Integrated management of anthracnose in chilli (*Capsicum annuum* L.)

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(2013-11-137)

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Integrated management of anthracnose in chilli (*Capsicum annuum* L.)

by

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2015

DECLARATION

I hereby declare that this thesis entitled '**Integrated management of anthracnose in chilli (*Capsicum annuum* L.)**' 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 University or Society.

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CERTIFICATE

Certified that this thesis entitled "**Integrated management of anthracnose in chilli (*Capsicum annuum* L.)**" is a record of research work done independently by Ms. Shilpa Treasa Chacko (2013-11-137) under my guidance and supervision that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

µm	Micro meter
µl	Micro litre
@	At the rate of
°C	Degree Celsius
CD	Critical difference
cm	Centimeter
DAT	Days after transplanting
DAI	Days after inoculation
et al.	And other co workers
Fig.	Figure
g	Gram
ha	Hectares
h.	Hours
g-1	Per gram
i.e.	that is
kg.	Kilogram
l.	Litre
m	Meter
mm	Milli meter
mg	Milli gram
Ml	Milli litre
sec	Seconds
sp. or spp.	Species (Singular and plural)
viz.	Namely
dia.	Diameter
D	Days

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Introduction

1. INTRODUCTION

Chilli, (*Capsicum annum* L.) is an annual herbaceous spice cum vegetable cum cash crop grown in both tropical and sub-tropical regions and belongs to family: Solanaceae. It is an essential ingredient of Indian cuisine because of its pungency, colour, flavour and aroma. In India chilli is cultivated in 7.94 lakhs ha, production is 1304 million tonnes and it's productivity is 1.6 million tonnes per ha (Kumar, 2013). Chilli suffers from many diseases caused by fungi, bacteria, viruses, nematodes and also abiotic stresses and among the fungal diseases damping off, anthracnose or fruit rot, powdery mildew and leaf spots are the most prevalent ones.

Athracnose or ripe fruit rot caused by *Colletotrichum capsici* (Syd.) Butler and Bisby is a wide spread problem limiting the profitable cultivation and seed production throughout the major chilli growing regions of India. The disease was reported for the first time in India by Sydow in 1913 from Coimbatore of Madras Presidency. *C. capsici* (Syd.) causes severe damage on chilli fruits in both pre and post harvest stages and these infections together account for more than 50% of the crop losses (Rahman *et al*., 2013 and Pakdeevaraporn *et al*., 2005).

C. capsici is a hemibiotrophic or facultative biotrophic fungus belonging to sub division Deuteromycotina and it can overwinter on alternative hosts such as other solanaceous or legume crops or plant debris and rotten fruits in the field (Pring *et al*., 1995). Apart from this *C. capsici* occur either externally or internally in chilli seeds (Mananadhar *et al*., 1995) and *Colletotrichum* species can survive in and on seeds as acervuli (Pernezny *et al*., 2003).

Fungicide application is one of the easiest and reliable approach to combat disease at commercial level. However, the frequent dependence on these agrochemicals has led to development of resistant strains, destruction of useful microorganisms and residue problems. At present, a number of new chemicals that is ecologically safe with lower toxic levels and which are required at lower concentration than the earlier fungicides are available. The results of this study will

help in identifying safer options for disease management through the screening of registered new generation fungicides.

Bailey (1987) recommended integrated management techniques, as no single specific management programme could eliminate the chilli anthracnose. Effective control of *Colletotrichum* diseases usually involves the use of a combination of cultural control, biological control, chemical control and intrinsic resistance (Wharton and Diéguez-Uribeondo, 2004). The present study envisages the integration of new generation fungicides with organic preparations and biocontrol agents to develop an ecofriendly integrated disease management practice.

In the light of the above, the present study was undertaken on the following aspects,

- Isolation and characterisation of the pathogen
- Symptomatology of the disease
- Seed borne nature of the pathogen
- Host range
- Survival of the fruit rot pathogen
- Isolation and *in vitro* testing of antagonists
- *In vitro* evaluation of bioagents, organic preparations and chemical fungicides
- *In vitro* seedling assay with different bioagents and fungicides
- Field evaluation of selected agents from seedling assay for disease management

Review of Literature

2. REVIEW OF LITERATURE

Chilli (*Capsicum annuum* L.) is a solanaceous vegetable cum spice crop, native to New World of tropics and subtropics was introduced into India from Brazil in sixteenth century by the Portuguese. It has a unique place in the world diet in its ripe dried form as spice as well as green fruit as vegetable. It has several medicinal properties and also used as counter irritant in neuralgia and rheumatic disorders. It is a good source of vitamin A (292 I.U per 100 g), vitamin C (111 mg per 100g) and thiamine (0.19 mg per 100 g). It is mainly cultivated for three constituents of fruits *viz*., capsaicin, capsanthin and oleoresin (Bosland and Votava, 2003 and Than *et al.*, 2008). Chilli is the fourth most important vegetable in the world (FAOSTAT, 2008). Twenty five per cent of world's total chilli production is contributed by India. In India chilli is cultivated in 7.94 lakhs ha, production is 1304 million tonnes and it's productivity is 1.6 million tonnes per ha (Kumar, 2013).

In India, chilli cultivation is mostly concentrated in southern states like Andhra Pradesh, Karnataka, Maharashtra, Orissa and Tamil Nadu occupying nearly 75 per cent of the total area under this crop. Though India stands first in chilli cultivation in the world, the productivity of dry chilli is very low (0.9 t/ha) compared to world average (2 t/ha) (Mesta *et al.*, 2007)

2.1. CHILLI ANTHRACNOSE: OCCURRENCE AND YIELD LOSS

Chilli crop is affected by several fungal, bacterial and viral diseases, of which chilli anthracnose causes considerable damage, inflicting severe quantitative and qualitative losses in India (Issac, 1992 and Anand *et al*., 2010). Chilli anthracnose caused by *Colletotrichum* sp. causes considerable economic loss by affecting both the yield and quality of the produce (Cannon *et al.,* 2012, Noireung *et al*., 2012). It is a serious problem for chilli in tropics and sub-tropics worldwide (Sharma *et al.* 2005, AVRDC, 2003). Halsted (1890) from New Jersey, USA reported anthracnose of chilli for the first time. *Gloeosporium piperatum* and *Colletotrichum nigrum* were

identified as causal agents of this disease. This disease was first reported from India by Sydow in 1913 from Coimbatore of the erstwhile Madras Presidency and *Vermicularia capsici* was identified as the causal agent. Anthracnose is also prevalent throughout the chilli growing areas of India (Gopinath *et al.*, 2006).

Under conditions favourable for the fruit rot disease development, pre and post harvest fruit losses up to 50 per cent have been reported (Higgins, 1930 and Smith Crossan, 1958). Chowdhury (1957) reported that this disease was quite serious and wide spread in Assam and the disease has been recorded from the states wherever chilli was grown resulting in a loss of 12 -30 per cent of the fruits.

Bansal and Grover (1969) during their studies on *Capsicum frutescens* L. reported that crop losses due to anthracnose disease ranged from 10-35 per cent in 1966 and 20-60 per cent during 1967 in six districts of Punjab and Haryana. The fruit rot was reported as one of the worst maladies in chilli which occurred in severe form in all the southern states leading to the yield loss up to 30 per cent (Durairaj, 1972). Thind and Jhooty (1985) reported that *C. capsici* was a predominant fungus in causing fruit rot of chilli and its incidence varied between losses 66-84 per cent. In Peninsular Malaysia, the reduction in yield of marketable fruits due to anthracnose generally ranges from 10-60 per cent depending on certain seasons of the year (Mah, 1987).

Anthracnose, a major problem on mature and ripening fruits, caused losses upto 50 per cent due to both pre-and post-harvest fruit decay (Hadden and Black, 1989). Yield losses in chilli due to fruit rot/dieback/anthracnose caused by C. *capsici* during the years 1985-1987 were estimated to be 27.17 per cent in Himachal Pradesh (Paul and Behl, 1990).

Howard *et al.* (1992) reported losses greater than 30 per cent in chilli production in United States due to anthracnose. Severe incidence of anthracnose disease on chilli fruits in Taiwan was reported by Manandhar (1995). Kannan *et al.*

(1998) reported that under suitable weather conditions, dieback and fruit rot caused by *C. capsici* cause yield loss up to 12 to 15 per cent from Karnataka, whereas, Verma (1999) reported that the fruit rot of chilli caused by *C. capsici* (Syd.) Butler and Bisby, was an important disease in field, transit, transport and storage in Andhra Pradesh.

The yield losses due to anthracnose varied from 10–60 per cent in different parts of India (Pandey and Pandey, 2006). Bagri *et al.* (2004) reported that fruit rot of chilli caused 10-15 per cent losses to mature fruits during transit and storage in Udaipur.

Oanh (2004) reported the sporadic occurrence of anthracnose in chilli growing areas of Thailand. Chilli fruit rot in India resulted in disease incidence levels ranging between 66 and 84 per cent, and incurring yield loss up to 12–50 per cent (Rahman *et al*., 2013). *C. capsici* (Syd.) causes severe damage on chilli fruits in both pre and post harvest stages and these infections together account for more than 50 per cent of the crop losses (Pakdeevaraporn *et al*., 2005). However, anthracnose disease caused by *C. capsici* (Syd.) is the most economically important constraint which is hampering production in major chilli growing regions of India, accounting for 12-25 per cent yield losses (Sharma *et al*., 2005). The fungus is both internally and externally seed-borne (Ramachandran *et al*., 2007).

Poonpolgul and Kumphai (2007) reported that chilli growers in Thailand had encountered severe yield losses up to 80 per cent due to anthracnose (*Colletotrichum* spp.) Apart from pre-harvest losses, fruit quality deterioration of chilli due to anthracnose range from 21-47 per cent (Rajapakse, 2002).

Suthin Raj and Christopher (2009) estimated that the loss due to fruit rot disease ranged from 8-60 per cent. Anamika (2014) conducted a survey to assess the incidence of anthracnose of chilli in five locations in Rewa Province and reported that the incidence ranged from 55.53 to 71.10 per cent.

In the *Colletotrichum* patho-system, different *Colletotrichum* species can be associated with anthracnose of the same host (Simmonds, 1965; Freeman *et al*., 1998; Cannon *et al*., 2000). Anthracnose of chilli has been shown to be caused by more than one *Colletotrichum* species 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; Golda, 2010).

The maximum development of fruit rot takes place at 28ºC and 95.7 per cent relative humidity (Singh, 2002). *C. capsici* and *C. gloeosporioides* have been reported to cause anthracnose disease in India, Indonesia, Korea and Thailand (Kim *et al*., 1999; Oh *et al*., 1999; Ahn *et al*., 2003; Oanh et al., 2004; Voorrips *et al*., 2004; Pakdeevaraporn *et al*., 2005; Gopinath *et al*., 2006).

2.2. ROLE OF WEATHER FACTORS ON ANTHRACNOSE DISEASE DEVELOPMENT

Environmental factors have a major role in the development of disease. When the relative humidity of the pepper microclimate ranges around 95-100 per cent and temperature 20-30°C, conidia of *Colletotrichum* spp. will germinate and form appresoria in a few hours and anthracnose lesions were formed (Dodd *et al.*, 1991 and Estrada *et al*., (1993).The maximum disease intensity of fruit rot in chilli by *C. capsici* on chilli plants was reported in December when temperature was between 18 ˚C and RH 75 per cent (Gupta *et al.*, 1983). Spores must have free water to germinate and the germinaton is negligible below 97 per cent RH. Spores are released from acervuli when there is abundance of moisture. Severity of disease is related to weather and the fungus is relatively inactive in dry weather (Hindorf, 2000). Mehrotra and Aggarwal (2003) observed that continuous rain or high humidity was

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

Pratibha, (2004) observed that die back occurred on foliage during August, September, October, November and December was 25.1, 42.6, 68.2, 61.2, and 53.2 per cent, respectively and it was observed that the maximum disease intensity was during the month of October when the leaf surface during the morning is wetted with dew deposition and is the most favourable time for the disease development.

2.3. ISOLATION OF THE PATHOGEN

Bharathi *et al.* (2004) isolated *C. capsici* from infected chilli seeds. The seeds were surface sterilized with 0.1per cent sodium hypochlorite solution for three min. and washed with three changes of sterile water, then plated on PDA and incubated at 25-28°C for seven days.

Oanh (2004) isolated *C. capsici* from chilli fruits by washing in running tap water and drying with tissue paper. The infected fruit tissue was cut out in a size of approximately three mm^2 with scalpel. Surface disinfection was made in a series of sodium hypochlorite one per cent (Clorox 10 per cent) for five minutes, then dipped into sterilized water for two minutes and once again dried on sterile filter paper. The tissues were placed on Potato Dextrose Agar (PDA) at the rate of four pieces per plate. After 5-10 d incubation at 25° C in alternative 12 h of darkness and light using UV light exposure.

Sharma *et al.* (2004) collected anthracnose infected leaves and plant materials which were surface disinfected and incubated on moistened sterile blotter discs. Than *et al.* (2008) isolated *C. capsici* from infected portion by touching with a sterilized loop and streaked on the surface of water agar plates which were then incubated overnight.

Sharma and Tripathi (2009) isolated *C. capsici* by washing fruits with rotten areas and placing them on carrot agar. Chadha *et al.* (2010) isolated *C. capsici* by surface sterilization with 0.1 per cent mercuric chloride solution for 30 sec and repatedly washing in sterile distilled water and then transferring onto PDA plates and incubated at 28° C for 5-7 days.

Choudhary *et al.* (2013) isolated *C. capsici* from infected chilli seeds by blotter method and agar plate method.

2.4. PATHOGENICITY

Rai and Chohan (1966) conducted pathogenicity test in chilli plants bearing twenty fruits by spraying with spore suspension of different isolates. The inoculated plants were placed in humid chamber. Kenchaiah (1975) proved the pathogenicity of the two isolates of *C. capsici* using both ripe and unripe fruits of *C. annum* and *C. fruitescens* and were pathogenic to their respective hosts and were cross inoculable.

Thind and Jhooty (1985); Singh *et al.* (2000) and Masoodi *et al.* (2013) conducted the pathogenicity test on chilli by detached fruit method. The fruits were washed and pin pricked gently with sterilized needle and then inoculated by placing uniform drop of spore suspension.

Suthin Raj and John (2006) inoculated *C. capsici* on 105 days old chilli plants. The plants kept in glass house were sprayed with sterile water followed by conidial suspension using atomizer in the late evening. The control plants were sprayed with sterile distilled water.

