and 24 days old. Exotoxin production was found to be more than endotoxin.

Nair and Ramakrishnan (1973) had reported the production of toxic metabolites by *C. capsici* (Syd.) Butler and Bisby and its role in leaf spot diseases of turmeric. The spot found by the exotoxin (culture filtrate) was smaller in size than that by endotoxin (concentrated toxic metabolite from mycelium), but both were similar in appearance.

2.3.2.3. Growth curve of *Colletotrichum gloeosporioides* in liquid media

For attaining maximum growth in liquid media *C. gloeosporioides* required different periods of incubation . *C. gloeosporioides* reached maximum growth after 10 days of incubation in Potato Dextrose Broth (PDB) (Hegde, 1986; Hegde et al ., 1990; Vinod Tasiwal and Benagi, 2009) while Ekbote (1994) observed that the fungus reached maximum growth after 12 days of incubation in (PDB) and Madan

(2004) reported that a period of 14 days was optimum for the growth of *C. gloeosporioides.*

Mesta (1996) found that *C. capsici* reached maximum mycelial dry weight on 15th day in (PDB).

2.3.3. Nutritional studies of *Colletotrichum spp.*

2.3.3.1. Effect of different carbon sources on growth and sporulation of *Colletotrichum spp.*

Earlier workers experimented with the carbon requirement of *Colletotrichum* for its growth and sporulation and came out with almost the same type of results with very few exceptions.

Ramakrishnan (1941); Durairaj (1956); Verma (1979); Naik (1985); Naik et al. (1988); Hegde et al. (1990); Reddy (2000) and Saxena (2002) reported that sucrose was the best source of carbon for the growth of *C. gloeosporioides.*

Glucose, dextrose and fructose supported good growth of *C. gloeosporioides* was reported by Naik et al. (1988); Hegde et al. (1990) and Sangeetha (2003).

There were contradictory reports on the requirement of mannitol as the source of carbon for the growth of *C. gloeosporioides.* When majority of earlier workers *viz*., Chaturvedi (1965); Reddy (2000); Manjunatha Rao and Rawal (2002); Sangeetha (2003) and Sangeetha and Rawal (2008) reported that mannitol was the best source of carbon for the growth of *C. gloeosporioides.* There was only one report by Binyamini and Shiffmann – Nadel (1972) that *C. gloeosporioides* showed poor development on mannitol. Starch supported good growth and sporulation was reported by Chaturvedi (1965) and Madan (2004).

Good sporulation of *C. gloeosporioides* was found in media supplemented with glucose, fructose, maltose, lactose, sucrose and mannitol as the sole source of carbon (Chaturvedi, 1965; Reddy, 2000; Manjunatha Rao and Rawal, 2002; Saxena, 2002; Sangeetha, 2003; Sangeetha and Rawal, 2008).

Singh (1970) and Tamil Vanan et al. (2005) obtained similar results while working with *C. capsici*. They reported that *C. capsici* grew better on dextrose followed by mannitol.

2.3.3.2. Effect of different nitrogen sources on growth and sporulation of *Colletotrichum spp.*

Organic nitrogen sources such as Urea, Peptone and Amino acids and inorganic nitrogen sources such as nitrates and nitrites of Potassium, Sodium and Ammonium and Ammonium phosphate were used by earlier workers for growing different species of *Colletotrichum* and obtained similar and contradictory results.

Asparagine, peptone and potassium nitrate supported good growth of *C. falcatum* while, ammonium sulphate and urea provided poor growth of *C. indicum* (Ramakrishnan, 1941) and Chaturvedi (1965). Durairaj (1956) reported that Ammonium phosphate supported maximum growth of *C. gloeosporioides* followed by organic nitrogen sources such as urea and asparagines where as Lai et al. (1993) reported that ammonia inhibited the growth of the pathogen which could utilize many nitrates and amino acids as a nitrogen source. Aspartic acid was found to be the best nitrogen source for growth (Chaturvedi, 1965; Wasantha Kumara and Rawal, 2008). Peptone and tyrosine were found to be a better nitrogen source for *C. gloeosporioides* isolated from arecanut (Hegde et al., 1990) and a poor growth was observed in the medium containing methionine.

Tandon and Chandra (1962) and Chaturvedi (1965) reported that several nitrogen compounds except nitrites, had supported varying degrees of growth of

C. gloeosporioides and the fungus could not grow on nitrites of potassium or sodium. Nitrites were toxic at lower pH values though they supported growth at alkaline medium. Tandon and Chandra (1962); Bilgrami and Verma (1978); Naik (1985); Naik et al. (1988); Ekbote (1994); Saxena (2002) and Wasantha Kumara and Rawal (2008) reported potassium nitrate as the best source for growth and sporulation of *C. gloeosporioides.* Ekbote (1994) reported that *C. gloeosporioides* utilized potassium nitrate more efficiently and ammonium nitrate less efficiently for growth and sporulation which were contradictory to Manjunatha Rao and Rawal (2002) and Sangeetha and Rawal (2008) reported ammonium nitrate as a better source for the growth and sodium nitrate favoured better sporulation.

Mishra and Mahmood (1960) found abundant sporulation of *C. capsici* on the medium containing peptone as a nitrogen source. Tamil Vanan et al. (2005) reported that all the isolates of *C. capsici* produced maximum radial growth on sodium nitrate containing medium while ammonium nitrate supported least growth.

2.3.4. Physiological requirements for growth and sporulation of *Colletotrichum spp.*

2.3.4.1 Effect of temperature on growth and sporulation of *Colletotrichum spp.*

Effect of temperature on the growth of *C. gloeosporioides* in culture media was studied by different workers and reported the optimum temperature requirement for growth and its sporulation, temperature range for good growth, and inhibitory levels of temperature. There are also reports on the temperature required for spore germination and appressoria production.

Optimum temperature requirement for growth of *C. gloeosporioides* reported by different workers are as follows. Optimum temperature at 25˚C (Rajak, 1983), 25 - 30˚C (Sangeetha, 2003), 20 – 30 ˚C (Naik, 1985 and Ruchi Garg et al., 2007), 25 - 35˚C (Hegde et al ., 1990), 30˚C (Quimio, 1973; Hegde, 1986; Yee and Sariah,

1993; Zhou Hui Ping et al., 2008; Masyahit et al., 2009; Vinod Tasiwal and Benagi, 2009), 32˚C (Madan, 2004), 29˚C (Ekbote, 1994), 28 to 30˚C (Wasantha Kumara and Rawal, 2008).

Optimum temperature for the sporulation of *C. gloeosporioides* reported by different workers were as follows. Optimum temperature at 30˚C (Sattar and Malik, 1939; Quimio, 1973; Yee Sariah, 1993 and Zhang Hai Ying et al., 2007), 25 - 28˚C (Sangeetha, 2003; Wasantha Kumara and Rawal, 2008), 20 - 24 ˚C (Kendrick and Walker, 1948; Slade et al., 1987).

Temperature requirement in culture for attaining the maximum growth was also reported by earlier workers. Hegde (1986) reported that *C. gloeosporioides* achieved maximum growth of at 30 ˚C and good growth at 20 to 35˚C. Bainik et al. (1998) reported that growth was maximum at 28˚C. Sangeetha (2003) recorded maximum growth of different mango isolates of *C. gloeosporioides* at a temperature range of 25 - 30˚C. Ruchi Garg et al. (2007) reported that the maximum radial growth of the fungus was 20 to 30˚C.

For spore germination of *C. gloeosporioides* the ideal temperature was 30˚C (Quimio, 1973). Estrada et al. (1993) reported that 20˚C was optimum for the production of appressoria for one isolate of *C. gloeosporioides* from mango while the other isolate required optimum temperature of 25˚C. Temperature above 30˚C recorded an inhibitory effect on growth of *C. gloeosporioides* (Kendrick and Walker, 1948; Slade et al., 1987). Masyahit et al. (2009) also recorded that at 35˚C the mycelial growth of *C. gloeosporioides* was inhibited.

2.3.4.2. Effect of p^H on the growth and sporulation of *C. gloeosporioides*

pH requirement for optimum growth, maximum growth and optimum range for good growth of *C. gloeosporioides* were recorded by earlier workers. Lilly and Barnett (1951) reported that a medium having pH values between 5 and 6 at the time

of inoculation was suitable for most fungi. According to them, fungi generally tolerate more acid than alkali.

C. gloeosporioides could grew and sporulate at a wide range of pH from 3 – 8.5 (Tandon and Chandra, 1962). Hegde (1986) reported that *C. gloeosporioides* grew well between the pH range of 5.0 to 7.0. Madan (2004) reported pH 5 for excellent growth of *C. gloeosporioides.*

Optimum pH recorded for growth of *C. gloeosporioides* was 6.0 by Tandon and Chandra (1962); Hegde (1986). Masyahit et al. (2009) obtained optimum pH for growth of *C. gloeosporioides* was at 5.5. Maximum growth was obtained at pH 5.5 (Chaturvedi, 1965), at pH 6.5 Ekbote (1994). Maccheroni et al. (2004) and Wasantha Kumara and Rawal (2008) reported that *C. gloeosporioides* grew well at pH 5 while sporulation was better at pH 6.

Kumarswamy (1983) and Angadi (1999) studied different pH levels on *C. capsici,* of which pH 6.0 was found to be optimum for the fungal growth where maximum conidia were germinated.

2.3.4.3. Effect of light intensities on growth and sporulation of *C. gloeosporioides*

Chowdhuary (1936 a) and Mishra and Siradhana (1980) found that continuous light or darkness was found to inhibit sporulation of *C. graminicola* (Ces) G. Wilson, but cultures exposed to alternate light and darkness were found to sporulate earlier and more conspicuous. They also noted that disease was more when the pathogen was exposed to diurnal light compared to continuous light or darkness. Ahmed (1982) reported that alternate cycles of light and darkness favoured both growth and sporulation of *C. capsici.*

Kamanna (1996); Sudhakar (2000); Yoon and Park (2001); Ashoka (2005); Narendra Kumar (2006) and Vinod Tasiwal and Benagi (2009) observed that exposure of *C.gloeosporioides* to alternate cycles of 12 hrs light and 12 hrs darkness resulted in maximum growth.

2.4. Effect of environmental factors on the incidence of disease by *Colletotrichum spp.*

Environmental factors play a major role in the development of disease epidemics. Wastie (1972) reported that a temperature range of 20 – 30 ˚C as the optimum for lesion development of *Colletotrichum gloeosporioides* on rubber leaves. Sarma and Nambiar (1976) reported that although shot hole incidence was found in cocoa through out the year in Kasaragod district of Kerala, the intensity was higher when a temperature range of 19 – 33 ˚C and relative humidity (RH) of 77 – 98 percentage prevailed. The relationships among rainfall intensity, duration and crop geometry and the dispersal of inoculum possibly lead to different levels of disease severity (Dodd et al., 1992). Bainik et al. (1998) reported that the incidence of *C. gloeosporioides* on mango fruit increased at temperature between 28 - 34.2 ˚C and RH between 70 - 87.2 per cent. The duration of the surface wetness appears to have the most direct influence on the germination, infection and growth of the pathogen on the host. Temperatures around 27˚C and 80 per cent RH are optimum for anthracnose disease development in Pepper (Roberts et al., 2001).

Gupta et al. (1983) observed that maximum disease intensity by *Colletotrichum capsici* on Capsicum plants occurred in December when temperature was 18 ˚C and RH 75 per cent in the field. Deshmukh and Kurundkar (2002) suggested that a linear relationship worked out between the disease intensity on the leaves, branches and on fruits may be used to predict green fruit yield of chilli. Mehrotra and Aggarwal (2003) reported that continuous rain or high humidity is

capable of causing fruit rot of chilli caused by *C. capsici* occurred during the end of September and the beginning of October.

2.5. Antagonistic Microorganisms for controlling *Colletotrichum spp.*

Biological control is an effective, eco-friendly and alternative approach to chemical fungicides for any disease management practice. The most exhaustively studied microorganisms as biocontrol agent is *Trichoderma* spp. The antagonistic potential of *Trichoderma* sp. was first demonstrated by Weindling (1932) on *Rhizoctonia solani* Kuhn, a soil borne plant pathogenic fungus.

Many fungal and bacterial microorganisms have been isolated from the phyllosphere and rhizosphere of healthy plants and their antagonistic activity against the pathogen has been studied by earlier workers. Different species of *Trichoderma viz., Trichoderma viride*, *T. harzianum, T. pseudokoningii, Gliocladium virens* and *Aspergillus niger, Aspergillus flavus* and bacteria like *P. fluorescens, Bacillus subtilis* were found antagonistic against *C. gloeosporioides* and *C. capsici* under *in vitro* condition. The results of application of these antagonists in the field for managing the diseases caused by different species of *Colletotrichum* have also been studied.

The potential for biological control of *Colletotrichum gloeosporioides* had been suggested as early as in 1976 by Lenne and Parbery (1976). Deshmukh and Raut (1992) reported that *T. harzianum* Rifai and *T. viride* Pers. overgrew colonies of *C. gloeosporioides* and *T. harzianum* was more aggressive than *T. viride.* Antagonistic effect of *T. viride, T. harzianum* and *Aspergillus niger* against *C. gloeosporioides* was reported by Patel (2000) and Santha Kumari (2002). Patel and Joshi (2001) and Raheja and Thakore (2002) reported that *Gliocladium virens* and *T. koningii* showed more mycelial inhibition of *C. gloeosporioides* compared to bacterial antagonist. They opined that this can be attributed to higher competitive

ability of these *Trichoderma* spp. Patel (2004) and Bhave (2005) found that maximum per cent inhibition (84.44 %) each by *T. viride* and *T. harzianum* against *C. gloeosporioides* while *Bacillus subtilis* showed 61.11 % and *P. fluorescens* showed 60.00 per cent inhibition when the test fungus was placed at the centre. The high antagonistic potential of *Trichoderma harzianum, T. tolyposporium and T. pseudokoningii against C. gloeosporioides* causal agent of cashew anthracnose, was studied by Medeiros and Menezes (1994) and Jebessa and Ranamukhaarachchi (2006). Bhuvneswari and Rao (2001); Adil Hussain et al. (2008); Jadhav et al. (2008) and Jadhav et al. (2009) recorded that maximum per cent inhibition of *C. gloeosporioides* was seen in *T. viride* (84.26 %) followed by *T. harzianum* (74.81 %) when the pathogen was placed at the centre. There was no sporulation of test fungus in the treatments of *Trichoderma* spp. Watve et al. (2009) reported that under *in vitro,* maximum per cent inhibition of *C. gloeosporioides* in colony dia. was achieved by *T. harzianum* (83.33 %) followed by *T. viride* (77.78 %) and *Bacillus subtilis* (77. 78 %). Vinod Tasiwal et al. (2009) noticed that maximum percentage reduction in colony growth of *C. gloeosporioides* causing anthracnose of papaya was observed in *T. virens* (60.87 %) followed by *T. koningii* (53.32 %), *T. harzianum* (51.89 %), *Bacillus subtilis* (50.97 %) and *T. viride* (50.11 %). Least growth inhibition percentage was observed in *P. fluorescens* (42.87 %).

Sariah Meon (1994) reported that on Potato dextrose agar, *B. subtilis* induced 64.6 % and 67.2 % inhibition in mycelial growth of *C. capsici* and *C. gloeosporioides,* respectively. Jeyalakshmi et al. (1998) reported that among the fungal antagonists*, Saccharomyces cerevisiae* exhibited maximum reduction of mycelial growth of *C. capsici* followed by *T. viride*. while, *B. subtilis* showed the maximum growth reduction followed by *P. fluorescens* isolate 27. *T. hematum* was found as a better biological control agent against *C. capsici* followed by *T. viride, T. harzianum, Gliocladium virens, Bacillus sp.* and *P. fluorescens* (Pathania et al., 2004). The strong antagonistic activity of *T. hamatum* (94.08 %), *T. harzianum*

(82.59 %) and *T. viride* (82.32 %) against *C. capsici* was reported by Chirame and Padule (2005) and Ushakiran et al. (2006). Mandeep Kaur et al. (2006) recorded that non volatiles produced by the *T. viride* reduced the mycelial growth of *C. capsici* by 52.5 % followed by *T. virens* (38. 12 %). They observed that the volatile metabolites of *T. virens* caused maximum growth reduction (72.73 %) whereas metabolites of *T. harzianum* caused only 41.28 % inhibition. Xiao Ye et al. (2007) observed that in dual culture, *Trichoderma harzianum* made contact with the *C. capsici* and subsequently the pathogen colonies were either overgrown or invaded by *T. harzianum* leading to the inhibition in pathogen growth, along with reduction in spore concentration.

Trichoderma spp. are able to effectively compete for surface area, thereby reducing pathogen infection success (Jeffries and Koomen, 1992). Soytong et al. (2005) found that application of bioproducts of *Chaetomium, Penicillium* and *Trichoderma* and powder formulation of these bioproducts significantly reduced disease levels of *C. gloeosporioides* causing anthracnose disease in grapes and incidence on leaves, twigs, and fruits when compared to those in the control. Watve et al. (2009) reported that in jatropha *T. harzianum* recorded 24.74 PDI of leaf spot caused by *C. gloeosporioides* with 21.45 per cent disease reduction as compared to carbendazim (0.1 %) which recorded the lowest leaf spot intensity of 7.38 PDI with highest per cent reduction (76.65 %) *in vivo*.

Trichoderma spp. are able to effectively control *C. capsici* infection in chilli (Pratibha Sharma et al. 2005). They reported that partially purified toxin of *T. harzianum* @ 1 % gave the greatest reduction in disease intensity of chilli fruit rot and this treatment reduced post harvest fruit rot upto 89.8 % followed by sorghum base @ 0.2 % and talc base @ 0.4 % formulations of *T. harzianum*. Pandey and Pandey (2005) found that *T. harzianum* was best giving 76.7 per cent seedling emergence in chilli.

The rhizobacteria based bio formulation (*Pseudomonas fluorescens* + *Bacillus subtilis* + neem + chitin) was found to be the best for reducing the fruit rot incidence besides increasing the plant growth and yield parameters under both greenhouse and field conditions (Bharathi et al., 2004). Ekbote (2005) reported that chilli seedlings (40 day old) treated for 30 min. in *P. fluorescens* (1%) showed superior effect in reducing fruit rot and die back than Contaf 25 EC @ 0.1%, Bavistin 50 WP @ 0.1% and Kitazin 48 EC (iprobenfos) @ 0.1%.

Gupta et al. (1991) observed that the growth inhibition of *C. lindemuthianum* on frenchbean by *T. viride, T. harzianum* and *Gliocladium virens* were 88.33, 44.66 and 31.66 per cent, respectively under *in vitro* condition. Ravi et. al. (1999) found that among six species of *Trichoderma*, *Gliocladium virens, Bacillus subtilis* and *P. fluorescens* tested against *C. lindemuthianum* in dual culture, *T. viride* recorded the maximum inhibition of mycelial growth followed by *T. harzianum*. Ravi et al. (2000) observed that both *T. viride* and its culture filtrate caused maximum inhibition of mycelial growth of *C. lindemuthianum.* He also observed that the culture filtrate exerted the maximum inhibition of *C. lindemuthianum* spore germination. Laxman (2006) found that among fungal bioagents tested, *T. harzianum* was the most effective for growth suppression of *C. truncatum* followed by *T. viride,* whereas among the bacterial antagonist *Bacillus subtilis* TNAU isolate showed maximum mycelial growth suppression. Begum et al. (2007) reported that *T. harzianum* was a strong inhibitor of *C. falcatum* under *in vitro* conditions.

2.5.1. Mechanism of parasitism of *Trichoderma spp.*

Brian and Mc Grawn (1945) reported that *T. viride* produced viridin which is a fungistatic antibiotic. Relationship between mycolytic enzymes, chitinases, β – 1, 3 glucanases produced by mycoparasitic fungi and their significance in fungal cell wall lysis and degradation have been well established (Elad et al., 1980). *Trichoderma hamatum* grow towards host hyphae and after contact, formed coils and

appressoria like structures from which penetration occurred (Chet et al., 1981). Papavizas (1985) reported that *Trichoderma* and *Gliocladium* were not only good sources of various toxic metabolites and antibiotics, but also of various enzymes as exo and endo glucanases, cellobiases and chitinases. Morshed (1985), Gupta et al. (1991) and Ravi et al. (2000) demonstrated the antagonism between *Trichoderma spp.* and *C. lindemuthianum.* They also noted that growth of *T. viride* was vigorous in dual culture and it effectively hyper parasitized the pathogen by penetrating and coiling its hyphae around *C. lindemuthianum* hyphae. Zeppa et al. (1990) reported that the volatile metabolites produced by *T. viride* were lactones, alcohols and terpene derivatives. Reeny (1995) reported that *T. viride* controlled *C. capsici* in vegetables by penetration of hyphal cells. A new tetracyclic diterpene $C_2OH_28O_2$ was isolated from the culture filtrate of a strain of *T. viride* that exhibited antifungal activity against *S. rolfsii* (Mannia et al., 1997). Godwin – Egein and Arinzae (2001) reported that the mechanisms of antagonism employed by *Trichoderma harzianum* against *F. oxysporum* were competition, lysis and hyperparasitism. Studies conducted by Umamaheshwari et al. (2002) showed that the volatile metabolites produced by *T. viride* was effective against *S. rolfsii,* the pathogen of jasmine wilt and the percentage disease inhibition was 61.11 per cent. *Trichoderma harzianum* isolate found to coil around and penetrate the hyphae of *C. gloeosporioides* (Anoop, 2002). Poornima (2007) reported the effectiveness of *Trichoderma harzianum* and *A. niger* against *C. gloeosporioides* causing leaf spot of Thippali.

2.5.2. Seed treatment with biocontrol agents

T. harzianum showed increase in seed germination and seedling vigour of lettuce seeds (Gopinath and Shetty, 1992). Sankar and Jeyarajan, (1996) reported that seed treatment with formulation of *Trichoderma* and *Gliocladium* controlled *Macrophomina phaseolina* in sesamum. Sorghum seeds treated with formulation of *T. harzianum* and *P. fluorescens* increased the field emergence (Raju et al., 1999).

Srinivas et al. (2006) reported that pure culture of *P. fluorescens* @ 1×10^8 cfu/g reduced the incidence of *Colletotrichum capsici* by 38 per cent whereas talcum powder formulation reduced the incidence by 22 % and 29 % @ 5g/kg and 10g/kg seeds respectively. Pure culture of *T. harzianum* @ 1×10^8 cfu/g reduced the incidence of *C. capsici* by 24 per cent whereas talc based formulation reduced the incidence of *C. capsici* by 12 % and 18 % @ 5g/kg and 10g/kg seeds respectively. He also observed that treatment of chilli seeds with pure culture of *P. fluorescens* @ 1×10^8 cfu/g increased the germination by 18 % whereas the talcum powder formulation of the same increased the germination by 13 % and 15 % @ 5g/kg and 10g/kg seeds respectively. Pure culture of *T. harzianum* @ 1×10^8 cfu/g increased the germination by 10 % and the formulation of the same increased the germination by 5 % and 7 % @ 5g/kg and 10g/kg seeds respectively. *P. fluorescens* pure culture @ 1×10^8 cfu/g increased the seedling vigour of chilli seeds by 13 per cent and talcum powder formulation increased the vigour by nine per cent and 11 % @ 5g/kg and 10g/kg seeds respectively. *T. harzianum* pure culture increased the vigour by seven per cent and the formulation by 4 % and 5 % @ 5g/kg and 10g/kg seeds respectively.

2.5.3. Mass multiplication of *Trichoderma spp.*

Papavizas et al. (1984) reported that for the biological control of plant pathogens, it is necessary to mass produce the promising antagonists rapidly in the form of spores, mycelia or mixture. The most suitable temperature to prolong shelf life of conidia and fermentor biomass propagules in pyrophyllite were -5 to 5°C (Mukherjee, 1991). Longer shelf life of *T. viride* was recorded with vermiculite wheat bran acid fermentor biomass formulation stored in milky white bags @ 2.05×10^6 cfu/g (Nakkeeran and Devi, 1997). The superiority of PDA over molasses, brewer's yeast etc. as a medium for biomass production was demonstated by Prasad et al. (1997). Talc and Kaolin were identified as better carriers of *T. harzianum* (Prasad and Rangeshwaran, 2000). Prasad et al. (2002) reported that conidial

formulation in talc based carrier retained optimum amounts of viable propagules (> 10^6 cfu/g) even after 180 days of storage at room temperature. Cowdung or FYM is an excellent substrate for mass multiplication of *T. harzianum* (Zaidi and Singh, 2004). Das et al. (2006) reported that talc based formulation exhibited a gradual declining trend in multiplication and sporulation of *T. harzianum* from 30 days onwards. Gade et al. (2008) reported that among the four different carrier materials tested talc was found as the best to retain maximum number of viable propagules of *Trichoderma spp*. upto 180 days of storage.

2.5.4. Method of application of *Trichoderma spp.*

Formulated products of biocontrol agents were applied to the plants by different methods. Aqueous suspension of conidia of *T. viride* and *T. polysporum* (Link ex Pers.) Rifai were used for spraying the plants in the field at early flowering to control storage rot in strawberry (Tronsmo and Dennis, 1977). De Oliveira et al. (1984) reported that the application of *T. harzianum* to the soil as conidial suspension @ 10^6 conidia per ml during the transplanting period significantly reduced the severity of white rot of garlic caused by *Sclerotium sepivorum* Berk. Foliar application of *T. harzianum* @ 10^8 spores per ml reduced the leaf spot of wheat caused by *Cochliobolus sativus* (Ito and Kuribayashi) Drechs. Ex. Dast. (Biles and Hill, 1988). Foliar spray of *T. harzianum* strain T -22 controlled powdery mildews on Catharanthus and pumpkins and *Botrytis cinerea* Pers. on strawberry and grapes (Harman, 2000). Sprays of *Trichoderma* spp. at ten days interval against web blight of urd bean decreased the disease severity (Sharma and Tripathi, 2001). Anoop (2002) reported that foliar as well as soil application of *T. harzianum* and *A. niger* were effective in reducing anthracnose of black pepper. The effect of antagonist alone and in combination with plant extract and chemicals was studied by Chandrasekaran and Rajappan (2002). They reported that individually *T. viride* at 0.4 per cent showed 50 and 52 per cent disease index of leaf anthracnose and pod blight

respectively but in combination with *Lawsonia inermis* at one per cent, alum at 0.1 per cent and *Trichoderma* at 0.4 per cent through seed treatment and foliar spray recorded 11 and 6 per cent PDI of leaf anthracnose and pod blight respectively. Dubey (2002 b) reported that foliar spray of *T.virens* and *T. viride* were efficient in increasing the grain yield in urd and mung beans. A significant increase in root length and number of root nodules over control were also observed. *T. viride* was applied as seed treatment (2g/kg seed), soil amendment and foliar spray against *R. solani* causing web blight of urd and mung bean (Dubey and Singh, 2004).

2.6. Plant extracts in management of diseases caused by *Colletotrichum spp.*

Shahidul Alam et al. (2002) revealed that out of the ten plant extracts tested, *Tagetes erecta* leaf and *Azadirachta indica* bark extracts were the most effective in inhibiting conidial germination of *C. gloeosporioides* after 5 - 30 min. of immersion and in 5 : 1.25 (w/v) concentration. But in the case of *Datura metel* seed extract, 50 % conidial germination of *C. gloeosporioides* was inhibited after 30 min. of immersion. Prabhakar et al. (2003) found that plant extracts of *Adenocalyma alleceum* and *Bougainvillea spectabilis* were most effective in inhibiting *C. gloeosporioides* followed by *Abutilon indicum, Acalypha indica*, *Agiratum sp., Allium cepa, Ocimum sanctum* and *Terminalia cepula.* Ogbebor et al. (2007) revealed that extracts of *Ocimum basilicum* L. and *Allium sativum* L. exhibited total inhibitory effects on the mycelial growth of *C. gloeosporioides* and on *in vivo* evaluation showed that treatment with 100 % *Ocimum basilicum* resulted in disease index of 31.7 % which was significantly lower than the control 65 % disease index at five per cent level of probability. Jadhav et al. (2008) reported that out of the seven plant extracts tested maximum per cent inhibition of *C. gloeosporioides* was achieved by *Allium sativum* extract at 10 per cent. Vinod Tasiwal et al. (2009) reported that among the nine plant extracts tested against *C. gloeosporioides, Lantana camara* at 7.5 per cent was found to be superior (45.54 %) followed by

turmeric at 7.5 per cent (40.73 %), onion at 7.5 per cent (38. 23 %), neem (kernel) at 7.5 per cent (32.33 %) and neem (leaf) at 7.5 per cent (23.70 %). Watve et al. (2009) reported neem leaf extract recorded maximum inhibition (78.15 %) against *C. gloeosporioides* followed by garlic (58.89 %), sadafuli (57.04 %), tulsi (55.93 %), onion (45.66 %) and Bougainvillea (40.37 %).

Sunil Kumar and Yadav (2007) reported that Phytoextract of *Azadirachta indica* (4 %) and *Allium sativum* (4 %) completely inhibited the growth and sporulation of *C. capsici* and *C. gloeosporioides* whereas *Datura stramonium* at this conc. showed only 67 per cent growth inhibition.

Shivapuri Asha et al. (1997) reported that leaf extracts of *Azadirachta indica, Allium sativum, Ocimum sanctum, Datura stramonium, Polyalthia longifolia, Vinca rosea* and *Withania somnifera* were effective in controlling *C. capsici.* Suhaila Mohamed et al. (1999) reported that ethanolic extracts of *Piper betle* between 0.01-1.0 mg ml^{-1} showed more stronger antifungal activity against seven fungi including *C.capsici* than 2.5 mg ml^{-1} prochloraz or 10 mg ml^{-1} clotrimazole . Gomathi and Kannabiran (2000) reported that out of the 23 aqueous leaf extracts screened against *C. capsici* and *Gloeosporium piperatum* infecting *Capsicum annuum*, the leaf extracts of *Solanum torvum, Datura metel* and *Prosophis juliflera* effectively inhibited the conidial germination and mycelial growth. Gurudatt Hegde et al. (2001) found that among the plant extracts *viz.*, *Ocimum*, Neem, Onion and Clerodendron @ 0.3 %, application of onion bulb extracts showed lowest spore germination and hence highest inhibition of *Colletotrichum capsici*. Roat et al. (2009) observed that among the seven partially purified plant products screened against *C. capsici in vitro* and *in vivo*, maximum inhibition of mycelial growth and spore germination was reported by *Bitter temru* fruit and *Datura stramonium* leaves.

Jeyalakshmi and Seetharaman (1998) reported that Palmarosa (*Cymbopogon martinii*) oil was the most effective one for reducing the fruit rot and dieback of chilli

followed by *Ocimum sanctum* leaf extract and neem oil. Saxena (1999) reported the efficacy of neem (*Azadirachta indica),* garlic (*Allium sativum*) and Tagak tagak (*Rhinocanthus nasuta)* at 5000 ppm on *Capsicum annuum* anthracnose was comparable with the fungicide carbendazim (Bavistin) at 100 ppm. Garlic extract performed well under room humidity, while Tagek tagek extract showed good control of chilli anthracnose under high moisture conditions. Neem extract minimized the 'ripe chilli fruit rot'. Bagri et al. (2004) reported that maximum reduction in severity of fruit rot was recorded with Emcop L (67.7 %) followed by Bitter temru fruit extract (66.4 %) and Datura leaf extract (53.3 %) at 0.1 % concentration in potted plants. Rahman et al. (2005) reported that among the botanicals, garlic extract was the most effective one to control *C. capsici*, followed by neem and ginger extract. Raj et al. (2006) found that among the plant products *viz., Allium sativum* (20 %), *Datura metel* (60 %), *Eucalyptus globulus* (60 %) and *Prosopis juliflora* (60 %), *A. sativum* recorded minimum disease incidence (24.1 %) followed by *E. globulus* (27.8 %) against chilli fruit rot caused by *C. capsici.*

The fungicidal spectrum of neem, *Azadirachta indica* has been attributed to Azadiractrachin which belongs to C 25 terpeniodes (Subramanian, 1993). Sawant (1999) observed that maximum inhibition of mycelial growth of *Colletotrichum lagenarium* was obtained by the extract of *Piper betle, Lawsonia inermis* and *Azadirachta indica*. Amadioha (2003) reported that extracts of *Piper nigrum* was the best in reducing the growth of *Colletotrichum lindemuthianum* in culture and in controlling the spread of the anthracnose disease of cowpea in the field followed by *Ocimum sanctum*. Sachin Gupta et al. (2005) reported that under *in vitro*, *Azadirachta indica* at 20 % was found to be the most effective plant species against *C. lindemuthianum* followed by *Murraya koenghii, Tagetes erecta, Allium cepa, Allium sativum*, *Ocimum sanctum* and *Lantana camara.* Anil Gupta et al. (2008) recorded that among four aqueous plant extracts, *viz.,* neem leaf, aonla leaf (*Phyllanthus emblica*), bougainvillea flower and marigold leaf (*Tagetes erecta*)

evaluated against *Colletotrichum lagenarium* by spore germination inhibition test inferred that neem leaf extract gave maximum spore (58.43 %) and mycelial growth inhibition (79.43 %), which was better than the fungicide mancozeb (0.1 %).

Ravi et al. (2000) found that botanicals (10 %) *viz.,* leaf extract of *L. inermis*, *Adenocalymma alliaceae*, *Azadirachta indica* tested against *C. lindemuthianum* performed better than 0.4 % talc formulations of *T. viride, P. fluorescens* and fungicides carbendazim (0.2 %) and mancozeb (0.4%). Anil Gupta et al. (2008) reported that under field conditions neem leaf extract at 50 % concentration gave significantly higher anthracnose disease control (79.29 %) as compared to untreated control and was on par with fungicide mancozeb (0.1 %).

2.7. Use of plant products in controlling *Colletotrichum spp.*

Amonkar and Baberhu (1971) and Singh and Singh (1980) reported that the active principle of *A. sativum* is a mixture of diallyl disulphide and diallyl trisulphide. Datar (1999) found that out of two rhizome and two bulb extracts tested against *Macrophomina phaseolina*, the extract of *Allium sativum* was most effective for controlling charcoal rot of Sorghum. Dubey (2002 a) reported that oil cakes especially groundnut cake and plant extracts were very effective for the control of web blight of urd and mung bean. Turmeric powder and sodium bicarbonate in 10:1 proportion was found inhibiting the growth of *Rhizoctonia solani* causing leaf blight of amaranthus (Priyadarsini, 2003). Eucalyptus oil (61.93 %), Garlic (60.13 %) and neem (57.14 %) were found most promising botanicals against *C. truncatum*, which showed higher inhibition of mycelial growth at 10 per cent concentration (Laxman, 2006). Kota et al. *(*2006) reported that maximum inhibition of mycelial growth and spore germination of *C. gloeosporioides* was observed in garlic bulb extract at 10 % concentration. Mina Koche et al. (2009) reported that under *in vitro*, *Azadirachta indica* seed extract (5 %) was effective among botanicals to check the mycelial growth of *C. dematium* (74.69 %).

2.8. Use of fungicides for controlling *Colletotrichum spp.*

Carbendazim and Benomyl being Benzimidazole group fungicide, they interfere with energy production and cell wall synthesis of fungi (Nene and Thapliyal, 1982). According to Davidse (1986) carbendazim induced nuclear instability by disturbing the mitosis and meiosis.

Jadhav et al. (2002) reported that Bordeaux mixture, mancozeb and carbendazim were effective in controlling mycelial growth of *C. gloeosporioides* causing leaf blight of papaya. Patel and Joshi (2002) found that carbendazim, propiconazole and difenconazole were effective against *C. gloeosporioides* causing leaf spot of turmeric. Karande et al. (2007) evaluated six fungicides against *C. gloeosporioides* and reported that bavistin, copper oxychloride and mancozeb completely inhibited the mycelial growth. Amarjit Singh et al. (2008) revealed that *in vitro* evaluation of carbendazim @ 100 µg/ml was the most effective fungicide for complete inhibition of the mycelial growth of *C. gloeosporioides*. Jadhav et al. (2008) reported that mancozeb + carbendazim (0.25 %), propiconazole (0.1 %), carbendazim (0.1 %), tricyclazole (0.15 %) showed 100 per cent inhibition of the *C. gloeosporioides*. Watve et al. (2009) found that best inhibition of growth and sporulation of *C. gloeosporioides* causing leaf spot of jatropha (100 per cent) under *in vitro* condition was observed in carbendazim (0.1 %), propiconazole (0.1 %), difenconazole (0.1 %) and copper oxychloride at 0.3 % concentration. Vinod Tasiwal et al. (2009) observed that 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 %).

Amarjit Singh et al. (2008) reported that under field conditions, Bavistin (0.1 %) was highly effective against *C.gloeosporioides* both on detached guava fruits as well as on fruits in field. Arasumallaiah and Rangaswamy (2008) revealed that among the six fungicidial treatments used to manage *C. gloeosporioides* in cashew,

spraying with chlorothalonil at 0.2 % effectively reduced the disease incidence to 7.64 %, followed by copper oxychloride at 0.3 % and carbendazim at 0.1 %, which reduced the disease to 7.97 and 8.17 % respectively.

Anil Kasyap et al. (2008) revealed that calixin (0.2 %), copper hydroxide (XL – 77) (0.3 %), copper hydroxide (XL – 57) (0.3 %) and mancozeb (0.3 %) completely inhibited the mycelial growth of *C. capsici*. This was followed by carbendazim (0.1 %) and chlorothalonil (0.3 %).

Kumar and Mahmood (1986) tested 12 different fungicides as seed treatment to control *C. capsici* causing die back and fruit rot of chilli and found that bavistin was best in controlling *C. capsici* and enhanced seed germination. Foltaf @ 0.2 % gave the most effective control of anthracnose and fruit rot of chilli followed by Fytolan @ 0.25 % and Bavistin at 0.1 % (Jayasekhar et al., 1987). Perane and Joi (1988) reported that combined seed treatment with Bavistin (Carbendazim) 0.2 % or Thiram (TMTD) 0.2 % followed by sprays of Dithane M - 45 (Mancozeb) 0.25 % or Bavistin 0.1 % were found most effective in reducing the incidence of fruit rot and die back of chilli and there by increasing the yield. Sulochana et al. (1992) reported that application of 1 % Bordeaux mixture, 0.3 % Ziride (Ziram) and 0.1 % Bavistin were equally effective in controlling the fruit rot of chilli. Bavistin at 0.1 % when applied once in nursery and again at one month and two months stage after transplanting gave the best control of anthracnose and ripe rot of chilli (Biswas, 1992). Sally et al. (1994) reported that seed treatment with Bavistin @ 0.05% followed by four sprays of Bavistin (0.05%) or Fytolan (0.3%) at three weeks interval 15 days after transplanting were effective in reducing the disease intensity of fruit rot of chilli. Best control of fruit rot and dieback of chilli was achieved with mancozeb at 0.2 % followed by carbendazim 0.2 % and fytolan (COC) 0.2 % (Ebenezar and Alice, 1996). Kumawat (1997) reported that the use of three sprays of mancozeb @ 0.25 % at 15 days interval showed highest reduction in chilli

anthracnose disease incidence, followed by thiophanate methyl, ziram and carbendazim. Hingole and Kurundkar (2004) reported that mancozeb + metalaxyl 72 WP (0.25 %) was the best in controlling *Colletotrichum capsici* and was significantly superior over the rest of fungicides. This was followed by propiconazole 10 EC (0.05 %) and carbendazim 50 WP (0.1 %), which was the most economical and profitable fungicide giving a cost: benefit ratio of 1: 15.36 as compared to the cost : benefit ratio of 1 : 2.92 in mancozeb + metalaxyl treatment. Raj et al. (2006) indicated that carbendazim (0.1 %) recorded minimum disease incidence of fruit rot of chilli (17.7 %) and gave the highest increase in fruit yield of 52.6 % over the untreated control.

Thakur and Khare (1990) reported that the best control of *Colletotrichum dematium* and *C. lindemuthianum* on *Vigna radiata* and highest yields were obtained in sprays of carbendazim (0.1 %) and triforine (0.15 %) in comparative field tests with 7 fungicides. Differences in carbendazim sensitivity of different isolates of *Gloeosporium ampelophagum* (Kumar and Thind, 1992) and other fungi to metalaxyl warrants that site specific systemic fungicides should be used with caution. Sharma Abhishek and Verma (2007) reported the efficacy of mancozeb, carbendazim and calixin *in vitro* for reducing disease intensity of anthracnose and fruit rot of mango. Mina Koche et al. (2009) reported that among the fungicides propiconazole (0.1 %), hexaconazole (0.1 %), penconazole (0.1 %) and carbendazim (0.1 %) were effective to inhibit the growth of *C. dematium.*

2.9. Growth promotion by antagonist *Trichoderma spp.*

The enhanced growth of tomato seedlings by *Trichoderma* treatment alone was reported by Kleifeld et al. (1983). The *T. harzianum* isolates stimulates plant growth even in the apparent absence of the pathogens because it interact directly with the plant by producing plant growth promoting active metabolites without interacting with pathogens (Windham et al. 1986). Mechanisms like production of hormone like metabolites and release of nutrients from soil organic matter and making it available

to plants by *Trichoderma* spp. were found to be involved in causing enhanced plant growth in different crops (Kleifeld and Chet, 1992; Altomare et al., 1999; Prasad et al., 2002). Inbar et al. (1994) observed that *T. harzianum* treated seedlings grow vigorously with higher chlorophyll content and were more resistant to damping off caused by *Pythium sp.* and *R. solani*. Singh et al. (1997) recorded that the growth of chickpea roots, shoots and leaves were enhanced in the presence of different fungal antagonists, with maximum growth in soil inoculated with *T. harzianum.* Increase in seedling and plant growth of chickpea due to soil application of *T. harzianum* prior to sowing was also reported by Sharma et al. (1999). Ganesan et al. (2000) found that application of *Trichoderma* significantly increased root dry weight in pepper cuttings when compared to untreated control. Karpagavalli and Ramabadran (2001) found that the application of *T. viride* and *T. harzianum* along with seed reduced the damping off incidence and improved the growth of root and shoot length and also dry matter production of tomato seedlings. Anith and Manomohandas (2001) reported that the combined application of *T. harzianum* and *Alcaligenes* also resulted in enhanced shoot weight in pepper. In addition to disease suppression, treatment with *T. harzianum* showed better yield in betelvine (Singh and Singh, 2002). Dubey (2002 b) reported that foliar spray of *T. virens* and *T. viride* increased the grain yield in urd and mung beans. A significant increase in root length and number of root nodules over control were also observed. Anandaraj et al. (2003) found that application of two strains of *P. fluorescens,* a strain of *Bacillus* and *T. harzianum* significantly enhanced growth of black pepper which resulted in increased number of nodes and consequently cuttings. The increase was 44.70 per cent over control for *P. fluorescens* and 38.90 per cent for treatment with a consortium of *P. fluorescens* and *T. harzianum.* Priyadarsini (2003) reported that the fungal antagonist *T. harzianum* enhanced the growth of amaranthus and successfully managed Rhizoctonia leaf blight disease.

MATERIALS

AND

METHODS

3. MATERIALS AND METHODS

The present investigation entitled "Ecofriendly management for fruit rot of Chilli (*Capsicum annuum* L.) caused by *Colletotrichum* spp." was carried out at College of Agriculture, Vellayani during the period 2008 – 2010. Chilli variety Vellayani Athulya was used in the study.

3.1. ISOLATION OF THE PATHOGEN

Chilli fruits showing initial stages of infection were collected from the crop museum of the College of Agriculture, Vellayani and used for the isolation of the organism. Isolations were done frequently from February 2009 to April 2010. The infected parts were cut into small bits, surface sterilized with 0.1per cent mercuric chloride solution for one min. and washed in three changes of sterile distilled water. These bits were placed on solidified Potato Dextrose Agar (PDA) medium in sterilized petri dishes. The dishes were incubated at room temperature (28 ± 2˚C). After three days, when the growth of the fungus was visible, mycelial bits were transferred aseptically to PDA slants. The slants were incubated at room temperature. Pure culture of the fungus was made by single spore isolation. From the isolations conducted, two fungal cultures showing slightly different cultural characters were obtained. The cultures obtained during the month of March 2009 and August 2009 were named as C_1 and C_2 respectively. Studies on morphological and cultural characters were conducted on both the cultures while studies including the nutritional and physiological factors, *in vitro* and *in vivo* management were conducted using the culture C_1. C_1 was the frequently obtained culture which is used throughout the study.

3.1.1. Single spore isolation

C_1 and C_2 cultures obtained after isolation were purified by single spore isolation (Johnston and Booth, 1983). Ten ml of two per cent water agar was poured

into sterile petri dishes and allowed to solidify. Dilute spore suspension was prepared in sterilized distilled water from seven days old culture. One ml of the suspension was spread uniformly on agar plate. These plates were incubated at 28 ± 2˚C for 12 h. The plates were examined under microscope to locate single isolated and germinated conidium and marked with ink on the surface of the dishes.

The growing hyphal tip portion was cut with the help of a cork borer under aseptic conditions and with an inoculation needle it was carefully transferred to PDA slants and incubated at 28 ± 2˚C. This culture was used for further studies.

3.1.2. Proving the pathogenicity

Pathogenicity of the two fungal cultures (C_1 and C_2) were proved following Koch's postulates. Chilli fruits grown in the field as well as those kept in the lab were artificially inoculated with seven day old culture of the fungus. Inoculated fruits were covered with polythene bags to maintain high humidity for disease development. After the symptoms were expressed, the fungus was reisolated from the fruit portions exhibiting typical symptoms of the disease and the morphological and cultural characters were studied.

3.1.3. Maintenance of the culture

The isolates were subcultured on PDA slants and allowed to grow at room temperature for seven days. The slants were kept in refrigerator at 5˚C. Subculturing was done once in a month. This culture was maintained and used throughout the study. Virulence of the fungus was maintained by passing through the host after every three months.

3.2. SYMPTOMATOLOGY

Symptoms of disease caused by both the cultures were studied by observing the naturally infected chilli fruits and also following the course of development of the disease under artificial inoculation.

3.3. MORPHOLOGICAL AND CULTURAL CHARACTERS OF THE PATHOGEN

3.3.1. Morphological characters

3.3.1.1. Colony characters

Five mm discs taken from both the fungal cultures C_1 and C_2 (of an actively growing seven day old sporulated culture) were inoculated separately at the centre of a 90 mm petri plate with sterile PDA and incubated at room temperature. Colony colour and characters were recorded after seven days.

3.3.1.2. Conidial characters

Size of the conidium, acervuli and setae of both the cultures were measured with a standardized microscope using micrometer. The average size and shape of 100 conidia were taken.

3.3.2. Cultural characters

3.3.2.1. Growth and sporulation of *C. gloeosporioides* (C_1) and *C. capsici* (C_2) on different solid media

The following culture media were used to study the growth of the fungi.

1. Richards' Agar (RA)
2. Potato Dextrose Agar (PDA)
3. Sabouraud's Agar (SA)

4. Czapek – Dox Agar (CDA)
5. Brown's Agar (BA)
6. Fries' Agar (FA)
7. Host Extract Agar (HEA)
8. Host Extract Dextrose Agar (HEDA)

The composition of the media used are given under Appendix I.

The media were prepared and sterilized by autoclaving at 15 pounds pressure for 20 min. which corresponds to a temperature of 121.6˚C. Fifteen ml of each medium listed above was poured into 90 mm dia. petri plates. After solidification, five mm discs of *C. gloeosporioides* from an actively growing culture were cut using a cork borer and a single disc was placed upside down at the centre of the petri dish. Each set of experiment was replicated thrice and the plates were incubated at room temperature. The measurement of the colony dia. was taken when the maximum growth was attained in any one of the media tested. Cultural characters such as colony dia., colony colour, type of margin and sporulation were also recorded.

The sporulation was categorized as follows.

Sl. No	Score	Category	Description
1	++++	Excellent	> 150
2	+++	Good	101 - 150
3	++	Fair	51 - 100
4	+	Poor	1 - 50
5	-	No sporulation	0

3.3.2.2. Growth and sporulation of *C. gloeosporioides* (C_1) and *C. capsici* (C_2) in different liquid media

The composition and preparation of different liquid media used were the same as that of solid media except that agar was not added. Thirty ml of different liquid media were added into each of 100 ml conical flasks. These flasks were then sterilized at 15 pounds pressure for 20 min. which corresponds to a temperature of 121.6˚C. The flasks were inoculated with five mm mycelial discs taken from periphery of seven day old culture and incubated at room temperature for 15 days. Each treatment was replicated thrice. The mycelia was filtered out and the dry mycelial weight was determined after drying the mycelia in an oven at 60 ˚C till constant weight attained. Sporulation in each treatment was recorded as described earlier.

3.3.2.3. Production of toxic metabolite by *C. gloeosporioides* (C_1) and *C. capsici* (C_2) in different liquid media

3.3.2.3.1. Effect of various media on toxic metabolite production of *C.gloeosporioides* on chilli fruit

The following liquid media were tested to assess their comparative merits in supporting the production of toxic metabolites by *C. gloeosporioides* (C_1).

1. Richards' Broth (RB)
2. Potato Dextrose Broth (PDB)
3. Czapek – Dox Broth (CDB)
4. Sabouraud's Broth (SB)
5. Host Extract Dextrose Broth (HEDB)
6. Fries' Broth (FB)

Thirty ml of each medium was taken in 100 ml conical flasks and sterilized by autoclaving at 15 pounds pressure for 20 min. The medium was inoculated with five mm disc of seven day old growth of *C. gloeosporioides*. For each treatment three replications were kept. After 15 days of incubation at room temperature, the culture was filtered through Whatman No. 42 filter paper. The comparative toxic activity of each of the filtrate was studied using the following bioassay technique.

Chilli fruits of uniform maturity were collected and surface sterilized by dipping in 0.1 per cent $HgCl_2$ solution for one min. followed by three washings with sterile distilled water. Culture filtrate (0.05 ml) was placed on the fruit after giving pin pricks. Control was kept by using sterilized water. The treated fruits were placed separately in sterilized polythene bags. A piece of moist cotton swab was placed inside the bag and mouth of the bag was tied. Pin pricks were given on the bag for aeration. The bagged fruits were incubated at room temperature for 72 h. Each treatment was replicated four times. The lesion size produced on the fruit were measured and recorded.

3.3.2.3.2. Effect of various media on toxic metabolite production of *C. capsici* on chilli fruit

The following liquid media were tested to assess their comparative merits in supporting the production of toxic metabolites by *C. capsici* (C_2).

1. Richards' Broth (RB)
2. Potato Dextrose Broth (PDB)
3. Czapek – Dox Broth (CDB)
4. Sabouraud's Broth (SB)
5. Host Extract Dextrose Broth (HEDB)
6. Fries' Broth (FB)
7. Host Extract Broth (HEB)
8. Brown's Broth (BB)

Thirty ml of each medium was taken in 100 ml conical flasks and sterilized by autoclaving at 15 pounds pressure for 20 min. The medium was inoculated with five mm disc of seven day old growth of *C. capsici* separately. For each treatment three replications were kept. After 15 days of incubation at room temperature, the culture was filtered through Whatman No. 42 filter paper. The comparative toxic activity of each of the filtrate was studied using the following bioassay technique.

Chilli fruits of uniform maturity were collected and surface sterilized by dipping in 0.1 per cent $HgCl_2$ solution for one min. followed by three washings with sterile distilled water. Culture filtrate (0.05 ml) was placed on the fruit after giving pin pricks. Control was kept by using sterilized water. The treated fruits were placed separately in sterilized polythene bags. A piece of moist cotton swab was placed inside the bag and mouth of the bag was tied. Pin pricks were given on the bag for aeration. The bagged fruits were incubated at room temperature for 72 h. Each treatment was replicated four times. The lesion size produced on the fruit were measured and recorded.

3.3.2.3.3. Exo and Endo toxic metabolite production of *C. gloeosporioides* and *C. capsici*

The fungus was grown on liquid Richards' medium for a period of 15 days at room temperature and the mycelial growth was filtered out through a previously weighed Whatman No. 1 filter paper. The mycelium was homogenized by adding water five times its weight (W/V). This homogenized mycelium was centrifuged at 1000 rpm for 15 min. The supernatant solution was taken and the pellets were discarded and again centrifuged at 1000 rpm for 15 min. The supernatant after second centrifugation was used as 'endo toxin' and the culture filtrate as 'exo toxin'. These two test solutions (endo toxin and exo toxin) were assayed using chilli fruits as mentioned in 3.3.2.3.1.

3.3.2.4. Compatibility of *C. gloeosporioides* and *C. capsici* on PDA under *in vitro* condition

PDA media was prepared and sterilized by autoclaving at 15 pounds pressure for 20 min. Fifteen ml of PDA medium was poured into 90 mm dia. petri plates. After solidification, agar disc of five mm dia. was cut from the edge of a vigorously growing seven day old sporulated culture of *C. gloeosporioides* and this was placed in the media two cm away from the periphery of petri plates. On the opposite side, two cm away from the periphery, five mm sized one week old culture disc of *C. capsici* cut from a vigorously growing culture was placed. Three replications were maintained. The plates were incubated at room temperature for six days and observations were made.

3.3.2.5. Combined and individual infection of *C.gloeosporioides* and *C. capsici* on detached chilli fruits

The healthy, uniformly matured detached chilli fruits of varieties Jwalamukhi, Jwalasakhi, Ujwala, Vellayani Athulya and Anugraha were screened against *C. gloeosporioides* and *C. capsici*. The detached fruits were surface sterilized by dipping in 0.1 per cent $HgCl_2$ solution for one min. followed by three washings with sterile distilled water. The treatments were as follows:

1) Combination of *C. gloeosporioides* and *C. capsici*
2) *C. gloeosporioides* alone
3) *C. capsici* alone

Each treatment was replicated three times. The fruits were artificially inoculated with the culture of the pathogen. For this conidial suspension of *Colletotrichum gloeosporioides* (10^6 conidia / ml) and *C. capsici* (10^6 conidia / ml) were taken from ten day old culture grown on Richards' broth medium. After giving

few injury by pin pricking on the fruits, the spore suspension of the pathogen was placed over the pin pricked area. The treated fruits were placed separately in sterilized polythene bags. A piece of sterilized moist cotton swab was placed inside the bag and mouth of the bag was tied. The perforated bagged fruits were incubated at room temperature. Lesions produced on the fruits were observed on the fourth day by taking the average of the dia. of lesions in two directions perpendicular to each other.

Further studies on growth phase, nutritional and physiological factors, *in vitro* and *in vivo* management were carried out using the frequently obtained culture *C. gloeosporioides* (C_1) only.

3.3.2.6. Growth phase of *C. gloeosporioides* (C_1) in liquid media

To study the growth phase of *C. gloeosporioides* (C_1) 30 ml of the Richards' broth was taken in 100 ml conical flasks and sterilized. The growth of the fungus was studied at different periods of incubation *viz.*, 6, 8, 10, 12, 14, 16, 18, 20 and 22 days. Five mm disc of *C. gloeosporioides* from an actively growing seven day old sporulated culture was introduced to each flask and incubated at 28 ± 2˚C. Three replications were kept for each treatment. After incubation for the required period the cultures were filtered through previously weighed Whatman No. 42 filter paper. The filter paper along with the mycelial mat were dried to a constant weight at 60˚C, cooled and weighed immediately on an analytical balance. The difference between final and initial weight of filter paper carrying the culture discs were taken as the weight of the mycelia. The data were analysed statistically.

3.3.3 Nutritional studies of *C. gloeosporioides* (C_1)

3.3.3.1. Effect of different carbon sources on growth and sporulation of *C. gloeosporioides*

Variations in growth and sporulation of *C. gloeosporioides* on different sources of carbon were studied in basal Richards' medium. The same quantity of carbon source used in basal Richards' medium was substituted by other carbon sources *viz.,* Mannitol, Dextrose, Starch and Lactose. Richards' medium without any carbon source served as control. Petri dishes containing different carbon sources were inoculated with five mm mycelial discs obtained from periphery of seven days old culture and incubated at room temperature for seven days. The radial growth in each case was determined by taking average of the colony dia. in two directions when the maximum growth was attained in any one of the media tested. The spore count was taken with haemocytometer.

3.3.3.2. Effect of different nitrogen sources on growth and sporulation of *C. gloeosporioides*

Both inorganic and organic forms of nitrogen were used for the study. The organic forms of nitrogen used were Asparagine, Urea, Casamino acid and the inorganic forms were Sodium nitrate, Calcium nitrate, Ammonium nitrate and Ammonium chloride. These were substituted for potassium nitrate in Richards' medium to give equivalent amount of nitrogen in each case. Fifteen ml of each medium listed above was poured into 90 mm dia. petri plates. After solidification, five mm discs of *C. gloeosporioides* from actively growing culture were cut using a cork borer and a single disc was placed upside down at the centre of the petri dish. Each set of experiment was replicated thrice and the plates were incubated at room temperature. Richard's media without any nitrogen source served as control. The measurement of the colony dia. was taken when the maximum growth was attained in any one of the media tested. Cultural characters such as colony dia., colony colour, type of margin and sporulation were also recorded.

3.3.4. Physiological factors affecting the growth of *C. gloeosporioides*

3.3.4.1. Effect of temperature on growth and sporulation of *C. gloeosporioides*

The growth of *C. gloeosporioides* was tested at different temperature *viz.,* 5, 10, 15, 20, 25, 30 and 35˚C. Richards' medium was used for the study. Five mm disc from an actively growing culture was cut and inoculated to solidified medium in petri plates and the plates were then placed in BOD incubators, for seven days at required temperature. Each treatment was replicated thrice. After incubation, radial growth from solid media was recorded by taking the average of the colony dia. in two directions.

3.3.4.2. Effect of p^H on growth and sporulation of *C. gloeosporioides*

C. gloeosporioides was grown on Richards' broth with selected p^H range of 3, 4, 5, 6, 7, 8 and 9. The p^H levels were adjusted by adding one normal alkali (NaOH) or acid (HCl). A seven day old five mm mycelial disc from an actively growing culture was inoculated separately into conical flasks containing 30 ml medium at different p^H levels. Three replications were maintained for each p^H level. These flasks were incubated at room temperature for ten days. The mycelium from each flask was harvested and dried in hot air oven till constant weight was reached. The dry weight of each treatment was recorded.

3.3.4.3. Effect of light and darkness on growth of *C. gloeosporioides*

The effect of light intensity on growth of *C. gloeosporioides* was studied by exposing the culture to the following treatments.

1) Alternate period of 12 h each under fluorescent light and 12 h darkness.
2) Continuous light for 24 h under fluorescent light.
3) Continuous darkness for 24 h by keeping in a closed wooden box.

Richards' broth was prepared in 100 ml conical flasks sterilized and was inoculated with five mm disc of *C. gloeosporioides*. Seven replications were maintained for each treatment. Observations on fungal growth were recorded after 15 days.

3.4. ISOLATION OF ANTAGONISTS FROM PHYLLOSPHERE AND RHIZOSPHERE OF CHILLI PLANTS

3.4.1. Isolation of antagonists from chilli phyllosphere

Serial Dilution plate technique was used for the isolation of mycoflora from the chilli leaf surface (Aneja, 2003). Disease free leaf samples collected from plants growing in the infected fields of College of Agriculture, Vellayani was used for the isolation. Cut five discs each of six mm dia. from every collected leaf using cork borer. Transferred 50 discs to 100 ml water blank and shaken for 20 min. in a rotary shaker. Ten gram of the leaf bits was transferred to 100 ml of sterile distilled water in a 250 ml conical flask. Transferred 10 ml of the suspension to 90 ml sterile water blank using a sterile pipette to make a 10^{-1} dilution. The contents were shaken for 2 – 3 min. Transferred 10 ml of the suspension to another 90 ml sterile water blank using another sterile pipette to make a 10^{-2} dilution. Transferred one ml aliquots from 10^{-1} and 10^{-2} dilution to sterile petri plate and poured melted and cooled Martin's Rose Bengal Agar medium and Nutrient Agar medium respectively in these plates. Replications were also maintained. The plates were then incubated at room temperature for seven days. Observed the Martin's Rose Bengal Agar plates after five to seven days and Nutrient Agar plates after two to three days of incubation for the appearance of fungi and bacteria. The fungal and bacterial colonies developed were examined and transferred to PDA and nutrient agar slants respectively. The fungal cultures were purified by the single hyphal tip method (Parmeter et al., 1969). The purified cultures were then stored in PDA and nutrient agar slants under refrigerated condition for identification and subsequent antagonism studies.

3.4.2. Isolation of antagonists from chilli rhizosphere

Soil samples were collected from the rhizosphere of healthy chilli plants from College of Agriculture, Vellayani. The rhizosphere mycoflora was obtained by the serial dilution plate technique (Johnson and Curl, 1972). Ten gram soil was weighed and mixed thoroughly in 100 ml sterile distilled water in 250 ml conical flasks to make a 10^{-1} dilution. From this it was serially diluted to get 10^{-4} and 10^{-6} dilutions. One ml each from 10^{-4} and 10^{-6} dilution were transferred aseptically into petri dishes and plated with melted and cooled Martin's Rose Bengal Agar medium and Nutrient Agar medium respectively. Replications were also maintained. Petri dishes were then incubated at room temperature for 48 – 72 h. The fungal as well as bacterial colonies developed were examined and transferred to PDA and nutrient agar slants respectively. The fungal cultures were purified by the single hyphal tip method (Parmeter et al., 1969). The purified cultures were then stored in PDA and nutrient agar slants under refrigerated condition for identification and subsequent antagonism studies.

3.5. *IN VITRO* MANAGEMENT OF *C. GLOEOSPORIOIDES*

3.5.1. *In vitro* screening of fungal antagonists against *C. gloeosporioides*

The fungal isolates obtained from the phyllosphere and rhizosphere were tested for their antagonistic effect on *C. gloeosporioides* by dual culture technique (Morton and Stroube, 1955). Agar disc of five mm dia. was cut from the edge of a vigorously growing seven day old sporulated culture of *C. gloeosporioides* and this was placed in the media two cm away from the periphery of petri plates. On the opposite side, two cm away from the periphery, five mm sized one week old culture disc of antagonistic fungus cut from a vigorously growing culture was placed. Three replications were maintained. The plates were incubated at room temperature for six

days. Control was kept with five mm disc of *C. gloeosporioides* at the centre of PDA plate. Antagonist that exhibited highest percentage inhibition was selected.

Percentage inhibition of mycelial growth was calculated using the formula (Vincent, 1927).

$$I = (C - T) / C \times 100$$

Where, I = Percentage inhibition of mycelial growth of the pathogen.

C = Growth of the pathogen in the control plates (mm).

T = Growth of the pathogen in dual culture (mm)

Colony development was observed and assessment on interactions between the antagonist and the pathogen were made. Observations on the interaction and its classification were made using the method of Purkayastha and Bhaswati Bhattacharyya (1982) with five groups as follows :

A - Homogenous - Free intermingling between pairing organisms.

B - Overgrowth - Pathogen overgrown by the test fungus.

C - Cessation of growth at the line of contact of the cultures.

D - Aversion - A clear zone of inhibition was observed between the two organisms.

E - Overgrowth - Test fungus overgrown by pathogens.

3.5.1.1. Identification of fungal antagonist of *C. gloeosporioides*

The selected fungal antagonists were identified by the slide culture technique (Riddle, 1974). Plain agar was melted and poured into sterile petri dishes to a thickness of two mm and after solidification, blocks of five mm^2 were cut out using a

sterile needle. One such block was placed at the centre of a sterile microscopic slide. All the four corners of the agar block were inoculated with one antagonistic fungal spore. The block was then covered with sterile cover slip. The slides were incubated separately in moist chamber for 48 h at room temperature. The cover slip was then gently lifted and were mounted with lactophenol cotton blue stain. The slides were then observed under microscope. Based on their spore morphology, they were identified at generic level. The species level identification of the antagonists were made at The Agharkar Research Institute, MACS, Pune.

3.5.2. *In vitro* screening of bacterial antagonist against *C. gloeosporioides*

Bacterial isolates obtained from the rhizosphere were tested for their antagonistic effect on *C. gloeosporioides* by the dual culture technique (Morton and Stroube, 1955). The bacterial isolate was streaked two cm away from the periphery of the petri plate and opposite to the bacterial isolate a five mm disc of the seven day old culture of *C. gloeosporioides* was placed two cm away from the periphery of the petri plate. Replications were maintained. Percentage inhibition of mycelial growth was calculated as in 3.5.1.

3.5.2.1. Identification of bacterial antagonist of *C. gloeosporioides*

The morphological and colony characters of collected bacterial isolates were studied by growing them on Nutrient Agar and Kings' B media. Gram staining, Catalase production, production of fluorescent pigment on Kings' B media and spore staining were conducted as per the procedure described in the Manual of microbiological methods by Society of American Bacteriologists (1957).

3.5.3. *In vitro* screening of antagonists against *C. gloeosporioides* on detached chilli fruits

The healthy, uniformly aged chilli fruits were surface sterilized by dipping in 0.1 per cent $HgCl_2$ solution for one min. followed by three washings with sterile distilled water. A five mm disc of the antagonist as well as the pathogen was placed together side by side on the chilli fruit, after giving pin pricks. Control was kept by placing only the pathogen. The treated fruits were placed separately in sterilized polythene bags. A piece of sterilized moist cotton swab was placed inside the bag and mouth of the bag was tied. The bagged fruits were incubated at room temperature and observations were made daily upto the ninth day. Each treatment was replicated four times and the treatment was compared with lesion size of the control fruits infected with *C. gloeosporioides.* Lesions produced on the fruits were assessed by taking the average of the dia. of lesions in two directions perpendicular to each other. The percentage reduction of lesion size was calculated by subtracting the lesion size of treated fruits from that on control and calculating the percentage reduction over control.

3.5.4. Studies on the selected fungal antagonist *T. harzianum*

3.5.4.1. Mycoparasitism of the selected fungal antagonist *T. harzianum* against *C. gloeosporioides*

The mechanism of mycoparasitism of the selected fungal antagonist on *C. gloeosporioides* was studied following the dual culture technique (Dennis and Webster, 1971 a). Melted PDA was poured in 90 mm sterile petri dishes and allowed to solidify. Sterilized cellophane discs of 90 mm dia. were placed over this so as to lie flat on the medium, using a pair of sterile forceps. An agar disc of five mm dia. containing the mycelium cut out from the margin of an actively growing culture of *C. gloeosporioides* was placed in the media 3 cm away from the periphery of the petri dish and a five mm agar disc of the test fungus (*T. harzianum)* was placed three cm away from it. The plates were incubated at room temperature (28 ± 2˚C) for 24 h.

Direct observations were carried out after incubation period under a light microscope. Microscopic observations for hyphal interactions were made by cutting out one sq. cm portions of cellophane containing the intermingling hyphal growth and mounting in lactophenol cotton blue stain.

3.5.4.2. Production of volatiles by *T. harzianum*

The method adopted by Dennis and Webster (1971 a) was followed for studying the effect of volatiles on the suppression of *C. gloeosporioides*. The petri dishes containing PDA were inoculated with selected fungal antagonist by placing five mm disc cut from an actively growing culture and incubated at room temperature. After three days, the lid of each plate was replaced by a petri plate bottom containing PDA inoculated with the pathogen. The two dishes were then sealed together with adhesive tape. Control was kept by keeping pathogen inoculated plate over the plate inoculated with the pathogen. Three replications were maintained in each case. After incubation the colony diameter of the test pathogen (*C. gloeosporioides*) was measured and compared with that of the control plates.

Percentage inhibition of mycelial growth was calculated using the formula (Vincent, 1927).

$$I = (C - T) / C \times 100$$

Where, I = Percentage inhibition of mycelial growth of the pathogen.

C = Growth of the pathogen in the control plates (mm).

T = Growth of the pathogen in treatment plates (mm)

3.5.4.3. Production of non volatiles by *T. harzianum*

The effect of non volatile substances produced by the selected antagonist is determined by following the methods of Dennis and Webster (1971 b). Twenty ml

PDA was poured into petri plates. A single cellophane disc was placed aseptically over PDA in each petri plate. Mycelial discs of five mm cut from the margin of selected fungal antagonist were placed in the centre of the cellophane tape. The plates were incubated at room temperature for one day. Then the cellophane along with adhering fungus was carefully removed and in its place a five mm mycelial disc of the test pathogen was placed. Instead of placing the fungal antagonist, controls were kept by keeping pathogen inoculated plate. Replications were maintained. After incubation the colony diameter of the test pathogen was measured and compared with that of the control plates. Percentage inhibition of mycelial growth was calculated as in 3.5.4.2.

3.5.4.4. Effect of culture filtrate of *T. harzianum* on *C. gloeosporioides*

Five mm culture disc of selected fungal antagonist was inoculated into 100 ml sterilized Potato Dextrose Broth (PDB) in 250 ml conical flask. The flask were then plugged tightly using cotton and incubated at room temperature for 15 days. The culture filtrate of selected fungal antagonist was obtained by filtering the broth through Millipore membrane filter. This was added to sterilized, melted PDA so as to get the desired concentration. The medium was poured into petri plates and the plates were inoculated at the centre with a five mm culture disc of the pathogen *C. gloeosporioides.* PDA plates inoculated with *C. gloeosporioides* but not amended with culture filtrate of antagonists served as check. The plates were then incubated at room temperature. Observations were made and percentage inhibition of mycelial growth was calculated as in 3.5.4.2.

3.5.4.5. Preparation of the talc based formulation of *T. harzianum* and its shelf life studies

The fermented biomass of selected antagonists *T. harzianum* was prepared by a slightly modified liquid fermentation process of Papavizas et al. (1984). Five

hundred ml of potato dextrose broth was taken in one litre conical flasks and autoclaved. It was inoculated with five mm culture disc of *T. harzianum* and incubated at room temperature for 15 days to obtain sufficient propagules in the mycelial mat. This liquid culture was mixed with one kilo gram sterilized talc powder which act as a carrier material and five grams of Carboxy Methyl Cellulose (sticker). This mixture was dried in shade for 72h, packed in polythene bags and stored at room temperature. For studying the shelf life of the formulated product, serial dilutions were prepared at regular intervals and the number of colony forming units were recorded initially after 10 days and at monthly intervals for six months.

3.5.5. *In vitro* screening of plant extracts against *C. gloeosporioides*

Seven plant extracts *viz.*, *Ocimum sanctum, Andrographis paniculata, Lantana camara, Datura stramonium, Azadirachta indica, Piper betle* and *Bouganvillea glabra* at three different concentrations (60, 80 and 100 %) were tested for their antimycotic behaviour against *C. gloeosporioides* following poisoned food technique (Nene and Thapliyal, 1982). Procedure for the preparation of plant extracts are detailed below.

Hundred gram fresh leaves from each plant collected from field were washed well and ground with 100 ml distilled water. The macerate was filtered through double layered cheese cloth and centrifuged at 3500 rpm for 20 min. The supernatant was filtered through Millipore membrane filter. Double strength PDA medium was supplemented aseptically with the different concentrations of each leaf extract. Fifteen ml sterilized medium was poured into each petri plate and inoculated with a five mm disc taken from the periphery of seven day old culture of *C. gloeosporioides* by placing it in the centre of the plate. The plates were incubated at room temperature. All the treatments were replicated thrice. A control was also maintained where no leaf extract was added to PDA. When complete mycelial

growth was observed in control plates, observation was taken from the treatment plates. Percentage inhibition of mycelial growth was calculated as in 3.5.4.2.

Plants used for preparation of leaf extracts

Sl. No.	**Plant (Common name)/ vernacular name**	**Scientific Name**
1	Betel vine (Vettila)	*Piper betle*
2	Holy basil (Thulsi)	*Ocimum sanctum*
3	Neem (Vep)	*Azadirachta indica*
4	Lantana (Poochedi)	*Lantana camara*
5	Datura (Ummam)	*Datura stramonium*
6	Andrographis (Kiriath)	*Andrographis paniculata*
7	Bougainvillea (Kadalasuchedi)	*Bouganvillea glabra*

Lower concentrations of the most effective leaf extract was tried further under *in vitro* condition.

3.5.6. In vitro screening of plant products against *C. gloeosporioides*

Four plant products *viz.*, Turmeric powder, Garlic Extract, Neem oil and Neem Seed Kernel Extract were tested against *C. gloeosporioides* following poisoned food technique (Nene and Thapliyal, 1982). Turmeric powder was mixed with Sodium bicarbonate in the ratio 10 : 1. The plant products used and their concentrations are given below.