For proving the pathogenicity, isolates of *C. capsici* were cultured on PDA for three days. Then 0.7 cm agar plug contained with mycelia of *C. capsici* was placed on pricked area on chilli fruit (*Capsicum anuum* L. var. *annuum*). All inoculated fruits were incubated in moist plastic chamber, kept at room temperature (25±2 ℃). Disease severity of anthracnose infection was recorded at five days after incubation (Sanders

and Korsten, 2003; Intana *et al*., 2007; Patilkulkarni, 2014; Mesta *et al*., 2007; Ratanacherdchai *et al*., 2009; Linu and Jisha, 2013; Kumar *et al*., 2015 and Rahman *et al*., 2013).

Manandhar *et al*. 1995 and Sangdee *et al*. 2011 inoculated chilli fruits with *C. capsici* and *C. gloeosporioides* by spot inoculation of 10 µl of conidial suspension (10^6 conidia per ml) and incubated at 28˚C and maintained high humidity.

Seedlings of five chilli varieties were planted in plastic tray for fifteen days until both cotyledons were fully expanded. Mycelium of *C. capsici* and *C. gloeosporioides* were ground in mortar and mixed with sterilized water to make up a liquid mixture. An inoculation site was made by needle puncture in the middle of cotyledons. A 15 µl droplet of liquid mycelium was dropped on each puncture with a micropipette. After 24 h of incubation period in moist chamber, seedlings were placed under open air environmental conditions in the net house. The symptom development on cotyledon was observed and the disease severity was recorded during three to seven DAI. Disease severity and plant-pathogen interaction was calculated as area of necrotic symptom (Oanh, 2004).

When the plants attained the age of 105 days and bearing the first flush of 25 day old ripe fruits, these fruits *in situ* were inoculated with the spore suspension (10^6 spores ml^{-1}) of the pathogen @ one ml per fruit using a fine hypodermic syringe (0.45 x 13mm / 26 x 1/2 inch) and water congestion was provided 24 h prior and after inoculation by covering with polythene bags and spraying with sterile distilled water inside (Christopher *et al.*, 2013).

Inoculation was done on chilli seedlings and fruits by the detached leaf assay procedure. Conidial suspension ($1x10^6$ conidia per ml) of twelve day old PDA grown cultures was sprayed on one month old chilli plants. The inoculated plants were covered with plastic bags for two days to maintain humidity. The plants were assayed for seven days after inoculation and continued to be so for up to 20 days, the presence

of the pathogen was further confirmed by incubating the leaves in moist chambers for five to seven days at 25 ± 2ºC and observed for the development of fungal growth (Nayaka *et al*., 2009).

Hartman and Wang (2011) sprayed a conidial suspension (10^6 conidia ml^{-1}) of C. *gloeosporioides* and *C. capsici* on surface of detached chilli fruits.

Each chilli fruit was injected once with 1 µl of the prepared conidial suspension in the middle of the fruit. The injection was performed using a microinjector which comprised of a Micro Syringe model 1705, dispenser PB600-1 (Hamilton, Switzerland), and a needle with one mm diameter and delivered a control number of conidia in each injection (1,000 conidia per µl) at 1 mm depth onto the pericarp. The inoculated chilli fruits were placed in a plastic box 20 x 30 x 10 cm^3 containing 500 ml distilled water and were incubated at room temperature (28-30°C), 100 per cent RH dark/ light 12/12 h for three days and 70 per cent RH dark/ light 12/12 h until nine days (Mongkolporn *et al*., 2014; Montri, 2008).

2.5. IDENTIFICATION OF THE PATHOGEN

The genus *Colletotrichum* belongs taxonomically to the sub division of Deuteromycotina (fungi imperfecti), class Coelomycetes, Order Melanconiales and family Melanconiaceae. The genus *Colletotrichum* was established by Corda (1831), for fungi characterized by hyaline, curved fusiform conidia and setose acervuli. After that followed a period of uncertainity as to the distinction between *Colletotrichum* and other genera such as *Vermicularia* and *Gloeosporium* (Jeffries *et al.,* 1990). Previously recogonized genus *Gloeosporium* Desm. et Mont was revised by Von Arx (1957). In few species, a perfect state was detected and described in the genus *Glomerella* (Stoneman) v. Schrenk et Spauld in the Division *Ascomycota* (Hindorf, 2000).

Conventional methods for identification and characterization of *Colletotrichum* species are based on morphological characteristics such as size and shape of conidia, existence of setae or presence of a teleomorph, and cultural characteristics such as colony colour, growth rate and texture (Smith and Black, 1991).

2.5.1. Morphological Characters

2.5.1.1. Colony Characters

C. capsici produced colonies with thin mat of mycelium with little aerial growth (Mc Govern, 1995). The mean colony diameter of *C. capsici* isolates varied from three to nine cm after seven days of incubation and took eight to fifteen days to complete the mycelial growth in nine cm petri plate. The colony colour of *C. capsici* isolates varied *viz.*, white, black, blackish white, black and grayish black and observed colonies with concentric rings on culture media with flat growth and raised colonies without any concentric rings (Christopher *et al.*, 2013). Patil *et al.* (2005) reported that the *C. capsici* had fluffy, raised colonies and produced acervuli in a scattered manner. Variation in colony colour and differences in radial mycelial growth of *C. capsici* isolates on Potato Dextrose Agar had been observed by Wijesekara and Agarwal (2006) and they reported that colonies had entire or wavy margins. Akhtar and Singh (2007) reported that *C. capsici* isolates exhibited ash to dark black colonies with presence of sickle shaped conidia and acervuli and also reported the differences in radial mycelial growth on Potato Dextrose Agar.

C. capsici isolates were divided into three groups according to the colony growth rate: the slow growing with growth rate less than 7.5 mm/day, medium growing with growth rate more than 7.5 to 9.5 mm/day and the fast growing with growth rate faster than 9.5 mm/day. The *C. capsici* isolates could be divided into four groups according to the surface mycelium: uniform, concentric rings, sector and irregular. Colony colour was divided into two groups whitish to grey and whitish to

brown. Conidia mass colour was divided into whitish to grey and orange (Montri, 2008).

C. *capsici* produced zigzag cottony or circular colonies on PDA with a color of greyish-white to dark grey on the ventral surface whereas the reverse of the colonies was mainly black. The colony diameter of different isolates ranged from 65 to 80 mm after seven days of incubation and produced white patches in between circles in some, whereas in other isolates formed ridges and furrows in concentric rings. The colony produced pink or saffron coloured conidial mass (Guldekar *et al.,* 2009). Kanchan and Biswas (2009) also reported that the isolates of *C. capsici* exhibited variation in respect of colony colour and growth pattern on Potato Dextrose Agar.

Various isolates of *C. capsici* produced cottony colonies on PDA with greyish-white to dark grey colour on the ventral surface, whereas, the reverse of the colonies was mainly black. The colony diameter of different isolates ranged from 65 to 80 mm after seven days incubation and produced zigzag cottony colonies or circular colonies (Sangdee *et al.,* 2011).

The colony colour of *C. capsici* varied from light to dark grey with whitish or brownish tinge. Most of the colonies had cottony or fluffy mycelial growth with regular to irregular margin. Among the cottony type colonies, suppressed growth and v-shaped pattern was also observed. Growth rate of fungus varied from 67.50- 32 mm after seven days (Masoodi *et al*., 2013).

C. capsici isolates varied in their cultural behavior ranging from cottony to fluffy, mostly suppressed with regular to irregular margin and the colour of colony ranged between white and grey. Growth rate of isolates was between 4 - 7.72 mm/day. The isolates were observed for colony reverse in terms of concentric ring and zones formed by acervuli. Most of them were without concentric ring and acervuli (Kumar *et al.,* 2015).

2.5.1.2. Conidial Size

Wijesekara and Agarwal (2006) reported that the size of conidia of *C. capsici* ranges from13.4-31.71 x 1.22- 6.1µm. Guldekar *et al*. (2009) observed that maximum conidial size of *C. capsici* was 28.54 x 3.06 µm whereas minimum was 18.21 x 5.26 µm. Sangdee *et al.* (2011) studied twelve isolates of *C. capsici* and reported that average length and width of conidia varied between 23.5 to 35.0 µm and 2.5 to 3.75 µm, respectively. Masoodi *et al*. (2013) has recorded that conidial size of *C. capsici* varies from 19.70-33.60 x 2.23-4.86 µm.

2.5.1.3. Conidial Shape

Wijesekara and Agarwal (2006); Montri, 2008 and Golda (2010) reported that *C. capsici* produces falcate conidia. The conidial shape of the different isolates of *C. capsici* was fusiform with both their ends pointed (Sangdee *et al.,* 2011). Masoodi *et al*. (2013) has recorded that *C. capsici* had fusiform to falcate type of conidia.

2.5.1.4. Acervuli and Setae

Maiello (1988) studied the initiation of acervuli of *C. capsici* on 12th day and observed that fructification initiated after three days of plating. Maximum dimension was noted as 228.04 x 163.80 µm while minimum was 159.40 x131.70 µm. Acervuli were rounded or elongated (Guldekar *et al*., 2009). Reena (2011) reported that acervuli of *C. capsici* were gregarious, abundant when young, circular to saucer shaped acervuli were first covered by host tissue, then erumpent and blackish covered by stiff divergent setae. Diameter of the acervuli measured 56.3-141.4 µm in host and 67-158.9 µm in culture. Masoodi *et al*. (2013) reported that acervuli production ranges from 32-55/5 mm disc.

Setae were dark in colour but paler at the apex, swollen at the base and tapering at the apex and more or less erect. Length of setae measured 44.7-142 µm in

host and 68.3-170.4 µm in culture (Reena, 2011). Setae measure 65-194.6 x 4.4-6.6 µm (Masoodi *et al*., 2013)

2.5.1.5. The perfect stage: *Glomerella cingulata*

Edgerton (1914) reported that *Glomerella*, the perfect stage of *Colletotrichum* have been developed in most cases under artificial conditions, either in pure culture on artificial media or on old dead pieces of the host or in few cases this stage have been found developing naturally. Stevens (1930) showed that irradiation with far ultra-violet light stimulated the production of *G. cingulata*. Kimati and Galli (1970) found that the perfect stage of *Colletotrichum* found most readily at temperatures between 15°C and 28°C and are favoured by slight acid conditions. Manandhar *et al.* (1986) reported that *Glomerella* can be produced on semi-synthetic media such as potato dextrose agar, lima bean agar, oat meal agar and sodium chloride yeast extract-sucrose agar.

Holliday (1980) reported that the perithecia of the genus *Glomerella* are pigmented, rounded or flask shaped, between 85 and 300 µm in dia. and with well developed ostioles which may be papillate or beaked. Mordue (1971) reported that within the perithecia are club-shaped or cylindrical, thin walled asci each of which contains eight randomly arranged ascospores. Tiffany and Gilman (1954) reported that the ascospores are aseptate, hyaline, fusiform and curved or rod shaped and 9-19 µm in length and 4-5 µm in width.

2.6. SYMPTOMATOLOGY

Anthracnose disease was reported as a major problem on mature chilli fruits, however, symptoms were also observed on foliage and immature fruits (Halsted, 1890; Dastur, 1921 and Higgins, 1926). Siddiqqui *et al*. (1977) and Kim *et al*. (1989) reported that the disease has been observed to occur in three phases *viz*: (i) Seedling blight or damping off prevalent in the nursery ii) Leaf spot and die back which is

initiated at different stages of growth and (iii) fruit rot in which mostly the ripe fruits are infected.

Anthracnose, deriving from a Greek word meaning ‘coal’, is the common term used for designating plant diseases characterized by very dark, sunken lesions, containing spores (Issac, 1992).

On leaves the disease appeared as small circular spots that coalesce to form large elliptical spots and under severe conditions, defoliation of affected plants occurs (Mc Govern, 1995).

The first outward sign of the fruit rot of chilli was the appearance of small black circular spot, in general, sharply defined but gradually diffused. Gradually, the lesion spread more in the direction of long axis which became elliptical. As the infection progressed, the spots get black, greenish black or dirty grey in colour, markedly delimited by a thick and sharp black outline enclosing a black or straw coloured area. Sometimes, two or more spots coalesced to form larger spots. When severely infected fruits were cut open, the lower surface of the skin was found to be covered with minute black spherical elevations. In advanced cases, seeds were covered by a mat of mycelium (Chowdhury, 1957).

The disease attacked the leaves, stems and flowers and was most damaging on mature ripe fruits. Disease symptoms were occasionally observed on green fruits as well. The first indication of the disease was the appearance of small, elliptical or oblong straw coloured, slightly sunken lesions on the surface of the mature fruit. This is followed by the development of black acervuli arranged in concentric rings, giving a target board appearance (Mah, 1987).

C. capsici attacked flowers and pods and caused stem die-back. Acervuli were produced as small grey to brown depressions on red ripe pods but not on immature green pods of chilli plants (Meon and Nik, 1988).

Mc Govern (1995) reported that the fruit rot began as small, round (1-2 mm dia.) and slightly depressed lesions that became surrounded by water soaked areas and the lesion enlarged to three cm in dia. became necrotic, wrinkled and covered with black acervuli. Zimand *et al*., 1996 observed that fruiting bodies and spores of *C. capsici* were abundantly produced on the black lesions.

Numerous acervuli were seen scattered on the discoloured area of the infected fruit. When a diseased fruit was cut open, the lower surface of the skin covered with minute, spherical, black stromatic masses or sclerotia of the fungus. A mat of fungal hyphae covered the seeds. Such seeds turn rusty in colour. Affected fruits were deformed, white in colour and lost their pungency. Ultimately, the diseased fruit shrivelled and dried (Than *et al*., 2008 and Rodeva, 2009). The pathogen also caused necrosis of tender twigs and the entire branch (Mesta, 2007) but, fruit rot was the most important symptom although the disease could damage other parts of the plant (Rodeva, 2009).

The symptoms of the disease appeared mostly on unripe fruits. Bleaching symptoms and lesions in concentric rings were seen on the fruit. The infected tissue formed a depression and the fruit shrinked. The spots on the tissue measure 20-40 mm in diameter (Ngullie, 2010).

2.6.1. Disease Symptoms on Ripe and Unripe Fruit

Ahmed (1982) reported that *C. capsici* infect chilli fruits in all stages of growth. Asuti and Suhardi (1986) reported that green or young chillies were more susceptible than half matured fruits. Adikaram *et al.* (1983) noticed appressoria were not well developed and infection hyphae remained quiescent on immature fruits. Basak *et al.* (1996) noticed *C. capsici* infection on young, mature and ripened chilli fruits but maximum was at ripened stage.

Colletotrichum gloeosporioides caused disease on unripe (green) and ripe (red) chilli fruits (Kim *et al*., 1989; Park *et al*., 1990), while the *C. capsici* infected immature fruits remained quiescent, developing symptoms after fruit ripening (Higgins, 1926; Adikaram *et al.*, 1982).

Maximum per cent disease index was observed on ripe red fruits (58 per cent) which were statistically on par with fruits turning from green to red (54.5 per cent) and green fruits recorded the least percentage disease incidence (22.5 per cent) (Hegde *et al.*, 2001). The red fruits recorded 50 per cent more fruit infection than green fruits. The stage at which chilli fruits turned from green to red was prone to infection and the infection continued severely when fruits turned red (Mesta *et al.*, 2007).

2.7. SEED BORNE NATURE OF *COLLETOTRICHUM* SPP.

The testing of chilli seeds by using both the blotter (29 per cent) and potato dextrose agar (37 per cent) showed infection of *C. capsici* to be well established both within and on the external surfaces of chilli seeds (Meon and Nik, 1988). *C. capsici* was isolated most frequently both by the blotter and potato dextrose agar, suggesting that the pathogen could be present on the seed surface as well as inside the seed (Grover and Bansod, 1970 and Rout and Rath, 1972). Padaganur and Naik (1991) isolated mainly *C. capsici* (75.5 per cent), *Fusarium* (16.25 per cent) and *Alternaria* (5 per cent) from diseased chilli fruits, while from apparently healthy fruits the proportions were *Alternaria* (31.75 per cent), *Aspergillus flavus* (16.5 per cent), *Fusarium* (14.5 per cent) and other fungi, including *C. capsici* (1.25 per cent).

Mesta (1996) also studied the seed mycoflora of chilli by standard blotter method and reported the presence of *C. capsici* and *Alternaria*, *Cercospora*, *Fusarium* and *Curvularia*.

Bhale *et al.* (2000) also revealed that standard agar plate method is better than blotter method in detecting the seed borne fungi of chilli. Solanki *et al.* (2001) reported the presence of *C. capsici, F. moniliforme*, *A. niger*, *A. flavus, Alternaria alternata* and *C. lunata* from chilli seed samples.

Kumudkumar *et al.* (2004) reported standard blotter method as best for the detection of *Colletotrichum* sp. from chilli seeds compared to other methods like visual inspection of dry seeds, seed washing test, agar plate method with PDA, agar plate with malt yeast and 2,4-D blotter method.

Balogun *et al*. (2005) reported that *P. digitatum, A. flavus* and *A. niger* from chilli seeds which were pathogenic on chilli fruits.

Seed-borne infections appear to be common in seed samples collected from affected fields (Nayaka *et al*., 2009). Vinay *et al.* (2009) confirmed the occurrence of *C. capsici,* species of *Cercospora, Alternaria, Penicillium* and *Aspergillus* on chilli seed samples collected from different chilli growing districts of northern Karnataka by standard blotter method and agar plate technique and revealed that *C. capsici* as the most predominant fungus encountered (71.24 per cent).

Thippeswamy *et al.* (2011) revealed that the important pathogenic fungi in chilli seeds were *C. capsici* (97.00 per cent), *A. solani*, (15.00 per cent), *A. alternata* (12.00 per cent), *F. solani* (20.00 per cent) and *F. oxysporum* (20.00 per cent) which were recorded in higher percentage by standard blotter method.

Kumari (2012) isolated *A. niger, A. flavus, A. alternata, F. moniliforme* and *Penicillium* sp. from chilli seeds by standard agar plate method and standard blotter method.

Chigoziri and Ekefan (2013) isolated a total of twenty fungal genera from chilli seeds collected from Ohimini Local Government Areas of Benue State using agar plate method and observed that *C. capsici, A. niger* and *A. flavus* were the most

frequently isolated fungi with 54.75 per cent, 44.00 per cent and 29.75 per cent occurrence, respectively.

2.8. SURVIVAL OF *COLLETOTRICHUM* SPP.

Survival of mycelia and stomata in colonized chilli seeds had been reported. *C. capsici* and *C. gloeosporioides* occur either externally or internally in chilli seeds (Manandhar *et al*., 1995). *Colletotrichum* species survive in and on seeds as acervuli and micro-sclerotia (Pernezny *et al*., 2003). It has been shown that the pathogen readily colonized the seed coat and peripheral layers of endosperm even in moderately colonized seeds. Heavily colonized seeds had abundant inter and intracellular mycelium 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). Diseased fruit were a source of inoculum, allowing the disease to spread from plant to plant within the field (Rodeva, 2009).

Depending up on the environmental conditions, facultative saprophytes like *Colletotrichum* sp. can survive in crop debris, soil etc. for varying periods. The survival of *C. capsici* (Syd.) Butler and Bisby causing anthracnose of betelvine, during unfavourable season were possible through competitive saprophytic ability (Dasagupta, 1989). The causal agent of anthracnose of arecanut, *C. gloeosporioides* was isolated from dried and diseased arecanut leaves and found that the pathogen was viable up to eight months (Hegde *et al.*, 1989). Palarpawar and Ghurde (1989) observed that *C. curcumae* (Syd.) Butler and Bisby retained upto nine months when naturally infected turmeric leaves were buried in the soil. *C. acutatum* survived in infected strawberry fruits for three months (Wilson *et al.*, 1992).

Eastburn and Gubler (1992) reported that survival of conidia of *C. acutatum* in plant debris and in soil varied depending on soil moisture and temperature. *C. lindemuthianum*, bean anthracnose overwintered four months in bean debris (Dillard

and Cobb, 1993). The survival of sorghum anthracnose pathogen was studied by Misra and Sinha (1996) and reported that *C. graminicola* survived for five months when infected leaves were stored in nylon bags buried in the soil while in infected tomato fruits, *C. coccodes* survived and served as the primary source of inoculum.

2.9. HOST RANGE

Wei and Cheo (1944) reported fruit rot and leaf spot caused by *C. capsici* from tomato. Singh (1995) reported fruit rot of brinjal caused by *C. capsici.* Mordue *et al.* (1971) reported the occurrence of *C. capsici* from tomato and brinjal fruits. Stem canker and dieback disease of pigeon pea by *C. capsici* was reported by Somani *et al*. (2000). Tripathi (1978) reported anthracnose in urdbean by *C. capsici.* Natural occurrence of anthracnose of mungbean caused by *C. capsici* was reported from *Vigna radiata* cultivars T44 and Jawahar 45 (Beniwal *et al*., 1985). Pring *et al*. (1995) tested the host range of *C. capsici* isolate from chilli and French bean and reported that *Colletotrichum* species can overwinter on alternative hosts such as other solanaceous or legume crops or plant debris and rotten fruits in the field. Water soaked lesions caused by *C. capsici* were observed on *Vigna radiata, V. mungo, V. unguiculata, Cicer arietinum* and *Cajanus cajani*. Pandey (2006) reported fruit rot pathogen *C. capsici* and observed large, water soaked, depressed, blackish brown lesions on tomato fruits.

Gautam (2014) reported necrotic lesions from French basil plant showing leaf blight symptoms caused by *C. capsici*. *C. capsici* has also been reported to cause brown blotch disease of cowpea (Emechebe, 1981) and anthracnose disease of cotton (Bailey, 1992), tomato (Mercer, 1975). Davis *et al.* (2005) reported *C. capsici* as leaf spot pathogen on *Coccinia grandis.*

2.10. ISOLATION AND IDENTIFICATION OF RHIZOSPHERE, PHYLLOSPHERE AND POMOPLANE ANTAGONISTS

The possibilities for biological control of pathogens of common leaf surface fungi have been explored by various workers (Rai and Singh 1980, Singh, 1995). Rani *et al.* (2007) found that maximum chilli phyllosphere fungi belonged to genera *Aspergillus* (70.10 per cent) followed by *Curvularia* (14.13 per cent), *Rhizopus* (8.15 per cent) and *Penicillium* (7.60 per cent). Basha *et al.* (2010) isolated 50 isolates of fungi and 44 isolates of bacteria from phyllosphere and pomoplane including plant pathogenic genera like, *Alternaria, Cercospora, Colletotrichum, Curvularia,* and other saprophytic fungi like *Penicillium, Aspergillus, Mucor, Gliocladium, Periconia* indicating that majority (75 per cent) of the phylloplane and pomoplane fungi isolated from chillies are pathogenic in nature and only 25 per cent of these fungi are of saprophytic nature. Tembhare *et al.* (2012) reported seven fungi belonging to five genera from phyllosphere of chilli variety Jwala including, *Aspergillus* (75 per cent), *Cladosporium* (13.37), *Rhizopus* (5.23 per cent), *Colletotrichum* (3.48 per cent) and *Paecilomyces* (2.9 per cent). Wadje and Deshpande (1979) also reported *Aspergillus, Rhizopus and Colletotrichum* from the phyllosphere of chilli plants.

2.11. *IN VITRO* MANAGEMENT STUDIES

2.11.1. Biocontrol Agents and Antagonists

The potential for biological control of *Colletotrichum gloeosporioides* had been suggested as early as in 1976 by Lenne and Parbery. 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. B. subtilis* has been used to control chilli diseases like fruit rot and die back (Jeyalakshmi *et al*, 1996). Jeyalakshmi and Seetharaman (1999) reported that *Trichoderma viride, T.*

harzianum and *T. koninigii* (Oudem) inhibited mycelial growth of *C. capsici* by 51.7, 56.6 and 42.5 per cent respectively.

Chidanandaswamy (2001) reported that *Pseudomonas fluorescens* was found to be superior in inhibiting the growth of the *C. capsici* causing leaf spot of turmeric followed by *T. harzianum* (Rifai) and *T. viride* (Pers) *in vitro*.

D'Souza *et al.* (2001) screened eight isolates of *T. harzianum* against *C. capsici* as a promising biocontrol agent under *in vitro* conditions by over growth of the antagonist within 5-6 days. Ramamoorthy and Samiyappan (2001) reported that *P. fluorescens* isolate pf 1 effectively inhibited the mycelial growth of *C. capsici in vitro* and decreased the fruit rot incidence in chilli under greenhouse conditions. Seed treatment plus soil application of talc based formulation of *P. fluorescens* isolate pf1 also effectively reduced the disease incidence.

Hegde *et al.* (2001) reported that *P. fluorescens* showed higher antagonistic activity against *C. capsici* recording mycelial growth inhibition of 54.38 per cent compared to the control *in vitro* conditions.

Wharton and Dieguez-Uribeondo (2004) tested the efficacy of biocontrol agents, *B. subtilis* and *Candida oleophila* against *C. capsici*. Srinivas *et al*. (2005) reported that antagonist *P. fluorescens* as seed treatment and as well as spray were found to be effective against *C. capsici.* Sharma *et al*. (2005) reported that *Trichoderma* species effectively controlled *C. capsici* infection in chilli.

Intana *et al*. (2007) tested three mutant and two wild type strains of *T. harzianum* for efficacy to inhibit and overgrow mycelia of *Colletotrichum capsici* on potato dextrose agar (PDA) at room temperature.

Suthin Raj and Christopher (2009) reported that four different chilli seeds infected by *C. capsici* were treated with biocontrol agents and evaluated for per cent reduction of *C. capsici*, seed germination and vigour index. It was found that the pure

culture of *P. flourescens* was effective in reducing *C. capsici* infection followed by pure culture of *T. harzianum.*

Tiwari *et al*. (2008) reported that antagonistic effect of isolates of *P. flourescens* and *T. harzianum* isolates was tested by dual culture technique against *C. capsici*.

Ekefan et *al*. (2009) evaluated four isolates of *T. harzianum and* showed that *T. harzianum* isolates significantly reduced colony radius of *C. capsici* compared to the control. Seed treatment with *T. harzianum* resulted in significantly lower per cent dead seedlings and higher seedling length compared to seeds inoculated with *C. capsici* alone.

Theerthagiri *et al.* (2009) reported that among the fungal and bacterial antagonists tested, three isolates of *T. viride* and *P. fluorescens* were very effective in inhibiting the mycelial growth of the pathogens *in vitro.* Bilal *et al. (*2010) reported that three bioagents (*T. viride, T. harzianum* and *Gliocladium virens*) were evaluated under *in vitro* and *in vivo* conditions against *C. lindemuthianum*. All the three antagonistic fungi caused significant inhibition of mycelial growth, maximum being with *T. viride* (69.21 per cent) followed by *T. harzianum* (64.20 per cent).Tembhare *et al.* (2012) found that *Aspergillus fumigatus* was more effective in inhibiting the mycelial growth (61.03 per cent) followed by *Paecilomyles variotii* (38.96 per cent).

The fungal species isolated from grass rhizosphere and found that the fungi exhibited large zones (> 50–60 mm) of inhibition to *C. capsici* include *Penicillium citrinum, P. chrysogenum, F. oxysporum, T. harzianum, T. koningii and Fusarium solani followed by P. rubrum, Phoma* sp. (Vasanthakumari *et al.*, 2013).

2.11. 2. Fungicides

Raju *et al*. (2007) reported that the mycelial growth of *C. capsici* isolates *viz.* Cc 12 and Cc 15 was inhibited by 87.60 and 86.90 per cent, respectively at one µg

while that of Cc 9 was inhibited by 98.00 per cent at 10 µg and 100.00 per cent at 25 µg of carbendazim.

Misra *et al.* (2008) tested eight fungicides under *in vitro* conditions and reported that all the *C. capsici* isolates were highly sensitive to one per cent bavistin (0.2 per cent), kavach (0.1 per cent) and (indofil M-45) providing more than 50 per cent inhibition.

Anand *et al*. (2010) reported that azoxystrobin treatment resulted in minimum fruit rot incidence (3.75 per cent). The mancozeb and carbendazim treatments resulted in 10.72 and 12.02 per cent fruit rot incidence, respectively.