Sl.No	Plant Product	Concentration (%)	
1.	Turmeric powder + Sodium bicarbonate (10:1)	0.075	0.15
2.	Garlic extract	5	10
3.	Neem oil	1	2
4.	Neem Seed Kernel Extract	2.5	5

Double strength PDA medium was prepared and sterilized. These products were then added independently to the media to get the required concentrations. After plating the media added with each plant product, a five mm culture disc of *C. gloeosporioides* was placed at the centre of the petri plate. The plates were then incubated at room temperature. Control plates were kept without adding plant products. Three replications were maintained for each treatment. When mycelial growth completely covered the surface of the media in control plates, observations on the inhibition of mycelial growth due to the use of plant products were recorded. Percentage inhibition of mycelial growth was calculated as in 3.5.4.2.

3.5.7. *In vitro* effect of Bavistin at various concentrations on the growth of *C. gloeosporioides*

Bavistin (Carbendazim) at three different concentrations (0.1, 0.05, 0.01%) were tested for their antimycotic behavior against *C. gloeosporioides* using poisoned food technique (Nene and Thapliyal, 1982). Double strength PDA medium was prepared and sterilized. Bavistin was then added independently to the media to get the required concentrations. After plating the poisoned media, a five mm culture disc of *C. gloeosporioides* was placed at the centre of the petri plate. The plates were

incubated at room temperature. Control plates were kept without adding Bavistin. Three replications were maintained for each treatment. When mycelial growth completely covered the surface of the media in control plates, observations were recorded. Percentage inhibition of mycelial growth was calculated as in 3.5.4.2.

3.5.8. *In vitro* evaluation of various combinations of selected antagonist + plant extract + plant product against *C. gloeosporioides*

The various combinations evaluated against *C. gloeosporioides* were as follows.

1) Selected antagonist + Selected plant extract + Selected plant product
2) Selected antagonist + Selected plant extract
3) Selected antagonist + Selected plant product
4) Selected plant extract + Selected plant product

The experiments were conducted using poisoned food technique (Nene and Thapliyal, 1982). Double strength PDA medium was prepared and sterilized. The combinations to be tested were added independently to the media to get the required concentrations. After plating the poisoned media, a five mm culture disc of *C. gloeosporioides* was placed at the centre of the petri plate. The plates were incubated at room temperature. Control plates were kept without adding amendments in the media. Three replications were maintained for each treatment. When mycelial growth completely covered the surface of the media in control plates, observations were recorded. Percentage inhibition of mycelial growth of *C. gloeosporioides* was calculated as in 3.5.4.2.

3.5.9. *In vitro* evaluation of selected plant extract and plant product individually and in combination against selected antagonist

In order to find out whether there is any inhibitory effect on the antagonist by combining it with the selected plant extract and selected plant product, when the combinations were sprayed on chilli plants, the following *in vitro* evaluation on the plant extract and plant product individually and in combination were conducted. The various treatments evaluated against the selected antagonist were as follows.

1) Selected plant extract + Selected plant product
2) Selected plant extract
3) Selected plant product

The experiment was conducted using poisoned food technique (Nene and Thapliyal, 1982).

Double strength PDA medium was prepared and sterilized. The selected plant extract and plant product individually and in combination were then added independently to the media to get the required concentrations. After plating the poisoned media, a five mm culture disc of selected antagonist was placed at the centre of the petri plate. The plates were incubated at room temperature. Control plates were kept without adding amendments in the media. Three replications were maintained for each treatment. When mycelial growth completely covered the surface of the media in control plates, observations were recorded. Percentage inhibition of mycelial growth of selected antagonist was calculated as in 3.5.4.2.

3.6. VARIETAL SCREENING

3.6.1. Screening of chilli varieties against *C. gloeosporioides*

Five chilli varieties released from Kerala Agricultural University *viz.*, Jwalamukhi, Jwalasakhi, Ujwala, Vellayani Athulya and Anugraha were screened against *C. gloeosporioides*. The varieties were grown in separate pots. When the plants were in the fruiting stage the fruits were artificially inoculated with the culture

of *C. gloeosporioides*. For this conidial suspension of *Colletotrichum gloeosporioides* (10^6 conidia / ml) was taken from ten days old culture grown on Potato Dextrose broth medium. After giving few injury by pin pricking on the fruits, the spore suspension of the pathogen was sprayed over the pin pricked area. To provide moisture a thin layer of moist cotton was placed over the inoculated region. To ensure humidity the plants were sprinkled with water and were covered with polythene cover having sufficient holes for aeration. Scoring for disease intensity was done using the 0 – 4 disease scale developed by Vishwakarma and Sitaramaiah (1986).

Disease severity scale	Fruit area affected (%)	Category
0	0	Immune
1	1 – 5	Resistant
2	6– 25	Moderately resistant
3	26 – 50	Susceptible
4	51 – 100	Highly susceptible

3.7. *IN VIVO* MANAGEMENT OF FRUIT ROT OF CHILLI CAUSED BY *C. GLOEOSPORIOIDES*

3.7.1. Preparation of pathogen inoculum and foliar application

The procedure described by Laha and Venkataramanan (2001) was adopted with slight modification. Five mm culture disc of *C. gloeosporioides* was inoculated in 250 ml conical flasks containing 100 ml sterile water enriched with 0.5 g peptone and incubated at room temperature. When the mycelial mat completely covered the

liquid surface, it was thoroughly agitated and the liquid was filtered through a muslin cloth. Foliar spray of *C. gloeosporioides* containing 10^6 conidia per ml was done 20 days after fruit formation. To ensure uniform infection of fruits by fruit rot, the plants were covered with polythene bags for two days to create a favourable microclimate for the successful infection by the pathogen.

3.7.2. *In vivo* management of fruit rot of chilli

A pot culture experiment was laid out in Completely Randomized Design (CRD) with four replications and nine treatments using the chilli variety Vellayani Athulya which was found highly susceptible to fruit rot under the screening trial. The experiment was conducted during December - April 2010 at College of Agriculture, Vellayani. The manuring and all the cultural practices were done as per package of practice recommendations (Crops : KAU, 2007). Before sowing seed treatment was given by treating the seeds with *Pseudomonas fluorescens* @ 10 g/Kg seed. The seeds were moistened by sprinkling water and treated with the talc based formulation of *P. fluorescens*. The treated seeds were dried in shade for half an hour and then used for sowing.

The treatments were selected based on the maximum inhibition of growth of *C. gloeosporioides* obtained in *in vitro* management studies. The best antagonist, best plant extract and best plant product and their combination were selected as treatments in this study. Talc based formulation of the selected antagonist *T. harzianum* was used for field spraying. The treatments were as follows.

T_1 - Seedling dip in antagonist at the time of transplanting stage + Foliar spray of antagonist

T_2 - Foliar spray with best plant extract.

T_3 - Foliar spray with best plant product.

T_4 - Seedling dip in antagonist at the time of transplanting stage + Foliar spray of antagonist + Foliar spray with best plant extract

T_5 - Seedling dip in antagonist at the time of transplanting stage + Foliar spray of antagonist + Foliar spray with best plant product

T_6 - Foliar spray with best plant extract + Foliar spray with best plant product

T_7 - Seedling dip in antagonist at the time of transplanting stage + Foliar spray of antagonist + Foliar spray with best plant extract + Foliar spray with best plant product.

T_8 - Foliar spray with Bavistin 0.05%.

T_9 - Untreated control.

Seedlings were dipped in the talc based formulation of the selected antagonist *T. harzianum* @ 500 g/ 1000 ml of water for 20 min. The treatments T_1, T_4, T_5 and T_7 were treated with this as seedling dip at the time of transplanting. The selected antagonist, plant extract and the plant product showing maximum antifungal activity against the pathogen under *in vitro* studies were used for *in vivo* studies. All the treatments were given two and seven days after artificial inoculation with the fruit rot pathogen. Bavistin at 0.05 % was given as foliar spray two days after artificial inoculation of the fruit rot pathogen. The following observations were recorded during the course of the experiment.

3.7.3. Disease intensity

The intensity of the fruit rot was recorded before the treatment application i.e. two days after artificial inoculation of the pathogen, after first treatment application (seven days after artificial inoculation of the pathogen), and after second treatment

application (twelve days after artificial inoculation of the pathogen). Scoring of the disease was done using the 0 – 4 disease scale developed by Vishwakarma and Sitaramaiah (1986). Score chart was described as in 3.6.1. (Plate 1).

Percentage disease index (PDI) of fruit rot was calculated using the formula developed by Mc Kinney 1923

$$\frac{\text{Sum of individual ratings} \times 100}{\text{Total number of fruit assessed} \times \text{Maximum disease category}}$$

3.7.4. Effect of application of ecofriendly materials on biometric parameters of chilli plant

After 80 days of transplanting, the treated plants were uprooted and the following observations were recorded.

3.7.4.1. Shoot length

Length of shoot from ground level to growing tip of each plant was measured and recorded.

3.7.4.2. Stem girth

Girth of the stem was measured using a thread and it was recorded.

3.7.4.3. Root length

Length of longest root of each plant was measured after uprooting.

3.7.4.4. Fresh plant weight

Fresh weight of plants were taken immediately after uprooting using an electronic balance and recorded.

3.7.4.5. Dry weight of plants

Plate 1. Disease scale for scoring fruit rot of chilli.

Dry weight of plants were taken after drying the samples to a constant weight in a hot air oven adjusted at 60˚C.

3.7.5. Effect of application of ecofriendly materials on fruit characters of chilli

Fruit characters like fruit length (cm), fruit girth (cm), fresh fruit weight (g) and fruit dry weight (g) were recorded. Length of fruit was measured from tip of the pedicel to tip of each fruit. Fruit girth in cm was also recorded. Fruits were collected from each plant and its fresh fruit weight was taken immediately using an electronic balance. Dry weight of fruits were taken after drying samples to a constant weight in a hot air oven adjusted at 60˚C.

3.7.6. Effect of application of ecofriendly materials on yield of chilli plant

The yield of plants include the number of fruits per plant and its mean weight.

3.8. STATISTICAL ANALYSIS

The data obtained from the studies conducted under laboratory and field conditions were subjected to analysis of variance (ANOVA) after appropriate transformations wherever needed.

EXPERIMENTAL

RESULTS

4. EXPERIMENTAL RESULTS

4.1. ISOLATION OF THE PATHOGEN

The pathogens causing fruit rot of chilli was isolated from the naturally infected chilli fruits. Two different fungal cultures (C_1 and C_2) were obtained from the infected plants. One type of culture (C_1) was obtained during March 2009 and another type (C_2) during August 2009. During isolations the frequently obtained culture was C_1. Both the cultures were isolated following standard procedure on Potato Dextrose Agar (PDA) medium.

4.1.1. Single spore isolation

Both the fungal cultures obtained were purified by single spore isolation procedure.

4.1.2. Proving the pathogenicity

Pathogenicity of both the cultures (C_1 and C_2) of the pathogen was proved following Koch's Postulate's on chilli fruits, variety Vellayani Athulya. Typical symptoms of fruit rot (circular or elliptical sunken necrotic lesion) was reproduced within two days. The pathogen was reisolated and proved the pathogenicity.

4.1.3. Maintenance of the culture

Both the cultures (C_1 and C_2) were subcultured frequently on PDA slants and were kept in refrigerator at 5˚C. Virulence of both fungi were maintained by passing through the host once in three months.

4.2. SYMPTOMATOLOGY

4.2.1. Under natural conditions

Symptomatology of both the isolates were almost similar. Small round (2 – 5 mm dia.) brown, circular or angular sunken lesions appeared on the fruit surface which later turned necrotic surrounded by water soaked area. These lesions spread in the direction of the long axis of the fruit and turned dark greyish. Individual lesions enlarged elliptically to about four cm in dia. and the fungus sporulated on the lesions as salmon pink coloured spore masses. Pinhead sized acervuli bearing setae developed on the lesion in a subcuticular or subepidermal fashion disrupting the outer epidermal cell wall. The acervuli were found arranged in concentric patterns on the necrotic tissue. As the infection progressed, the spots were markedly delimited by a thick and sharp outline enclosing a lighter black or straw coloured area. When the diseased fruit was cut open, the lower surface of the skin was covered with minute, elevated, spherical, black, stromatic masses of the fungus. Severe infection resulted in change of fruit colour from red to straw or dirty greyish white. Infected seeds turn rusty in colour. Affected fruits get wrinkled and deformed. Finally, the diseased fruits were shrivelled and dried. The infected fruits either remained on the plant or dropped off. The variety Vellayani Athulya was prone to fruit rot infection with the culture (C_1) and (C_2) Symptoms occurred 15 – 20 days after fruit formation till harvest and extended upto post harvest stage (Plate 2).

4.2.2. Under artificial conditions

Dark yellowish to brown, circular or elliptical sunken lesions, followed by the production of greyish white mycelium with salmon coloured masses of conidia appeared on fruit surface which spread in the direction of the long axis. A mat of fungal hyphae covered the seeds within three to four days. Infected seeds turned rusty in colour. After 8 – 9 days of artificial inoculation the entire fruit get rotted. The formation of acervuli and setae could not be observed under artificial conditions. Infection with C_1 and C_2 occurred at all stages of the fruit (Plate 3).

Plate 2. Symptoms under natural infection.

Plate 3. Symptoms under artificial infection.

4.3. MORPHOLOGICAL AND CULTURAL CHARACTERS OF THE PATHOGEN

4.3.1. Morphological characters

Morphology of the two cultures (C_1 and C_2) were studied on PDA.

4.3.1.1. Colony characters

On PDA, C_1 appeared white which gradually turned to dark greyish white as it grew older. Aerial mycelia grew evenly felty with salmon pink conidial pustules at the centre of the colony. Diurnal zonations were not so prominent on the upper surface. Setae and acervuli were found in the culture. A distinct olivaceous grey zone alternated with rosy buff zone was observed on the reverse side of the colony (Plate 4, Table 1).

On PDA, C_2 appeared creamy white which gradually turned light greyish white as it grew older. Salmon pink coloured conidial pustules appeared at the centre of the colony. Thin mat of mycelium were seen with less aerial growth. Diurnal zonation of dense and sparse development were prominent on upper side of the culture. Reverse side of the colony were brownish grey to black. Setae and acervuli were found in the culture (Table 1). C_1 grew faster than C_2.

4.3.1.2. Conidial characters

4.3.1.2.1. Conidial morphology of the isolate C_1

On PDA, the mycelial growth consisted of branched and septate hyphae. Conidia were borne on elongated phialides in acervular conidiomata or on solitary fertile hyphae. Conidia were one celled, straight, cylindrical with obtuse ends, sometimes slightly tapered with rounded apex and truncate base, hyaline, aseptate with centrally placed oil globules (Plate 5, 6 and 7). Conidia germinated in glucose

Table 1. Colony and conidial morphology of *Colletotrichum gloeosporioides and C. capsici* on PDA

Sl. No.	**Characters**	***C. gloeosporioides***	***C. capsici***
1.	Type of growth	Evenly felty	Felty
2.	Colony colour	Uniformly dark greyish white	Creamy white turned into greyish white
3.	Colony forms	Circular	Circular
4.	Type of margin	Entire	Entire
5.	Elevation	Slightly raised	Slightly raised
6.	Spore mass colour	Salmon pink	Salmon pink
7.	Diurnal zonations	Not prominent	Prominent
8.	Mycelial dry weight (mg/30ml)	272.73 mg	584.26 mg
9.	Length of conidia (µm)	19.26	25.68
10.	Breadth of conidia (µm)	4.7	4.92
11.	Shape of conidia	straight, cylindrical with obtuse ends, hyaline, aseptate, centrally placed oil globule	Hyaline, unicellular, falcate, centrally placed oil globule.
12.	Length of setae (µm)	124.12	179.76
13.	Breadth of setae (µm)	6.42	6.63
14.	No. of setae / acervulus	23	57
15.	Mycelium width (µm)	3.85	4.28
16.	Diameter of Acervulus (µm)	158.36	175.48

solution (1 %) as thin, hyaline germ tubes (Plate 10). Conidiophores were hyaline and cylindrical (Plate 9). Average conidial size was 19.26 × 4.7 µm and were within the range of 17.12 – 21.4 × 4.28 – 5.35 µm. Acervuli bearing setae were round to elongated or irregular, brown, and measured 158.36 µm in dia. and were within the range of 89.88 – 252.52 µm (Plate 8). Setae abundant, brown, straight to slightly curved, 1 – 4 septate, swollen at the base and tapering towards the apex, average size of the setae was 124.12 × 6.42 µm and were within the range of 77.04 – 171.24 × 5.13 – 7.49 µm. From the cultural and conidial morphology of the isolate C_1 collected during March 2009 was identified as *Colletotrichum gloeosporioides* (Table 1).

4.3.1.2.2. Conidial morphology of the isolate C_2

On PDA, the mycelial growth (Plate 11) consisted of branched and septate hyphae. Conidia were borne singly at the tips of the hyaline cylindrical conidiophores or on solitary fertile hyphae (Plate 13). Conidia were hyaline, unicellular and curved with narrow ends (falcate conidia) and contained a centrally placed oil globule (Plate 14). Average size of the conidia were 25.68 × 4.92 µm and were within the range of 18.40 – 29.96 × 4.28 – 5.99 µm. Acervuli bearing setae were round to elongated or irregular and dark brown, measured 175.48 µm in dia. within a range of 111.28 – 338.12 µm. Setae were abundant and dark brown, 1 – 5 septate, slightly curved, rigid, hardy, swollen at the base, slightly tapered towards the paler acute apex (Plate 12). Average size of setae were 179.76 × 6.63 µm within the range of 102.72 – 231.12 × 5.56 – 7.66 µm. Based on these characters the isolate C_2 collected during August 2009 was identified as *Colletotrichum capsici* (Table 1).

4.3.2. Cultural characters

4.3.2.1. Growth and sporulation of *C. gloeosporioides* (C_1) and *C. capsici* (C_2) on different solid media

Colletotrichum gloeosporioides

Plate 4. Growth of *Colletotrichum gloeosporioides* on PDA.

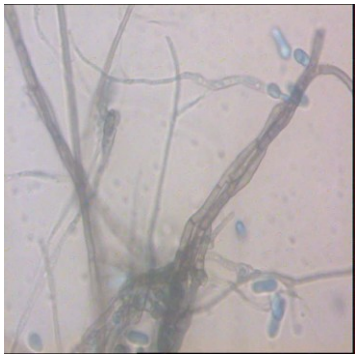


Plate 5. Conidia and Hyphae

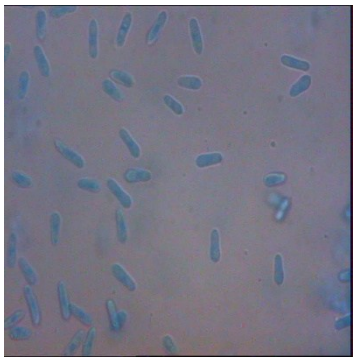


Plate 6. Conidia (40X)

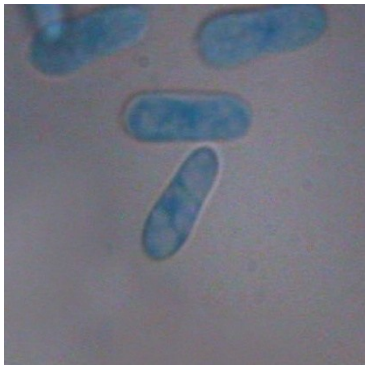


Plate 7. Conidia (100X)

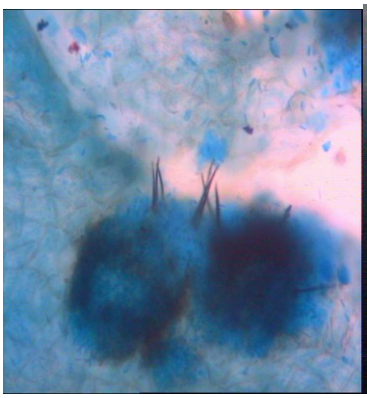
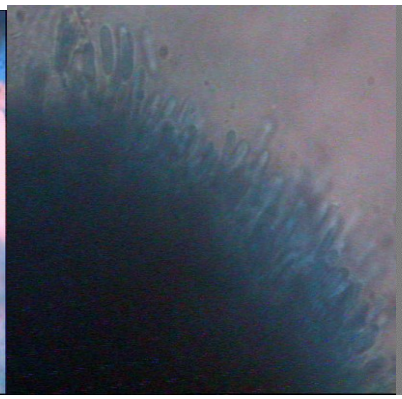


Plate 8. Acervulus carrying setae



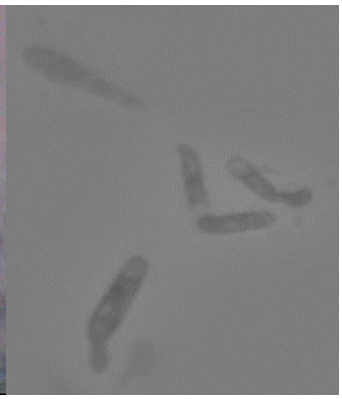


Plate 9. Conidiophores bearing conidia

Colletotrichum capsici

Plate 11. Growth of *Colletotrichum capsici* on PDA.

Plate 12. Acervulus bearing setae

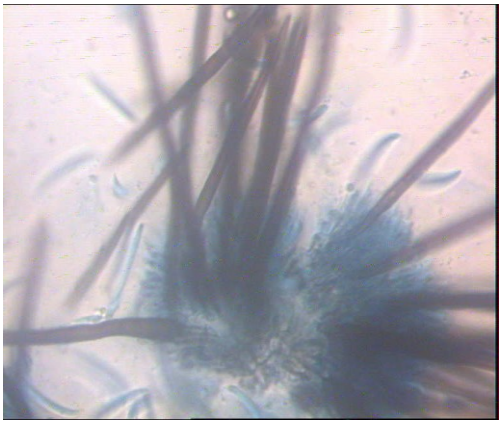


Plate 13 Conidiophores bearing conidia

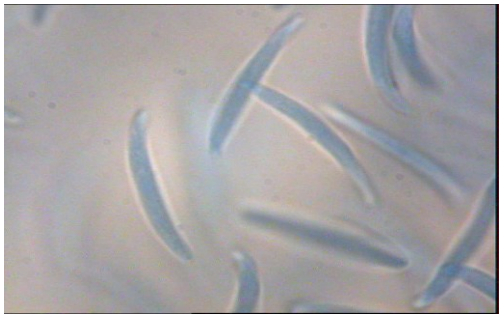


Plate 14. Conidia (100 X)

4.3.2.1.1. Growth and sporulation of *C. gloeosporioides* on different solid media

The diversity in cultural and morphological characters of *C. gloeosporioides* was studied on eight different solid media under laboratory conditions. The data are presented in Table 2.

Maximum radial growth was obtained on Richards' agar medium (90.00 mm) within six days and was significantly superior to all the other media tested. This was followed by Potato dextrose agar (88.83 mm), Sabouraud's agar (87.83 mm), Czapek - Dox agar (86.67 mm), Brown's agar (80.00 mm), Fries' agar (76.33 mm) and Host extract agar (72.16 mm). Minimum growth was observed on Host extract dextrose agar medium (68.17 mm).

Sporulation was obtained in all the eight media tested. Excellent sporulation of the fungus was recorded on Sabouraud's agar, Potato dextrose agar and Richards' agar media. Sporulation was fair on Czapek - Dox agar and Host extract agar. With respect to mycelial colour, it varied from dull white to grey. The growth varied from slightly raised to slightly fluffy with smooth and entire margins. The growth of the fungus on PDA was circular, evenly felty, greyish white with entire margin showing diurnal zonations. Mycelial growth on Sabouraud's agar was like a felted mat with circular entire margins having salmon pink conidial pustules at the centre of the colonies. On Richards' agar, the fungus produced dull white, slightly fluffy, circular growth having smooth and entire margins. Mycelial growth on Host extract dextrose agar, Fries' agar and Brown's agar were greyish white in colour having smooth, circular, entire margins with good sporulation. On Czapek - Dox agar and Host extract agar the fungus produced dull white to greyish white coloured mycelial growth with slightly raised smooth, circular, entire margin having fair sporulation (Plate 15, Fig.1).

Table 2. Growth and sporulation of *C. gloeosporioides* on different solid media.

Sl. No.	1.	2.	3.	4.	5.	6.	7.	8.
Media	Richards' agar	Potato dextrose agar	Sabouraud's agar	Czapek-Dox agar	Brown's agar	Fries' agar	Host extract agar	Host extract dextrose agar
Growth characters	Good growth, evenly fluffy with dull white, diurnal zonations, circular, slightly raised colony with entire margin, reverse of colony appeared light salmon pink in colour.	Good growth, evenly felty with greyish white, diurnal zonations, circular, entire margin, reverse of colony appeared in the form of distinct olivaceous grey zonations alternated with rosy buff zonations	Aerial mycelium even, felted mat with salmon pink conidial pustules evident at the centre of the colony, dark grey centre with white circular and entire margin, diurnal zonations, reverse of the colony light grey in colour.	Evenly felty with greyish white, circular, slightly raised, entire margin, reverse of colony appeared dark grey in colour.	Greyish white, loosely textured colonies, appressed, circular, entire margins, diurnal zonations, reverse of colony light grey in colour.	Felty, dark grey centre with white smooth entire margin, slightly raised, circular, reverse of colony appeared smoky grey in colour.	Evenly felty with greyish white, circular, entire margin, slightly raised, reverse of the colony uncoloured .	Evenly felty, dark grey centre with white smooth, entire margin, slightly raised, circular, diurnal zonations, reverse of the colony light grey in colour.
Sporulation	++++	++++	++++	++		+++	++	+++
***Radial growth(mm)**	90.00	88.83	87.83	86.67	80.00	76.33	72.16	68.17
CD at 5%	0.684	* Mean of three replication						

++++ Excellent sporulation, +++ Good sporulation, ++ Fair sporulation, + Poor sporulation, - no sporulation.

4.3.2.1.2. Growth and sporulation of *C. capsici* on different solid media

The diversity in cultural and morphological characters of *C. capsici* was studied on eight different solid media. The data are presented in Table 3.

Maximum radial growth was observed on Potato dextrose agar (90.00 mm) within seven days and was significantly superior to all the other media tested. This was followed by Richards' agar (88.17 mm), Czapek - Dox agar (86.5 mm) and Brown's agar (85.50 mm). Growth on Host extract agar (84.17 mm) and Fries' agar (83.67 mm) were statistically on par with each other followed by Sabouraud's agar (79.17 mm). Minimum growth was observed on Host extract dextrose agar (76.00 mm).

Sporulation was obtained in all the eight media tested. Excellent sporulation of the fungus was recorded on Potato dextrose agar, Czapek - Dox agar, Host extract agar and Fries' agar. Sporulation was good on Richards' agar and Host extract dextrose agar. Sporulation was fair in Brown's agar. With respect to mycelial colour, it varied from dull white to dark grey. In all the eight media the growth varied from slightly raised with smooth and entire margins. The growth of the fungus on PDA was flat, circular, greyish white with entire margin showing diurnal zonations and with excellent sporulation. Mycelial growth on Fries' agar was light greyish white in centre with circular entire dark grey outer margins having salmon pink conidial pustules arranged in a concentric manner. On Czapek - Dox agar the fungus showed greyish white coloured mycelial growth with smooth entire margin having salmon pink conidial masses at the centre of the colony whereas growth on Host extract agar was with distinct concentric zonations of salmon pink conidial masses. On Richards' agar, the fungus produced inner greyish white with outer dull white growth, circular with smooth and entire margins. Mycelial growth on Host extract dextrose agar was dark grey in colour with smooth, circular, entire margins and having good sporulation. Brown's agar produced dull white mycelial growth with smooth circular

Table 3. Growth and sporulation of *C. capsici* on different solid media.

Sl.No	1.	2.	3.	4.	5.	6.	7.	8.
Media	Richards' agar	Potato dextrose agar	Sabouraud's agar	Czapek- Dox agar	Brown's agar	Frie's agar	Host extract agar	Host extract dextrose agar
Growth characters	Good growth, inner greyish white with outer dull white margins concentric zonations are prominent, circular, smooth flat and entire margin, reverse of colony appeared dark grey in colour.	Good growth, greyish white, concentric zonations, circular, entire, smooth margin, reverse of colony appeared as dark grey alternated with light grey zonations, salmon pink conidial pustules are seen at the centre of the colony.	Loose textured aerial mycelium having dirty white colonies. No diurnal zonations are seen, Poor sporulation, circular and flat entire margins, reverse of colony was uncoloured.	Greyish white, concentric zonations not so prominent, salmon pink coloured pustules seen at the centre of the colony, circular, smooth and have entire margins, reverse of colony appeared as smoky grey in colour.	Off white mycelium with concentric zonations are prominent , smooth, circular, and entire flat margins, reverse of colony was light grey in colour.	Inner light greyish white mycelium with outer dark grey margins, salmon pink conidial pustules are arranged in concentric patterns, reverse of colony was dark grey in colour.	Dirty greyish white, colony with circular, entire and smooth margins, diurnal zonations are seen, salmon pink coloured conidial pustules are arranged in concentric patterns, reverse of colony was dark brown in colour.	Distinct diurnal zonations with dark greyish mycelium, circular smooth and entire margins, reverse of colony was dark grey in colour.
Sporulation	+++	++++	+	++++	++	++++	++++	+++
*** Radial growth (mm)**	88.17	90.00	79.17	86.50	85.50	83.67	84.17	76.00
CD at 5 %	0.638	* Mean of three replication						

++++ Excellent sporulation, +++ Good sporulation, ++ Fair sporulation, + Poor sporulation, - no sporulation.

entire margin. Dirty white loose textured mycelial growth having poor sporulation was seen on Sabouraud's agar (Plate 16, Fig. 1).

4.3.2.2 Growth and sporulation of *C. gloeosporioides* (C_1) and *C. capsici* (C_2) in different liquid media

4.3.2.2.1. Growth and sporulation of *C. gloeosporioides* in different liquid media

Dry mycelial weight and sporulation of *C. gloeosporioides* in eight different liquid media after 15 days of growth was recorded and the data are presented in Table 4. Significant differences in growth and sporulation were recorded in liquid media. Richards' broth was the best liquid media for growth of the fungus and recorded dry mycelial weight of 284.23 mg from 30 ml broth in 15 days. Significantly high growth was recorded in this media compared to all the other media tested. The dry weight of mycelium recorded from other media were 272.73 mg from Potato dextrose broth (PDB), 263.3 mg from Sabouraud's broth, 258.43 mg from Czapek - Dox broth, 254.73 mg from Fries' broth, 178.67 mg from Brown's broth and 129.23 mg from Host extract dextrose broth. Significant differences in growth was recorded between the different media tested. The minimum growth was observed in Host extract broth (95.5 mg).

Sporulation was excellent in Fries' broth, PDB and Richards' broth with numerous salmon pink conidial masses. Good sporulation was observed in Czapek - Dox broth, Sabouraud's broth, Host extract dextrose broth and Brown's broth. Fair sporulation was seen in Host extract broth (Plate 17, Fig. 2).

4.3.2.2.2. Growth and sporulation of *C. capsici* in different liquid media

Dry mycelial weight and sporulation of *C. capsici* in eight different liquid media after 15 days of growth was recorded and the data are presented in Table 5.

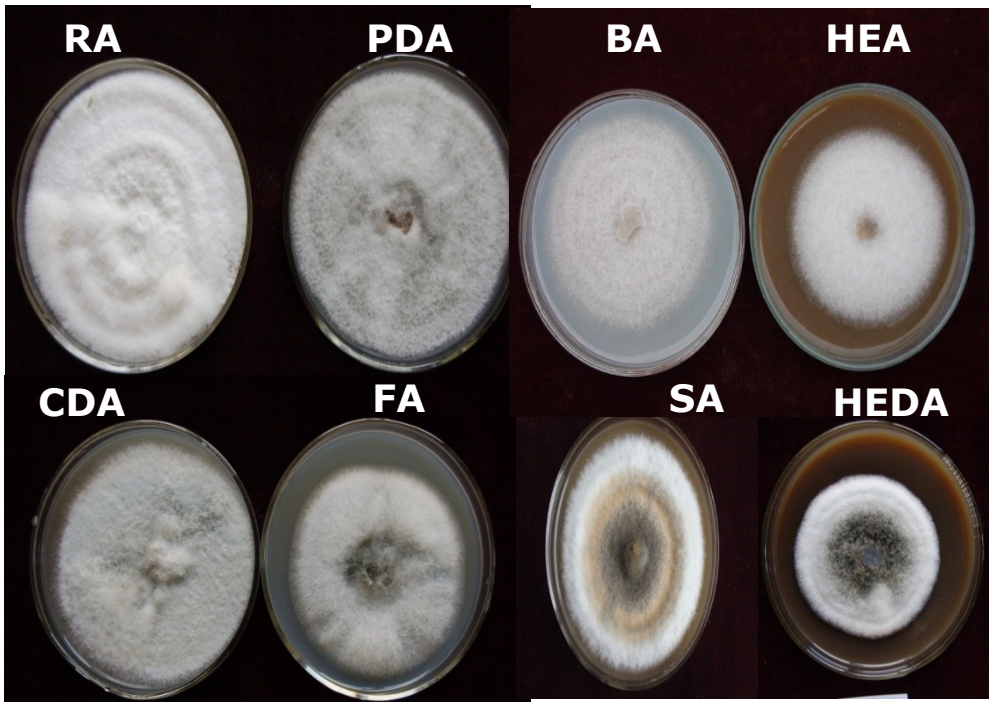


Plate 15. Growth of *C. gloeosporioides* on different solid media.

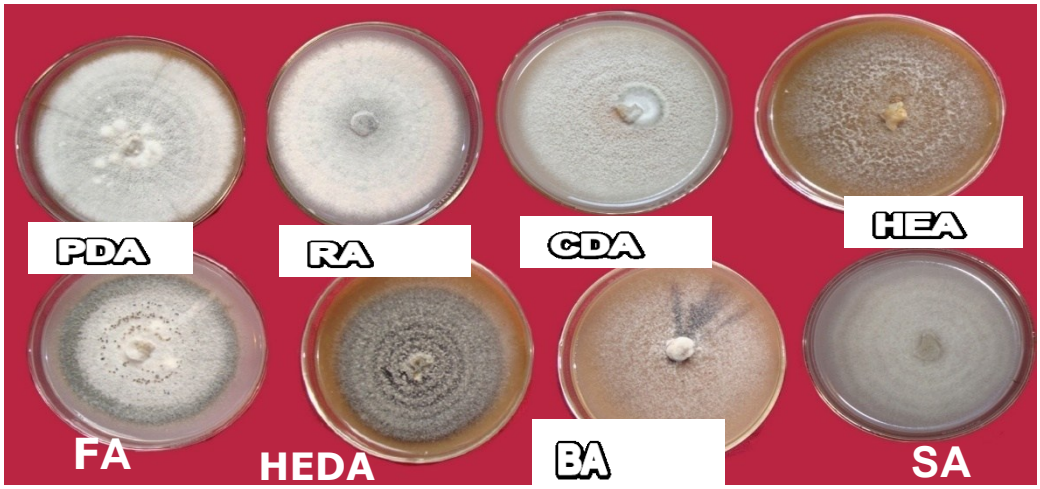


Plate 16. Growth of *C. capsici* on different solid media.

PDA - Potato Dextrose Agar

RA - Richards' Agar

CDA - Czapek Dox Agar

HEA - Host Extract Agar

HEDA - Host Extract Dextrose Agar

FA - Fries' Agar

BA - Brown's Agar

SA - Sabouraud's Agar

Table 4. Growth and sporulation of *C. gloeosporioides* in different liquid media

Sl. No.	Media	Growth characters	Dry mycelial weight * (mg)	Sporulation #
1.	Richards' broth	Off white, good growth, fair sporulation	284.23	+ +++
2.	Potato dextrose broth	Greyish white, good growth, excellent sporulation.	272.73	+ + + +
3.	Czapek's broth	Greyish white	258.43	+ + +
4.	Sabouraud's broth	Greyish white	263.3	+ + +
5.	Host extract broth	Grey, poor sporulation	95.5	++
6.	Host extract dextrose broth	Greyish black with salmon pink conidial masses, good sporulation.	129.23	+ + +
7.	Fries' broth	Dark greyish white with prominent salmon pink conidial masses, excellent sporulation.	254.73	+ + + +
8.	Brown's broth	Greyish white, good sporulation.	178.67	+ + +
CD at 5 %			0.153	

* Mean of three replication.

\# ++++ Excellent sporulation, +++ Good Sporulation, ++ Fair sporulation, + Poor sporulation , - No sporulation

Table 5. Growth and sporulation of *C. capsici* in different liquid media

Sl.No.	Media	Growth characters	Dry mycelial weight * (mg)	Sporulation#
1.	Richards' broth	Good growth, excellent sporulation with initially off white and later turned into dark grey to black mycelium.	528.67	+ +++
2.	Potato dextrose broth	Good growth, excellent sporulation, salmon pink conidial pustules are seen.	584.27	+ + + +
3.	Czapek's broth	Blackish grey mycelium with excellent sporulation	501.60	++++
4.	Sabouraud's broth	Dark greyish black mycelium with fair sporulation	467.70	+ +
5.	Host extract broth	Dark grey mycelium, poor growth with poor sporulation	67.30	+
6.	Host extract dextrose broth	Dark greyish mycelium with salmon pink conidial pustules are seen	262.20	+++
7.	Fries' broth	Off white mycelium with salmon pink conidial pustules are seen	366.73	++++
8.	Brown's broth	Greyish white mycelium with good sporulation.	96.50	+++
CD at 5 %			0.375	

* Mean of three replication

\# ++++ Excellent sporulation, +++ Good Sporulation, ++ Fair sporulation, + Poor sporulation, - No sporulation

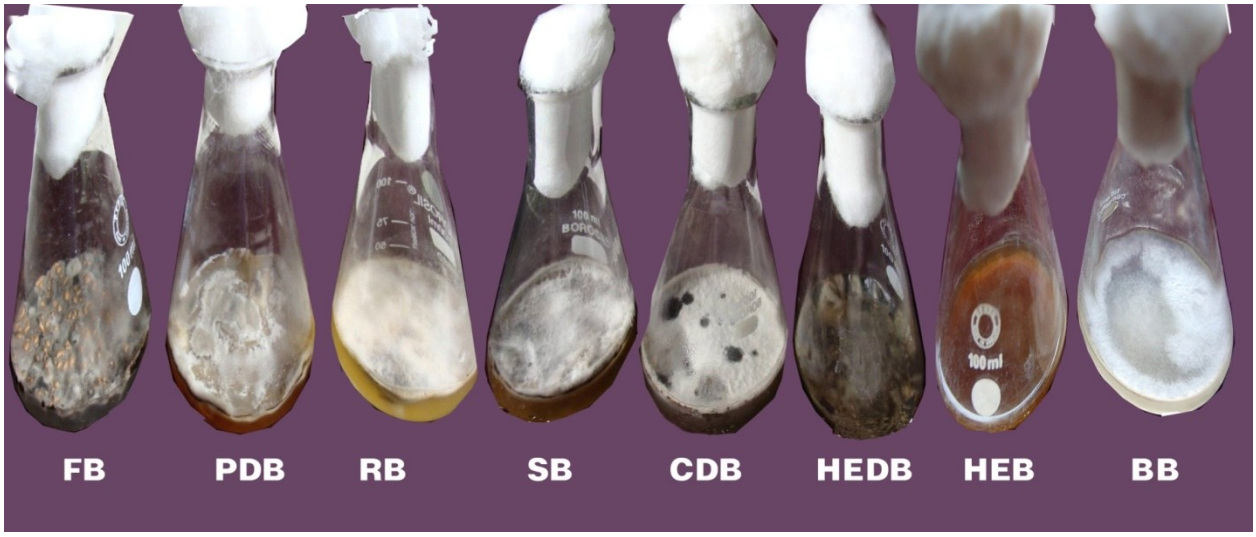


Plate 17. Growth of *C. gloeosporioides* in different liquid media.

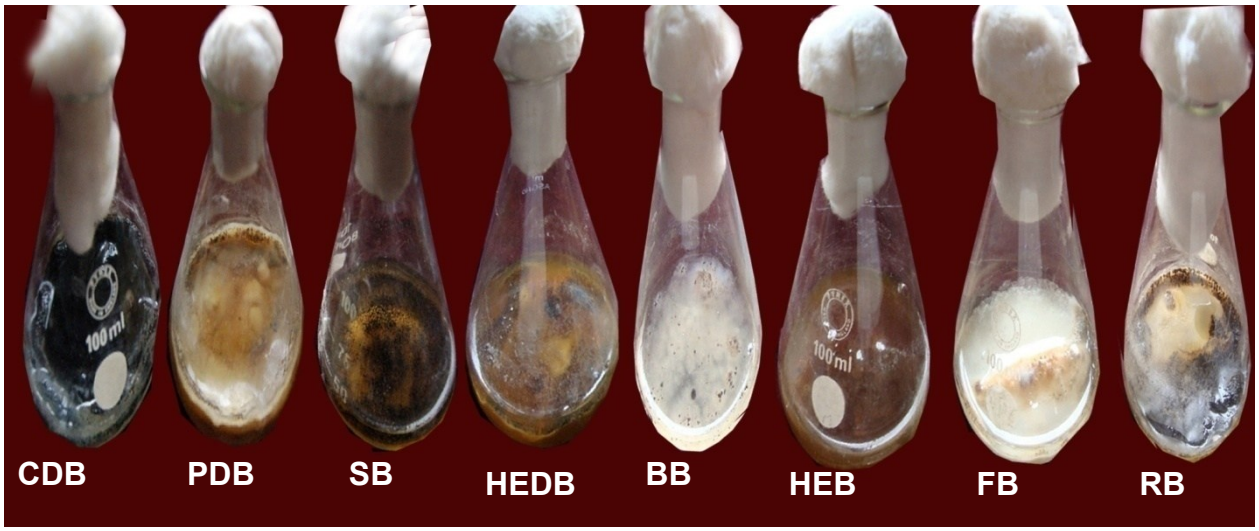


Plate 18. Growth of *C. capsici* in different liquid media.

PDA - Potato Dextrose Agar

RA - Richards' Agar

CDA - Czapek Dox Agar

HEA - Host Extract Agar

HEDA - Host Extract Dextrose Agar

FA - Fries' Agar

BA - Brown's Agar

SA - Sabouraud's Agar

Significant differences in growth and sporulation were recorded in the liquid media. Potato dextrose broth (PDB) was the best liquid media for growth of the fungus which recorded 584.27 mg dry weight of the mycelium from 30 ml PD broth in 15 days. Significantly high growth was recorded by PDB compared to all the media tested. This was followed by Richards' broth, Czapek - Dox broth, Sabouraud's broth, Fries' broth, Host extract dextrose broth and Brown's broth with dry weight of the mycelium 528.67 mg, 501.60 mg, 467.70 mg, 366.73 mg, 262.20 mg, 96.50 mg respectively. Significant differences were observed between different liquid media tested. The minimum growth was observed in Host extract broth (67.30 mg).

Sporulation was excellent in Fries' broth, PDB, Czapek - Dox broth and Richards' broth with numerous salmon pink conidial masses. Sporulation was good in Host extract dextrose broth and Brown's broth. Fair sporulation was seen in Sabouraud's broth and poor sporulation was found in Host extract broth (Plate 18, Fig. 2).

.4.3.2.3. Production of toxic metabolite by *C. gloeosporioides* (C_1) and *C. capsici* (C_2) in different liquid media

The comparative toxic metabolite activity of the culture filtrate was studied by conducting bioassay on chilli fruits.

4.3.2.3.1. Effect of various media on toxic metabolite production of *C.gloeosporioides* on chilli fruit

Observations on the effect of the toxin produced by *C. gloeosporioides* on chilli fruit variety Vellayani Athulya are presented in Table 6. Size of lesions formed after three days by the culture filtrate containing toxin of the fungus was maximum in the case of Richards' broth which was on par with Sabouraud's broth. This was

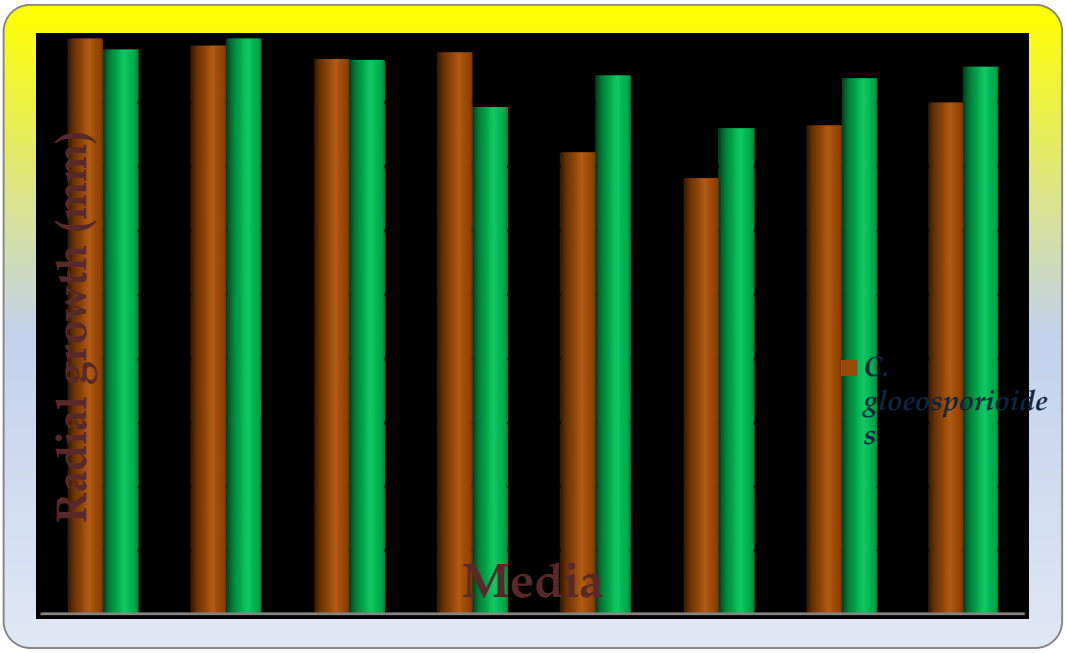


Fig. 1. Growth of *C. gloeosporioides* and *C. capsici* on different solid media

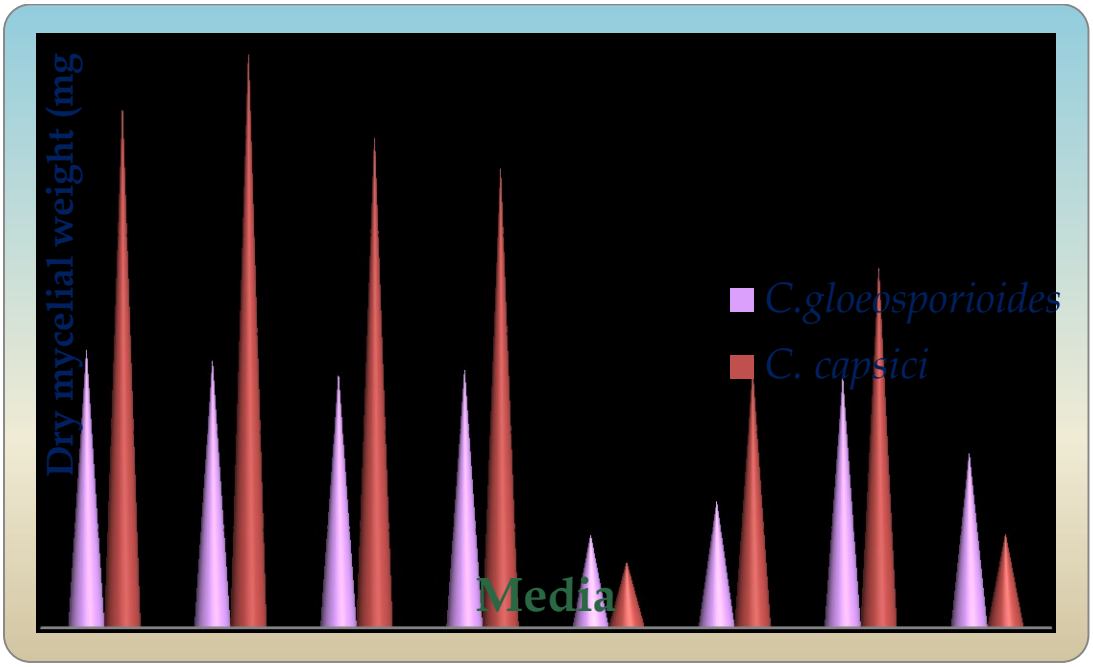


Fig. 2. Growth of *C. gloeosporioides* and *C. capsici* in different liquid media

Table 6. Effect of toxic metabolite of culture filtrate of *C. gloeosporioides* on chilli fruit (Vellayani Athulya)

Sl.No.	Media	Lesion size (cm) after 3 days*.	Symptoms
1.	Richards' culture filtrate	3.62	Lesions are light brown surrounded by water soaked areas.
2.	Sabouraud's culture filtrate	3.47	Inner portions are dark brown while outer portions take light brown colour.
3.	Host extract dextrose culture filtrate	2.8	Inner lesions are dark brown surrounded by light brown water soaked areas.
4.	Fries' culture filtrate	2.35	Lesions appear as dark brown
5.	Potato dextrose culture filtrate	2.13	Lesions appear as dark brown surrounded by water soaked areas.
6.	Czapek - Dox culture filtrate	1.58	Inner portions are light brown surrounded by dark brown areas.
7.	Control (Sterile water)	0	No lesions.
CD at 5 %		0.163	

* Mean of three replication

followed by Host extract dextrose, Fries', Potato dextrose and Czapek - Dox culture filtrate when compared with sterile water control.

Lesions were light brown and water soaked spreading an area of 3.62 cm. when the culture filtrate from Richards' broth was artificially inoculated on the fruits. The lesion length produced by the toxic metabolite produced by the pathogen (C_1) on different media *viz.,* Sabouraud's, Host extract dextrose, Fries', Potato dextrose and Czapek - Dox broth were 3.47, 2.8, 2.35, 2.13 and 1.58 cm respectively. Colour of the lesions produced by toxin from different media were also slightly different. The lesions were observed as dark brown in the centre with outer margin light brown in Sabouraud's, Host extract dextrose but it was on the reverse in Czapek – Dox broth lesions (Plate 19). .

4.3.2.3.2. Effect of various media on toxic metabolite production of *C. capsici* on chilli fruit

Observations on the effect of toxin produced by *C. capsici* on chilli fruit variety Vellayani Athulya are presented in Table 7. Size of lesions formed after three days by the culture filtrate containing toxin of the fungus was maximum in Fries' culture filtrate (4.20 cm) followed by Potato dextrose (3.85 cm), Host extract (3.57 cm) and Richards' (3.10 cm). Host extract dextrose (2.80 cm) was statistically on par with Czapek – Dox (2.77 cm) culture filtrate. This was followed by Brown's (2.28 cm) and Sabouraud's (1.45 cm) culture filtrate when compared with sterile water control.

The colour of the lesions produced by different culture filtrates vary slightly. Lesions were sunken and dark brown in the centre surrounded by light brown margin in Fries' whereas uniformly dark brown sunken lesions were observed in Potato dextrose and Host extract culture filtrate. Inner portion of lesions were light brown surrounded by water soaked margins in Richards' and Host extract dextrose culture

Table 7. Effect of toxic metabolite of culture filtrate of *C. capsici* on chilli fruit (Vellayani Athulya)

Sl. No.	Media	Lesion size (cm) after 3 days*.	Symptoms
1.	Fries' culture filtrate	4.20	Inner sunken dark brown surrounded by light brown margins.
2.	Potato dextrose culture filtrate	3.85	Dark brown sunken lesions.
3.	Host extract culture filtrate	3.57	Dark brown sunken lesions.
4.	Richards' culture filtrate	3.10	Inner portion of the lesions appear as light brown surrounded by water soaked areas.
5.	Host extract dextrose culture filtrate	2.80	Light brown sunken lesions surrounded by water soaked areas
6.	Czapek - Dox culture filtrate	2.77	Light brown sunken inner lesions surrounded by dark brown margin.
7.	Brown's culture filtrate	2.28	Sunken dark brown lesions surrounded by water soaked areas.
8.	Sabouraud's culture filtrate	1.45	Sunken light brown inner portions which are delimited by dark brown margin.
9.	Control (Sterile water)	0	No lesions.
	CD at 5 %	0.090	

* Mean of three replication

filtrate whereas in Czapek - Dox it was light brown sunken inner area surrounded by dark brown margin. Sabouraud's and Brown's culture filtrates produced sunken dark brown lesions surrounded by water soaked areas (Plate 20).

4.3.2.3.3. Exo and Endo toxic metabolite production of *C. gloeosporioides* and *C. capsici*

Observations on the effect of exo and endo toxin by both species of *Colletotrichum* on symptom/ lesion development on host fruit are presented in Table 8 and 9. It was observed that brown sunken necrotic spotting of three to four centimeter on the surface of fruits was obtained within three days by exotoxin (culture filtrate) of *C. gloeosporioides* after 72 h. whereas the lesion size was smaller with endotoxin (concentrated toxic metabolite from mycelium). In *C. capsici* also the exotoxin produced bigger lesions than endotoxin. As the incubation period increased, there was a proportionate increase in the size of lesion.

4.3.2.4. Compatibility of *C. gloeosporioides* and *C. capsici* on PDA under *in vitro* condition

C. gloeosporioides and *C. capsici* grew individually without intermingling of hyphae on PDA. Both the fungus ceased their growth at the point of contact. More growth was observed in *C. gloeosporioides* than in *C. capsici.*

4.3.2.5. Combined and individual infection of *C.gloeosporioides* and *C. capsici* on detached chilli fruits

The healthy, uniformly mature chilli fruits collected from varieties Jwalamukhi, Jwalasakhi, Ujwala, Vellayani Athulya and Anugraha which were screened against *C. gloeosporioides* and *C. capsici* individually and in combination are presented in Table 10.

Combined infection of *C. gloeosporioides* + *C. capsici* was higher on detached chilli fruits which was statistically significant when compared to infection by

Plate 19. Symptoms produced by *C. gloeosporioides* culture filtrate on chilli fruits (var. Vellayani Athulya).

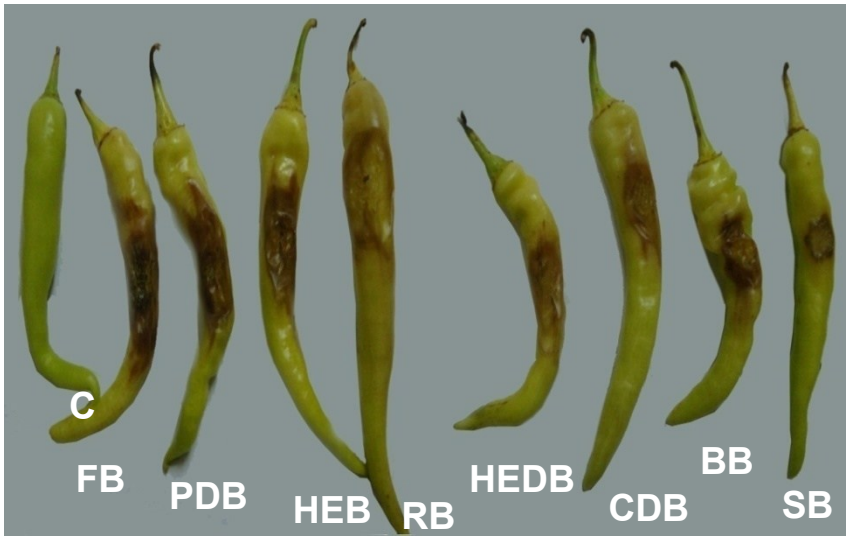


Plate 20. Symptoms produced by *C. capsici* culture filtrate on chilli fruits (var. Vellayani Athulya).

PDA - Potato Dextrose Agar

RA - Richards' Agar

CDA - Czapek Dox Agar

HEA - Host Extract Agar

HEDA - Host Extract Dextrose Agar

FA - Fries' Agar

BA - Brown's Agar

SA - Sabouraud's Agar

Table 8. Effect of exo and endo toxic metabolite of *C. gloeosporioides* on detached chilli fruits.

Sl. No.	Treatment	Lesion size in (cm) after three days *	
		Exotoxin #	Endotoxin #
1.	Culture filtrate from Richards' broth	++++	+++
2.	Richards' broth	-	-
3.	Sterile water (control)	-	-

Table 9. Effect of exo and endo toxic metabolite of *C. capsici* on detached chilli fruits.

Sl. No.	Treatment	Lesion size in (cm) after three days *	
		Exotoxin #	Endotoxin #
1.	Culture filtrate from Richards' broth	+++++	++++
2.	Richards' broth	-	-
3.	Sterile water (control)	-	-

* Mean of three replication

\# - No symptoms/lesions

\+ Symptoms/lesions upto 1 cm.

++ Symptoms/lesions between 1- 2 cm

+++ Symptoms/lesions between 2- 3 cm

++++ Symptoms/lesions between 3 – 4 cm

+++++ Symptoms/lesions more than 4 cm

Table 10. Combined and individual infection of *C.gloeosporioides* and *C. capsici* on detached chilli fruits of KAU varieties.

Sl. No.	Chilli Varieties	Lesion size (cm) on fruit*			
		***C.gloeosporioides* infection**	***C. capsici* infection**	***C.gloeosporioides* + *C. capsici***	Mean
1.	Vellayani Athulya	2.02 (1.42)#	2.83 (1.68)	3.55 (1.88)	2.8 (1.66)
2.	Jwalasakhi	1.42 (1.19)	2.03 (1.42)	2.87 (1.69)	2.10 (1.44)
3.	Jwalamukhi	1.08 (1.04)	1.29 (1.14)	2.15 (1.47)	1.50 (1.22)
4.	Anugraha	0.83 (0.91)	0.87 (0.93)	1.29 (1.14)	0.99 (0.99)
5.	Ujwala	0.51 (0.72)	0.57 (0.75)	0.79 (0.89)	0.62 (0.79)
	Mean	1.17 (1.06)	1.17 (1.19)	2.13 (1.42)	2.13 (1.42)
CD at 5 % between three pathogens			0.016		
CD at 5 % between treatments			0.036		
CD at 5 % between varieties					0.021

***** Mean of three replication

(Figures given in parenthesis are transformed values).

C. capsici and *C. gloeosporioides* alone. Variety Vellayani Athulya was highly susceptible to fruit rot infection compared to Jwalasakhi, Jwalamukhi, Anugraha and Ujwala (Plate 21).

Further studies on growth phase, nutritional and physiological factors, *in vitro* and *in vivo* management were carried out using the frequently obtained culture *C. gloeosporioides* (C_1) only.

4.3.2.6. Growth phase of *C. gloeosporioides* (C_1) in liquid media

The experiment was conducted to ascertain the number of days required for maximum growth of the fungus in broth by monitoring the dry mycelial weight. The results are represented in Table 11.

There was significant difference in dry weight of mycelium obtained by different incubation periods. The dry mycelial weight of *C. gloeosporioides* gradually increased from sixth day of incubation (248.51 mg) and reached maximum on 12^{th} day (512.69 mg) and was significantly superior over all other incubation periods *viz.,* 6, 8, 10, 14, 16, 18, 20 and 22^{nd}. Declining trend in growth was observed from 14^{th} day onwards (Plate 22, Fig. 3).

4.3.3. Nutritional studies of *C. gloeosporioides* (C_1)

4.3.3.1. Effect of different carbon sources on growth and sporulation of *C. gloeosporioides*

Carbon is the most important nutrient and an essential structural component of the frame work of the fungal cell. It's requirement and utilization by *C. gloeosporioides* was studied with five different carbon sources using Richard's media as the basal medium and the data are presented in Table-12.

Table 11. Growth phase of *C.gloeosporioides* in Richards' broth

Sl. No.	Incubation period	Dry mycelial weight* (mg)
1.	6	248.51
2.	8	326.73
3.	10	484.53
4.	12	512.69
5.	14	497.50
6.	16	463.52
7	18	328.86
8.	20	287.49
9.	22	263.29
CD at 5 %		0.404

***** Mean of three replication

Table 12. Growth and sporulation of *C. gloeosporioides* in different carbon sources.

Sl. No.	Basal Richards' media with Carbon source	Growth Characters	Radial growth* (mm)	Sporulation $\times 10^4$ conidia/ml #
1.	Sucrose	Greyish white, concentric zonations prominent, round smooth, raised having entire margins, thick fluffy growth, reverse of colony appeared as light salmon pink in colour.	90.00	118.5
2.	Mannitol	Off white having circular smooth and entire margins, concentric zonations present, less fluffy growth, reverse of colony appeared as grey in colour.	86.33	89.5
3.	Dextrose	Thick fluffy growth, white, circular, smooth, entire margins, reverse of colony appeared as light salmon pink in colour.	85.57	106.5
4.	Starch	Greyish white, circular, smooth and having entire margins, reverse of colony appeared as smoky grey in colour.	84.00	81.25
5.	Lactose	Off white, slightly fluffy, circular, smooth and entire margins, reverse of colony appeared as light greyish white in colour.	58.00	74
6.	Control	Loose textured dirty white sparse growth of mycelium, No sporulation was seen, reverse of colony appeared uncoloured.	78.83	0
		CD at 5 %	0.602	1.556

***** Mean of three replication
# Mean of four replication

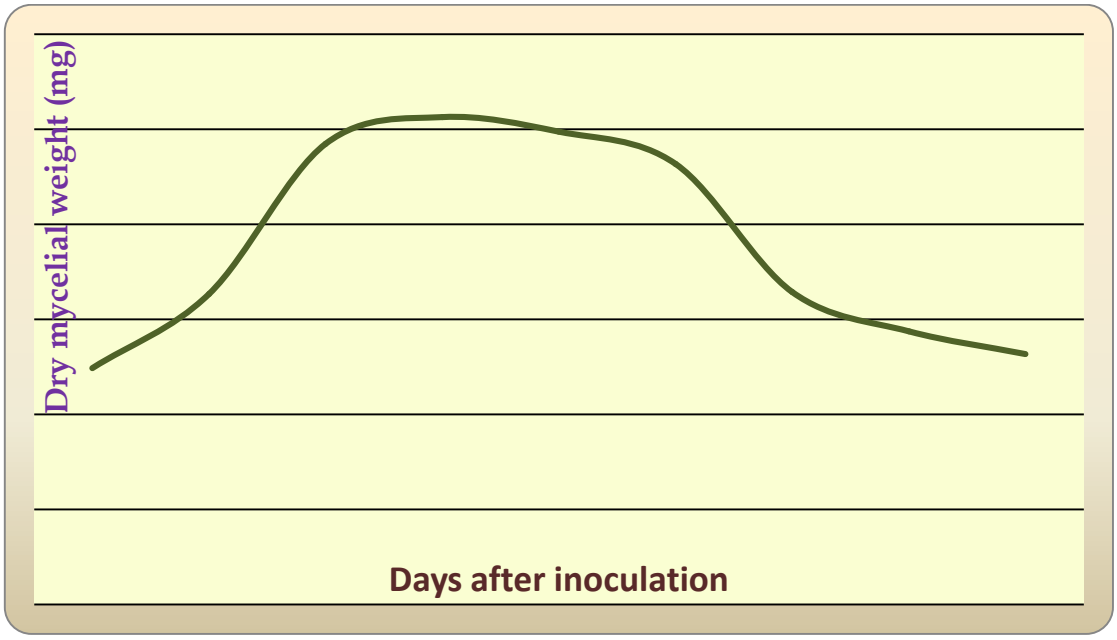


Fig 3. Growth curve of *C. gloeosporioides* in Richards' Broth.

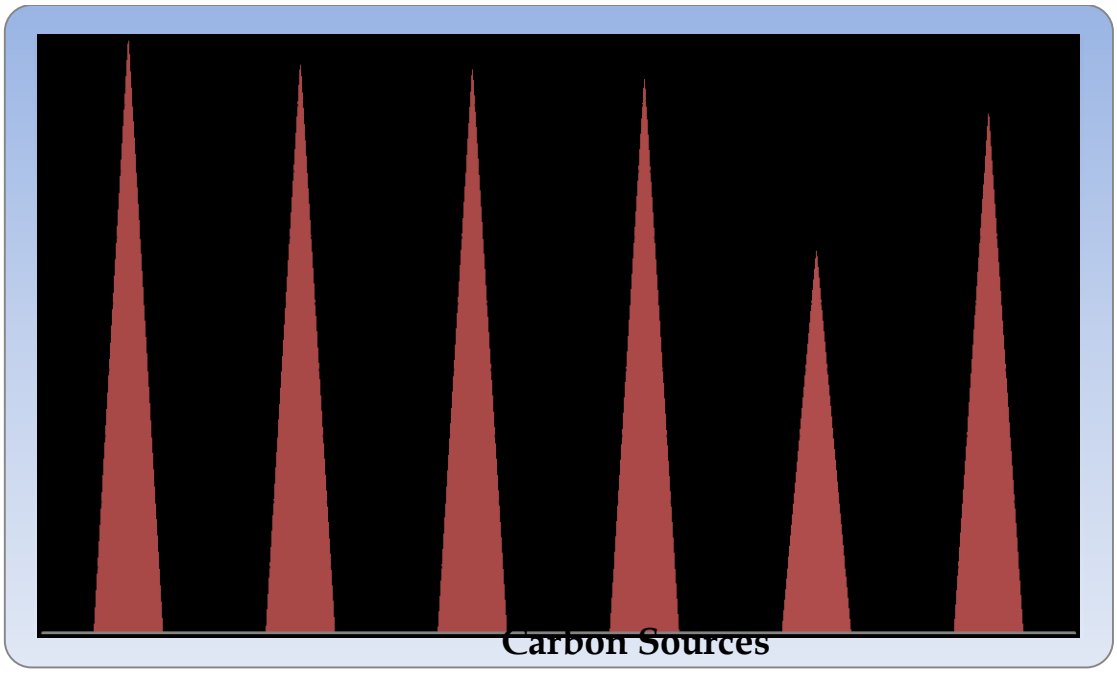


Fig. 4. Growth of *C. gloeosporioides* on Richards' Agar with different Carbon sources

1. Vellayani Athulya
2. Jwalasakhi
3. Jwalamukhi
4. Anugraha
5. Ujwala

Plate 21. Combined and individual infection of *C.gloeosporioides and C. capsici* on chilli fruits of different varieties.

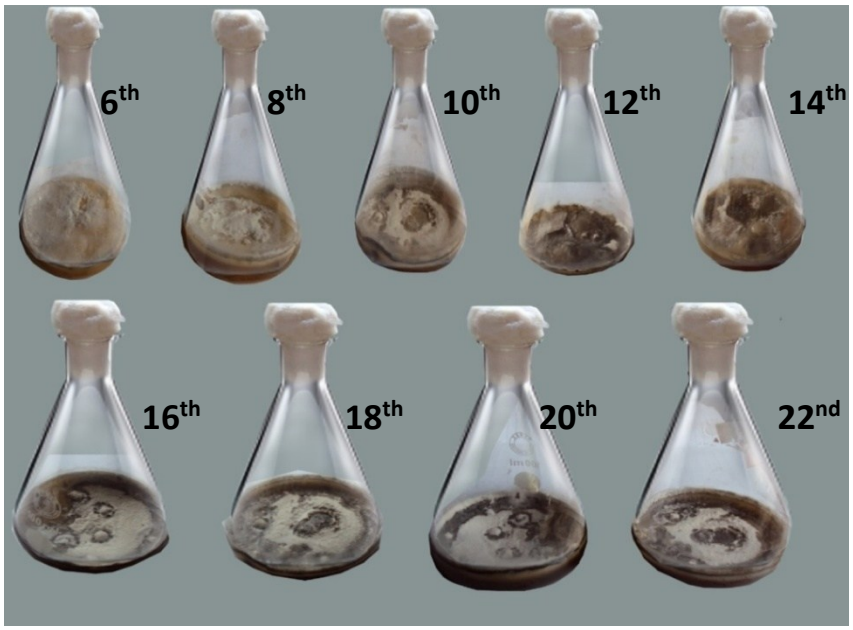


Plate 22. Growth of *C. gloeosporioides* in Richards' Broth at different intervals.

There were significant differences in the radial growth of *C. gloeosporioides* in various carbon sources. Among the five carbon sources sucrose was the best one recording the maximum radial growth of 90.00 mm followed by Mannitol with 86.33 mm growth but it was significantly less compared to sucrose. The radial growth recorded in Dextrose and Starch were 85.57 mm and 84.00 mm respectively which were also significantly less than sucrose and mannitol. Though radial growth in Lactose (58.00 mm) was less when compared to control without carbon (78.83 mm), the mycelial growth appeared as off white and slightly fluffy in lactose media whereas in control the growth was sparse and dirty white. Culture colour in different carbon sources varied slightly. In Richards' medium with Dextrose as carbon source culture appeared pure white in colour, but it was greyish white in sucrose and starch and off white in mannitol and lactose (Plate 23 and Fig. 4).

Heavy sporulation was observed in sucrose media as the source of carbon and no sporulation was observed in media without any carbon source. There was significant difference in sporulation between sucrose and dextrose. Media with mannitol and starch showed moderate sporulation. Sporulation in media with lactose as carbon source was almost 1/3rd less when compared to the best carbon source, sucrose.

4.3.3.2. Effect of different nitrogen sources on growth and sporulation of *C. gloeosporioides*

Nitrogen is an important component required for protein synthesis and other vital functions. Its requirement for *C. gloeosporioides* was studied using different organic and inorganic sources and the results are presented in Table 13 and 14.

Significant differences in growth of *C. gloeosporioides* was recorded in Richards' media supplemented with various organic nitrogen sources. Asparagine was the best utilized nitrogen source as it recorded the maximum growth of 90.00

Table 13. Growth and sporulation of *C. gloeosporioides* in different organic nitrogen sources.

Sl. No.	Basal Richards' media with Organic Nitrogen Source	Growth characters	Radial growth* (mm)	Sporulation $\times 10^4$ conidia/ml #
1.	Asparagine	Off white, circular, smooth and entire margin, reverse of colony appeared as smoky grey in colour.	90.00	125.25
2.	Casamino acid	Greyish white, circular smooth, slightly raised, fluffy growth and entire margins, reverse of colony appeared as light grey in colour.	86.88	117.5
3.	Urea	White, concentric zonations, smooth and entire margins, reverse of colony appeared with concentric zonations alternated with off white and light grey in colour.	81.88	102.75
4.	Control	Dirty white, loose textured, sparse growth, smooth and entire margins, poor sporulation was seen, reverse of colony uncoloured.	85.53	4.5
CD at 5 %			0.418	1.751

***** Mean of four replication
# Mean of four replication

Table 14. Growth and sporulation of *C. gloeosporioides* in different inorganic nitrogen sources.

Sl. No.	Basal Richards' media with Inorganic Nitrogen Source	Growth characters	Radial growth* (mm)	Sporulation × 10^4 conidia/ml #
1.	Potassium nitrate	Off white, smooth, circular and entire margins, reverse of colony appeared dark grey in colour	90.00	120.33
2.	Sodium nitrate	Off white, circular, smooth and entire margins, reverse of colony appeared smoky grey in colour.	82.33	104.33
3.	Calcium nitrate	Off white, circular, smooth and entire margins, reverse of colony appeared with distinct concentric zonations of alternate rosy buff and grey colour.	82.00	91
4.	Ammonium nitrate	Greyish white, smooth, slightly raised, entire margins, reverse of colony appeared with concentric zonations of light brown and dark grey colour.	68.83	73
5.	Ammonium chloride	Dark grey mycelium, irregular, rough, wavy margin, reverse of colony appeared light grey in colour.	31.83	17
6.	Control	Dirty white, loose textured, sparse growth, smooth, and entire margins, poor sporulation was seen, reverse of colony appeared as uncoloured.	85.53	5.33
		CD at 5 %	0.813	1.453

* Mean of three replication
# Mean of three replication

mm. Casamino acid with 86. 88 mm radial growth recorded next but it was significantly lower than Asparagine. Urea recorded (81.88 mm) significantly low growth compared to control (85.53 mm). In culture, mycelial colour was white in Richards' media supplied with urea, off white in Asparagine and greyish white in casamino acid. In control only sparse loose textured dirty white mycelial growth was observed (Plate 24, Fig. 5).