Pardhi *et al.* (2011) found that all *C. capsici* isolates were highly sensitive to chlorothalonil and showed maximum per cent growth inhibition against isolate Cc 1 (81.09 per cent), Cc 2 (80 per cent), Cc 3 (82.59 per cent), Cc 4 (84.81 per cent) and Cc 5 (85.53 per cent) followed by propiconazole. Boonyapipat (2013) conducted *in vitro* assay of fungicides against *C. capsici* and recorded that captan at 2,000 ppm inhibited the mycelial growth with an average radius of 0.9 cm and captan at 1,500 ppm with an average radius of 2.26 cm.

Shinde (2012) reported that mancozeb @ 300 ppm concentration showed highest inhibitory effect on *C. capsici* and recorded maximum inhibition and least mycelial dry weight.

Barhate *et al*. (2012) reported that among seven fungicides tested, benomyl (0.25 per cent) was found most effective, which inhibited 90 per cent growth of *C. capsici* followed by propiconazole (0.1 per cent), hexaconazole (0.1 per cent), mancozeb (0.2 per cent), carbendazim (0.05 per cent) and chlorothalonil (0.2 per cent) with 86.66 , 85.55 , 84.44, 73.33, 68.88 and 65.55 per cent growth inhibition over control, respectively.

Ahiladevi *et al*. (2014) reported that azoxystrobin at 0.12 per cent was very effective in reducing the mycelial growth, dry weight, spore germination and germ tube elongation of about 83.27, 69.78, 81.39 per cent and 78.18 µm, respectively.

2.11.3. Panchagavya

Joseph and Sankarganesh (2011) reported that among the samples from panchagavya, the 1000 µl dilution alone showed 100 per cent antifungal activity, whereas, the remaining dilutions (500, 100 µl) showed moderate antifungal activity but there was no antifungal activity at lower dilutions (10 µl).

Kumar *et al*. (2010) reported that Maha Pancha Gavya (MPG), a concoction made from five cow products was tested for its toxicity against *Pythium aphanidermatum* (Edson) Fitz. and its antagonists at 5, 10, 25, and 50 per cent concentration in *in vitro* to find out if it can be used in integration for the control of damping-off in tomato in nursery beds. MPG was very effective inihibiting the growth of *P. aphanidermatum.*

Panchagavya at 10 and 20 per cent provided 5.6 per cent mycelial inhibition of *C. capsici*. A combination of neem oil and panchagavya at 10 and 20 per cent inhibited the mycelial growth of *C. capsici* 60.55 and 67.7 per cent, respectively (Reena, 2011).

Adhao (2013) observed that panchagavya was able to suppress the growth of *Fusarium oxysporum*. The superior antifungal activity of panchagavya was recorded at 4 per cent followed by 2, 6, 8 and 10 per cent as compared to control.

The result of the *in vitro* experiment showed that, panchagavya gave 100 per cent inhibition of the growth of the *P. aphanidermatum* at all the concentrations tested (Anees, 2014)

2.11.4. Jeevamruth

Jeevamruth at 10 and 20 per cent inhibited the mycelial growth of *C. capsici* by 5.6 and 11.2 per cent, respectively. A combination of neem oil and panchagavya at 10 and 20 per cent inhibited the mycelial growth of *C. capsici* 56.24 and 61.6 per cent, respectively (Reena, 2011).

2.11.5. Fish Amino Acid

Anees (2014) reported that the fish amino acid gave 100 per cent mycelial inhibition of *P. aphanidermatum* only at 10 per cent concentration followed by 5 per cent concentration providing 23 per cent inhibition.

2.12. SEED TREATMENT AND SEEDLING VIGOUR

Seed treatment with fungicides is well known for the control of seed borne fungi (Mills and Wallace, 1970 and Abou- Heilan, 1984). Bavistin followed by Vitavax and Thiram have been also been reported to be effective seed dressing fungicide against seedborne *C. capsici* (Mridha and Siddique, 1989; Mirdha and Choudhary, 1990; Mishra, 1988).

Kenchaiah (1975); Kumar and Mahmood (1986); Mallaraju and Swamy (1988) tested 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 and mancozeb were found to be highly effective in inhibiting growth of C. *capsici.*

Mathur and Gurjar (2002) reported that the treatment of chilli seeds with pure cultures of *P. fluorescens* at the rate of 10^8 cfu g^{-1} increased the germination by 17.2 per cent whereas the *T. harzianum* pure culture increased the seed germination by 13.5 per cent.

Bharathi *et al.* (2004) evaluated the antagonistic strains and revealed that *B. subtilis* had the highest effect on seedling vigour (1404.96) and germination per cent (96.00) followed by *P. fluorescens* (Pf-1).

Joi *et al.* (2004) reported that the chemical fungicides bavistin and captan increased the vigour by 0.8 and 4.1 per cent, respectively and *P. fluorescens* pure culture 10^8 cfu g^{-1} increased the seedling vigour of chilli seeds by 13.7 per cent and *T. harzianum* pure culture increased the vigour by 12.1 per cent was found to be quite effective in controlling an anthracnose rot of chillies and enhanced seedling quality.

Srinivas (2005) reported that pure culture of *P. fluorescens* at the rate of 10^8 cfu g^{-1} reduced the incidence by 38 per cent whereas talcum powder formulation reduced the incidence by 22 and 29 per cent at the rate of 5 g kg^{-1} and 10 g kg^{-1} seeds respectively. Pure culture of *T. harzianum* at the rate of 1 x 10^8 cfu g^{-1} reduced the incidence by 24 per cent whereas talcum based formulation of the same at the rate of 5 g kg^{-1} and 10 g kg^{-1} reduced the incidence by 12 and 18 per cent respectively. The chemical fungicides bavistin, dithane M-45 and captan appeared less effective and reduced the incidence of *C. capsici* by only 7, 5 and 8 per cent, respectively.

Karande *et al.* (2007) also reported that the chemical fungicides namely bavistin, and captan appeared to be less effective and reduced the incidence of *C. capsici* in chilli seedlings by only 15.3 and 20.1 per cent, respectively.

Kumar *et al.* (2010) reported that seed treatment with *T. viride* completely eliminated the seed borne fungal pathogens of pigeon pea including *C. dematium.*

Suthin Raj and Christopher *et al.* (2009) reported that seed treatment with a pure culture of *P. fluorescens* (10^8 cfu g^{-1}) reduced the *C. capsici* incidence by 42.7 per cent and treatment with pure culture of *T. harzianum* (10^8 cfu g^{-1}) reduced the incidence by 38.7 per cent.

Mandeep *et al.* (2006) reported that the effect of seed dressing with bioagents on seed borne infection of chilli fruit rot fungus showed no significant difference in seed germination in infected and healthy seed lots, though it was higher in treated seeds. However, there was a significant difference in the emergence of healthy seedlings in bioagents treated seed lots. *T. viride* was most effective in reducing the seed infection though it was statistically on par with *T. viride* and *T. virens.* Untreated infected seeds gave rise to rotted seedlings and formation of necrotic lesions on primary leaves possessing acervuli of the fungus.

Choudhary *et al.* (2013) assessed the efficacy of different bioagents for the control of *C. capsici* infection on seedling health and reported that the maximum per cent seed germination (82.35 per cent) was recorded in seeds treated with *T. viride* and pre and post emergence mortality was minimum in case of *T. polysporum* (2.65 and 6.10 per cent) followed by *T. viride* (6.00 and 6.80 per cent). Among the fungicides tested, bavistin (0.1 per cent) was most effective with 93.00 per cent (68.00 per cent in control) seed germination with no pre or post emergence mortality and increased seedling vigour index of 506.85 which was 306.00 in control followed by captan (91.01 per cent) and indofil M-45 (77.5 per cent).

Hamid *et al.* (2014) carried out a study with different treatments of bioagents viz. *T. harzianum, P. fluorescens, T. viride* on chilli seeds and concluded that seed treatment with *T. harzianum* and *T. harzianum* + *P. fluorescens* promoted the establishment of seedlings (90 per cent) compared to control (60 per cent) and reported that seed treatment with bioagents minimized establishment of pathogens on seeds thereby improving germination and seedling vigour index.

2.13. *IN VIVO* MANAGEMENT ANTHRACNOSE OF CHILLI

Bailey (1987) recommended integrated management techniques, as no single specific management program could not eliminate the chilli anthracnose disease. Effective control of *Colletotrichum* diseases usually involves the use of a

combination of cultural control, biological control, chemical control and intrinsic resistance (Wharton and Diéguez- Uribeondo, 2004).

2.13.1. Biocontrol Agents and Antagonists

Bharathi *et al.* (2004) reported that PGPR mixed bioformulation of *P. fluorescens* (pf1) + *B. subtilis* + neem + chitin was found to be the best for reducing the fruit rot incidence of chilli besides increasing the plant growth and yield parameters under both greenhouse and field conditions.

Ekbote (2005) reported that treatment of 40 day old chilli seedlings with *P. fluorescens* solution (one per cent) reduced the incidence of dieback and fruit rot and increased the yield of chilli compared to the control and the seedling dip treatment for 30 min was found most effective.

Yadav (2008*)* reported that seedling treatment with 20 g of *Trichoderma* mixed with one litre of water helped in control of anthracnose of chilli caused by *C. capsici.*

Benagi *et al.* (2009) reported that seed treatment with *T. viride, T. hamatum, T. viride, T. harzianum* and *P. fluorescens* recorded higher seed germination and effective against anthracnose of chilli and *P. fluorescens* @ 10 g/l could be used as seedling dip and sprays for management chilli anthracnose.

Ngullie *et al.* (2010) evaluated the efficacy of plant antagonists and fungicide and revealed that spraying with *T. viride* (2 per cent) showed a disease reduction of 61.41 per cent followed by *P. fluorescens* (58.10 per cent). However, the fungicide bavistin (0.1 per cent) with 80.84 per cent disease reduction ranked first.

Park *et al.* (1990) observed that there was a greater reduction of anthracnose infection caused by *C. acutatum* on matured fruits treated with BS07 when compared to untreated control. Narasimhan *et al.* (2013) reported that the *B. subtilis* formulation

significantly reduced the incidence of anthracnose in chilli fruits caused by *C. gloeosporioides* by 65.30 per cent.

Rahman *et al.* (2013) reported that the *Trichoderma* strains significantly reduced anthracnose disease severity on chilli fruits by 69.52-81.39 per cent.

2.13. 2. Fungicides

Raju *et al*. (1982) reported that captan (0.15 per cent) at 15 days intervals gave the maximum reduction of fruit rot of chilli and highest yield (1491 kg ha^{-1}). Ebenezar and Alice (1996) observed that superior control of fruit rot and die back in chilli was achieved with mancozeb at 0.2 per cent followed by carbendazim at 0.2 per cent and fytolan at 0.2 per cent. Jayasekhar *et al*. (1987) reported that captan @ 0.2 per cent gave the most effective control of chilli anthracnose followed by copper oxychloride @ 0.25 per cent and carbendazim at 0.1 per cent. Mallaraju and Swami (1988) found that fungicides like zineb, mancozeb and carbendazim were effective chemicals for the control of chilli anthracnose. Das and Mohanty (1988) reported control of anthracnose and fruit rot of chilli with carbendazim and dithane M- 45.

The fungicide traditionally recommended for anthracnose management in chilli is manganese ethylenebisdithiocarbamate (maneb) (Smith, 2000), although it does not consistently control the severe form of anthracnose on chilli fruit. The strobilurin fungicides azoxystrobin (quadris), trifloxystrobin (flint), and pyraclostrobin (cabrio) have recently been labeled for the control of anthracnose of chilli, but only preliminary reports are available on the efficacy of these fungicides against the severe form of the disease (Alexander and Waldenmaier, 2002; Lewis and Miller, 2003).

To control the fruit rot of chilli, six fungicides were tested under field conditions and it was observed that minimum per cent disease index up to 7.73, 9.33

and 12.00 was recorded in bavistin and captan recorded 10.80, 13.06 and 14.40 per cent, respectively 120 DAT (Singh and Razdan, 2000).

Greenhouse and field experiments were conducted to study fruit rot of chilli caused by *C. capsici* disease control by spraying propiconazole (0.1, 0.05, 0.025 per cent), difenoconazole (0.05 and 0.025 per cent) and carbendazim (0.1 per cent) and observed that application of propiconazole at 0.1 per cent caused a dramatic reduction of disease incidence by 70 per cent when compared to difenoconazole at 0.05 per cent (58 per cent) and carbendazim at 0.1 per cent (44 per cent). Additionally, the fruit yield increased in the range of 86, 63 and 60 per cent for propiconazole, difenoconazole and carbendazim, respectively, when compared to unsprayed controls (Gopinath *et al*., 2006).

Mistry *et al*. (2008) reported minimum fruit rot intensity in chilli plants treated with carbendazim (0.05 per cent) followed by chlorothalonil (0.2 per cent), foltaf (0.20 per cent) and benomyl (0.025 per cent) and *T. viride* (10^8 cfu).

Salkinkop *et al.* (2008) reported the beneficial microorganisms from panchagavya and their establishment in the use of compost that improved the plant growth, crop yield and suppressed plant pathogens.

Mesta *et al.* (2009) reported that a field experiment was conducted during kharif 2006 and 2007 at Agricultural Research Station, Devihosur, with seven treatments and three replications including seedling dip and spray of *P. fluorescence* @10g l^{-1} in various combinations with chemicals, viz., carbendazim and hexaconazole. Seed treatment with carbendazim (0.2 per cent)+seedling dip in *P. fluorescence* at 45, 60 DAT+ 2 spray hexaconazole 75 and 90 DAT recorded least disease incidence for fruit rot (20.60 PDI), highest yield(8.00 q ha^{-1}) higher net returns. Maximum inhibition (22.71 per cent) was observed in case of anthracnose affected chilli plants treated with propiconazole (0.1 per cent) (Raj *et al*., 2013

2.13. 3. Organic Preparations

Meena *et al.* (2000) did not find any pest and diseases in the crops sprayed with Panchagavya. The *Pseudomonas* (45×10^3 cfu ml^{-1}) and saprophytic yeasts (35×10^4 cfu ml^{-1}), contained in panchagavya which might have contributed to plant protection because the presence of *Pseudomonas* on plant surfaces have been found to induce the production of pathogensis related protein, siderophores, antibiotics in groundnut and rice.

Suresh (2008) reported that the foliar spraying of cow urine, neem oil, *Pseudomonas*, panchagavya @ 2 to 5 ml l^{-1} of water alternatively at regular intervals control the anthracnose of chilli.