In the case of inorganic nitrogen sources, the fungus recorded maximum growth on Potassium nitrate (90.00 mm). Culture colour in different nitrogen sources varied. All the other inorganic sources recorded significantly lower growth than control. Control recorded 85.53 mm growth. Growth of the fungus in sodium nitrate (82.33 mm) was on par with calcium nitrate (82.00 mm). Ammonium nitrate (68.83 mm) was followed next with Ammonium chloride (31.83 mm). In Richards' medium with Potassium nitrate, Sodium nitrate and Calcium nitrate as inorganic nitrogen sources the culture appeared off white in colour, it was greyish white in Ammonium nitrate and dark grey in Ammonium chloride (Plate 25, Fig. 5).

In all the media with organic nitrogen sources *viz.*, Asparagine, Casamino acid and urea there was heavy sporulation. But in inorganic nitrogen sources Potassium nitrate and Sodium nitrate recorded good sporulation. Moderate sporulation was recorded with Calcium nitrate and Ammonium nitrate. Sporulation was sparse in Ammonium chloride supplemented media. Poor sporulation was recorded in media without any organic and inorganic nitrogen sources.

4.3.4. Physiological factors affecting the growth of *C. gloeosporioides*

4.3.4.1. Effect of temperature on growth and sporulation of *C. gloeosporioides*

The fungus *C. gloeosporioides* was grown on Richards' agar medium at different temperature *viz.,* 5, 10, 15, 20, 25, 30, and 35˚C to know the suitable

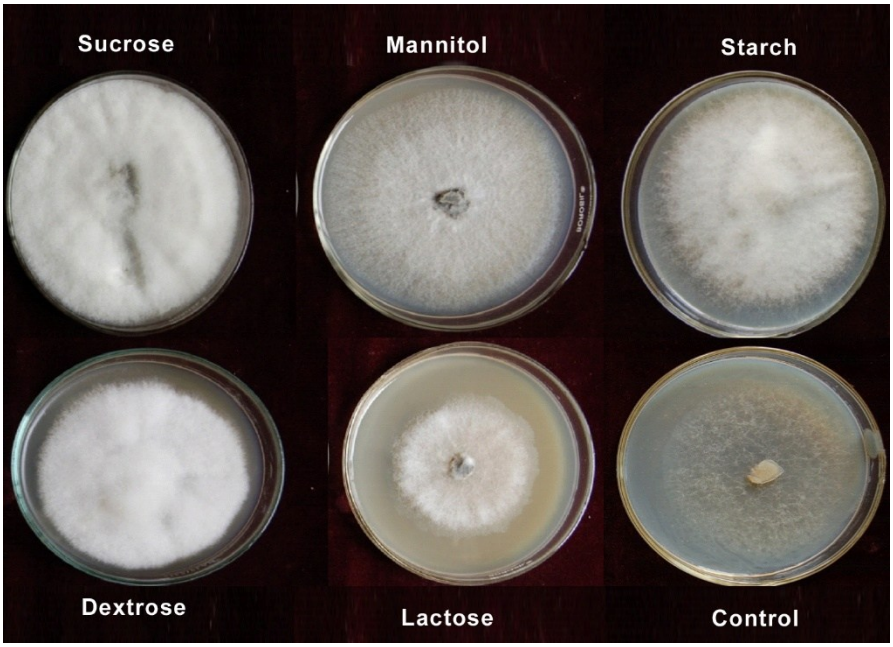


Plate 23. Growth of *C. gloeosporioides* on Richards' Agar with different Carbon sources

Plate 24. Growth of *C. gloeosporioides* on Richards' Agar with different Organic Nitrogen Sources

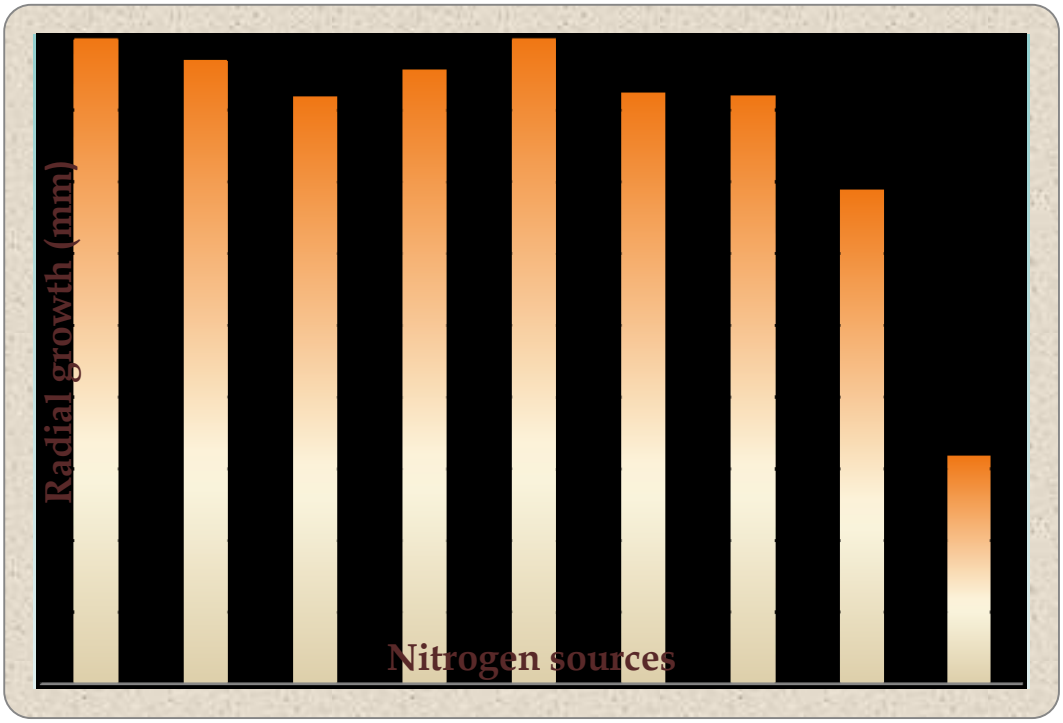


Fig. 5. Growth of *C. gloeosporioides* on Richards' Agar with different nitrogen sources

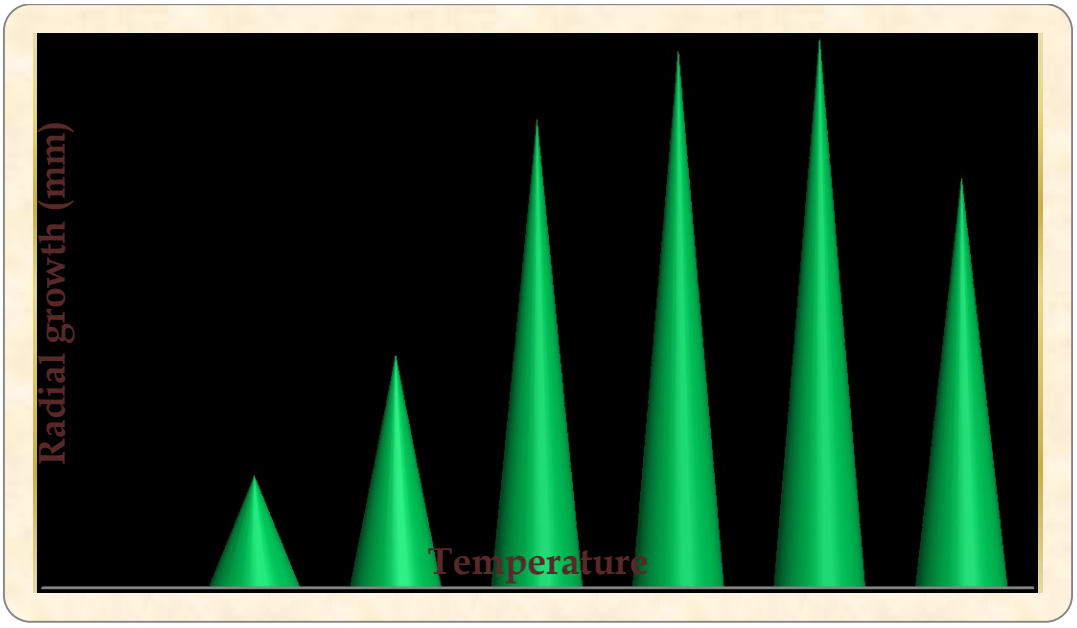


Fig. 6. Growth of *C. gloeosporioides* on Richards' Agar at different temperature

temperature requirement for their maximum radial growth and sporulation (Table 15).

Maximum growth was recorded at a temperature of 30˚C (90.00 mm), indicating that 30˚C was the optimum temperature for growth of the fungus. Growth at 25˚C was 88.00 mm but it was significantly lower than the growth at 30˚C. Twenty degree Celsius and 35˚C favoured good growth while at 15˚C and 10˚C the growth was poor. The fungus did not grow at 5˚C (Plate 26, Fig. 6.).

The fungus recorded excellent sporulation at 30˚C and 25˚C. Good sporulation occurred at 20˚C and fair at 35˚C, while poor sporulation was recorded at 10˚C and 15˚C. The fungus did not grow and sporulate at 5˚C.

4.3.4.2. Effect of p^H on growth and sporulation of *C. gloeosporioides*

The fungus was grown in Richards' medium at different p^H levels and observations were recorded on dry mycelial weight and sporulation. The results are presented in Table-16.

Optimum p^H was 6.0 for the growth of *C. gloeosporioides.* The fungus recorded maximum dry mycelial weight of 586.80 mg/30 ml Richards' broth at p^H 6.0 and was significantly superior over the other p^H levels tested followed by p^H level of 5.0 (532.60 mg). The fungus grew in a moderate level at p^H 4 and 7 with dry mycelial weight of 495.83 mg and 409.43 mg respectively. At p^H 8 (289.70 mg) the dry mycelial weight was just half of that at p^H 6. At p^H 3 the dry mycelial weight was 248.43 mg. The least dry mycelial weight was recorded at p^H 9 (137.00 mg), almost $1/5^{th}$ of that of optimum p^H (Plate 27, Fig.7).

A similar trend was observed in sporulation. The optimum p^H for best sporulation was at p^H 6 and 5. Good sporulation was recorded at p^H 4 and 7. At P^H 3, 8 and 9 poor sporulation was observed.

Table 15. Growth and sporulation of *C. gloeosporioides* at different temperature.

Sl. No.	Temperature (˚C)	Radial growth (mm) in Richards' medium *	Sporulation#
1.	5	0.00	-
2.	10	18.33	+
3.	15	38.17	+
4.	20	76.67	+++
5.	25	88.00	++++
6.	30	90.00	++++
7.	35	67.17	++
	CD at 5 %	0.689	

Table 16. Growth and sporulation of *C. gloeosporioides* at different p^H.

Sl. No.	P^H	Mycelial dry weight (mg) in Richards' medium *	Sporulation#
1.	3	248.43	+
2.	4	495.83	+++
3.	5	532.60	++++
4.	6	586.80	++++
5.	7	409.43	+++
6.	8	289.70	+
7.	9	137.00	+
	CD at 5 %	0.523	

*Mean of three replication

\# ++++ Excellent sporulation, +++ Good Sporulation, ++ Fair sporulation, + Poor sporulation , - No sporulation

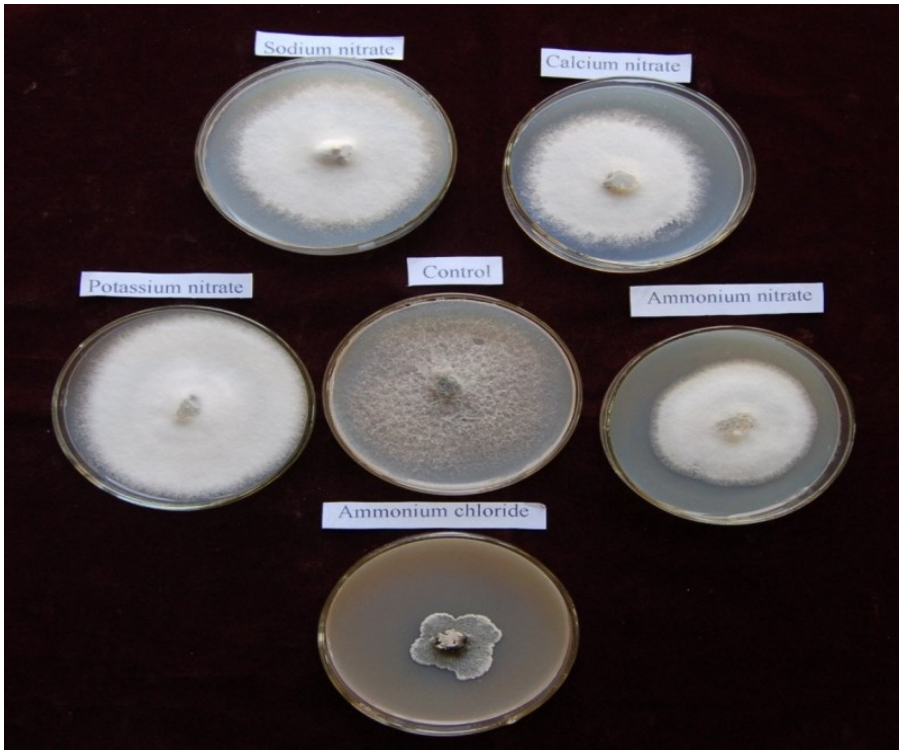


Plate 25. Growth of *C. gloeosporioides* on Richards' Agar with different Inorganic Nitrogen Sources.

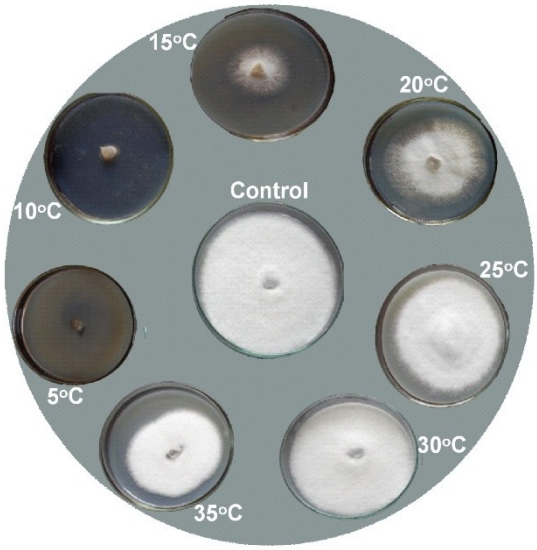


Plate 26. Growth of *C. gloeosporioides* on Richards' Agar at different temperatures.

4.3.4.3. Effect of light and darkness on growth of *C. gloeosporioides*

The effect of light on growth of the fungus was studied in Richards' broth. The results are presented in Table 17.

Exposure of fungal culture to alternate cycles of 12 hr each under fluorescent light and 12 hr darkness resulted in maximum dry mycelial weight of the fungus to the tune of 583.57 mg. Similarly, exposure of fungal culture to continuous light resulted in production of dry mycelial weight of 466.53 mg. The least dry mycelial weight of 342.47 mg was observed in the conical flasks containing fungal culture which was exposed to continuous darkness (Plate 28).

4.4. ISOLATION OF ANTAGONISTS FROM PHYLLOSPHERE AND RHIZOSPHERE OF CHILLI PLANTS

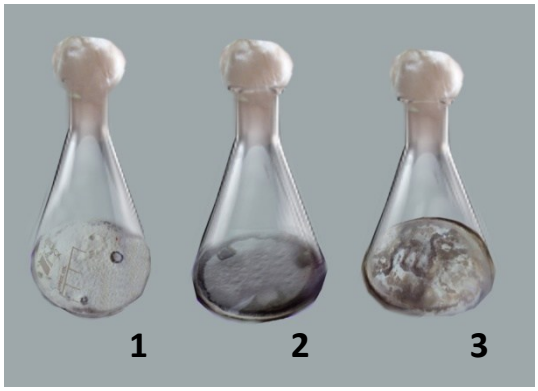
4.4.1. Isolation of antagonists from chilli phyllosphere

From the chilli phyllosphere, two fungi were obtained having antagonistic property against the pathogen *C. gloeosporioides*. They were later identified as *Penicillium* sp. and *Aspergillus niger* (Table 18).

4.4.2. Isolation of antagonists from chilli rhizosphere

From the rhizosphere, three fungi and two bacteria were obtained having antagonistic property against the pathogen *C. gloeosporioides*. The fungi were later identified as *Trichoderma harzianum, Gliocladium virens* and *Aspergillus flavus*. The bacteria were identified as *Bacillus* sp. and *Pseudomonas fluorescens* (Table 18).

Plate 27. Growth of *C. gloeosporioides* in Richards' broth at different p^H



1. Continuous darkness

2 Continuous light

3 Alternate period of 12h light and 12h darkness

Plate 28. Growth of *C. gloeosporioides* under light and darkness.

Table 17. Effect of light and darkness on growth of *C. gloeosporioides*.

Sl. No.	Treatments	Mycelial dry weight (mg)
1.	Alternate cycles of 12 hr light and 12 hr darkness	583.57
2.	Continuous light	466.53
3.	Continuous dark	342.47
CD at 5 %		0.324

***** Mean of seven replication

Table 18. Fungal and Bacterial antagonists collected from Phyllosphere and Rhizosphere

Source	Organisms obtained
Phyllosphere of chilli	**1.** *Aspergillus niger*
	2. *Penicillium* sp.
Rhizosphere of chilli	**1.** *Pseudomonas fluorescens*
	2. *Trichoderma harzianum*
	3. *Gliocladium virens*
	4. *Aspergillus flavus*
	5. *Bacillus* sp.

4.5. *IN VITRO* MANAGEMENT OF *C. GLOEOSPORIOIDES*

4.5.1. *In vitro* screening of fungal antagonist against *C. gloeosporioides*

All the antagonists significantly suppressed the growth of *C. gloeosporioides* (Table 19, Fig. 8). *Trichoderma harzianum* was the most effective with 94.81 % inhibition of the pathogen. *T. harzianum* had over grown and completely suppressed the growth of *C. gloeosporioides* within five days when they were placed in PDA media at five cm apart. *Gliocladium virens, Aspergillus flavus, Aspergillus niger* and *Penicillium* sp. showed cessation of growth at the line of contact of antagonist and the pathogen in six days of incubation. *T. harzianum* was followed by *Gliocladium virens* with 88.88 % growth inhibition of the pathogen. *Aspergillus flavus* showed an inhibition of 85.56 % and the least inhibition was observed with *Penicillium* sp. 82.59 % (Plate 29).

4.5.1.1. Identification of fungal antagonist of *C. gloeosporioides*

Five different fungi were obtained from rhizosphere and phyllosphere of healthy chilli plants having antagonistic activity against *C. gloeosporioides*. They were identified based on the spore morphology, colony and cultural characters. The fungal antagonists identified were *Aspergillus niger, Aspergillus flavus, Penicillium* sp., *Trichoderma harzianum* Rifai. and *Gliocladium virens* Mitler, Giddens and Foster. *Trichoderma harzianum* and *Gliocladium virens* were identified by the fungal culture identification centre, Agharkar Research Institute, MACS, Pune.

4.5.2 *In vitro* screening of bacterial antagonist against *C. gloeosporioides*

The efficiency of the two bacterial isolates obtained from the rhizosphere of chilli in inhibiting the growth of *C. gloeosporioides* under *in vitro* condition was studied by dual culture technique. The bacterial isolates varied in their ability in inhibiting the pathogen.

Table 19. Percentage inhibition of *C. gloeosporioides* by antagonist in dual culture.

Sl. No.	Microbial antagonist	Radial growth of *C. gloeosporioides* (cm) in dual culture #	% inhibition over control *	Type of interactions
1.	*T . harzianum*	0.47	94.83 (76.82)	B•
2.	*Gliocladium virens*	1.00	88.88 (70.49)	C•
3.	*Aspergillus niger*	1.16	87.04 (68.88)	C
4.	*Aspergillus flavus*	1.30	85.56 (67.64)	C
5.	*Penicillium* sp.	1.57	82.59 (65.31)	C
6.	*Pseudomonas fluorescens*	2.00	77.78 (61.85)	
7.	*Bacillus* sp.	1.10	87.78 (69.51)	
8.	Control.	9.00	-	
	CD at 5 %		0.744	

*Mean of three replication

B• - Over growth - Pathogen overgrown by test fungus
C• - Cessation of growth at the line of contact of the cultures
(Figures given in parenthesis are transformed values).

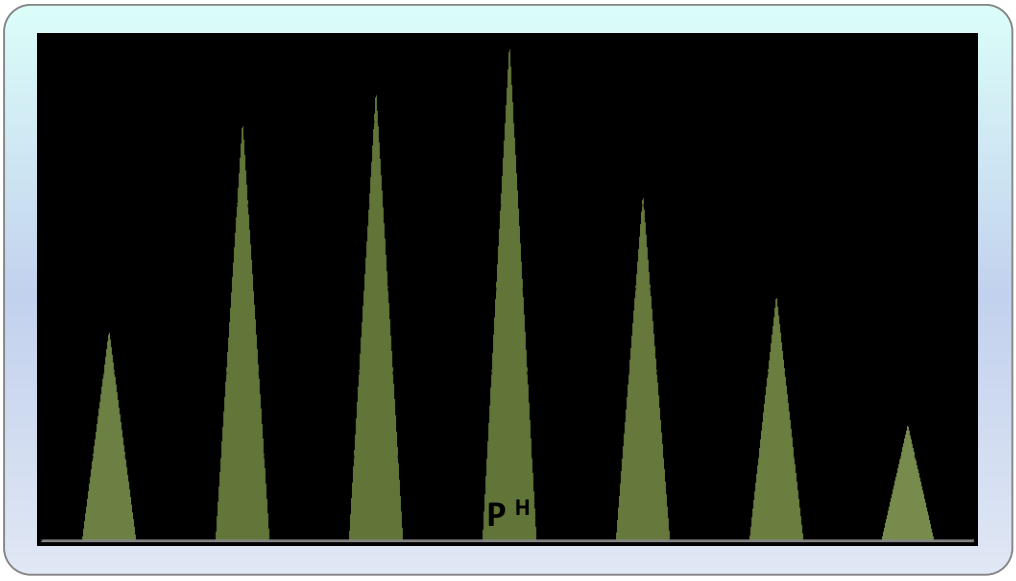


Fig. 7. Growth of *C. gloeosporioides* in Richards' Broth at different p^H

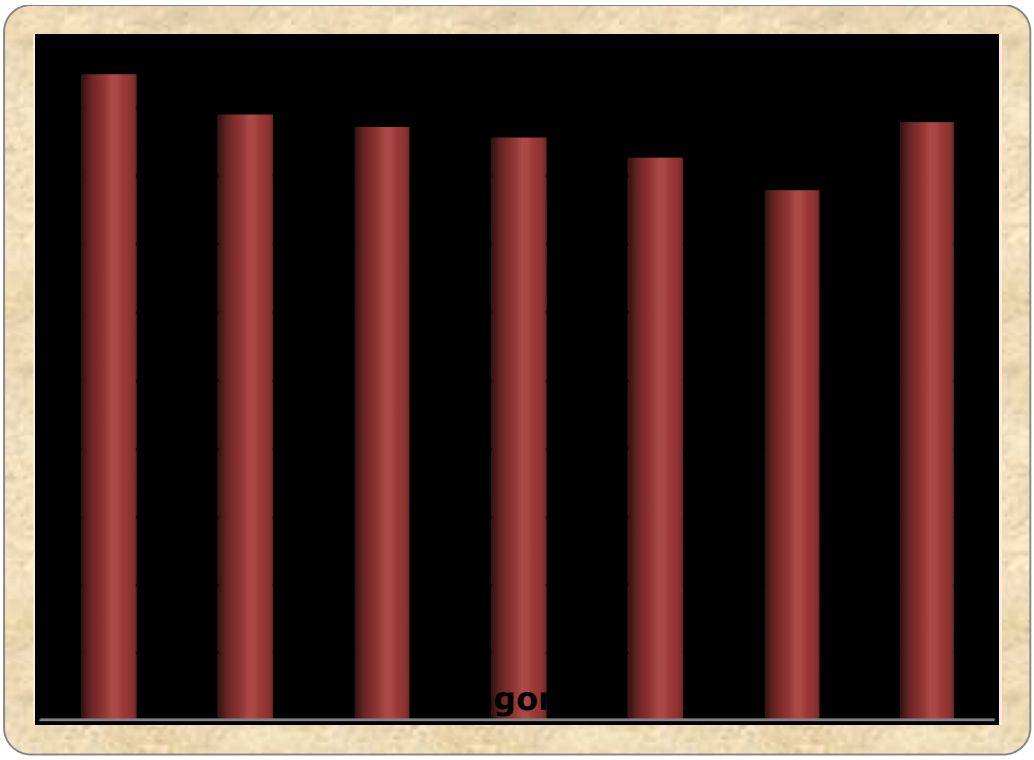


Fig. 8. *In vitro* inhibition of *C. gloeosporioides* by antagonists.

Bacillus sp. and *Pseudomonas fluorescens* showed statistically significant difference in their antagonistic property against *C. gloeosporioides* (Table 19). *Bacillus* sp. inhibited 87.78 % growth of *C. gloeosporioides* whereas *Pseudomonas fluorescens* showed 77.78 % growth inhibition (Plate-30).

The per cent growth inhibition by the bacterial and fungal isolates were compared. Between the two bacterial isolates, *Bacillus* sp. exhibited significantly high inhibition of growth of the pathogen than *Pseudomonas fluorescens*. *Bacillus* sp. was statistically on par with *Aspergillus niger*. Among the fungal and bacterial antagonists tested, *Trichoderma harzianum* which showed the maximum suppression of pathogen was selected for *in vivo* experiment (Fig. 8).

4.5.2.1. Identification of bacterial antagonist of *C. gloeosporioides*

The isolates having fluorescence around them were selected and they were subjected to various tests as indicated under materials and methods. From this they were found as gram –ve rod, catalase positive and produced raised, entire, slimy colonies on King's B medium and identified as *Pseudomonas fluorescens.* The other bacteria was identified as *Bacillus* sp. based on colony morphology, bacterial shape, gram staining and spore staining. The bacterium was gram +ve, rod shaped with endospore, producing flat colonies with tree like margins on nutrient agar.

4.5.3. *In vitro* screening of antagonists against *C. gloeosporioides* on detached chilli fruits

Among the various antagonists, *Trichoderma harzianum* produced minimum infection on the fruit surface, followed by *Gliocladium virens*. The lesion developed by *Bacillus* sp. was on par with *Gliocladium virens*. *Pseudomonas fluorescens, Aspergillus niger* and *Penicillium* sp. significantly inhibited disease development (Table 20, and Plate 31).

Table 20. Screening of microbial antagonists against *C. gloeosporioides* on detached chilli fruit (var. Vellayani Athulya).

Sl. No.	Microbial Antagonists	Third day		Sixth day		Nineth day	
		Fruit rot Lesion Size (cm) *	% reduction over control	Fruit rot Lesion Size (cm)*	% reduction over control	Fruit rot Lesion Size (cm)*	% reduction over control
1.	*Trichoderma harzianum*	0.88	65.08	1.00	81.92	1.25	89.25
2.	*Gliocladium virens*	1.33	47.22	1.48	73.24	1.62	86.07
3.	*Bacillus* sp.	1.42	43.65	1.58	71.43	1.72	85.21
4.	*Pseudomonas fluorescens*	1.58	37.30	1.8	67.45	1.93	83.40
5.	*Aspergillus niger*	1.85	26.59	2.28	58.77	4.42	61.99
6.	*Aspergillus flavus*	1.87	25.79	2.63	52.44	4.92	57.69
7.	*Penicillium* sp.	1.95	22.62	2.92	47.19	5.88	49.44
8.	Control	2.52		5.53		11.63	
	CD at 5 %	0.122		0.114		0.119	

*Mean of three replication

Plate 29. Inhibition of *C. gloeosporioides* by fungal antagonists

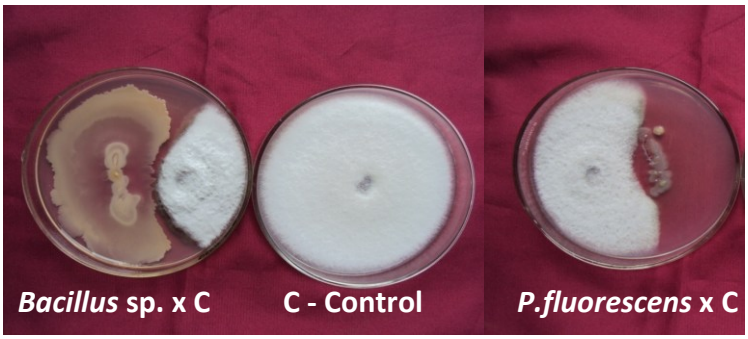


Plate 30. Inhibition of *C. gloeosporioides* by bacterial antagonists.

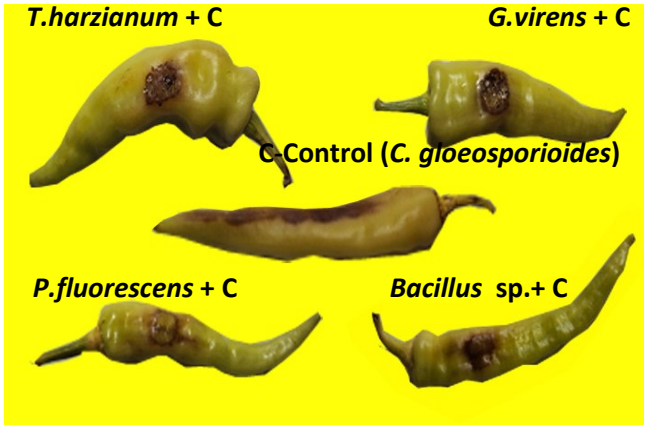


Plate 31. Lesion on Vellayani Athulya fruits by inoculation with antagonist and *C.gloeosporioides.*

4.5.4. Studies on the selected fungal antagonist *T. harzianum*

Among the five fungal and two bacterial antagonists, the isolate which showed maximum percentage inhibition against *C. gloeosporioides* was *T. harzianum.*

4.5.4.1. Mycoparasitism of the selected fungal antagonist *T. harzianum* against *C. gloeosporioides*

Trichoderma harzianum Rifai. inhibited the mycelial growth of *C. gloeosporioides* in dual culture. Contact between *Trichoderma harzianum* and *C. gloeosporioides* was established in the dual culture after 24 h of incubation. At first they grew in intimate contact with the hyphae of *C. gloeosporioides* and then coiled around the pathogen, resulting in emptying the contents of hyphae. Ultimately pathogenic hyphae shrivelled and got killed. The antagonist completely overgrew and killed the pathogen within five days of incubation (Plate 32).

4.5.4.2. Production of volatiles by *T. harzianum*

Volatile compound released from the cultures of *T. harzianum*. inhibited colony growth of *C. gloeosporioides.* The percentage inhibition of *C. gloeosporioides* by volatiles was 37.78. (Table 21, Plate 33).

4.5.4.3. Production of non volatiles by *T. harzianum*

The results showed that the fungal antagonists *T. harzianum*. inhibited the growth of *C. gloeosporioides* by the production of non volatile antifungal substances. The percentage inhibition of *C. gloeosporioides* when exposed to the volatiles was found to be less compared to that by non volatile compounds. The growth of *C. gloeosporioides* was inhibited by 51.11 per cent by the non volatiles produced by *T. harzianum* (Table 21, Plate 33).

4.5.4.4. Effect of culture filtrate of *T. harzianum* on *C. gloeosporioides*

The diffusible antibiotics in the culture filtrate of *T. harzianum* significantly restricted the growth of *C. gloeosporioides* at 10 % concentration (57.78 %), 5 % concentration (28.61 %) and 1 % concentration (5.27 %). The data are presented in Table 22.

4.5.4.5. Preparation of the talc based formulation of *T. harzianum* and its shelf life studies

Talc based formulation of *T. harzianum* was prepared and kept in polythene bags as indicated in materials and methods. Viable propagules of *T. harzianum* in talc based formulation stored at room temperature was recorded by serial dilution technique. The population was found to reduce gradually with length of storage. The population count after 10 days of incubation was 166.25×10^4 cfu/g. After 30 days of storage, the number of propagules were reduced to 143.25×10^4. After 6 months of storage, maximum number of viable propagules observed were 22.75×10^4 cfu/g (Table 23, Fig. 9).

4.5.5. *In vitro* screening of plant extracts against *C. gloeosporioides*

Seven plant extracts *viz., Piper betle, Ocimum sanctum, Azadirachta indica, Lantana camara, Datura stramonium, Andrographis paniculata* and *Bouganvillea glabra* at three different concentrations (60, 80 and 100 %) were evaluated in the laboratory for their efficacy against *C. gloeosporioides* through poisoned food technique (Table 24, Fig. 10).

Among the seven plant extracts evaluated, *Datura stramonium* was found to be the best in inhibiting the mycelial growth (90.71 %) of *C. gloeosporioides* and was significantly superior to all other plant extracts tested. The remaining six plant extracts also showed significantly higher inhibition of mycelial growth of

Table 21. Effect of volatiles and non volatiles of *Trichoderma harzianum* on growth of *C.gloeosporioides*

Treatments	Growth of the Pathogen (cm)*	% inhibition over control
Volatiles of *T. harzianum*	5.6	37.78
Non volatiles of *T. harzianum*	4.4	51.11
Control	9.0	-

*Mean of three replication

Table 22. Effect of *Trichoderma harzianum* culture filtrate on growth of *C.gloeosporioides*

Sl. No.	Culture filtrate of *T. harzianum* concentration (%)	Growth of the Pathogen (cm)*	% inhibition over control*
1.	1	8.53	5.27 (49.46)
2.	5	6.43	28.56 (32.32)
3.	10	3.80	57.78 (13.27)
CD at 5 %			0.892

*Mean of four replication

Table 23. Effect of Talc on the population of *Trichoderma harzianum*.

Days of incubation	**Population of *Trichoderma harzianum* (× 10^4 cfu/g)**
10^{th}	166.25
30^{th}	143.25
60^{th}	108.50
90^{th}	95.00
120^{th}	67.75
150^{th}	43.00
180^{th}	22.75
CD at 5 %	1.079

*Mean of four replication

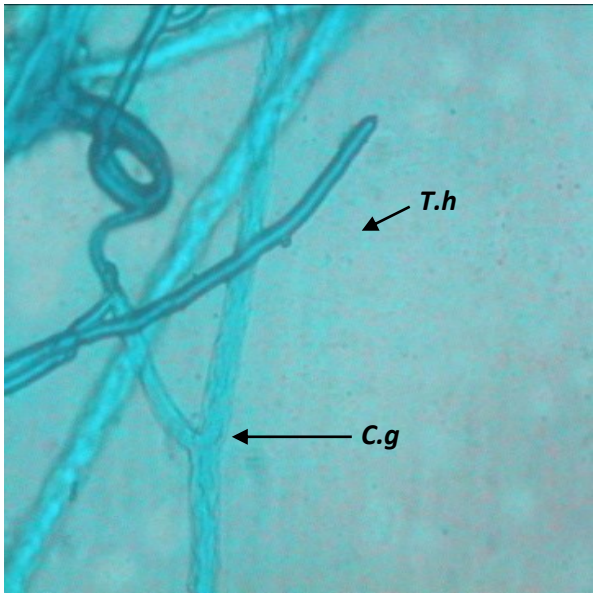


Plate 32. Hyphal coiling of *T. harzianum* (*T. h*) on *C. gloeosporioides* (*C. g*).

Plate 33. Inhibition of growth of *C. gloeosporioides* by volatiles and non volatiles of *T. harzianum*.