Yadav (2008) reported that seed treatment with jeevamruth and beejamruth controlled anthracnose of chilli caused by *C. capsici.*

Nagaraja (2009) reported that seed treatment with organics, beejamruth was found effective with 84 per cent seed germination and highest vigour index of 1211 and there was a gradual reduction in incidence of fruit rot of chilli.

Materials and Methods

3. MATERIALS AND METHODS

3.1. ISOLATION OF CHILLI ANTHRACNOSE PATHOGEN AND PURIFICATION OF THE ISOLATES

3.1.1. Isolation of the Pathogen

Chilli fruits and leaves having fruit rot and leaf spot symptoms collected from chilli growing fields of College of Agriculture, Vellayani were used for isolation of the pathogen. Isolation from the fruit and leaf portion were done separately. Isolations were done following Christopher *et al.* (2013) by cutting small pieces from the advancing margin of lesions which were then immersed in 0.1 per cent mercuric chloride for thirty sec, washed three times in sterile distilled water and blotted dry before placing on potato dextrose agar medium. The mycelium coming out of the tissues were sub-cultured to another petri plate containing sterile potato dextrose agar to obtain pure cultures of the nine pathogen isolates and incubated at room temperature.

3.1.2. Single Spore Isolation

The isolates of *Colletotrichum capsici* and *Colletotrichum gloeosporioides* were purified by single spore isolation (Dhingra and Sinclair, 1985). The spore suspension was prepared by placing a small piece of mycelium with conidia of *C. capsici* and *C. gloeosporioides* aseptically into separate test tubes containing sterile distilled water and shook vigorously. After that, one loop full of spore suspension was streaked on Water Agar (WA) in a zig-zag manner. The inoculated WA plates were incubated at room temperature for 24-48 h. After that, well isolated colonies arising from single conidia were sub cultured to new PDA plates. The inoculated plates were incubated at room temperature for 6-7 days and used for further identification studies.

The growing hyphal tip was cut with the help of cork borer under aseptic conditions and with an inoculation needle it was carefully transferred to PDA slants and incubated at 25±2°C. This culture was used for further studies.

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 refrigerated 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 the pathogen through the host after every three months.

3.2. PATHOGENICITY STUDIES

3.2.1. Proving the Pathogenicity

The pathogenicity of the fungal isolates was proven following the Koch's postulates and the most virulent isolate was selected for further study. For this, chilli fruits (Vellayani Athulya) were artificially inoculated with seven day old culture of the fungus by pin prick method. Prior to inoculation, all fruits were swabbed with 70 per cent (v/v) ethanol to reduce surface contamination and left for air-dry in laboratory by 3-4 min Fruits were then wounded by gentle pricking with sterilized needle. Inocula were prepared by culturing each isolate on PDA at room temperature (approximately 25±2°C) for seven days. Mycelial bits were cut from actively sporulating areas near to colony periphery by using a sterilized cork borer and placed over the wounded part of chilli fruits. A thin layer of moist cotton was kept on that area and a control was kept without pin prick. Inoculated fruits were kept in moisture chamber at room temperature (25±2°C) to maintain humidity for the disease development. After the symptoms were expressed, the fungus was re isolated from the fruit portions exhibiting typical symptoms of the disease. The experiments were done using a Completely Randomized Design with four replications. The

observations were taken as lesion diam. of tested fruits (cm) (Ratanacherdchai *et al.*, 2003).

3.2.2. Virulence Rating

The isolates were subjected to virulence rating based on the size of the lesion formed on fruits and the most virulent isolate was selected for further studies. The isolates were grouped into three categories based on lesion size as follows: 0-0.5 cm or 25 per cent of fruit infected (low virulent isolates), 0.51-2 cm or 50 per cent of fruit infected (moderately virulent isolates) and > 2 cm or 75 per cent of fruit infected (highly virulent isolates) (Ratanacherdchai *et al.*, 2010).

3.3. SYMPTOMATOLOGY

Symptoms of the disease caused by *C. capsici* and *C. gloeosporioides* alone and the symptoms produced in combination of both the pathogens were studied by observing the naturally infected chilli fruits and also following the course of the development of the disease under artificial inoculation.

3.4. IDENTIFICATION OF THE PATHOGEN

3.4.1. Morphological Characters

The fungal morphology of *C. capsici* and *C. gloeosporioides* was observed with regard to the growth rate and morphological character of the colony

3.4.1.1. Colony Characters

Five mm disc taken from all the fungal isolates were inoculated separately at the centre of a 90 mm sterile petri plate with sterile PDA and incubated at room temperature (25±2℃). Observations on the radial growth and growth rate of mycelia, colony colour, texture, appearance, growth pattern, margin, number of days taken for

spore mass production and spore mass colour of colonies were recorded after seven days.

3.4.1.2. Conidial Characters

After sporulation, shape, length and breadth of conidia, size of acervuli and number of setae per acervuli of both the isolates were measured with Motic BA 2.10 compound microscope under 400 X objective magnification.

3.4.1.3.Characters of Perfect Stage

Five mm disc taken from all the fungal isolates were inoculated separately at the centre of a 90 mm sterile petri plate with sterile PDA and incubated at room temperature (25±2℃) for three months and microscopic observations were taken for the presence of asci and ascospores Manandhar *et al.* (1986)

3.4. ASSAY OF INFECTED SEEDS

3.4.1. Standard Blotter Method

Naturally infected chilli fruits were collected from chilli growing fields of College of Agriculture, Vellayani and the seeds were extracted from the fruit. Four hundred seeds of each sample were tested by employing standard blotter method with eight replications in Completely Randomized Design. Blotting paper of 90 mm size were moistened with distilled water and placed in 90 mm sterilized petriplates after draining excess water. Seeds were placed at the rate of 25 seeds per petriplate at equal distance in each petriplate. The plates were incubated at room temperature (25±2°C) under alternate cycles of 12 h NUV light and darkness. After eight days of incubation, the seeds were observed under Motic BA 2.10 compound microscope under 400 X objective magnification.for the presence of fungi. Infection levels were recorded as the percentage of infected seeds in a sample (ISTA, 2000).

3.4.2. Standard Agar Plate Method

Naturally infected chilli fruits were collected from different chilli growing fields of College of Agriculture, Vellayani. Seeds were extracted from the fruit. Four hundred seeds of each sample were tested by employing standard agar plate method with eight replications. The seeds were surface sterilized for 45 sec in one per cent solution of sodium hypochlorite to remove surface fungi. Seeds were placed at the rate of 25 seeds per petri plate containing 20 ml of two per cent water agar. The petri plates were incubated for seven days at room temperature (25±2°C). The seeds were observed under Motic BA 2.10 compound microscope under 400 X objective magnification for the presence of fungi. Infection levels were recorded as the percentage of infected seeds in a sample (Vinay, 2010).

3.5. SURVIVAL OF THE PATHOGEN IN CROP DEBRIS

The surviving ability of *C. capsici* in crop debris was studied under *in vitro* and *in vivo* conditions. For *in vitro* studies *C. capsici* infected portions collected from field were brought to the laboratory, kept at room temperature (25±2°C) in paper cover and weekly isolations were done on PDA medium. The plates were incubated at room temperature (25±2°C). After eight days of incubation, the fungi were identified by preparing slides and viewing under compound microscope (40 X) (Anoop, 2000).

For studying the surviving ability of *C. capsici* under *in vivo* condition, *C. capsici* infected samples were buried in collected field soil taken in plastic bags at 30 cm depth. Weekly isolation was carried out from buried infected portion on PDA medium. After 8 days of incubation at 25±2°C and fungi were identified by viewing under Motic BA 2.10 compound microscope under 400X objective magnification as earlier for the presence of the pathogen (Anoop, 2000).

3.6. HOST RANGE OF THE PATHOGEN IN CROPS AND COMMON WEEDS

A survey was conducted in chilli fruit rot disease endemic areas of College of Agriculture, Vellayani to study the host range of the pathogen in other vegetable crops and common weeds grown in those areas. The plant showing fruit rot and leaf spot symptoms were collected in paper covers and brought to the laboratory for isolation and fungal organism was identified by preparing slides and viewing spores under Motic BA 2.10 compound microscope under 400 X objective magnification.for the presence of the pathogen.

In vitro inoculation was conducted to study the host range of the pathogen in some crops (Table 1) and weeds (Table 2) in and around the chilli fields. For this, the crops and weeds seen in the disease affected chilli field were inoculated with conidial suspension of the pathogen (Pring *et al.,* 1995). Un-inoculated control of each plant was also maintained. Symptoms were observed and re-isolation was done to prove the pathogenicity of *C. capsici.*

3.7. ISOLATION OF PHYLLOSPHERE, POMOPLANE AND RHIZOSPHERE MICROORGANISMS

The mycoflora and bacterial genera of phyllosphere, pomoplane and rhizosphere of the disease free chilli plants among fruit rot infected chilli plants in the field was studied. The dilution plate technique was followed for the isolation of fungal and bacterial antagonists from rhizosphere (Johnson and Curl, 1972) pomoplane (Basha *et al*., 2010) and phyllosphere (Tembhare *et al*., 2012).

3.7.1. Isolation of Fungal and Bacterial Antagonists from Pomoplane of Chilli

Chilli fruits were collected from the disease free plants in the fruit rot affected chilli fields of College of Agriculture, Vellayani. Ten g of fruit was transferred to 99 ml sterile distilled water in 250 ml conical flasks and shaken for 20 min in a rotary

Table 1. Crops selected for artificial inoculation of the pathogen

Sl No.	Name of the crop	Scientific name
2.	Brinjal	*Solanum melongena* L.
3.	Ivy gourd	*Coccinia indica* L.
4.	Green gram	*Vigna mungo* L.
5.	Sesame	*Sesamum indicum* L.
6.	Black gram	*Vigna radiata* L.
7.	Tomato	*Solanum lycopersicum* L.
8.	Sweet potato	*Ipomea batatus* L.

Table 2. Weeds selected for artificial inoculation of the pathogen

Sl No.	Name of the weed	Common name
1.	*Amaranthus viridis* L.	Slender Amaranth
2.	*Cynodon dactylon* L.	Dog's tooth grass
3.	*Cyperus rotundus* L.	Purple nut sedge
4.	*Cleome rutidospermum* L.	Fringed spider flower
6.	*Panicum repens*	Torpedo grass
7.	*Richardia scabra* L.	Rough Mexican clover
8.	*Commelina benghalensis* L.	Tropical spiderwort

shaker. From this, the 10^{-4} dilution was prepared for isolation of fungal antagonists. One ml of 10^{-4} was plated using pour plate method on Martin's Rose Bengal Agar medium. For isolation of bacterial antagonists, 10^{-6} dilution was prepared. One ml of 10^{-6} was plated by following pour plate method on Nutrient Agar medium. The plates were incubated at room temperature (25±2°C) for three to four days. After the incubation, fungal and bacterial colonies were examined and transferred to plates and subsequently purified. The purified cultures of the predominant fungi and bacteria were then stored under refrigerated conditions for identification and subsequent studies for antagonism (Basha *et al*., 2010).

3.7.2. Isolation of Fungal and Bacterial Antagonists from Phyllosphere of Chilli

Chilli leaves were collected from the disease free plants in the fruit rot affected chilli fields of College of Agriculture, Vellayani. One g of leaf was transferred to 99 ml sterile distilled water in 250 ml conical flasks and shaken for 20 min in a rotary shaker. From this, the 10^{-4} dilution was prepared for isolation of fungal antagonists. One ml of 10^{-4} was plated using pour plate method on Martin's Rose Bengal Agar medium. For isolation of bacterial antagonists, 10^{-6} dilution was prepared. One ml of 10^{-6} was plated by following pour plate method on Nutrient Agar medium. The plates were incubated at room temperature (25±2°C) for three to four days. After the incubation, fungal and bacterial colonies were examined and transferred to plates and subsequently purified. The purified cultures of the predominant fungi and bacteria were then stored under refrigerated conditions for identification and subsequent studies for antagonism (Tembhare *et al*., 2012).

3.7.3. Isolation of Fungal and Bacterial Antagonists from Rhizosphere of Chilli

Rhizosphere soil along with roots was collected from the disease free plants in the fruit rot affected chilli fields of College of Agriculture, Vellayani. One g of rhizosphere soil was transferred to 99 ml sterile distilled water in 250 ml conical

flasks and shaken for 20 min in a rotary shaker. From this, the 10^{-4} dilution was prepared for isolation of fungal antagonists. One ml of 10^{-4} was plated using pour plate method on Martin's Rose Bengal Agar medium. For isolation of bacterial antagonists, 10^{-6} dilution was prepared. One ml of 10^{-6} was plated by following pour plate method on Nutrient Agar medium. The plates were incubated at room temperature (25±2°C) for three to four days. After the incubation, fungal and bacterial colonies were examined and transferred to plates and subsequently purified. The purified cultures of the predominant fungi and bacteria were then stored under refrigerated conditions for identification and subsequent studies for antagonism (Johnson and Curl, 1972).

3.8. *IN VITRO* SCREENING OF THE FUNGAL ISOLATES AGAINST *COLLETOTRICHUM CAPSICI* BY DUAL CULTURE TECHNIQUE.

The fungal isolates obtained by serial dilution techniques were primarily evaluated for their antagonistic potential against *C. capsici* by cross culture method followed by Tembhare *et al*. (2012) inoculating four fungal isolates at the four corners of a petri plate at a distance of 3.5 cm from the centre of the nine cm petri plate. The pathogen being a very slow grower, mycelial discs of five mm diameter taken from the actively growing margins of seven days old cultures of the pathogen was inoculated at the centre of the petri plates two days before the inoculation of isolated fungal antagonist. On the second day after incubation five mm mycelial discs cut from the three days old culture of the isolated fungi were placed a distance of 3.5 cm from the centre of the nine cm petri plate. The procedure was repeated in all plates. Control plates were also maintained with only *C. capsici.* The plates were incubated at room temperature (25±2°C). Observations were recorded on radial growth, over growth and inhibition zone.

The fungal isolates that showed maximum antagonistic action towards the pathogen were selected for final screening by dual culture technique (Mishra, 2010)

for confirmation. For that, mycelial discs of five mm diam. taken from the actively growing margins of seven days old culture of the pathogen *C. capsici* were placed on PDA plates opposite to the colony of the fungal antagonist at a distance of 2.5 cm from the periphery of the culture plate. Control plates were also maintained with only *C. capsici.* The plates were incubated at room temperature (25±2°C). Four replications were maintained for each treatment. Observations were recorded on radial growth, over growth and inhibition zone.