Table 24. Effect of various plant extracts on the growth of *C. gloeosporioides.*

Sl. No	Plant Extracts	Growth of the Pathogen (cm) at 60% concentration*	% inhibition over control	Growth of the Pathogen (cm) at 80% concentration *	% inhibition over control	Growth of the Pathogen (cm) at 100 % concentration*	% inhibition over control	Mean	
								Growth of the Pathogen (cm)	% inhibition over control
1.	*Ocimum sanctum*	4.85	30.71 (33.64)	3.37	51.90 (46.07)	2.48	64.52 (53.42)	3.57	49.04 (44.38)
2.	*Andrographis paniculata*	3.45	50.71 (45.39)	2.65	62.14 (52.00)	1.88	73.09 (58.73)	2.66	61.98 (52.04)
3.	*Lantana camara*	3.2	54.28 (47.44)	1.53	78.09 (62.07)	0.83	88.10 (69.79)	1.86	73.49 (59.77)
4.	*Datura stramonium*	1.95	72.14 (58.12)	0.00	99.99 (90.00)	0.00	99.99 (90.00)	0.65	90.71 (79.37)
5.	*Azadirachta indica*	5.75	17.89 (25.01)	4.85	30.71 (33.64)	4.08	41.65 (40.18)	4.89	30.08 (32.94)
6.	*Piper betle*	5.95	14.99 (22.77)	5.65	19.28 (26.04)	4.95	29.28 (32.75)	5.52	21.18 (27.18)
7.	*Bougainvillea glabra*	5.3	24.27 (29.50)	3.75	46.42 (42.93)	2.68	61.66 (51.72)	3.91	44.12 (41.39)
8.	Control	7.0		7.0		7.0		7.0	
CD at 5 % between concentrations					0.892				
CD at 5 % between extracts									0.515

(Figures given in parenthesis are transformed values)
*Mean of three replication

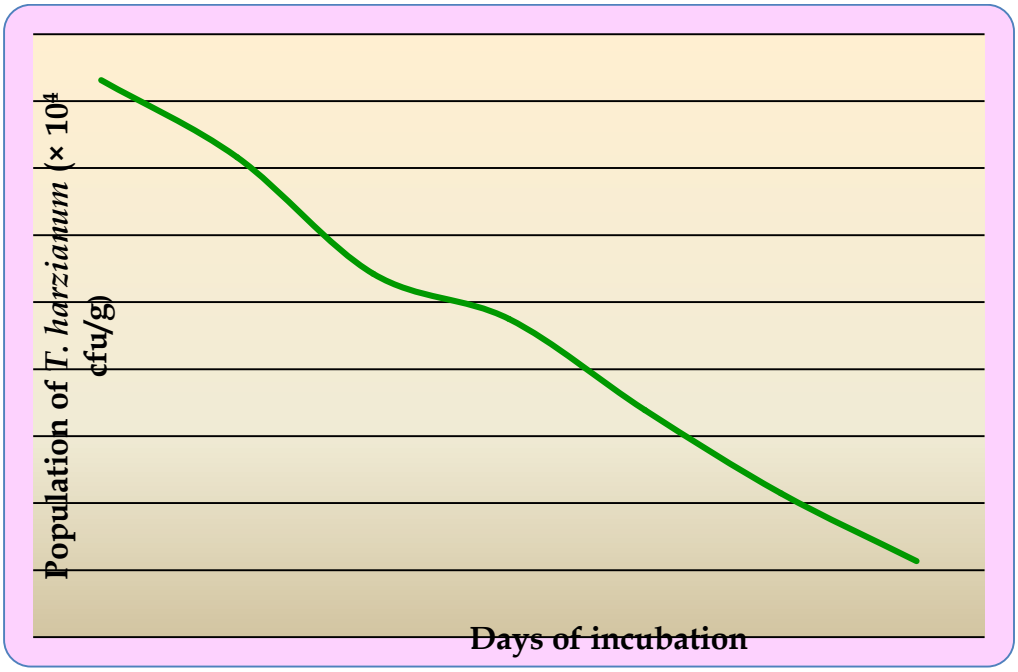


Fig. 9. Influence of storage period on the population of *T. harzianum*.

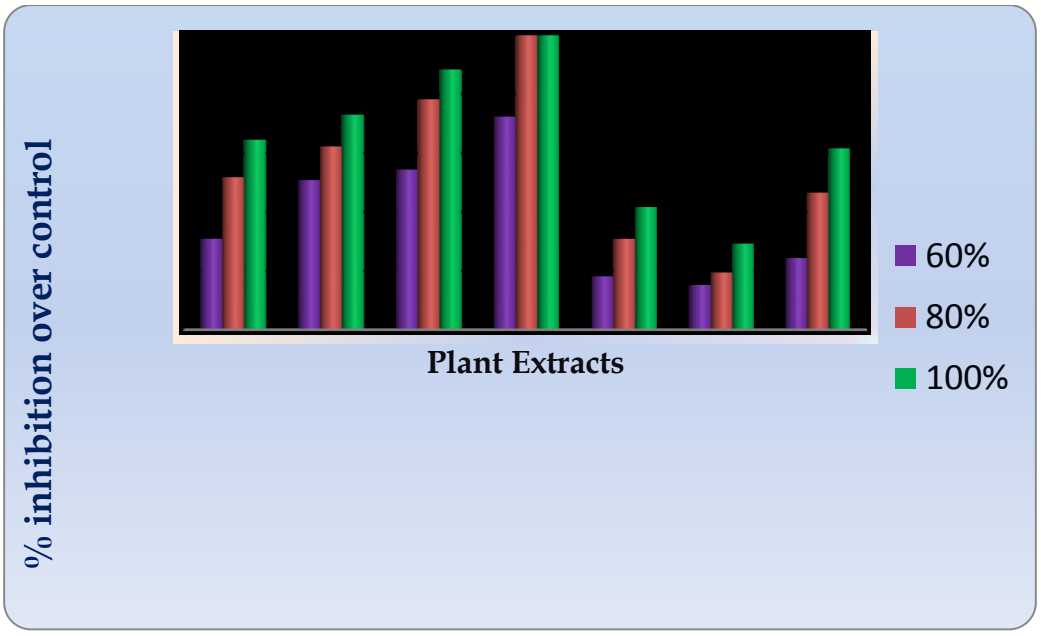


Fig. 10. Inhibition of *C. gloeosporioides* by different plant extracts

C. gloeosporioides. They were *Lantana camara, Andrographis paniculata, Ocimum sanctum, Bougainvillea glabra, Azadirachta indica, Piper betle* with 73.49 %, 61.98 %, 49.04 %, 44.12 %, 30.08 %, 21.18 % inhibitions respectively.

Among the seven plant extracts at 100 % concentration evaluated, *Datura stramonium* was found to be the best with cent per cent inhibition of mycelial growth of *C. gloeosporioides* and was significantly superior to all other extracts tested. The extracts of *Lantana camara, Andrographis paniculata, Ocimum sanctum*, *Bougainvillea glabra* and *Azadirachta indica* exhibited 88.10, 73.09, 64.52, 61.66 and 41.65 per cent inhibitions respectively. Least inhibition of mycelial growth of *C. gloeosporioides* was recorded in *Piper betle* (29.28 %). All the plant extracts at 100 % concentration significantly inhibited the growth of *C. gloeosporioides.*

A similar trend was observed when all the seven plant extracts were evaluated at 80 and 60 per cent concentrations against *C. gloeosporioides.*

Among the different concentrations of the different plant extracts, *Datura stramonium* leaf extract (99.99 %) at 80 and 100 per cent concentration was on par with each other giving cent per cent inhibition of the mycelial growth of *C. gloeosporioides* and were significantly superior to all other plant extracts tested. *Lantana camara* leaf extract at 100 % concentration (88.10 %) and 80 % concentration (78.09 %) exhibited significantly lower effect than *Datura stramonium* at 100 and 80 % concentration. *Andrographis paniculata* at 100 per cent concentration (73.09 %) was on par with *Datura stramonium* at 60 % concentration (72.14 %) and showed significantly lower effect than *Lantana camara* at 100 and 80 % concentration. Next best plant extract in inhibiting the growth of *C. gloeosporioides* was *Ocimum sanctum* at 100 per cent concentration (64.52 %). *Andrographis paniculata* at 80 % concentration (62.14 %) was on par with *Bougainvillea glabra* at 100 per cent concentration (61.66 %). *Lantana camara* leaf extract at lowest concentration of 60 % (54.28 %) showed less effect than

Bougainvillea glabra at 100 per cent concentration. *Ocimum sanctum* at 80 % concentration (51.90 %) was on par with *Andrographis paniculata* at 60 % concentration (50.71 %). *Bougainvillea glabra* at 80 % concentration (46.42 %) and *Azadirachta indica* at 100 % concentration (41.65 %) showed significantly lesser effect than *Andrographis paniculata* at 60 % concentration. *Azadirachta indica* at 80 % concentration and *Ocimum sanctum* at 60 % concentration were on par with *Piper betle* at 100 per cent concentration (29.28 %) and showed significantly less effect than *Andrographis paniculata* at 60 % concentration. *Bougainvillea glabra* at 60 %, *Piper betle* at 80 % and *Azadirachta indica* at 60 % concentration showed significantly lower effect than *Andrographis paniculata* at 60 % concentration. Though least inhibition of mycelial growth of *C. gloeosporioides* was exhibited by *Piper betle* at 60 per cent concentration (14.99%), this leaf extract was statistically significant in its effectiveness in checking the growth of *C. gloeosporioides (*Plate 34).

Lower concentrations (*viz.,* 40, 20, 10 and 5 %) of the most effective leaf extract *Datura stramonium* tested under *in vitro* condition are presented in Table 25 and Plate-35. *Datura stramonium* at 40, 20, 10 and 5 % concentration were also effective in inhibiting the growth of *C. gloeosporioides* and the per cent inhibition recorded were 55.18, 40.71, 36.96 and 28.75 respectively. As the concentration decreases, its effect is also found decreasing (Plate 35).

4.5.6. *In vitro* screening of plant products against *C. gloeosporioides*

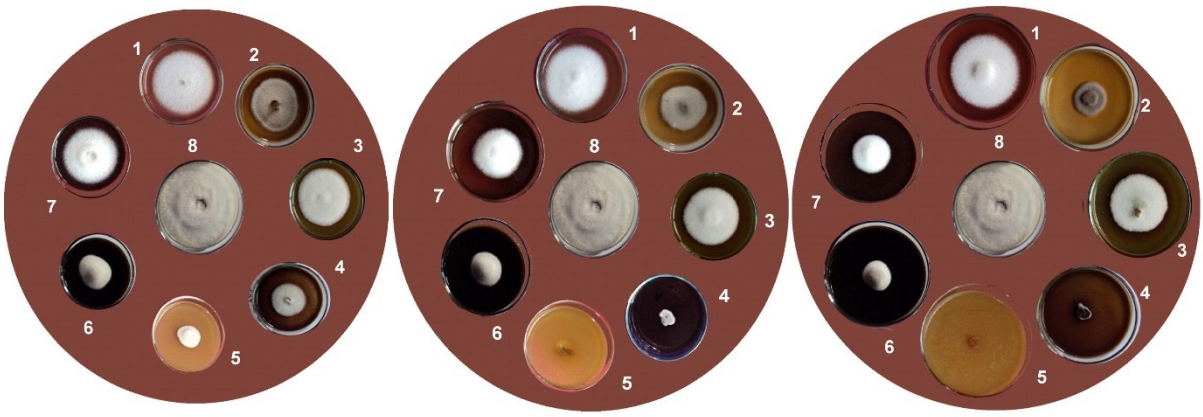
Four plant products *viz.*, Turmeric powder + Sodium bicarbonate (10:1), Garlic Extract, Neem oil and Neem Seed Kernel Extract at two different concentrations were evaluated for their efficacy against *C. gloeosporioides* using poisoned food technique (Table 26).

Table 25. Percentage inhibition of *C. gloeosporioides* by *Datura stramonium* leaf extract.

Sl. No.	*Datura stramonium* leaf extract Concentration (%)	Growth of the Pathogen (cm)	% inhibition over control
1.	40	3.14	55.17 (7.43)
2.	20	4.15	40.70 (6.38)
3.	10	4.41	36.96 (6.08)
4.	5	4.98	28.74 (5.36)
5.	control	9.0	
CD at 5 %			0.101

(Figures given in parenthesis are transformed values).

*Mean of four replication



1. *Piper betle*
2. *Ocimum sanctum*
3. *Azadirachta indica*
4. *Lantana camara*
5. *Datura stramonium*
6. *Andrographis paniculata*
7. *Bougainvillea glabra*
8. Control

Plate 34. Inhibition of growth of *C. gloeosporioides* by plant extracts (60, 80 and 100 %).

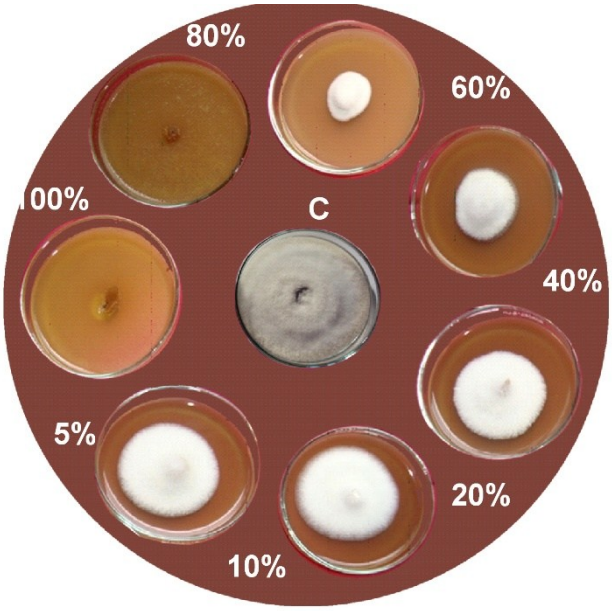


Plate 35. Inhibition of growth of *C. gloeosporioides* by *Datura stramonium.*

Table 26. Effect of various plant products on the growth of *C. gloeosporioides.*

Sl. No	Plant Product	Concentration (%)	Growth of the Pathogen* (cm)	inhibition over control (%)	Mean	
					Growth of the Pathogen (cm)	% inhibition over control
1.	Turmeric powder + Sodium bicarbonate (10 : 1)	0.15	6.87	23.69 (29.12)	7.18	20.18 (26.60)
		0.075	7.5	16.67 (24.09)		
2.	Garlic bulb extract	10	2.23	75.19 (60.10)	3.4	62.23 (52.33)
		5	4.57	49.26 (44.56)		
3.	Neem oil	2	5.8	35.55 (36.59)	6.25	30.55 (33.47)
		1	6.7	25.55 (30.35)		
4.	Neem seed kernel extract	5	7.22	19.81 (26.42)	7.87	12.59 (19.89)
		2.5	8.52	5.36 (13.38)		
5.	Control		9.0		9.0	
	CD at 5 % between concentrations			0.842		
	CD at 5 % between plant products					0.595

* Mean of three replication

(Figures given in parenthesis are transformed values)

Among the plant products, Garlic extract gave highest percentage growth inhibition of 62.23 and was significantly superior to all other plant products tested. All the four plant products were effective in checking the growth of *C. gloeosporioides* when compared to control. However Neem oil (30.55 %) was nearly 50 per cent less effective than Garlic extract. 10 : 1 proportion of Turmeric powder and Sodium bicarbonate was significantly less effective (20.18%) than neem oil. Least inhibition of mycelial growth of *C. gloeosporioides* was observed in Neem Seed Kernel Extract (12.59 %).

Among the different concentrations of different plant products tested, Garlic at 10 % concentration gave the highest growth inhibition (75.19 %) followed by 2 % Neem oil (35.55 %). In general, higher the concentration of plant products, higher was the reduction of mycelial growth of the pathogen (Plate 36).

4.5.7. *In vitro* effect of Bavistin at various concentrations on the growth of *C. gloeosporioides*

Complete growth inhibition was observed at 0.1 % and 0.05 % concentration of Bavistin tested. Bavistin at 0.01 % concentration exhibited growth inhibition of 81.11 % (Table 27, Plate 37).

4.5.8. *In vitro* evaluation of various combinations of selected antagonist + plant extract + plant product against *C. gloeosporioides*

This experiment was conducted to find out the inhibitory effect of the combination of selected antagonist, plant extract and plant product on the mycelial growth of *C. gloeosporioides*. The four different combinations *viz.,* talc based formulation of *Trichoderma harzianum* (1 %) + *Datura stramonium* leaf extract (20 %) + Garlic bulb extract (10 %), talc based formulation of *Trichoderma harzianum* (1 %) + *Datura stramonium* leaf extract (20 %), talc based formulation of *Trichoderma harzianum* (1 %) + Garlic bulb extract (10 %) and *Datura stramonium*

Table 27. Percentage inhibition of *C.gloeosporioides* by different concentrations of Bavistin.

Sl. No.	Bavistin Concentrations (%)	Growth of the Pathogen (cm)*	% inhibition over control
1.	0.1	0	0
2.	0.05	0	0
3.	0.01	7.3	81.11
4.	Control	9.0	

*Mean of three replication

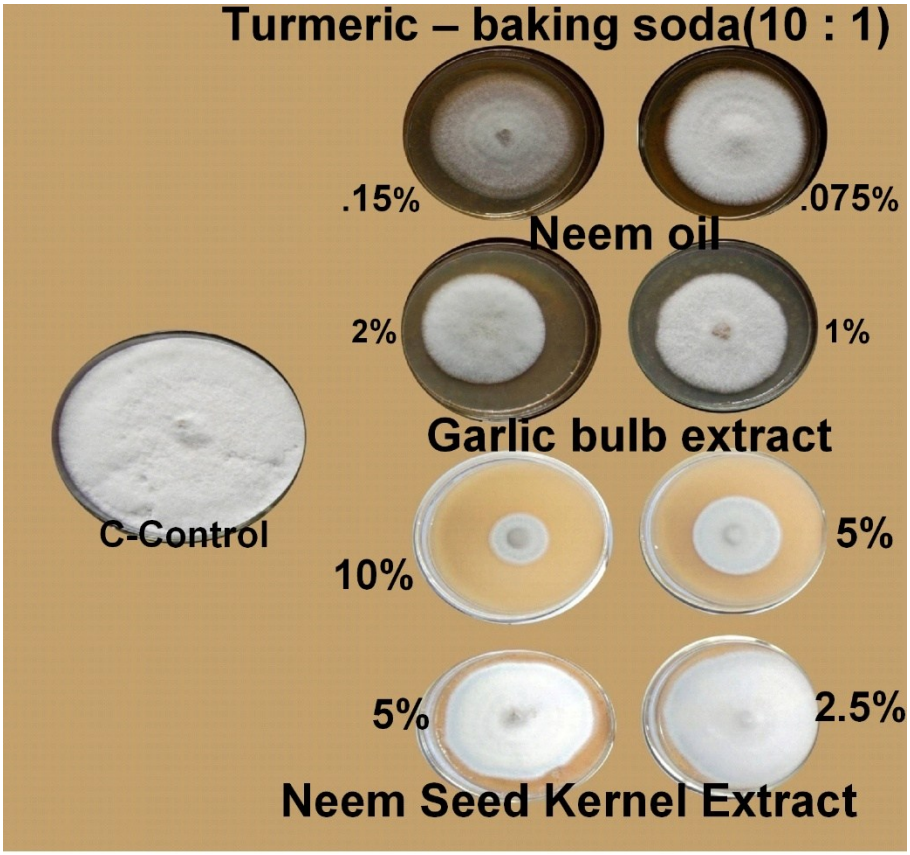


Plate 36. Inhibition of growth of *C. gloeosporioides* by plant products.

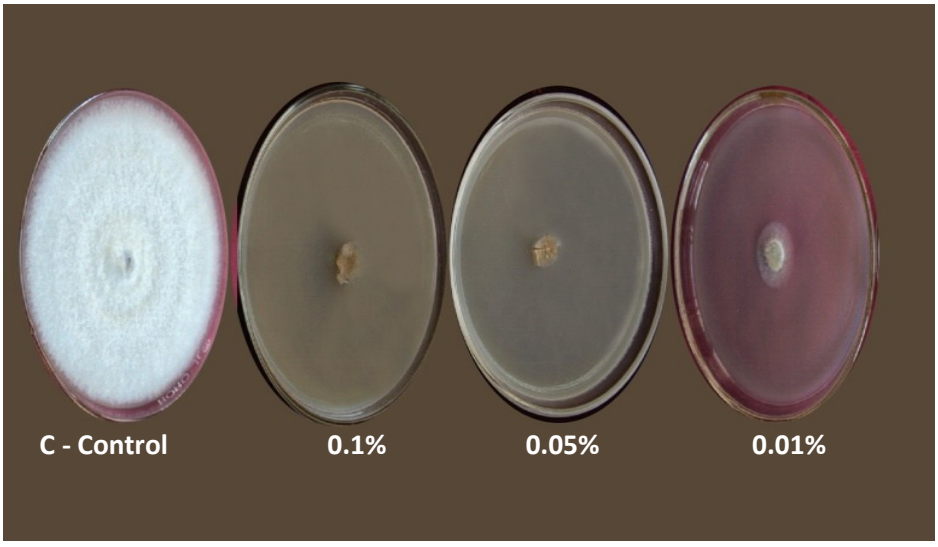


Plate 37. Inhibition of growth of *C. gloeosporioides* by Bavistin.

leaf extract (20 %) + Garlic bulb extract (10 %) were tested against *C. gloeosporioides.* In all the four combinations, *C. gloeosporioides* showed cent percent reduction in mycelial growth. These results reveal the fact that *T. harzianum* is compatible with the botanicals and that the combinations acted synergistically against the pathogen *C. gloeosporioides* (Plate 38).

4.5.9. *In vitro* evaluation of selected plant extract and plant product individually and in combination against selected antagonist

This experiment was conducted to find out whether there is any inhibitory effect of selected plant extract and plant product and its combination on the antagonist. *Trichoderma harzianum* grew well in the media incorporated with *Datura stramonium* leaf extract (20 %) + Garlic bulb extract (10 %) concentration within five days indicating that the antagonist is compatible with *Datura stramonium* leaf extract + Garlic bulb extract. *Trichoderma harzianum* also grew well without any inhibition in the media which is incorporated with *Datura stramonium* leaf extract at 20 % and Garlic bulb extract at 10 % concentration. From these results it is evident that the selected antagonist is compatible with the selected plant extract and selected plant product and such a combination is selected for further *in vivo* studies (Plate-39).

4.6. VARIETAL SCREENING

4.6.1. Screening of chilli varieties against *C. gloeosporioides*

In order to select a susceptible variety for conducting the *in vivo* experiment on management of fruit rot of chilli, a screening trial was conducted using the chilli varieties released by KAU. Five chilli varieties released from Kerala Agricultural University *viz.*, Jwalamukhi, Jwalasakhi, Ujwala, Vellayani Athulya and Anugraha were screened against *C. gloeosporioides.* (Table 28).

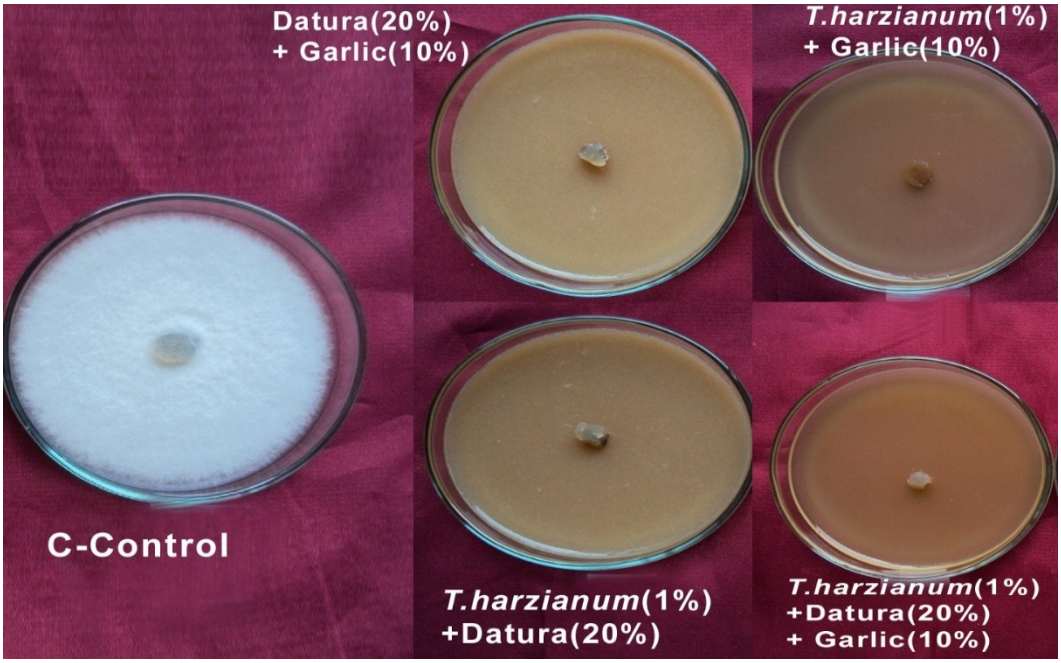


Plate 38. Inhibition of growth of *C. gloeosporioides* by combinations of extracts of Datura, Garlic and *T. harzianum* formulation.

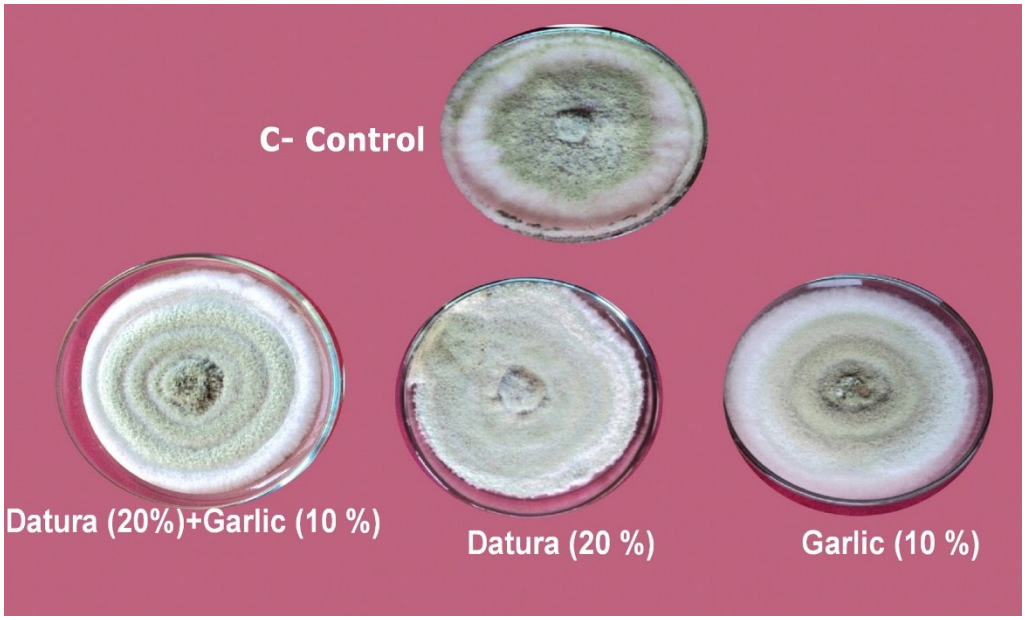


Plate 39. Effect of extracts of Datura and Garlic and their combination on *T. harzianum*

Table 28. Screening of chilli varieties against *C. gloeosporioides.*

Sl. No.	Disease Severity scale	Fruit surface area affected (%)	Varieties	Disease Incidence* (%)	Disease index* (PDI)	Category
1.	0	0				Immune
2.	1	1-6	Ujwala	5.88 (14.03)	3.82 (11.27)	Resistant
3.	2	6 – 25	Anugraha	14.25 (22.17)	16.15 (23.68)	Moderately Resistant
4.	3	25 – 50	Jwalamukhi	35.00 (36.26)	38.49 (38.33)	Susceptible
5.	4	50 – 100	Jwalasakhi	66.59 (54.67)	79.07 (62.75)	Highly Susceptible
			Vellayani Athulya	84.34 (66.66)	90.13 (71.66)	
CD at 5 %				4.621	2.706	

(Figures given in parenthesis are angular transformed values)

* Mean of four replication

None of the varieties were found immune to fruit rot disease. The variety Ujwala showed resistant reaction with 3.82 per cent disease index whereas Anugraha was moderately resistant with 16.15 disease index. Jwalamukhi was susceptible with fruit rot disease index of 38.49 per cent. Jwalasakhi and Vellayani Athulya were highly susceptible to fruit rot infection showed a disease index of 79.07 and 90.13 per cent respectively (Plate 40, Fig. 11).

.4.7. *IN VIVO* MANAGEMENT OF FRUIT ROT OF CHILLI CAUSED BY *C. GLOEOSPORIOIDES*

4.7.1. Preparation of pathogen inoculum and foliar application

Conidial suspension of *C. gloeosporioides* containing 10^6 conidia / ml prepared in 0.5 % peptone water was sprayed on chilli plants 20 days after fruit formation.

4.7.2. *In vivo* management of fruit rot of chilli

Based on the results of the *in vitro* management studies, the best antagonist *T. harzianum*, best plant extract *Datura stramonium* leaf extract and the best plant product Garlic bulb extract and their combinations were selected as treatments for the *in vivo* study. *T. harzianum,* was formulated into talc based formulation for field spraying. The following were the nine treatments and their schedule of application.

1. **Vellayani Athulya**
2. **Jwalasakhi**
3. **Jwalamukhi**
4. **Anugraha**
5. **Ujwala**

Plate 40. Fruit rot infection by *C. gloeosporioides* on chilli varieties released by KAU

Treatments	**Particulars**	**Schedule of Application**	**Dose**
T_1	Talc based formulation of *Trichoderma harzianum*	• Seedling root dip at the time of transplanting • Foliar spray two Days After artificial Inoculation (DAI) of the pathogen. • Foliar spray seven DAI	500 g/l 1 % 1 %
T_2	*Datura stramonium* leaf extract	• Foliar spray two DAI • Foliar spray seven DAI	20 % 20 %
T_3	Garlic bulb extract	• Foliar spray two DAI • Foliar spray seven DAI	10 % 10 %
T_4	T_1 + T_2	• Seedling root dip at the time of transplanting with *T. harzianum* • Foliar spray by the combination of *T. harzianum* and *D. stramonium* two DAI. • Foliar spray by the combination of *T. harzianum* and *D. stramonium* seven DAI.	500g/l 1 % + 20 % 1 % + 20 %
T_5	T_1 + T_3	• Seedling root dip at the time of transplanting with *T. harzianum* • Foliar spray by the combination of *T. harzianum* and garlic bulb extract two DAI. • Foliar spray by the combination of *T. harzianum* and garlic bulb extract seven DAI.	500g/l 1 % + 10 % 1 % + 10 %

T_6	T_2 + T_3	• Foliar spray by the combination of *D. stramonium* and garlic bulb extract two DAI. • Foliar spray by the combination of *D. stramonium* and garlic bulb extract seven DAI.	20 % + 10 % 20 % + 10 %
T_7	T_1 + T_2 + T_3	• Seedling root dip at the time of transplanting with *T. harzianum* • Foliar spray by the combination of *T. harzianum* + *D. stramonium* leaf extract + garlic bulb extract two DAI. • Foliar spray by the combination of *T. harzianum* + *D. stramonium* leaf extract + garlic bulb extract seven DAI.	500g/l 1 % + 20 % +10 % 1 % + 20 % +10 %
T_8	Bavistin 0.05 %	• Foliar spray two DAI.	0.05%
T_9	Pathogen inoculated control	• Water spray.	

4.7.3. Disease intensity

Conidial suspension of *C. gloeosporioides* containing 10^6 conidia / ml was sprayed on chilli plants 20 days after fruit formation. Two days after foliar spraying with the pathogen, all the inoculated fruits showed incidence of the disease in the form of water soaked lesions around the pin pricks.

The percent disease index (PDI) was calculated based on the damage caused by fruit rot pathogen on the chilli fruits using the formula developed by Mc Kinney during 1923. Data on disease index before treatment application, after first treatment application (seven days after artificial inoculation of the pathogen) and after second treatment application (twelve days after artificial inoculation of the pathogen) are given in (Table 29, Plate 41 and Fig. 12).

Table 29. Effect of ecofriendly materials on disease intensity of fruit rot of chilli.

Treatment		Disease index before treatment application*	Disease index after first treatment application*	Disease index after second treatment application*	Disease reduction (%)
T_1	*Trichoderma harzianum* talc based formulation (1 %)	36.59 (35.57)	31.74 (34.28)	30.76 (33.67)	55.87
T_2	*Datura stramonium* leaf extract (20 %)	28.01 (22.07)	26.44 (30.93)	36.04 (36.88)	48.29
T_3	Garlic bulb extract (10 %)	34.56 (32.20)	30.25 (33.35)	33.16 (35.14)	52.42
T_4	T1 + T2	32.16 (28.36)	32.69 (34.86)	30.76 (33.67)	55.87
T_5	T1 + T3	35.19 (33.24)	35.09 (36.31)	29.79 (33.07)	57.25
T_6	T2 + T3	35.43 (33.63)	31.71 (34.25)	30.25 (33.35)	56.56
T_7	T1 + T2 + T3	33.97 (31.24)	29.31 (32.76)	27.40 (31.55)	60.69
T_8	Bavistin 0.05 %	33.07 (29.79)	27.88 (31.85)	25.48 (30.30)	63.45
T_9	Untreated Control	26.66 (20.14)	36.53 (37.17)	69.72 (56.59)	
	CD at 5 %	1.594	1.543	1.466	

(Figures given in parenthesis are angular transformed values) * Mean of four replication

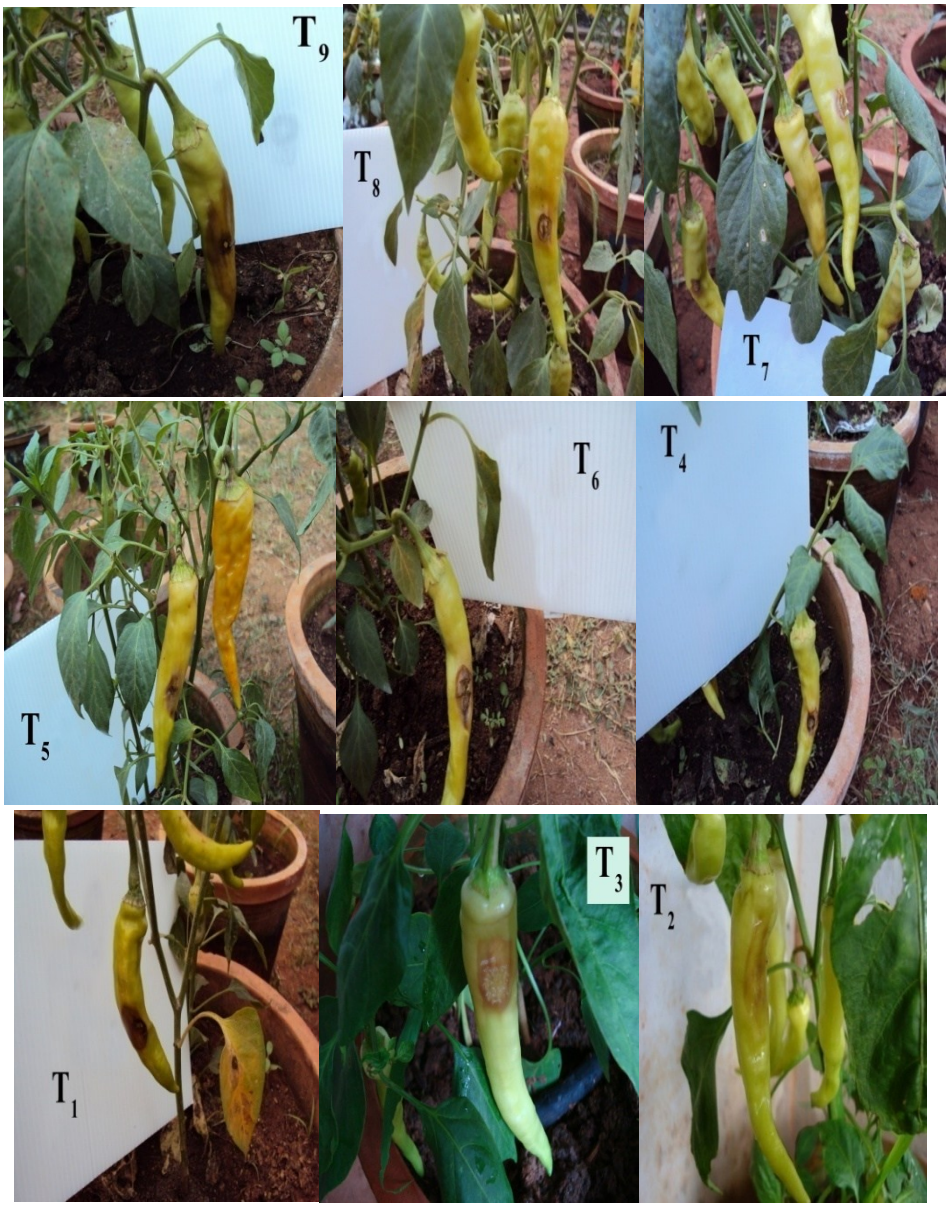


Plate 41. Effect of ecofriendly materials on disease intensity of fruit rot of chilli.

T1 - Talc based formulation of *T. harzianum*

T2 - *Datura stramonium* leaf extract

T3 - Garlic bulb extract

T4 - T1 + T2

T5 - T1 + T3

T6 - T2 + T3

T7 - T1 + T2 + T3

T8 - Bavistin @ 0.05 %

T9 - Control.

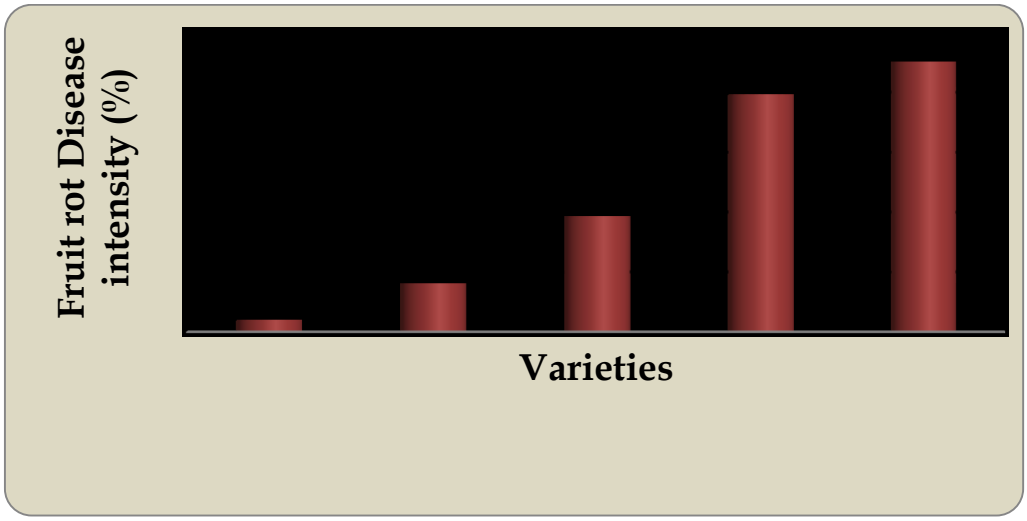
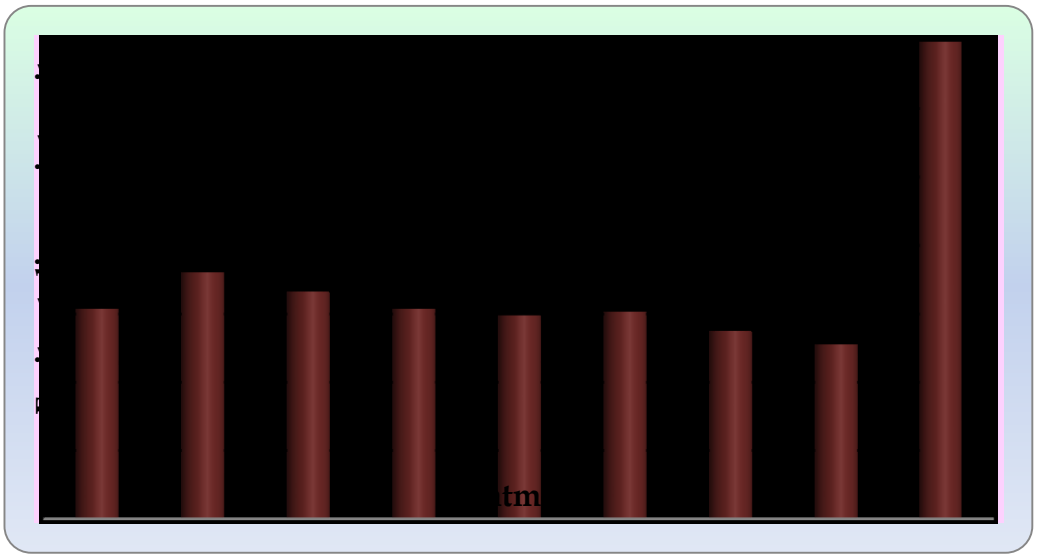


Fig. 11. Reaction of chilli varieties against *C. gloeosporioides.*



T1 - Talc based formulation of *T. harzianum*

T2 - *Datura stramonium* leaf extract

T3 - Garlic bulb extract

T4 - T1 + T2

T5 - T1 + T3

T6 - T2 + T3

T7 - T1 + T2 + T3

T8 - Bavistin @ 0.05 %

T9 - Control.

Fig. 12. Effect of ecofriendly materials on disease intensity of fruit rot of chilli.

First treatment application was given, two days after artificial inoculation. All the treatments recorded significantly low disease intensity compared to control. Plants sprayed with Datura leaf extract (T_2) with PDI of 26.44 and Bavistin as check (T_8) with 27.88 PDI were statistically on par with each other. T_8 (Bavistin as check) was on par with T_7 (talc based formulation of *T. harzianum* + Datura leaf extract + Garlic bulb extract) with 29.31 PDI. Plants which received treatments T_7, T_3 (Garlic bulb extract) and T_6 (Datura leaf extract + Garlic bulb extract) were also statistically on par with each other with PDI of 29.31, 30.25 and 31.71 respectively. Plants sprayed with Garlic bulb extract (T_3) was on par with Datura leaf extract + Garlic bulb extract (T_6), talc based formulation of *T. harzianum* (T_1) and talc based formulation of *T. harzianum* + Datura leaf extract (T_4) with 30.25, 31.71, 31.74 and 32.69 PDI respectively. DI of 32.69 per cent recorded after spraying with talc based formulation of *T. harzianum* + Datura leaf extract (T_4) was statistically on par talc based formulation of *T. harzianum* + Garlic bulb extract (T_5) with 35.09 per cent.

Second treatment application was given five days after first treatment application. It was observed that application of all the treatments gave significant control of the disease. Maximum control of the disease of 25.48 PDI was obtained by spraying Bavistin as check (T_8). But it was found that spraying talc based formulation of *T. harzianum* + Datura leaf extract + Garlic bulb extract (T_7) with 27.40 PDI was statistically on par with spraying Bavistin 0.05 % in controlling fruit rot disease. All the other treatments including spraying talc based formulation of *T. harzianum* + Garlic bulb extract (T_5) with PDI of 29.79, Datura leaf extract + Garlic bulb extract (T_6) with 30.25 and talc based formulation of *T. harzianum* + Datura leaf extract (T_4) 30.76 were statistically on par with each other and with talc based formulation of *T. harzianum* (T_1) with 30.76. All these treatments showed significantly lower effect in reducing the disease intensity compared to T_7 (talc based formulation of *T. harzianum* + Datura leaf extract + Garlic bulb extract). Treatments T_3 (Garlic bulb extract) and T_2 (Datura leaf extract) showed significantly lower

effect in reducing the disease intensity when compared to the combination sprays. Among the eight different treatments the lowest effect after two sprayings was recorded by *Datura stramonium* leaf extract.

Bavistin recorded 63.45 per cent disease reduction followed by T_7 (talc based formulation of *T. harzianum* + Datura leaf extract + Garlic bulb extract) 60.69 %. T_5 (talc based formulation of *T. harzianum* + Garlic bulb extract) recorded 57.25 % whereas T_6 (Datura leaf extract + Garlic bulb extract) recorded 56.56 %. Lowest percentage disease reduction was observed in T_2 (Datura leaf extract) 48.29 %.

4.7.4. Effect of application of ecofriendly materials on biometric parameters of chilli plant

4.7.4.1. Shoot length

All the treatments had significant influence on increasing the shoot length of chilli plants. Treatments involving *T. harzianum* showed maximum influence on shoot length. The maximum shoot length of 45.53 cm was obtained in T_7 (talc based formulation of *T. harzianum* + Datura leaf extract + Garlic bulb extract) followed by 43.70 cm in T_5 (talc based formulation of *T. harzianum* + Garlic bulb extract), 42.38 cm in T_4 (talc based formulation of *T. harzianum* + Datura leaf extract), and 40. 85 cm in T_1 (talc based formulation of *T. harzianum*). T_8 (Bavistin) applied plants recorded a shoot length of 35.85 cm. T_6 (Datura leaf extract + Garlic bulb extract) showed a shoot length of 32.78 cm. Shoot length recorded by treatments T_2 (Datura leaf extract) which was on par with T_3 (Garlic bulb extract) was 32.13 cm. In control plants the shoot length recorded was the least with 29.85 cm. (Table 30, Plate 42 and Fig. 13).

4.7.4.2. Stem girth

As in shoot length all the treatments significantly influenced the stem girth also. The treatments involving *T. harzianum* showed maximum influence in increasing the stem girth. The maximum stem girth of 4.2 cm was observed in T_7 followed by T_5, T_4 and T_1 with 4.00, 3.8 and 3.70 cm respectively. Stem girth recorded by T_8 was 3.48 cm. All the other treatments recorded lower stem girth than that recorded by T_8 which was statistically on par with that of T_6. The least stem girth was recorded in control plants (Table 30).

4.7.4.3. Root length

As in shoot length and stem girth all the treatments significantly influenced the root length also. The treatments involving *T. harzianum* showed maximum influence on root length. All the treatments were significantly different from each other in root length. The maximum root length of 33.78 cm was obtained in T_7 (talc based formulation of *T. harzianum* + Datura leaf extract + Garlic bulb extract) followed by T_5 (talc based formulation of *T. harzianum* + Garlic bulb extract) with 30.10 cm, T_4 (talc based formulation of *T. harzianum* + Datura leaf extract) with 28.40 cm and T_1 (talc based formulation of *T. harzianum*) with 25.65 cm. Bavistin recorded 23.60 cm root length. Treatments in T_6 (Datura leaf extract + Garlic bulb extract), T_3 (Garlic bulb extract) and T_2 (Datura leaf extract) recorded lower root length than that in Bavistin. The lowest root length was recorded by control plants. (Table 30, Plate 43 and Fig. 13).

4.7.4.4. Fresh plant weight

Fresh plant weight was also influenced by various treatments. All the treatments were significantly different from each other in fresh plant weight. Maximum influence in fresh plant weight was recorded in treatments involving *T. harzianum*. Maximum fresh weight of 154.65 g was recorded in T_7 followed by

Table 30. Effect of ecofriendly materials on biometric characters of chilli plant.

Treatments		Shoot length (cm)*	Stem girth (cm)*	Root length (cm)*	Fresh plant weight (g)*	Dry plant weight (g)*
T_1	*Trichoderma harzianum* talc based formulation (1 %)	40.85	3.7	25.65	129.33	49.61
T_2	*Datura stramonium* leaf extract (20 %)	32.13	3.15	19.38	97.29	32.79
T_3	Garlic bulb extract (10 %)	32.13	3.25	20.73	105.5	37.86
T_4	T1 + T2	42.38	3.83	28.40	138.6	55.65
T_5	T1 + T3	43.70	4.00	30.10	149.43	62.62
T_6	T2 + T3	32.78	3.43	22.93	114.83	41.38
T_7	T1 + T2 + T3	45.53	4.2	33.78	154.65	68.86
T_8	Bavistin 0.05 %	35.85	3.48	23.60	117.33	44.48
T_9	Inoculated Control	29.85	3.00	17.5	89.43	28.93
	CD at 5 %	0.110	0.080	0.136	0.171	0.105

* Mean of four replication

Plate 42. Effect of ecofriendly materials on shoot length of chilli plants

Plate 43. Effect of ecofriendly materials on root length of chilli plants.

T1 - Talc based formulation of *T. harzianum*

T2 - *Datura stramonium* leaf extract

T3 - Garlic bulb extract

T4 - T1 + T2

T5 - T1 + T3

T6 - T2 + T3

T7 - T1 + T2 + T3

T8 - Bavistin @ 0.05 %

T9 - Control.

T_5 with 149.43 g, T_4 with 138.6 g, T_1 with 129.33 g, T_8 recorded 117.33 g fresh weight. All the other treatments (T_6, T_3 and T_2) recorded lower fresh weight than Bavistin. Lowest fresh weight was recorded by control plants (Table 30).

.4.7.4.5. Dry weight of plant

As observed under fresh plant weight maximum dry weight was recorded in treatments involving *T. harzianum*. All the treatments recorded significantly more dry weight than control plants and all the treatments were significantly different from each other in dry weight of plants. Maximum dry weight of 68.86 g was recorded in T_7 followed by T_5, T_4 and T_1 with 62.62 g, 55.65 g, and 49.61 g respectively. Bavistin sprayed plants recorded 44.48 g dry weight. T_6, T_3 and T_2 recorded lower dry weight than in T_8. Lowest dry weight was recorded by control plants. The data are presented in Table 30.

4.7.5. Effect of application of ecofriendly materials on fruit characters of chilli

Fruit characters like fruit length (cm), fruit girth (cm), fresh fruit weight (g) and fruit dry weight (g) were recorded. All the fruit characters were significantly influenced by the treatments. Bavistin exhibited a significant influence on fruit characters unlike that observed under the biometric characters of the plant. Maximum influence on fruit characters was recorded by T_7 followed by T_8. The influence on fruit characters recorded by other treatments were in the decreasing order, T_5, T_4, T_1, T_6, T_3 and T_2 respectively. The results are presented in Table 31.

4.7.6. Effect of application of ecofriendly materials on yield of chilli

All the treatments recorded significantly higher yield when compared to control. All the treatments were significantly different from each other in yield. Maximum yield was obtained from T_7 (talc based formulation of *T. harzianum* + Datura leaf extract + Garlic bulb extract) with 462.28 g/plant followed by T_8

Table 31. Effect of ecofriendly materials on fruit characters of chilli.

Treatments		Fruit length (cm)*	Fruit girth (cm)*	Fresh Fruit weight* (g)	Dry fruit weight (g)*	No. of fruits / plant*	Yield/ plant (g)*	Increase in yield over control (g)
T_1	*Trichoderma harzianum* talc based formulation (1 %)	14.68	7.70	14.78	3.61	21.75	321.28	72.62
T_2	*Datura stramonium* leaf extract (20 %)	13.45	7.23	13.67	3.14	19.75	269.93	21.27
T_3	Garlic bulb extract (10 %)	13.90	7.38	14.04	3.31	21.00	294.74	46.08
T_4	T1 + T2	15.03	7.98	15.86	3.93	23.25	368.64	119.98
T_5	T1 + T3	15.53	8.13	16.16	4.06	24.00	387.84	139.18
T_6	T2 + T3	14.58	7.55	14.69	3.59	21.25	312.08	63.42
T_7	T1 + T2 + T3	16.05	8.50	16.97	4.67	27.25	462.28	213.62
T_8	Bavistin 0.05 %	15.78	8.28	16.78	4.23	25.00	419.39	170.73
T_9	Inoculated Control	12.65	7.05	13.09	2.91	19.00	248.66	-
	CD at 5 %	0.120	0.078	0.162	0.048	0.669	10.398	

* Mean of four replication

(Bavistin) with 419.39 g/plant. The yield obtained from other treatments were in the order T_5 (talc based formulation of *T. harzianum* + Garlic bulb extract), T_4 (talc based formulation of *T. harzianum* + Datura leaf extract), T_1 (talc based formulation of *T. harzianum*), T_6 (Datura leaf extract + Garlic bulb extract), T_3 (Garlic bulb extract) and T_2 (Datura leaf extract) with yield of 387.84 g, 368.64 g, 321.28 g, 312.08 g, 294.74 g and 269.93 g/plant respectively. T_9 the untreated control recorded the lowest yield of 248.66 g/plant (Table 31, Fig. 14).

The increase in yield obtained from T_7 was 213.62 g/plant and from T_8 - Bavistin was 170.73 g/plant when compared to control T_9. About 50 % increase was obtained from T_7 when compared to control. The lowest increase over control was recorded in T_3 with 46.08 g/plant followed by T_2 with 21.27 g/plant.

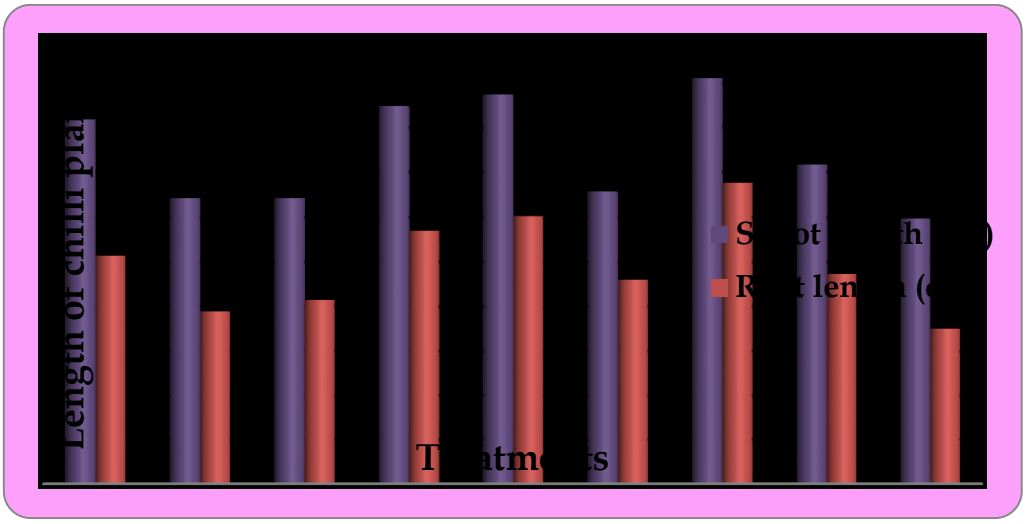


Fig. 13. Effect of ecofriendly materials on shoot and root length of chilli plants

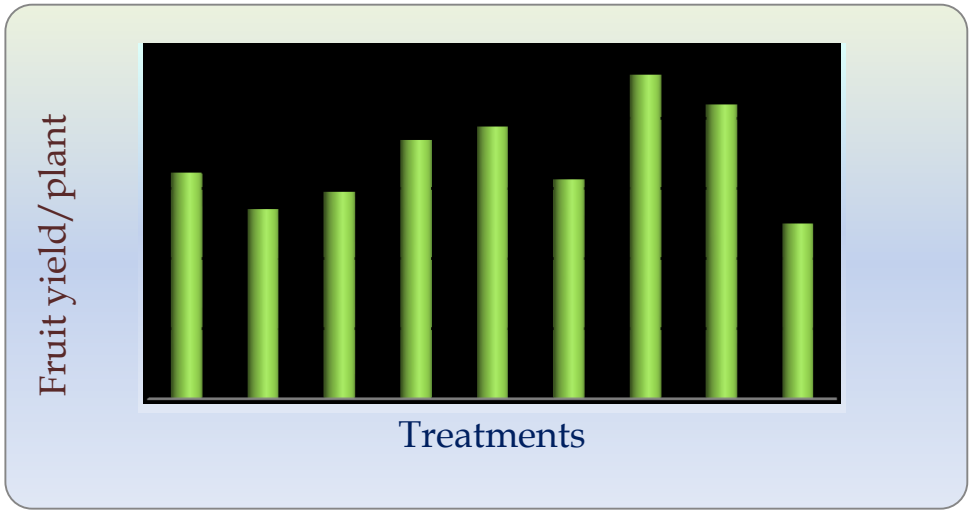


Fig. 14. Effect of ecofriendly materials on the chilli fruit yield/plant

T1 - Talc based formulation of *T. harzianum*

T2 - *Datura stramonium* leaf extract

T3 - Garlic bulb extract

T4 - T1 + T2

T5 - T1 + T3

T6 - T2 + T3

T7 - T1 + T2 + T3

T8 - Bavistin @ 0.05 %

T9 - Control.

DISCUSSION

5. DISCUSSION

The efficacy of ecofriendly management practices using biocontrol agents, plant extracts and plant products for the management of fruit rot of chilli, the most commonly cultivated spice cum vegetable crop of Kerala was investigated in the present study. Anthracnose of chilli was first reported from New Jersey, USA, by Halsted in 1890 who described the causal agents as *Gloeosporium piperatum* and *Colletotrichum nigrum*. These taxa were then considered as synonyms of *C. gloeosporioides* by Von Arx (1957). Chilli anthracnose was first reported in India by Sydow in the year 1913 from Coimbatore of the erst while Madras Presidency. In India, anthracnose disease is a major problem and is one of the significant economic constraints to chilli production (Ramachandran et al., 2007).

On tissue isolation, two different isolates were obtained from the infected chilli fruits variety Vellayani Athulya. One isolate (C_1) was obtained during March 2009 which was later identified as *C. gloeosporioides* and another type of isolate (C_2) during August 2009 which was later identified as *C. capsici*. *C. gloeosporioides* and *C. capsici* as the pathogens of fruit rot of chilli were recorded by earlier workers. Apart from *C. capsici* (Syd.) Butler and Bisby and *C. gloeosporioides* (Penz) Penz. and Sacc. (Verma, 1973) reported another two species, *viz., C. graminicola,* and C. *atramentarium* as the pathogens of chilli anthracnose from India. Ramachandran et al. (2007) reported that of the different species of *Colletotrichum, viz., C. capsici, C. gloeosporioides* and *C. acutatum* causing anthracnose in chilli, *C. capsici* was the most predominant species in the major chilli growing states *viz.,* Karnataka and Andhra Pradesh in India. Selvakumar (2007) reported that in addition to *C. capsici,* in north eastern India *C. dematium*, *C. gloeosporioides, C. graminicola* and *C. atramentarium* were also found to be the associated pathogens of chilli anthracnose. Mohan Rao et al. (2007) reported that *C .capsici* was the predominant species in Karnataka, Tamil Nadu, and Maharashtra while *C. gloeosporioides* was predominant in Andhra Pradesh. In the present investigation under Kerala

conditions, *C. gloeosporioides* was most prevalent during the months of February to July and *C. capsici* was prevalent during the months of August to January. Though *C. gloeosporioides* was reported as the pathogen of chilli anthracnose from India (Verma, 1973; Ramachandran et al., 2007; Selvakumar, 2007 and Mohan Rao et al., 2007) this is the first report from Kerala.

During the period from February – July 2009 an average temperature of 32˚C, rainfall of 120.45 cm and relative humidity (RH) of 89.5 per cent was prevailing in the premises of College of Agriculture, Vellayani. At that period isolations conducted from fruit rot affected chilli *C. gloeosporioides* was frequently obtained. Similar report were made by Bainik et al. (1998) reported that the incidence of *C. gloeosporioides* on mango fruit increased at temperature between 28 - 34.2 ˚C and RH between 70 - 87.2 per cent. *C. capsici* was isolated during the period from August 2009 – January 2010 from fruit rot of chilli. The average temperature prevailing during that period was 30.2˚C, rainfall of 157.75 cm and RH of 90 per cent. Similar findings were made by earlier workers. Chowdhury (1957 b) reported that optimum temperature for the growth of *C. capsici* was around 28˚C and 92 % RH. According to Misra and Mahmood (1960), maximum chilli fruit rot disease development takes place at 28˚C and 95.7 % RH. Continuous rain or high humidity is capable of causing fruit rot of chilli by *C. capsici* during the end of September and the beginning of October (Mehrotra and Aggarwal, 2003). The results of the present investigation revealed that a hot climate with an average maximum temperature of 32˚C and rainfall of 120.45 cm was favourable for the predominance of *C. gloeosporioides* and the cool climate with an average maximum temperature of 30.2˚C and rainfall of 157.75 cm for *C. capsici.*

Chilli anthracnose usually develops under high humid conditions when rain occurs after the fruits have started to ripen with reported losses of up to 84 % (Thind and Jhooty, 1985). Fruit rot of chilli incited by *C. capsici* is reported to cause yield

losses up to 30.7 % in Tamil Nadu (Sujathabai, 1992), 8 – 27% in Maharashtra (Datar, 1995), 10 – 30 % in Punjab (Rai and Chohan, 1996), and 25 – 48 % in Karnataka (Ekbote, 2001). Anthracnose occurs both as pre harvest or post harvest decay of mature fruits which accounts for more than 50 % of the yield losses (Poulos, 1992; Bosland and Votava, 2003; Pakdeevaraporn et al., 2005; Ramachandran et al., 2007). In Thailand, the marketable yield loss due to anthracnose amounted to 10 – 80% of the crop production (Poonpolgul and Kumphai, 2007).

Present investigation showed that under natural condition, symptoms produced by both *C. gloeosporioides* and *C. capsici* were almost similar. Small round (2 – 5mm dia.) brown, circular or angular sunken lesions appeared on the fruit surface which later turned necrotic surrounded by a water soaked area. These lesions spread in the direction of the long axis of the fruit and turned dark greyish. Individual lesions enlarged elliptically to about four cm in dia. and the fungus sporulated on the lesions as salmon pink coloured spore masses. Pinhead sized acervuli bearing setae developed on the lesion in a subcuticular or subepidermal fashion disrupting the outer epidermal cell wall. The acervuli were arranged in concentric patterns on the necrotic tissue. As the infection progressed, the spots were markedly delimited by a thick and sharp outline enclosing a lighter black or straw coloured area. Similar observations were made by Mc Govern (1995); Roberts et al., 2001 and Pandey and Pandey (2006) who were of the opinion that fruit rot began as small, round (1 - 2 mm dia.), brown, and slightly depressed lesions that became surrounded by water soaked areas. Individual lesion enlarged concentrically to about three cm in dia. and became wrinkled and covered with black acervuli. Total fruit rot often occurred due to the coalescence of multiple lesions. Typical anthracnose symptoms on chilli fruit include sunken necrotic tissues, with concentric rings of acervuli. Similar findings were also recorded by earlier workers (Palmateer and Ploetz, 2007; Gupta et al., 2009).

Present investigation showed that chilli fruits (var. Vellayani Athulya) were prone to fruit rot infection with *C. gloeosporioides* from 15 days after fruit formation till harvest and extended to post harvest stage while infection with *C. capsici* occurred when the green fruits turned into red and extended to post harvest stage. Same type of observations were made by (Hong and Hwang, 1998 and Kim et al., 1999). They reported that *C. capsici* generally caused disease on ripe red fruit, while *C. gloeosporioides* and *C. acutatum* produced disease both on young and mature green fruits. Kim et al. (2004) reported that different species cause diseases of different organs of the chilli plant; for example, *C. acutatum* and *C. gloeosporioides* infect chilli fruits at all developmental stages, but usually not the leaves or stems, which are mostly damaged by *C. coccodes* and *C. dematium*. Similar findings were made by (Hegde et al., 2001; Rajapakse and Ranasinghe, 2002; Mesta et al., 2007).

Under artificial conditions both *C. gloeosporioides* and *C. capsici* produced dark yellowish to brown, circular or elliptical sunken lesions on the fruit surface followed by the production of greyish white mycelium with salmon coloured masses of conidia. The infection spread in the direction of the long axis. A mat of fungal hyphae covered the seeds. Infected seeds turned rusty. Infection occurred at all developmental stages of the fruit.

On PDA culture growth of *C. gloeosporioides* appeared white in colour which gradually turned to dark greyish white as it grew older. Aerial mycelia grew evenly felty with salmon pink conidial pustules at the centre of the colony. Diurnal zonations were not so prominent on the upper surface. Setae and acervuli were found in the culture. A distinct olivaceous grey zone alternated with rosy buff zone on the reverse side of the colony was observed. The present finding was in tune with the findings of (Mc Govern, 1995; Mohan Rao et al., 2007; Gupta et al., 2009).

On PDA culture growth of *C. capsici* appeared creamy white which gradually turned light greyish white as it grew older. Salmon pink coloured conidial pustules

appeared at the centre of the colony. Thin mat of mycelium was seen with less aerial growth. Diurnal zonation of dense and sparse development were prominent on upper side of the culture. Reverse side of the colony were brownish grey to black. Setae as well as acervuli were found in the culture medium. The present findings was in accordance with the findings of (Misra and Dutta, 1963; Mc Govern, 1995; Jeyalakshmi and Seetharaman, 1999; Chander Mohan et al., 2006; Mohan Rao et al., 2007).

Measurement of mycelium, conidium, acervuli and setae of both *C. gloeosporioides* and *C. capsici* were made during the present investigation. The mycelial growth of C_1 consisted of branched and septate hyphae. Conidia were borne on elongated phialides in acervular conidiomata or on solitary fertile hyphae. Conidia were one celled, straight, cylindrical with obtuse ends, sometimes slightly tapered with rounded apex and truncate base, hyaline, aseptate with centrally placed oil globules. Conidia germinated in glucose solution (1 %) as thin, hyaline germ tubes. Similar findings was made by (Sunil Kumar and Yadav, 2007). Average conidial size was 19.26 × 4.7 µm and were within the range of 17.12 – 21.4 × 4.28 – 5.35µm. Conidiophores were hyaline and cylindrical. Acervuli bearing setae were round to elongated or irregular, brown, and measured 158.36 µm in dia. and were within the range of 89.88 – 252.52 µm. Setae were abundant, brown, straight to slightly curved, 1 – 4 septate, swollen at the base and tapering towards the apex, average size of the setae was 124.12 × 6.42 µm and were within a range of 77.04 – 171.24 × 5.13 – 7.49 µm. From the cultural and conidial morphology the isolate C_1 was identified as *Colletotrichum gloeosporioides.* These observations were in tune with Yee and Sariah (1993) who reported that *C. gloeosporioides* produced cylindrical conidia with obtuse ends, hyaline, aseptate, uninucleate and measured 4 – 24 × 2 - 6 µm which were formed in setose or globose acervuli. The shape of the acervuli ranged from round to elongated to irregular and measured 60 – 250 µm in dia. Setae were sparse to profuse, dark brown to black, straight to slightly curved, 1-

4 septate, swollen at the base and tapering towards the apex, 70 – 165 µm long. Rohana Wijesekara and Agarwal (2006); Palmateer and Ploetz (2007) and Gupta et al. (2009) also obtained same type of result.

The mycelial growth of C_2 consisted of branched and septate hyphae. Conidia were borne singly at the tips of the hyaline cylindrical conidiophores or on solitary fertile hyphae. Conidia were hyaline, unicellular and curved with narrow ends (falcate conidia) and contained a centrally placed oil globule. Average size of the conidia were 25.68 × 4.92 µm and were within the range of 18.40 – 29.96 × 4.28 – 5.99 µm. Acervuli bearing setae were round to elongated or irregular and dark brown and measured 175.48 µm in dia. and were within the range of 111.28 – 338.12 µm. Setae were abundant and dark brown, 1 – 5 septate, slightly curved, rigid, hardy, swollen at the base, slightly tapered towards the paler acute apex. Average size of setae were 179.76 × 6.63 µm and were within the range of 102.72 – 231.12 × 5.56 – 7.66 µm. Based on these studies the isolate C_2 collected during August 2009 was identified as *Colletotrichum capsici.* Similar findings were made by Sutton (1992) who reported the size of conidia of *C. capsici* as 18 – 23 × 3.5 – 4.0 µm. Rohana Wijesekara and Agarwal (2006) described it as 13.41 – 31.71 × 1.22 – 6.1 µm while Ruchi Garg et al. (2007) reported it as 22.5 × 3.3µm. Jameel Akhtar and Singh (2007) reported that conidial size of *C. capsici* was measured 25.27 – 26.15 × 3.14 – 3.67 µm. Jameel Akhtar et al. (2008) noted that conidia of *C. capsici* were falcate, fusiform with acute apices, and narrow truncated. They were one celled, hyaline and uninucleate. The conidia measured 16 – 30 × 2.5 – 4 µm in size and acervuli were rounded, elongated, approximately 350 µm in dia. Setae were abundant, brown, 1 – 5 septate, rigid, hardy swollen at the base, slightly tapered towards the paler acute apex. They were up to 250 µm long and 5 – 8 µm wide.

The present investigations revealed variations in the colony growth of *C.gloeosporioides* on different culture media. This may be due to variations in the

nutritional requirements of the fungus. Maximum radial growth of *C. gloeosporioides* was obtained on Richards' agar medium (90.00 mm) in six days and was significantly superior to all the other eight media tested. Excellent sporulation of the fungus was recorded on Sabouraud's agar, Potato dextrose agar and Richards' agar media. These observations were in agreement with (Hiremath et al., 1993; Ekbote, 1994; Ekbote et al., 1997; Vinod Tasiwal and Benagi, 2009).

Maximum radial growth of *C. capsici* was observed on Potato dextrose agar medium (90.00 mm) in seven days and was significantly superior to all the other eight media tested. Excellent sporulation of the fungus was recorded on Potato dextrose agar, Czapek - Dox agar, Host extract agar and Fries' agar media. The results are in confirmation with that of Mesta (1996).

Fungi in general utilized a wide range of nutrients as energy source. Richards' broth was the best liquid media for growth of *C. gloeosporioides* and recorded 284.23 mg dry weight of the mycelium from 30 ml broth in 15 days. Sporulation was excellent in Fries' medium, PDB and Richards' broth with numerous salmon pink conidial masses. The present investigation was exactly similar to the findings of (Ekbote, 1994; Ekbote et al., 1997; Vinod Tasiwal and Benagi, 2009).

Potato dextrose broth (PDB) was the best liquid media for growth of the *C. capsici* with 584.26 mg dry weight of the mycelium from 30 ml PD broth in 15 days. Sporulation was excellent in Fries' medium, PDB, Czapek-Dox medium and Richards' broth with numerous salmon pink conidial masses.

The comparative toxic activity of the culture filtrate was studied by conducting bioassay on chilli fruits. Size of lesions formed by the culture filtrate of *C. gloeosporioides* containing toxin of the fungus was maximum in the case of Richards' broth which was on par with Sabouraud's. Similar findings were made by Sharma and Sharma (1969).

Size of lesions formed by the culture filtrate of *C. capsici* containing toxin of the fungus was maximum in the case of Fries' broth.

C. gloeosporioides and *C. capsici* produced lesions on detached chilli fruits. As the incubation period increases, there was a proportionate increase in the size of the lesion. The size of the lesion produced by exotoxin (culture filtrate) was more than endotoxin (concentrated toxic metabolite from mycelium) in both species after 72 h. The results are in agreement with that of Sharma and Sharma (1969) who opined that exotoxin production was found to be more than endotoxin in the case of *C. gloeosporioides*.

C. gloeosporioides and *C. capsici* grow individually without intermingling of hyphae on PDA. Both the fungus ceased their growth at the point of contact. More growth was observed in *C. gloeosporioides* than in *C. capsici.*

Combined infection of *C. gloeosporioides* + *C. capsici* produced more lesion size compared to infection by *C. capsici* and *C. gloeosporioides* alone. The variety Vellayani Athulya was highly susceptible to fruit rot infection compared to other four varieties released from KAU *viz.*, Jwalasakhi, Jwalamukhi, Ujwala and Anugraha.

The conidial morphology, colony characters and culture growth leading to identification of the fungi were conducted on both C_1 and C_2 *viz., C. gloeosporioides* and *C. capsici*. Further studies like growth phase, nutritional and physiological factors, *in vitro* and *in vivo* management were conducted using isolate C_1 which was identified as *C. gloeosporioides* during the course of investigation. C_1 was the first and frequently obtained isolate with which the work was initiated and continued till the end of the study.

The growth phase of *C. gloeosporioides* in Richards' broth was recorded upto 22 days of incubation. The dry mycelial weight of *C. gloeosporioides* gradually

increased from sixth day of incubation (248.51 mg) and reached maximum on 12th day (512.69 mg). Significant increase in growth was recorded each day from 6th upto 12th day of incubation. Declining trend in growth was observed from 14th day onwards indicating autolysis. Lilly and Barnett (1951) also reported autolysis after maximum growth where cellular enzymes begin to digest the various cell constituents. Similar finding was recorded by Ekbote (1994) who observed that *C. gloeosporioides* reached maximum growth after 12 days of incubation in PDB.

Carbon is the most important nutrient required by fungi and it is the essential structural component of the fungal cell. Among the five carbon sources tested for growth of *C. gloeosporioides*, sucrose recorded the maximum radial growth (90.00 mm) followed by Mannitol. This finding is in agreement with the studies of (Naik et al., 1988; Hegde et al., 1990; Reddy, 2000; Saxena, 2002). Heavy sporulation was recorded with sucrose as the source of carbon followed by dextrose and no sporulation was observed in media without any carbon source. Similar finding was reported by (Reddy, 2000; Manjunatha Rao and Rawal, 2002; Saxena, 2002; Sangeetha, 2003; Sangeetha and Rawal, 2008).