Per cent inhibition of the pathogen over control was calculated by adopting the formula (Vincent, 1927),

$$I = C\text{-}T / C \times 100$$

I – Per cent growth inhibition

C – Growth in control

T - Growth in treatment

The most efficient fungal antagonists from dual culture experiment was selected for morphological characterization and used for evaluating the efficacy of disease suppression during *in vitro* seedling assay.

3.9. IDENTIFICATION AND MORPHOLOGICAL CHARACTERIZATION OF SELECTED FUNGAL ANTAGONISTS

The fungal antagonists showing maximum *in vitro* mycelial inhibition of *Colletotrichum capsici* were selected for morphological identification. For the morphological characterization of selected fungal antagonists, single spore isolation technique for fungal purification and slide culture technique (Riddel, 1950) for morphological identification were followed.

3.9.1. Single Spore Isolation

Single spore isolation technique (Dhingra and Sinclair, 1985) described under 3.1.1 was used for the purification isolated *Trichoderma* sp. and *Penicillium* sp. The inoculated plates were incubated at room temperature (25±2°C) for 3-5 days and used as inoculum for further identification studies.

3.9.2. Slide Culture Technique

Slide culture technique (Riddel, 1950) was used for the species level identification of isolated *Trichoderma* sp. and *Penicillium* sp. Petri plates containing a filter paper, two pieces of glass rods, two cover slips and one microscopic slide were autoclaved. Using a sterile scalped blade, 5mm wide blocks of plain agar medium were cut and placed two 5mm agar block at two sides of the slide by using an inoculation needle. Then inoculated the four corners of the agar block with mycelium of fungal antagonist and placed the cover slips over the agar block. Wet the filter paper with little sterile water to form a damp chamber. The slide culture units were incubated at room temperature (25±2°C). The observation was taken 48 h after inoculation. The size of conidiophore was measured under microscope.

3.10. *IN VITRO* SCREENING OF THE BACTERIAL ISOLATES AGAINST *COLLETOTRICHUM CAPSICI*

Five mm mycelial disc cut from the seven days old culture of the pathogen *Colletotrichum capsici* was placed on the centre of the PDA plates and two streaks were done with bacterial isolate on both the sides perpendicular to the disc 2.5 cm apart as described by Ngullie *et al.* (2010). Control plates were also maintained with only *Colletotrichum capsici.* The plates were incubated at room temperature (25±2°C). Four replications were maintained for each treatment. Observations were recorded on radial growth and inhibition zone.

Per cent inhibition of the pathogen over control was calculated by adopting the formula (Vincent, 1927),

$$I = C\text{-}T / C \times 100$$

I – Per cent growth inhibition

C – Growth in control

T - Growth in treatment

The most efficient bacterial antagonists from dual culture experiment was selected for morphological characterization and used for evaluating the efficacy of disease suppression during *in vitro* seedling assay.

3.10.1. Identification of Bacteria

The bacterial isolate obtained through serial dilution was streaked on nutrient agar medium in sterile petri plates. The bacterial isolate obtained were subjected to gram staining technique to identify the bacterium. Gram staining was done based on the technique devised by Gram (1884). Bacterial smear was prepared on a glass slide. The smear was fixed by heating and a drop of crystal violet was poured on the heat fixed smear. The excess stain was washed off in water after 2-4 min. Gram's iodine was poured and waited for 1-2 min. The excess stain was washed off in running water. The bacterial cells were stained with 95 per cent ethanol so as to decolourise the stain, then the bacterial cells were counter stained using safranin (Appendix II) and after two min excess stain was washed out. A drop of cedar oil was dropped into the slide and was observed under oil immersion objective (100 X).

Endospore staining was done based on the method described by Shaffer and Fulton, 1950. The smear of the bacteria was prepared and was heat fixed. The slide was placed on the rim of a beaker containing boiling water with the smear on the

upper side. The smear was flooded with malachite green (Appendix II) when the water in the beaker started boiling. The slide was left for 3-4 min. The slide was taken and excess stain was drained by washing under tap water. The smear was counter stained using safranin and washed after 30 sec. A drop of cedar oil was dropped into the slide and observed under oil immersion objective (100 X).

3.11. *IN VITRO* EVALUATION OF CHEMICAL FUNGICIDES AGAINST *COLLETOTRICHUM CAPSICI*

The *in vitro* evaluation of selected fungicides against *Colletotrichum capsici* was done by using Poisoned food technique (Nene and Thapliyal, 1993). Seven commercially available chemicals including four systemic, two contact and one combination fungicide were used for the screening. The efficacy of each fungicide was tested at three concentrations i.e. at field concentration, lower dose than field concentration and higher dose than field concentration. The fungicides and the concentrations tested by poisoned food technique are given in the table 3.

In order to study this, 50 ml distilled water and 50 ml double strength PDA medium were taken in separate 250 ml conical flask and sterilized. Added desired concentration of fungicide in to the 50 ml sterile distilled water and swirled well. Then the fungicide mixed with 50 ml melted and cooled double strength PDA to get desired concentration. Thereafter 20 ml of the poisoned medium was poured in to sterilized petriplate (nine cm diam.) under aseptic conditions in Laminar Air flow inoculation chamber and allowed to solidify. The same procedure was repeated for all the fungicides.

Each plate was inoculated in the centre with five mm diameter disc cut from the seven days old test *C. capsici* culture individually under aseptic conditions and incubated at room temperature (25±2°C).

Table 3. The fungicides and the concentrations tested by poisoned food technique

Treatment	Fungicide name	Trade name	Lower dose (%)	Field dose (%)	Higher dose (%)	Nature of fungicide
T1	Difenoconazole	Score	0.01	0.05	0.1	Systemic
T2	Propiconazole	Tilt	0.05	0.1	0.15	Systemic
T3	Chlorothalonil	Kavach	0.05	0.1	0.15	Contact
T4	Azoxystrobin	Amistar	0.05	0.1	0.15	Systemic
T5	Captan +Hexaconazole	Taqat	0.05	0.1	0.15	Combination fungicide
T6	Carbendazim	Bavistin	0.01	0.05	0.1	Systemic
T7	Mancozeb	Indofil M-45	0.1	0.2	0.3	Contact

Unamended PDA plates inoculated with *Colletotrichum capsici* served as checks. Radial growth of the test isolates was recorded after seven days of incubation. Per cent inhibition of mycelial growth over control was calculated using the formula (Vincent, 1927)

$$I = C\text{-}T / C \times 100$$

Where,

I = per cent inhibition.

C = growth of *C. capsici* in unamended medium.

T = growth of *C. capsici* in amended medium.

3.12. *IN VITRO* EVALUATION OF ORGANIC PREPARATIONS AGAINST *COLLETOTRICHUM CAPSICI*

3.12.1. Preparation of Panchagavya

Panchagavya was prepared by following the steps described in the organic Package of Practice, KAU (2009). Cow dung - seven kg and cow ghee- one kg were mixed in a clean plastic bucket thoroughly both in morning and evening hours and keep aside for three days. After three days, cow urine – ten litres and water - ten litres were added. The mixture was keep for 15 days with regular mixing both in morning and evening hours. After 15 days, added cow milk – three litres, cow curd - two litres, tender coconut water - three litres, jaggery - three kg and well ripened poovan banana - 12 numbers. Then keep the bucket under shade by covering with mosquito proof net and stirred the content twice a day both at morning and evening. The stock solution was used for disease management studies after 30 days.

3.12.2. Preparation of Fish Amino Acid

Fish amino acid was prepared by following the steps described by Weinert *et al*. (2014) with slight modifications, mixing one kilogram of sardine fish (*Sardina pilchardus*) with one kg of jaggery (brown sugar) in a plastic can and keep the plastic can under shade condition and covered the mouth with paper and tie it with the string. Keep the can undisturbed for 25 days. After 25 days, the content was filtered through muslin cloth and stored in the same can. The filtered content was used in the field by mixing with water (five ml per litre of water).

3.12.3. Preparation of Jeevamruth

Jeevamruth was prepared by following the steps described by Chadha *et al.* (2012). Mix the ingredients cow dung- one Kg, cow urine - one L, jaggery – 200 g, pulse flour – 200 g, fertile soil – 100 g and water ten L in a drum with the help of a wooden stick. Swirl the mixture 2-3 times per day regularly for 5-7 days for proper fermentation. The preparation was diluted and used for spraying @ 2.5 per cent.

3.12.4. *In vitro* Evaluation of Organic Preparations

The *in vitro* evaluation of organic preparations i.e. pachagavya, fish amino acid and jeevamruth against *Colletotrichum capsici* was done by using Poisoned food technique (Nene and Thapliyal, 1993). The concentrations tested were 2.5, 5 and 10 per cent for all the organic preparations.

In order to study this, 47.5 ml, 45 ml and 40 ml PDA medium in three separate 250 ml conical flask were taken and sterilized. The organic preparations were filtered initially with Whatman filter paper and then filter sterilized by passing through bacterial filter (0.22 µm) before adding to the media. Added desired amount (2.5 ml, 5 ml, and 10 ml) of filter sterilized organic preparations in to the desired amount (47.5 ml, 45 ml and 40 ml) of melted PDA to get desired concentration.

There after 20 ml of the amended medium was poured in to sterilized petri plate under aseptic conditions in Laminar Air flow inoculation chamber and allowed to solidify. The same procedure was repeated for all concentrations of all the organic preparations.

Each plate was inoculated in the centre with five mm diameter disc cut from the seven days old *C. capsici* culture under aseptic conditions and incubated at room temperature (25±2°C).

Unamended PDA plates inoculated with *Colletotrichum capsici* served as control. Radial growth of the pathogen at all concentrations was recorded after seven days of incubation. Per cent inhibition of growth over control was calculated using the formula (Vincent, 1927),

$$I = C\text{-}T / C \times 100$$

Where,

I = per cent inhibition.

C = growth of *C. capsici* in unamended medium.

T = growth of *C. capsici* in amended medium.

3.13. SEEDLING ASSAY OF DIFFERENT AGENTS FOR THE CONTROL OF THE DISEASE ON SEEDLINGS

The selected fungal and bacterial antagonists, the fungal endophyte *Piriformospora indica,* the KAU released biocontrol agents *Pseudomonas fluorescens* and *Trichoderma harzianum* and seven commercially available fungicides were assessed for the management of the disease on seedlings at the recommended concentration (Table 4 and Table 5).

Table 4. The fungicides and the concentration used for seedling assay

Treatment	Chemical fungicide	Dose (%)
T1	Difenoconazole	0.05
T2	Propiconazole	0.1
T3	Azoxystrobin	0.1
T4	Chlorothalonil	0.1
T5	Mancozeb	0.2
T6	Carbendazim	0.05
T7	Captan +Hexaconazole	0.1

Table 5. Bio agents used for seedling assay

Treatment	Bio agent	Dose
T1	*Penicillium citrinum*	10^6 cfu ml^{-1}
T2	*Trichoderma viride*	10^6 cfu ml^{-1}
T3	*Bacillus* sp.	10^8 cfu/ml
T4	*Pseudomonas fluorescens*	10^8 cfu/ml
T5	*Piriformospora indica*	10^6 cfu ml^{-1}
T6	KAU talc based formulation of *Pseudomonas fluorescens*	10 g Kg^{-1}
T7	KAU talc based formulation of *Trichoderma*	20 g kg^{-1}

Chilli seeds were smeared with the spore suspension (10^6 conidia/ ml) of *C. capsici*. After 24 h of inoculation, the seeds were treated with cell suspension of *P. fluorescens, Bacillus* sp. and conidial suspension of *T. viride, P. citrinum, P. indica* at the rate of 10^8 cfu $ml^{-1}g^{-1}$ by mixing seeds with five ml of cell or conidial suspension. Similarly talc based formulations of *P. fluorescens* (28 $x10^7$ cfu g^{-1}) and *T. harzianum* (19 X 10^7 cfu g^{-1}) in the form of slurry were treated to chilli seeds at the rate of two g kg^{-1} and 20 g kg^{-1}, respectively. Commercially available fungicides were used for the seed treatment at recommended concentration. After 24 h of treatment, the seeds were air-dried. Treated and untreated seeds were placed between paper rolls in four replicates of about ten seeds each for germination. The rolls were kept at 25 ± 2°C (Suthin Raj *et al.,* 2009). The disease incidence was assessed based on per cent seedling mortality. Observations were recorded on seed germination, seedling mortality and seedling vigour index of seedlings after 15-20 days.

The formula used for the calculation of percentage seedling mortality given below (Suleiman, 2010):

$$\text{Percentage seedling mortality} = \frac{\text{No. of seedlings dead}}{\text{Total no. of seedlings}} \times 100$$

Seedling vigour was also calculated by formula suggested by Abdul and Anderson (1973).

Vigour index = (root length in mm + shoot length in mm) x per cent germination.

3.14. STUDIES ON *IN VIVO* SUPRESSION OF FRUIT ROT OF CHILLI.

A pot culture experiment was conducted at Department of Plant Pathology to evaluate the efficacy of the selected treatments (Table 6) in suppressing the fruit rot of chilli under natural conditions. Chilli variety, Vellayani Athulya was used for the pot

Table 6. Selected treatments for pot culture study

	Treatments	Dose
	Biocontrol agents	
T1	*Bacillus* sp.	10^8 cfu/ml
T2	*Penicillium citrinum*	10^6 conidia/ml
T3	*Trichoderma viride*	10^6 conidia/ml
T4	*Pseudomonas fluorescens*	10^8 cfu/ml
	Organic preparations	
T5	Panchagavya	2.5%
T6	Jeevamruth	2.5%
	Chemical fungicides	
T7	Difenoconazole	0.05%
T8	Propiconazole	0.1%
T9	Azoxystrobin	0.1%
T10	Captan +Hexaconazole	0.1%
T11	Carbendazim	0.05%
Positive checks		
T12	Talc based formulation of *Pseudomonas fluorescens*	20%
T13	Talc based formulation of *Trichoderma harzianum*	10%
T14	Mancozeb	0.2%
T15	Inoculated control	

culture experiment. The seeds were sown in pro-trays filled with potting mixture consisting of vermicompost and coir pith in 1: 1 ratio. The chilli seedlings were transplanted to the pot filled with potting mixture (sand, soil and cow dung in 1: 1: 1 ratio) after the emergence of first true leaves. Each pot contained one plant and three replication of each treatment were maintained. The design followed was CRD. The treatments were given as foliar application after the fruits exhibited symptoms after the inoculation of the pathogen.