Nitrogen is an important component required for protein synthesis and other vital functions. Significant differences in growth of *C. gloeosporioides* was recorded in Richards' media supplemented with various organic nitrogen sources. Asparagine was the best utilized nitrogen source as it recorded the maximum growth (90.00 mm) followed by Casamino acid. In the case of inorganic nitrogen sources, the fungus recorded maximum growth on Potassium nitrate (90.00 mm) followed by sodium nitrate (82.33 mm) which was on par with Calcium nitrate (82.00 mm). This was followed by Ammonium nitrate (68.83 mm). Similar studies on good growth of *C. gloeosporioides* where Asparagine and Potassium nitrate were used as nitrogen source was reported by Ramakrishnan (1941) and Durairaj (1956). Heavy sporulation was recorded in Asparagine, Casamino acid and urea. In Potassium

nitrate and Sodium nitrate also profuse sporulation was recorded. Potassium nitrate was reported as the best source for growth and sporulation of *C. gloeosporioides* (Naik, 1985; Naik et al., 1988; Saxena, 2002; Wasantha Kumara and Rawal, 2008). Chaturvedi (1965) and Ekbote (1994) reported that nitrates were comparatively better sources than ammonium compounds for the growth of *C. gloeosporioides*.

Temperature affects almost every function of fungi, including growth, spore germination and reproduction. Maximum growth was recorded at a temperature of 30˚C (90.00 mm), indicating that 30˚C was the optimum temperature required for growth of the fungus on Richards' agar medium. The results are in confirmation with (Hegde, 1986; Yee and Sariah, 1993; Zhou Hui Ping et al., 2008; Masyahit et al., 2009; Vinod Tasiwal and Benagi, 2009). The fungus recorded excellent sporulation at 30˚C and 25˚C. The results are in agreement with that of (Yee and Sariah, 1993; Sangeetha, 2003; Zhang Hai Ying et al., 2007; Wasantha Kumara and Rawal, 2008).

Hydrogen ion concentration is one of the most important factors influencing the growth of the fungi. The p^H of the medium determines the rate and amount of growth and many other life processes (Lilly and Barnett, 1951). According to them, fungi generally tolerate more acid than alkali. Optimum p^H was 6.0 for the growth of *C. gloeosporioides.* At p^H 6.0 the fungus recorded maximum dry mycelial weight of 586.80 mg in 30 ml of Richards' broth. Similar observations were recorded by Tandon and Chandra (1962) and Hegde (1986). For best sporulation the optimum p^H was 6 and 5. The results are in confirmation with that of Maccheroni et al. (2004) and Wasantha Kumara and Rawal (2008).

Exposure of fungal culture to alternate cycles of 12 h each under fluorescent light and 12 h darkness resulted in maximum dry mycelial weight of the fungus (583.57 mg) in Richards' broth. Similar observations were made by (Sudhakar,

2000; Yoon and Park, 2001; Ashoka, 2005; Narendra Kumar, 2006; Vinod Tasiwal and Benagi, 2009).

Hingole and Kurundkar (2004) reported that mancozeb + metalaxyl 72 WP (0.25 %) controlled *Colletotrichum capsici* but carbendazim 50 WP (0.1 %) was the most economical and profitable fungicide giving a cost : benefit ratio of 1 : 15.36 as compared to the cost : benefit ratio of 1 : 2.92 in mancozeb + metalaxyl treatment. However, residues of these fungicides in chilli pose a serious threat to human beings and to the environment. The above mentioned problems envisages the need for an ecofriendly approach like use of biocontrol agents, plant extracts and plant products for the management of diseases in chilli.

During the present investigation, native fungi isolated from phyllosphere and rhizosphere of chilli were tested for their antagonistic effect on the pathogen. Based on the conidial morphology and colony characters the fungal isolates were identified in the laboratory. Two of the fungal isolates were sent to Agharkar Research Institute, MACS, Pune for further identification and they were identified it as *Trichoderma harzianum* Rifai and *Gliocladium virens* Mitler, Giddens and Foster. Under *in vitro* studies, *T. harzianum* Rifai was the most effective antagonist (94.81 %) in suppressing the growth of *C. gloeosporioides*. In the present investigation, it was observed that *T. harzianum* overgrown and completely suppressed *C. gloeosporioides* within five days of inoculation**.** Deshmukh and Raut (1992) reported that *T. harzianum* Rifai and *T. viride* Pers. overgrew colonies of *C. gloeosporioides* and *T. harzianum* was more aggressive than *T. viride.* The high antagonistic potential of *T. harzianum, T. tolyposporium and T. pseudokoningii against C. gloeosporioides* causal agent of cashew anthracnose, was studied by Medeiros and Menezes (1994). Antagonistic effect of *T. harzianum* and *T. viride* against *C. gloeosporioides* causing leaf spot disease in turmeric was reported by

Patel (2000). *T. harzianum* isolates coil around and penetrate the hyphae of *C. gloeosporioides* (Anoop, 2002; Poornima, 2007). Mode of action of *Trichoderma* spp. in suppressing *Colletotrichum* spp. of fruit rot of chilli was extensively studied by earlier workers. *T. harzianum* and *Aspergillus niger* against *C. gloeosporioides* causing anthracnose disease in black pepper was reported by Santha Kumari (2002). *T. hematum* was found as a better biological control agent against *C. capsici* followed by *T. viride, T. harzianum, Gliocladium virens, Bacillus sp.* and *P. fluorescens* (Pathania et al., 2004). Watve et al. (2009) reported that under *in vitro* conditions, maximum per cent inhibition of *C. gloeosporioides* in colony dia. was achieved by *T. harzianum* (83.33 %) followed by *T. viride* (77.78 %) and *Bacillus subtilis* (77. 78 %).

One of the bacterial antagonists obtained from the rhizosphere of chilli plants were identified by morphological characters, gram staining, catalase test and fluorescence test as *Pseudomonas fluorescens.* Another bacterial sp. obtained from the rhizosphere was identified as *Bacillus* sp. based on the morphological characters, gram staining, catalase test and endospore staining. Among the bacterial antagonists tested *Bacillus* sp. inhibited the growth of *C. gloeosporioides* with an inhibition per cent of 87.78 followed by *Pseudomonas fluorescens* (77.78) per cent. Similar results were reported by (Bravo, 1993; Sariah Meon, 1994; Rahman et al., 2007).

In the present investigation *T. harzianum* followed by *Gliocladium virens* showed more mycelial inhibition compared to bacterial antagonist *viz., Bacillus* sp. These observations were in agreement with the earlier workers. Patel and Joshi (2001) and Raheja and Thakore (2002) reported that *T. virens* and *T. koningii* showed more mycelial inhibition of *C. gloeosporioides* compared to bacterial antagonist. Patel (2004) and Bhave (2005) found that maximum per cent inhibition (84.44 %) each by *T. viride* and *T. harzianum* against *C. gloeosporioides* while

Bacillus subtilis showed 61.11 % and *P. fluorescens* 60.00 % inhibition when the test fungus was placed at the centre. Watve et al. (2009) reported that under *in vitro* conditions, maximum per cent inhibition of *C. gloeosporioides* in colony dia. was achieved by *T. harzianum* (83.33 %) followed by *T. viride* (77.78 %) and *Bacillus subtilis* (77. 78 %). Vinod Tasiwal et al. (2009) noticed that maximum percentage reduction in colony growth of *C. gloeosporioides* causing anthracnose of papaya was observed in *T. virens* (60.87 %) followed by *T. koningii* (53.32 %), *T. harzianum* (51.89 %), *Bacillus subtilis* (50.97 %) and *T. viride* (50.11 %). Least growth inhibition percentage was observed in *P. fluorescens* (42.87 %). This can be attributed to higher competitive ability of *Trichoderma* spp.

Contact between *T. harzianum* and *C. gloeosporioides* was established in the dual culture after 24 h of incubation. At first they grew in intimate contact and then *T. harzianum* coiled around the pathogen, resulting in emptying the contents of hyphae. Ultimately pathogen hyphae shrivelled and got killed. The antagonist completely overgrew and killed the pathogen within five days of incubation. Similar observations were made by Chet et al. (1981) who reported that *T. hamatum* grew towards host hyphae and after contact, formed coils and appressoria like structures from which penetration occurred. Morshed (1985); Gupta et al. (1991); Reeny (1995); Ravi et al. (2000); Anoop (2002) also showed the mechanism of antagonism employed by *Trichoderma* spp. against *Colletotrichum* spp. were competition, lysis and hyperparasitism.

Trichoderma harzianum produce both volatile and non volatile compounds. The percentage inhibition of *C. gloeosporioides* when exposed to volatiles produced by *T. harzianum* was less when compared to non volatiles. Papavizas (1985) reported that *Trichoderma* and *Gliocladium* were not only good sources of various toxic metabolites and antibiotics, but also of various enzymes as exo and endo

glucanases, cellobiases and chitinases. Zeppa et al. (1990) and Umamaheshwari et al. (2002) reported that the volatile metabolites produced by *T. viride* are lactones, alcohols and terpene derivatives. A new tetracyclic diterpene $C_2OH_28O_2$ was isolated from the culture filtrate of a strain of *T. viride* that exhibited antifungal activity against *S. rolfsii* (Mannia et al., 1997). Mandeep Kaur et al. (2006) recorded that non volatiles produced by the *T. viride* reduced the mycelial growth of *C. capsici* by 52.5 % followed by *T. virens* (38. 12 %).

Shelf life study on talc based formulation of *T. harzianum* had shown that viable propagules of *T. harzianum* was reduced with length of incubation at room temperature (28 ± 2˚C). The population count after 10 days of incubation was 166.25 × 10^4 cfu/g. A decline in the number of propagules was observed from 30 days onwards. After 6 months of storage, number of viable propagules observed were 22.75 × 10^4 cfu/g. Similar observations were made by Prasad et al. (2002) who reported that conidial formulation in talc based carrier retained optimum amounts of viable propagules (> 10^6 cfu/g) even after 180 days of storage at room temperature. Das et al. (2006) reported that talc based formulation exhibited a gradual declining trend in multiplication and sporulation of *T. harzianum* from 30 days onwards. Gade et al. (2008) reported that among the four different carrier materials tested, talc was the best to retain maximum number of viable propagules of *Trichoderma* spp. after 180 days of storage. Talc and Kaolin were identified as better carriers of *T. harzianum* (Prasad and Rangeshwaran, 2000).

Among the seven plant extracts evaluated *in vitro* against *C. gloeosporioides*, *Datura stramonium* exhibited 90.71 per cent mycelial growth inhibition and was significantly superior to all the other plant extracts tested. Between plant extracts also, significant difference was observed in mycelial growth inhibition. *Lantana camara, Andrographis paniculata, Ocimum sanctum, Bougainvillea glabra, Azadirachta indica* and *Piper betle* recorded 73.49, 61.98, 49.04, 44.12, 30.08, 21.18

percentage inhibitions respectively. Crude extracts from plant materials have been found to significantly inhibit mycelial growth of many pathogenic fungi (Owolade and Osikanlu, 1999). In the present study, *Datura stramonium* leaf extract (99.99 %) at 80 and 100 per cent concentration were statistically on par with each other giving cent per cent inhibition of the mycelial growth of *C. gloeosporioides* and were significantly superior to all the other plant extracts tested. Lower concentrations of *Datura stramonium* at 40, 20, 10 and 5 % were also effective in inhibiting the growth of *C. gloeosporioides* and the per cent inhibition recorded were 55.18, 40.71, 36.96 and 28.75 respectively. Plant extracts in suppressing the growth of *Colletotrichum* spp. affecting fruit rot of chilli was extensively studied by various workers. Shivapuri Asha et al. (1997) reported that leaf extracts of *Azadirachta indica, Allium sativum, Ocimum sanctum, Datura stramonium, Polyalthia longifolia, Vinca rosea* and *Withania somnifera* were effective against *Colletotrichum* spp. causing fruit rot of chilli. Similar observations were made by (Gomathi and Kannabiran, 2000; Gurudatt Hegde et al., 2001; Shahidul Alam et al., 2002; Prabhakar et al., 2003; Ogbebor et al., 2007; Sunil Kumar and Yadav, 2007). Suhaila Mohamed et al. (1999) reported that ethanolic extracts of *Piper betle* between 0.01-1.0 mg ml^{-1} concentration showed more stronger antifungal activity against *Colletotrichum* spp. causing fruit rot of chilli. Yadav et al. (2007) reported that *Solanum torvum, Datura metel, Prosopis juliflera* and *Allium sativum* at 100 % concentration proved strong antifungal activity against *Colletotrichum* spp. Roat et al. (2009) observed that among the seven partially purified plant products screened against *C. capsici in vitro* and *in vivo*, maximum inhibition of mycelial growth and spore germination was reported by *Bitter temru* fruit and *Datura stramonium* leaves. Watve et al. (2009) reported the *in vitro* effect of neem leaf extract (78.15 %) garlic (58.89 %), sadafuli (57.04 %), tulsi (55.93 %), onion (45.66 %) and Bougainvillea (40.37 %) against *Colletotrichum gloeosporioides*.

Among the plant products, Garlic bulb extract gave highest percentage growth inhibition of 62.23. This was significantly superior to all the other plant products tested. All the four plant products showed significant differences between each other and between concentrations in inhibiting the pathogen. Neem oil exhibited 30.55 percentage inhibition, 10 : 1 proportion of Turmeric powder + Sodium bicarbonate 20.18 percentage and Neem Seed Kernel Extract 12.59 percentage inhibition. Amonkar and Baberhu (1971) and Singh and Singh (1980) reported that the active principle of *A. sativum* was a mixture of diallyl disulphide and diallyl trisulphide. Kota et al., 2006; Laxman, 2006 and Jadhav et al., 2008 reported that maximum inhibition of mycelial growth and spore germination of *C. gloeosporioides* was observed in garlic bulb extract at 10 % concentration. Mina Koche et al. (2009) reported that under *in vitro* conditions, *Azadirachta indica* seed extract (5 %) was effective among botanicals tried to check the mycelial growth of *Colletotrichum* spp.

In the present investigation, complete growth inhibition of *C. gloeosporioides* was obtained by Bavistin (0.05 %) and (0.1 %). Bavistin at 0.01 % concentration gave 81.11 per cent growth inhibition. Similar results were reported by (Jadhav et al., 2002; Karande et al., 2007; Amarjit Singh et al., 2008; Jadhav et al., 2008; Watve et al., 2009). Vinod Tasiwal et al. (2009) observed that among systemic fungicides tested carbendazim was successful in completely inhibiting the growth of *C. gloeosporioides* at all three concentrations i.e. 0.05 %, 0.10 % and 0.15 %.

In order to select a susceptible variety for conducting the *in vivo* experiment on management of fruit rot of chilli, a screening trial was conducted using the released KAU chilli varieties. Five chilli varieties released from Kerala Agricultural University *viz.,* Jwalamukhi, Jwalasakhi, Ujwala, Vellayani Athulya and Anugraha were screened against *C. gloeosporioides*. None of the varieties were immune to fruit rot disease. The variety Ujwala showed resistant reaction with 3.82 per cent disease i

ndex whereas Anugraha was moderately resistant with 16.15 per cent disease index. Jwalamukhi was in the susceptible category with fruit rot disease index of 38.49 per cent. Jwalasakhi and Vellayani Athulya were highly susceptible to fruit rot infection and recorded per cent disease index of 79.07 and 90.13 respectively.

Complete inhibition of growth of *C. gloeosporioides* was obtained under *in vitro* evaluation by using combinations of *Datura stramonium* leaf extract (20 %) + *Allium sativum* bulb extract(10 %), talc based formulation of *T. harzianum* (1 %)+ *Datura stramonium* leaf extract (20 %), talc based formulation of *T. harzianum* (1 %) + *Allium sativum* bulb extract (10 %) and talc based formulation of *T. harzianum* (1 %) + *Datura stramonium* leaf extract (20 %) + *Allium sativum* bulb extract (10 %).

Based on the *in vitro* studies, *T. harzianum* (1 %), *Datura stramonium* leaf extract (20 %) and *Allium sativum* bulb extract (10 %) were selected for *in vivo* studies. The antagonist *T. harzianum* grew without showing any inhibition in medium containing *Datura stramonium* leaf extract (20 %) + *Allium sativum* bulb extract (10 %), *Datura stramonium* leaf extract (20 %) alone and *Allium sativum* bulb extract (10 %) alone. Hence there was no problem of compatibility by combining the antagonist with *Datura stramonium* leaf extract and *Allium sativum* bulb extract for field spraying.

Based on the results of the *in vitro* management studies, the treatments were selected for *in vivo* management study. Under *in vitro* conditions, crude extracts of *Datura stramonium* at 80 and 100 % concentration completely inhibited the growth of *C. gloeosporioides*. In order to avoid flower fall and immature fruit drop, field sprays of crude extracts at 80 and 100 % were not included. Owolade et al. (2003) reported that crude extracts of *Acalypha ciliata* and *Carica papaya* totally inhibit the mycelial growth of *C. capsici* at 80 and 100 % concentration. But fields sprays of these crude extracts at 20 % concentration significantly reduced the disease

incidence and severity of brown blotch of cowpea. In the present investigation the best antagonist *T. harzianum* (1 %), best plant extract *Datura stramonium* leaf extract (20 %) and the best plant product Garlic bulb extract (10 %) and their combinations were tested under *in vivo* condition against fruit rot of chilli.

The disease index was calculated based on the damage caused by fruit rot pathogen on the chilli fruit using the formula developed by Mc Kinney (1923). Disease index was calculated before the treatments, five days after first and second treatment application. The lowest disease intensity was shown by T_8 (Bavistin 0.05 %). Similar findings were reported by Jayasekhar et al. (1987); Perane and Joi (1988); Sulochana et al. (1992). They reported that application of 1 % Bordeaux mixture, 0.3 % Ziride (Ziram) and 0.1 % Bavistin were equally effective in controlling the fruit rot of chilli. Bavistin (Carbendazim) at 0.1 % when applied once in nursery and again at one month and two months stage after transplanting gave the best control of anthracnose and ripe rot of chilli. (Biswas, 1992). Sally et al. (1994) reported that seed treatment with Bavistin @ 0.05% followed by four sprays of Bavistin (0.05%) or Fytolan (0.3%) at three weeks interval 15 days after transplanting was effective in reducing the disease intensity of fruit rot of chilli. Arasumallaiah and Rangaswamy (2008) found that under field conditions, Bavistin (0.1 %) was highly effective both on detached chilli fruits as well as on fruits in field.

In the present study it was observed that Bavistin 0.05 % was on par with T_7 (talc based formulation of *T. harzianum* + Datura leaf extract + Garlic bulb extract). Almost all the treatments involving the antagonist *T. harzianum* gave better control of the disease than treatments without *T. harzianum*. *Trichoderma* spp. are able to effectively compete for surface area, thereby reducing pathogen infection success (Jeffries and Koomen, 1992). Anoop (2002) reported that foliar as well as soil application of *T. harzianum* and *A. niger* were effective in reducing

C. gloeosporioides in black pepper. Sharma et al. (2004) reported that six sprayings of *T. harzianum* toxin (1 %) alternated with six sprayings of Neemarin at (4 %) concentration at 10 days interval till harvest reduce the post harvest and storage losses caused by *C. capsici.* Pratibha Sharma et al. (2005) reported that partially purified toxin of *T. harzianum* at 1 % concentration gave the greatest reduction in disease intensity of fruit rot of chilli and this treatment reduced post harvest fruit rot upto 89.8 % followed by sorghum based and talc based formulations of *T. harzianum* @ 0.2 and 0.4 per cent respectively. Watve et al. (2009) reported that under *in vivo* assay against *C. gloeosporioides*, *T. harzianum* recorded the leaf spot intensity of 24.74 PDI with 21.45 per cent disease reduction as compared to carbendazim (0.1 %) which recorded the highest reduction (76.65 %) of leaf spot intensity (7.38 PDI).

Saxena (1999) reported that the efficacy of neem (*Azadirachta indica),* garlic (*Allium sativum*) and Tagak tagak (*Rhinocanthus nasuta)* at 5000 ppm on *Capsicum annuum* was comparable with the fungicide carbendazim (Bavistin) at 100 ppm. Bagri et al. (2004) reported that maximum reduction in severity of fruit rot was recorded with Emcop L (67.7 %) followed by Bitter temru fruit extract (66.4 %) and Datura leaf extract (53.3 %) preparation at 1/1000 concentration in potted plants. Raj et al. (2006) found that among the plant products [*Allium sativum* (20 %), *Datura metel* (60 %), *Eucalyptus globulus* (60 %) and *Prosopis juliflera* (60 %)], *A. sativum* recorded minimum disease incidence (24.1 %) followed by *E. globulus* (27.8 %) against chilli fruit rot caused by *Colletotrichum spp.* Roat et al. (2009) observed that under *in vivo* condition, *Bitter temru* fruit and *Datura stramonium* leaves exhibited maximum control of fruit rot and dieback of chilli.

The maximum increase in shoot length, stem girth, root length, fresh plant weight and dry plant weight was observed for treatment with T_7 (talc based formulation of *T. harzianum* + Datura leaf extract + Garlic bulb extract) followed by

T_5 (talc based formulation of *T. harzianum* + Garlic bulb extract), T_4 (talc based formulation of *T. harzianum* + Datura leaf extract), and T_1 (talc based formulation of *T. harzianum*) in comparison to untreated control. All the above treatments include the antagonist *T. harzianum* which showed a positive trend towards shoot length, stem girth, root length, fresh plant weight and dry plant weight. Indeed growth promoting activity of *T. harzianum* is a well established fact. Windham et al. (1986) reported that the *T. harzianum* isolates stimulated plant growth even in the apparent absence of the pathogens because it interact directly with the plant by producing plant growth promoting active metabolites without interacting with pathogens. Singh et al. (1997) and Sharma et al. (1999) recorded that the growth of chickpea roots, shoots and leaves were enhanced due to soil application of *T. harzianum.* Ganesan et al. (2000) found that application of *Trichoderma* significantly increased root dry weight in pepper cuttings when compared to untreated control. Dubey (2002 b) reported that foliar spray of *T.virens* and *T. viride* increased the root length and number of root nodules over control. Priyadarsini (2003) reported that the fungal antagonist *T. harzianum* was effective in managing the disease and enhancing the growth of amaranthus.

Highest yield per plant (462.28 g) was recorded in T_7 (talc based formulation of *T. harzianum* + Datura leaf extract + Garlic bulb extract) followed by T_8 (Bavistin as check) (419.39 g). Foliar spray of *T.virens* and *T. viride* were reported to be efficient in increasing the grain yield in urd and mung beans (Dubey, 2002 b). The efficacy of carbendazim (0.1 %) in reducing the disease incidence to 17.7 % and increasing the fruit yield by 52.6 % over untreated control was reported by Raj et al. (2006).

From the results of the present investigation it could be recommended that a seedling dip with *Trichoderma harzianum* @ 500 g/1000 ml of water for 20 min. at the time of transplanting and two foliar spray at five days interval with a

combination of talc based formulation of *T. harzianum* (1 %) + *Datura stramonium* leaf extract (20 %) + Garlic bulb extract (10 %) is effective for managing fruit rot of chilli incited by *C. gloeosporioides* under field conditions. Further field experiments should be done to confirm the results of the study. The results will definitely help chilli growers who are desirous of following organic plant protection measures to build a non hazardous, pesticide free, pollution free environment for the healthy living conditions.

SUMMARY

6. SUMMARY

Fruit rot of chilli is one of the most important disease affecting the crop in Kerala. The climatic conditions prevailing in the state are congenial for the spontaneous occurrence and spread of the disease. The pathogen with its high variability and wide host range has made it difficult to develop disease resistance. The chemical control measures though proven effective is costly and poses severe threat to human health and environment. Therefore the present investigation was carried out with the objective of evolving an ecofriendly management practice for the disease using biocontrol agents, plant extracts and plant products.

The fruit rot pathogens were isolated from chilli plants grown in the crop museum, COA, Vellayani and were made use of in the study. The study involves symptomatology of the disease under natural and artificial conditions. In both situations, symptoms appeared as small brown sunken circular necrotic lesions with concentric rings of acervuli. The lesions enlarged elliptically and the fruit get shrivelled. During the present investigation, two species of *Colletotrichum viz., C. gloeosporioides* and *C. capsici* were isolated and the pathogenicity of the disease was proved. Conidial, morphological and cultural characters of both the organisms were studied. Conidia of *C. gloeosporioides* were cylindrical, straight with obtuse ends whereas that of *C. capsici* were falcate.

Growth in different solid and liquid media, carbon sources, nitrogen sources, temperature and pH were studied. Best solid medium for the growth of *C. gloeosporioides* was Richards' Agar whereas the best liquid medium was Richards' broth, best carbon source - sucrose, best organic nitrogen source - Asparagine, best inorganic nitrogen source - Potassium nitrate, optimum temperature 30˚C, optimum pH 6.0, optimum light intensities - alternate cycles of 12 h each under fluorescent light and 12 h darkness. *C. gloeosporioides* reached maximum growth after 12 days of incubation in Richards' broth.

Fungal antagonists obtained from the chilli phyllosphere were *Penicillium* sp.*, Aspergillus niger* and from the rhizosphere *Trichoderma harzianum, Gliocladium virens, Aspergillus flavus*. Bacterial antagonists obtained from the rhizosphere were *Bacillus* sp. and *Pseudomonas fluorescens*. The best antagonist *T. harzianum* obtained under *in vitro* screening by dual culture technique was selected for the *in vivo* study. *T. harzianum* grew in intimate contact with the hyphae of *C. gloeosporioides* and then coiled around the pathogen, resulting in emptying the contents of the *C. gloeosporioides* hyphae. Ultimately pathogen hyphae shrivelled and got killed. The antagonist completely overgrew and killed the pathogen within five days of incubation. The percentage inhibition of *C. gloeosporioides* exposed to the volatiles was found to be less when compared to that by diffusible compounds. Talc based *T. harzianum* was formulated and its shelf life was found to be more than 180 days.

Leaf extracts at 60, 80 and 100 per cent concentrations from *Piper betle, Ocimum sanctum, Azadirachta indica, Lantana camara, Datura stramonium, Andrographis paniculata* and *Bougainvillea glabra* were tested against *C. gloeosporioides* under *in vitro* conditions by poisoned food technique and maximum inhibition of the pathogen was obtained in *Datura stramonium* at 80 and 100 per cent concentration. Lower concentrations of *Datura stramonium* were also tested (40, 20, 10 and 5 %) and it was found that as the concentration decreased, its effect decreased.

Among the plant products *viz.,* Turmeric powder, Garlic bulb extract, Neem Seed Kernel Extract and Neem oil tested against the pathogen, highest inhibition was obtained in Garlic bulb extract at 10 per cent concentration.

Combinations of the biocontrol agent, plant extract and plant product were tested against the pathogen and it was observed that *T. harzianum* (1 %) + *Datura*

stramonium (20 %) + Garlic bulb extract (10 %) completely inhibited the growth of *C. gloeosporioides*.

Among the five KAU released chilli varieties *viz.*, Jwalamukhi, Jwalasakhi, Ujwala, Anugraha and Vellayani Athulya screened against fruit rot, none of the varieties were found immune to the disease. Vellayani Athulya was the most susceptible one to the disease and it was selected for *in vivo* management trial.

For *in vivo* management of fruit rot of chilli a pot culture experiment in CRD with four replications and nine treatments *viz.*, the selected antagonist *T. harzianum* (1 %), plant extract *Datura stramonium* (20 %), plant product Garlic bulb extract (10 %) individually and their combinations along with standard fungicidal check Bavistin @ 0.05 % and unsprayed control was laid out at COA, Vellayani using the highly susceptible chilli variety Vellayani Athulya. Among the nine treatments, seedling dip with talc based formulation of *T. harzianum* 500 g per 1000 ml of water for 20 min. and two foliar sprays at five days interval with a combination of talc based formulation of *T. harzianum* @ 1 % + *Datura stramonium* leaf extract @ 20 % + Garlic bulb extract @ 10 % was found best for the management of fruit rot of chilli caused by *C. gloeosporioides.*

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* Originals not seen.

APPENDIX

APPENDIX – I

Composition of different media

Potato Dextrose Agar

Pealed and sliced potato	:	200 g
Dextrose	:	20.00 g
Agar	:	20.00 g
Distilled water	:	1000 ml

Richards' Agar

KNO_3	:	10.00 g
KH_2PO_4	:	5.00 g
$MgSO_4$. $7H_2O$	:	2.50 g
$FeCl_3$	:	0.02 g
Sucrose	:	50.00 g
Agar	:	20.00 g
Distilled water	:	1000 ml

Czapek – Dox Agar

Sucrose	:	30 g
$NaNO_3$	:	2 g
K_2HPO_4	:	1 g

$MgSO_4$. $7H_2O$	:	0.5 g
KCl	:	0.5 g
$FeSO_4$	:	0.01 g
Agar	:	20.00 g
Distilled water	:	1000 ml

Sabouraud's Agar

Glucose	:	40.00 g
Peptone	:	10.00 g
Agar	:	20.00 g
Distilled water	:	1000 ml

Host Extract Agar

Chilli leaves	:	200.00 g
Agar	:	20.00 g
Distilled water	:	1000 ml

Host Extract Dextrose Agar

Chilli leaves	:	200.00 g
Dextrose	:	20.00 g
Agar	:	20.00 g
Distilled water	:	1000 ml

Brown's Agar

Glucose	:	2.00 g
K_2HPO_4	:	1.25 g

Asparagine	:	2.00 g
$MgSO_4$. $7H_2O$	:	0.75 g
Agar	:	20.00 g
Distilled water	:	1000 ml

Fries' Agar

$(NH_4)_2NO_3$	:	1.00 g
KH_2PO_4	:	1.00 g
$MgSO_4$	:	0.50 g
NaCl	:	100.00 mg
$CaCl_2$	:	130.00 mg
Sucrose	:	30.00 g
$MnSO_4$. $7H_2O$	:	10.00 mg
$FeSO_4$	:	20.00 g
Boric acid	:	1.00 mg
$CuSO_4$	:	0.10 mg
$ZnSO_4$	:	0.01 mg
Distilled water	:	1000 ml

King's medium B (KMB)

Peptone	:	20 g
K_2HPO_4	:	1.50 g
$MgSO_4$. $7H_2O$	:	1.50 g
Glycerol	:	10.00 ml
Distilled water	:	1000 ml

pH	:	7.2

Martin's rose Bengal agar

Dextrose	:	10.00 g
Peptone	:	5.00 g
KH_2PO_4	:	1.00 g
$MgSO_4$. $7H_2O$	:	0.50 g
Rose Bengal	:	33 mg/L
Streptomycin	:	30.00 mg
Agar	:	20.00 g
Distilled water	:	1000 ml

Nutrient Agar

Beef extract	:	3.00 g
Peptone	:	5.00 g
NaCl	:	8.00 g
Distilled water	:	1000 ml

APPENDIX – II

Meteorological data from 2009 – 2010.

Month	Max. Temp	Mini. Temp	Max. RH	Mini. RH	Rainfall
February-2009	31.7	23.5	89.9	77.7	0.0
March	33.3	25.8	89.0	66.1	12.6
April	33.0	26.8	88.2	67.1	39.9
May	32.9	25.8	89.1	72.5	305.6
June	31.0	24.0	90.3	81.1	174.0
July	30.1	24.0	90.5	83.7	190.6
August	30.2	24.1	87.5	81.7	74.9
September	29.6	24.2	91.0	85.0	114.2
October	30.4	24.2	90.2	84.3	100.9
November	29.5	23.8	90.6	83.4	485.7
December	30.7	23.7	90.4	85.7	51.6
January-2010	31.2	23.5	89.7	83.7	119.2

ECOFRIENDLY MANAGEMENT FOR FRUIT ROT OF CHILLI (*CAPSICUM ANNUUM* L.) CAUSED BY *COLLETOTRICHUM* SPP.

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ABSTRACT

ECOFRIENDLY MANAGEMENT FOR FRUIT ROT OF CHILLI (*CAPSICUM ANNUUM* L.) CAUSED BY *COLLETOTRICHUM* SPP.

Chilli belonging to the family Solanaceae having Mexican centre of origin is an important spice cum vegetable crop grown for its pungent fruits. Besides being a rich source of spicy flavour and colour, they are free of cholesterol, low in sodium, rich in vitamin A, C, E, folic acid and potassium. India is a major producer, exporter and consumer of chilli. The area and production of chilli in the country is 7.57 lakh ha and 11.67 lakh tonnes. In Kerala, the area under cultivation is 14,000 ha with a productivity of 1000 Kg/ha.

Chilli anthracnose / fruit rot was first reported in India by Sydow in the year 1913 from Coimbatore of erst while Madras Presidency. Anthracnose occurs both as pre harvest and post harvest decay of mature fruits and account for more than 50 % of the crop losses.

Since 1940, chemical fungicides were used for the control of chilli diseases. The indiscriminate usage of a wide range of fungicides has invited many undesirable problems such as development of fungal resistance, toxic residues in the produce, environmental pollution and escalating costs in vegetable production. So there is an urgent need to find out an effective, alternative methods of disease control, which was less harmful to human beings and environment. The objective of the present investigation was to evolve an ecofriendly management strategy to control fruit rot of chilli using biocontrol agents, plant extracts and plant products.

The study involves symptomatology of the disease under natural and artificial conditions. In both the situations, symptoms appeared as small brown sunken circular necrotic lesions with concentric rings of acervuli. The lesions enlarged elliptically and the fruit get shrivelled. During the present investigation, two species of *Colletotrichum viz., C. gloeosporioides* and *C. capsici* were isolated and the

pathogenicity of the disease was proved. Conidial, morphological and cultural characters of both the organisms were studied. Conidia of *C. gloeosporioides* were cylindrical, straight with obtuse ends whereas that of *C. capsici* were falcate.

Growth in different solid and liquid media, carbon sources, nitrogen sources, temperature and pH were studied. Best solid medium for the growth of *C. gloeosporioides* was Richards' Agar whereas the best liquid medium was Richards' broth, best carbon source - sucrose, best nitrogen source - Asparagine, best inorganic source - Potassium nitrate, optimum temperature 30˚C and optimum pH 6.0.

Fungal antagonists obtained from the chilli phyllosphere were *Penicillium* sp*., Aspergillus niger* and from the rhizosphere *Trichoderma harzianum, Gliocladium virens, Aspergillus flavus*. Bacterial antagonists obtained from the rhizosphere was *Bacillus* sp. and *Pseudomonas fluorescens*. The best antagonist obtained under *in vitro* screening by dual culture technique *T. harzianum*, was selected for the *in vivo* study. Talc based formulation of *T. harzianum* was made and its shelf life was found to be more than 180 days.

Leaf extracts at 60, 80 and 100 % concentrations from *Piper betle, Ocimum sanctum, Azadirachta indica, Lantana camara, Datura stramonium, Andrographis paniculata* and *Bougainvillea glabra* were tested against *C. gloeosporioides* under *in vitro* conditions by poisoned food technique and maximum inhibition of the pathogen was obtained in *Datura stramonium* at 80 and 100 % concentration. Lower concentrations of *Datura stramonium* were also tested (40, 20, 10 and 5 %) and it was found that as the concentration decreases, its effect is also found decreasing.

Among the plant products *viz.,* Turmeric powder, Garlic bulb extract, Neem Seed Kernel Extract and Neem oil tested against the pathogen, highest inhibition was obtained from Garlic bulb extract at 10 % concentration.

Combinations of the biocontrol agent, plant extract and plant product were tested against the pathogen and it was observed that *T. harzianum* (1 %) + *Datura stramonium* (20 %) + Garlic bulb extract (10 %) completely inhibited the growth of *C. gloeosporioides*.

Five chilli varieties released from KAU were screened against the disease and found that none of the varieties were found immune to the disease and the variety Vellayani Athulya was found to be the most susceptible one. This variety was selected for the *in vivo* management trial.

For *in vivo* management of fruit rot of chilli a pot culture experiment in CRD with four replications and nine treatments was laid out at College of Agriculture, Vellayani. The treatments used were *T. harzianum* (1 %), *Datura stramonium* (20 %), Garlic bulb extract (10 %) individually and in combinations with standard fungicidal check Bavistin @ 0.05 % and unsprayed control. The variety Vellayani Athulya was used for the *in vivo* experiment. Among the treatments seedling dip with talc based formulation of *T. harzianum* 500 g per 1000 ml of water for 20 min. and two foliar sprays at five days interval with a combination of talc based formulation of *T. harzianum* @ 1 % + *Datura stramonium* leaf extract @ 20 % + Garlic bulb extract @ 10 % was found best for the management of fruit rot of chilli caused by *C. gloeosporioides.*