3.14.1. Preparation of Pathogen Inoculum

The fruit rot pathogen, *C. capsici* was mass multiplied in potato dextrose broth by inoculating with mycelial discs of five mm dia. cut from edges of actively growing five to seven day old culture of *C. capsici* and incubated at room temperature.

3.14.2. Application of Inoculum

For application of the pathogen on chilli plants, the conidial suspension of *C. capsici* was prepared by harvesting mycelial mats from fourteen day old culture raised in potato dextrose broth. Mats were suspended in sterile distilled water (SDW) and homogenised in a warring blender for one minute and strained through double layered muslin cloth and diluted with SDW in such a manner to contain 10^6 conidia ml^{-1}. Then it was sprayed using a hand sprayer on to the plants bearing 10 day old fruits (Golda, 2010).

3.14.3. Application of Treatments

Foliar spraying of the selected treatments at recommended dose was done at fortnightly intervals after five days of inoculation.

The following observations of chilli plants were recorded during the course of the experiment.

3.14.4. Disease Incidence

Observations on fruit rot incidence were taken from the next day of inoculums application.

Disease incidence was calculated using the formula (Anamika *et al*., 2014):

$$\text{Disease incidence} = \frac{\text{Number of fruits affected}}{\text{Total number of fruits}} \times 100$$

3.14.5. Disease Intensity

Observation on fruit rot disease intensity was recorded after inoculum application and at weekly intervals after the treatment application. Scoring of the disease was done using the score chart developed by Vishwakarma and Sitaramaiah (1986) after careful study of the disease and development. Size of the lesion was taken into account for devising the scale. Based on this a 0-4 scale has been devised (Plate-1, Table 7).

The percentage disease index was calculated using the formula given by Mc Kinney (1923).

$$\text{Per cent Disease Index} = \frac{\text{Sum of grades of each leaf}}{\text{No: of leaves assessed}} \times \frac{100}{\text{Maximum grade used}}$$

Table 7. Score chart used for assessing fruit rot of chilli

Grade	Description
0	Healthy
1	1 - 5 per cent of the fruit infected
2	5-25 per cent of the fruit infected
3	26-50 per cent of the fruit infected
4	51-100 per cent of the fruit infected

Plate 1. Score chart used for assessing fruit rot of chilli

Biometric observations such as plant weight, plant height, root length, root weight, no. of nodules, plant dry weight, dry root weight and yield were also recorded.

Results

4. RESULTS

The present study on the 'Integrated management of anthracnose in chilli (*Capsicum annuum* L.)' was conducted during the period 2013-2015 at the Department of Plant Pathology, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala to evolve an integrated management for anthracnose in chilli. The results obtained from the laboratory and field experiments are summarized below:

4.1. ISOLATION OF CHILLI ANTHRACNOSE PATHOGEN AND PURIFICATION OF THE ISOLATES

4.1.1 Isolation of the Pathogen

The pathogens causing the fruit rot disease in chilli, *Colletotrichum capsici* and *Colletotrichum gloeosporioides* were isolated from the infected leaves and fruits showing typical anthracnose symptoms. The samples were collected from different chilli growing fields of College of Agriculture, Vellayani during the surveys and the isolation was done following standard procedures mentioned under 3.1.

Five isolates of *C. capsici* and two isolates of *C. gloeosporioides* were obtained from fruit while two isolates of *C. gloeosporioides* were obtained from leaves (Table 8). The isolates were serially numbered from C1 to C9 and were subjected to pathogenicity studies for selecting the most virulent isolate of the pathogen for further studies.

The climatic parameters including average temperature, average RH and average rainfall around College of Agriculture, Vellayani during the year 2013 at the time of collection of diseased samples were recorded (Table 9, Plate 2) during 2013 and observed that during August to October months an average rainfall of 10-15 cm, relative humidity 89.70 per cent (RH) and 29 ˚C temperature was prevailing in the premises of College of Agriculture, Vellayani and during this period, *C. capsici* was frequently isolated from the infected samples.

Table 8. Isolates collected from different locations

Isolate	Time of sample collection	Location	Parts affected	Description of symptom
C1	August 2013	Instructional farm, Vellayani	Leaf	Necrosis on affected part of leaf
C2	September 2013	Instructional farm, Vellayani	Unripe fruit	Concentric circles around the lesion on fruit , wrinkled and deformed fruit
C3	September 2013	Dept. of Olericulture, Vellayani	Leaf	Necrotic spot on leaf and an yellowing around it
C4	September 2013	Instructional farm, Vellayani	Ripe fruit	Concentric circles around the lesion on fruit, pinhead sized acervuli present
C5	October 2013	Dept. of Olericulture, Vellayani	Ripe fruit	Concentric circles around the lesion on fruit, pinhead sized acervuli present
C6	October 2013	Dept. of Olericulture, Vellayani	Unripe fruit	Water soaked lesion on affected tissue followed by necrosis
C7	October 2013	Instructional farm, Vellayani	Unripe fruit	Water soaked lesion on fruit
C8	October 2013	Instructional farm, Vellayani	Ripe fruit	Concentric circles around the lesion on fruit
C9	October 2013	Crop museum, Vellayani	Ripe fruit	Necrosis on affected part of leaf

Table 9. Weather data during 2013

Month	Maximum temperature (^{0}C)	Minimum temperature (^{0}C)	MaximumRH (%)	Minimum RH (%)	Total Rainfall (mm)
January	31.1	25.7	92.3	77.7	19.4
February	30.5	25	92.7	79.1	33.9
March	30.4	24.7	90.9	79	7.6
April	29.7	24.2	92.9	80.4	24.2
May	30.1	24.2	90.4	76.7	19.4
June	29.9	24.2	91.6	73.6	140.6
July	29.2	23.5	95.3	85.9	170.2
August	29.4	23.5	88.6	77.3	100.7
September	29.7	24	89.7	79.6	70.4
October	29.8	24	94	80.9	149.9
November	29.9	23.9	87.6	84.1	206.6
December	29.2	23.9	96.1	79.3	80

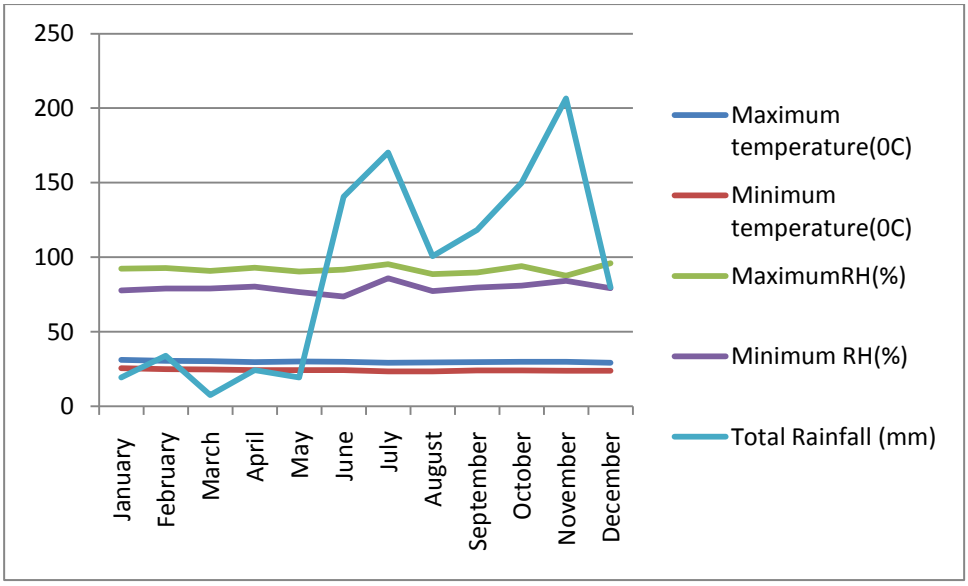

Plate 2. Weather data during 2013

4.1.2. Single Spore Isolation

The cultures of *C. capsici* and *C. gloeosporioides* mentioned under 4.1 were purified by single spore isolation method and pure cultures were obtained and maintained on PDA.

4.2. PATHOGENICITY STUDIES

4.2.1. Proving of Pathogenicity

The pathogenicity of the different isolates of *C. capsici and C. gloesporioides* was confirmed by proving Koch's postulates. The pathogen was artificially inoculated on the mature green fruits and ripe fruits by pinprick method. Between the nine isolates, C2 isolate showed the largest lesion compared to all other isolates. The isolates C1, C2, C3, C4, C5 and C8 took only three days for the initiation of symptoms. The symptoms appeared as small brown water soaked lesion which gradually enlarged and covered the fruit surface. The isolate C3 took maximum time of six days for symptom expression. The leaves of chilli were inoculated with *C. capsici* (C2), *C. gloeosporioides* (C6) alone and in combination and among these, the isolate C2 produced comparatively larger spots (Plate 3A-3C). Similarly, combined and individual inoculation of C2 and C6 on chilli fruits revealed that the isolate C2 (*C. capsici*) exhibited larger lesions compared to the other inoculations (Plate 4, Table 10). Re- isolation of the pathogen from the artificially inoculated fruits and leaves yielded *Colletotrichum* sp. identical to the original culture.

4.2.2. Virulence Rating

All the isolates were subjected to virulence rating based on the size of the lesion developed on inoculated fruit (Table 11). All the nine isolates tested i.e., C1 to C9, showed varying levels of lesion size on unripe fruits and ripe fruits (Plate 5 and 6). Based on the above observations, the isolate C2 which produced a lesion of 4.34 cm on unripe fruit and five cm on ripe fruit was rated as the most virulent pathogenic

(A) *C. capsici*

(B) ***C. gloeosporioides***

(C) *C. gloeosporioides* + *C. capsici*

Plate 3. Symptoms produced on chilli leaves by combined and individual inoculation of *C. capsici and C. gloeosporioides*

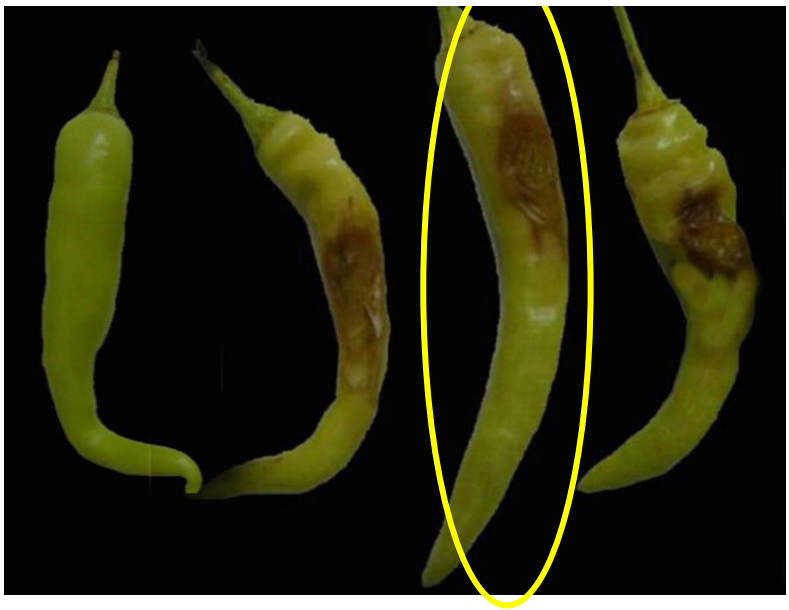

Plate 4. Symptoms produced on chilli fruits by combined and individual inoculation of *C. capsici and C. gloeosporioides*

Table 10. Combined and individual inoculation of *C.capsici and C.gloeosporioides* on fruit and leaf

Treatments	Lesion size on leaves (cm)*	Lesion size on fruits (cm)*
C. gloeosporioides	1.15[c]	2.03[c]
C. capsici	2.05[a]	4.00[a]
C. capsici+ *C. gloeosporioides*	1.52[b]	3.10[b]
CD at 5%	0.07	0.10

*Mean of four replications

Table 11. Virulence rating of different isolates of the pathogen causing fruit rot of chilli

Isolate	Pathogenicity	Days for symptom expression	Virulence rating	Lesion size (cm)*	
				Unripe fruit	Ripe fruit
C1	Pathogenic	3	+	1.34^{g}	1.60^{h}
C2	Pathogenic	3	+++	4.34^{a}	5.00^{a}
C3	Pathogenic	6	+	1.17^{h}	1.72^{g}
C4	Pathogenic	3	++	3.20^{c}	3.60^{c}
C5	Pathogenic	3	+	1.55^{f}	2.10^{f}
C6	Pathogenic	4	++	3.54^{b}	4.50^{b}
C7	Pathogenic	4	+	2.34^{e}	3.17^{e}
C8	Pathogenic	3	+	2.73^{d}	3.55^{d}
C9	Non-pathogenic	-	-	0^{i}	0.00^{i}
CD (0.05)				0.17	0.16

*Mean of four replications

\+ : 25% of fruit infected

\+ + : 50% of fruit infected

\+ + + : 75% of fruit infected

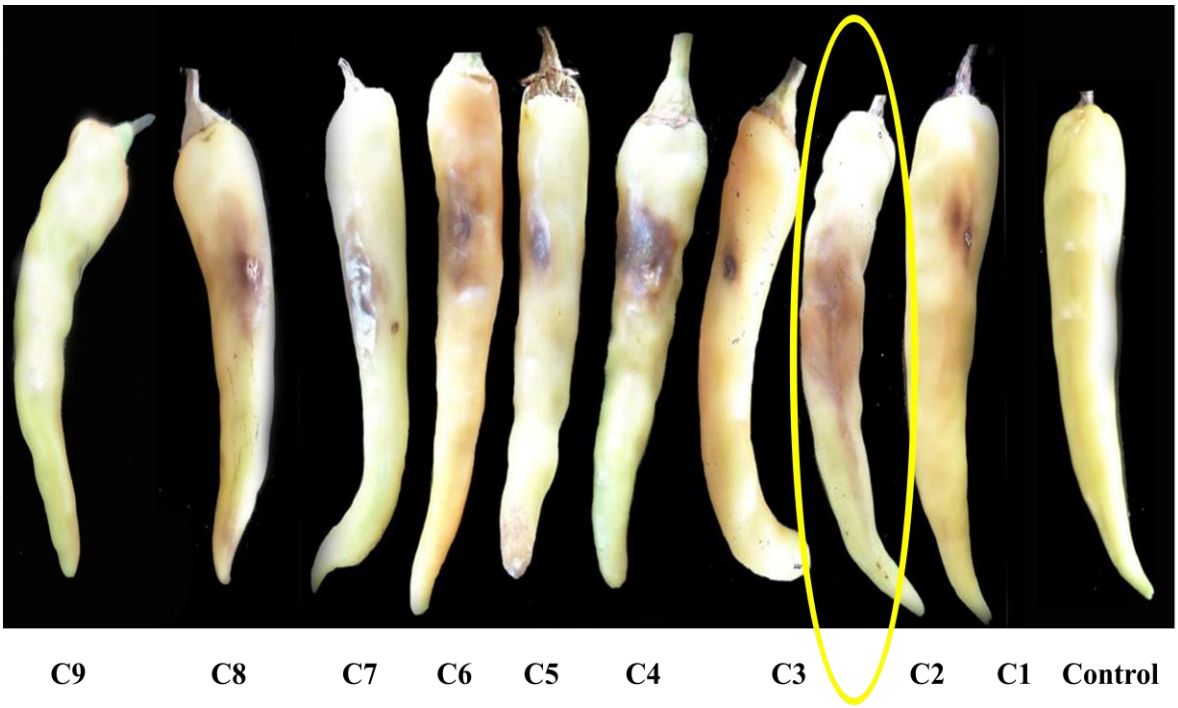

Plate 5. Symptoms produced by different isolates of *Colletotrichum* spp. on unripe fruits

Plate 6. Symptoms produced by different isolates of *Colletotrichum* spp. on ripe fruits

isolate and selected for further studies. Re-isolation of the pathogen from the artificially inoculated fruit resulted in the pathogen culture identical to the original culture.

4.3. SYMPTOMATOLOGY OF CHILLI ANTHRACNOSE

Fruit rot disease appeared as small round black or brown water soaked lesion (0.5 cm) on the fruit surface (Plate 7A). Then the water soaked area gets replaced by necrotic tissue (Plate 7B). Gradually, the lesion enlarged in size in the longitudinal axis of the fruit (Plate 7C). This elliptical spot enlarged up to 3- 7.5 cm. As the necrosis progressed the lesions became concentric (Plate 7D) and small black pin head sized structures of which each one is a cluster of acervuli seen arranged concentrically on the lesion (Plate 7E). Occasionally, scattered arrangements of acervuli were also observed (Plate 7F). These acervuli emerged from the epidermis of the fruit by disrupting the fruit skin. When the diseased fruit was cut open, the lower surface of the skin was covered with minute, elevated, and black masses of the fungus. The severely infected seeds will be black, covered by white or black mycelial growth. When the whole fruit was infected, fruits became wrinkled, deformed, shrivelled and dried (Plate 7G). The reddish colour of the fruit changed to straw colour. Symptoms were observed on ripe fruit as well as on unripe fruit (Plate 7H and 7I) but, the ripe fruits were infected more severely than the green fruits.Occasionally symptoms appeared on petioles showing elongated brown necrotic spots. *C. capsici* and *C. gloeosporioides* produced similar symptoms on chilli fruit. Finally, severely infected fruits droped off from the plant.

On leaves, small brown spots (0.2-0.3 cm) appeared which enlarged (three cm) and coalesce with other spots (Plate 8A). As the infection progressed, the spots became either black or grey in colour and they were delimited by a thick and sharp outline enclosing a straw coloured area. Later, the delimited area become necrotic and tissue disentigrated exhibiting shot hole symptom (Plate 8B).

(A) Water soaked lesion on fruit

(B) Infected fruits showing necrotic lesion on fruit

(C) Enlarging lesion on the longitudinal axis and concentric circles on the fruit

(D) Concentric circles on fruit

Plate 7. Symptoms of chilli anthracnose on fruit under field conditions

(E) Concentric arrangement of acervuli on fruit

(F) Scattered arrangement of acervuli on fruit

(G) wrinkled and deformed fruit

Plate 7. Symptoms of chilli anthracnose on fruit under field conditions contd.

(H) Unripe fruits showing fruit rot symptom

(I) Ripe fruits showing fruit rot symptom

Plate 7. Symptoms of chilli anthracnose on fruit contd.

(A) Infected leaves showing leaf spot symptom

(B) Infected leaves showing disentigration of necrotic part

Plate 8. Symptoms of anthracnose disease on chilli leaves

Plate 9. Progressive death of the twigs starting at the tip: 'die-back' symptom

At severe stage, progressive death of twigs starting at the tips i.e. 'die-back' symptom (Plate 9) appeared resulting in complete destruction of the plant.

4.4. IDENTIFICATION OF THE PATHOGEN ASSOCIATED WITH CHILLI ANTHRACNOSE

4.4.1. Morphological Characters

Morphological characters of the different isolates were studied by growing them on PDA as mentioned under 4.4 (Table 12, Plate 10).

4.4.1.1. Colony Characters

The isolates C1, C2, C3, C5 and C6 had suppressed cottony mycelial growth with regular margin and took 5-7 days for completing the mycelial growth in nine cm dia. petri plate. The colour of these isolates varied from white to grey whereas the isolate C3 had a brownish tinge. The surface mycelium of the isolate C2 was uniform whereas concentric ring pattern was observed in C1, C3, C5 and C6. Reverse side of the colony were brownish grey to black. All the isolates of *C. capsici* showed sporulation on PDA media after 10 DAI and produced salmon pink coloured spore mass whereas the number of days taken for spore mass production ranged from 10-20 days.

The isolates C4, C7 and C8 had greyish white to dark grey colour. Aerial mycelium had cottony, tufty growth and produced salmon pink coloured or creamy white spore mass at the centre of the colony and the number of days taken for spore mass production ranged from 10-20. Reverse side of the colony were unevenly white to grey or dark (Plate 11). The isolate C5 had a distinct olivaceous grey zone alternated with rosy buff zone was observed on the reverse side of the colony.

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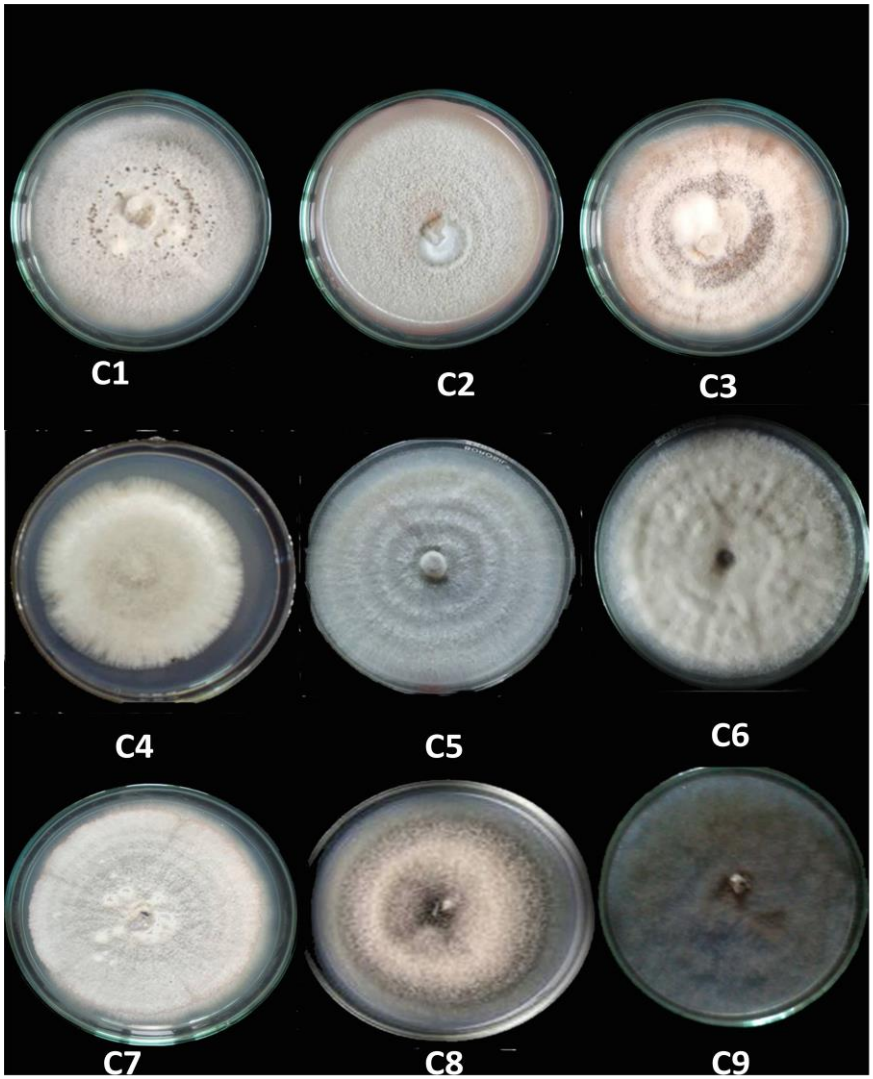


Plate 10. *Colletotrichum* spp. isolates collected from different locations

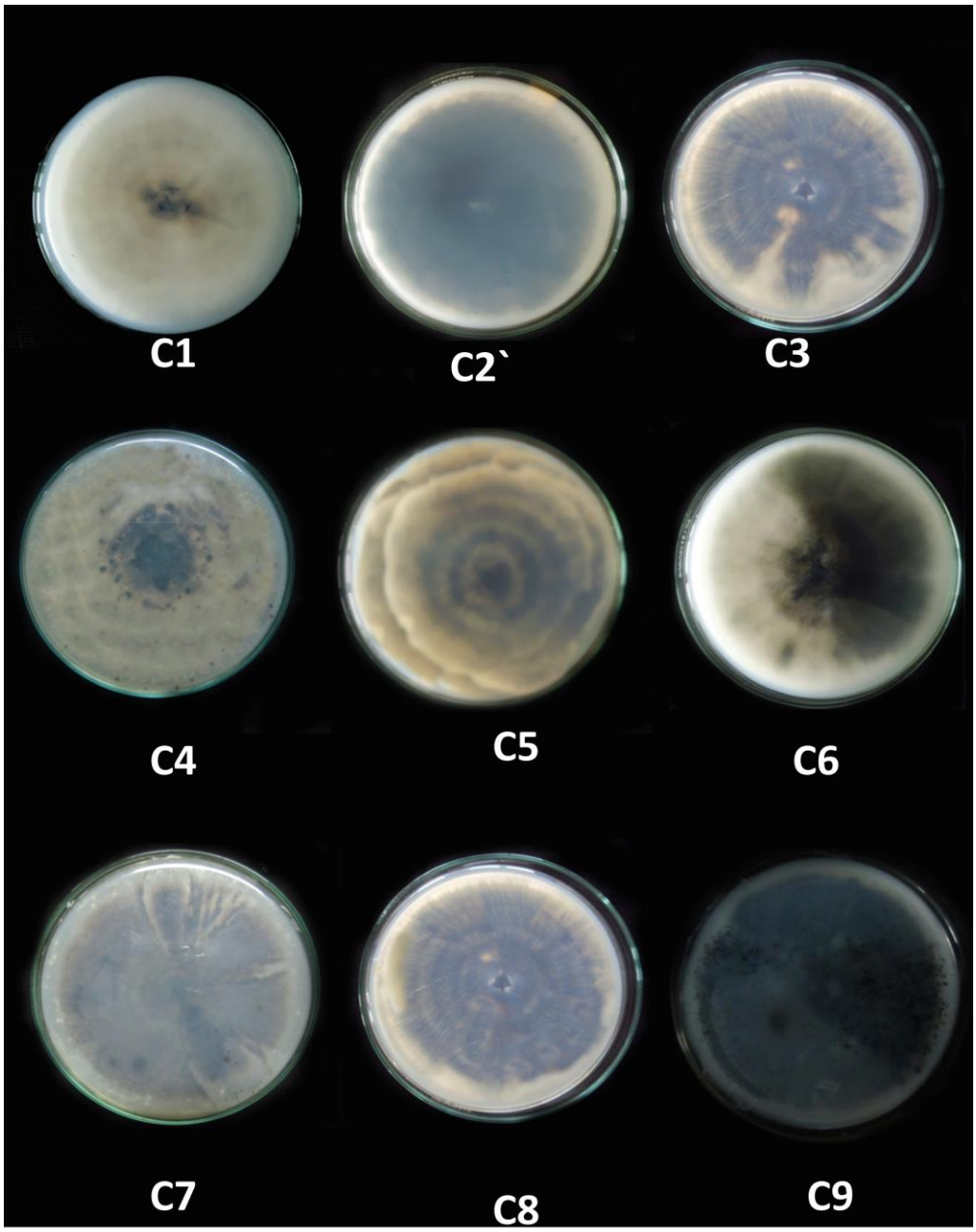


Plate 11. Growth of different isolates when viewed from the reverse side of petriplate

Table 12. Colony characters of *C. capsici*

Isolate No.	DTCP	AGR	Growth pattern	Colony colour (Upper)	Colony colour (lower)	Spore mass colour	DTSP
C1	6	1.37	Sparse	White to grey	Light grey	Salmon pink	12.50
C2	7	1.22	Sparse	White to grey	Grey to black	Salmon pink	18.00
C3	7	1.22	Sparse	White to grey	Grey to black	Salmon pink	10.00
C4	6	1.37	Sparse	White to grey	Grey	Salmon pink	14.23
C5	7	1.2	Dense	White to grey	Grey to black	Creamy white	20.05
C6	6	1.35	Dense	White to grey	Grey to black	Salmon pink	10.00
C7	7	1.38	Dense	White	Grey to black	Salmon pink	15.9
C8	6	1.21	Dense	White to grey	Grey to black	Salmon pink	16.10
C9	6	1.32	Dense	Black	Black	Salmon pink	17.05

DTCP - Days taken to cover 9 cm petridish
AGR - Average growth rate (cm/day)
DTSP - Days taken for spore mass production

4.4.1.2. Conidial Characters

On PDA, the mycelial growth consisted of branched and septate hyphae. Conidia borne singly at the tip of hyaline cylindrical conidiophores or on solitary fertile hyphae. Conidia were hyaline, unicellular and curved with narrow ends and contained a centrally placed oil globule (Plate 12 A). The average conidial size was 25.68 x 4.92 µm and were within the range of 18.40 - 29.96 x 4.28 - 5.99 µm. Acervuli bearing setae were round to elongated or irregular, brown and measured 175.48 µm in diameter and were within the range of 111.28 - 338.12 µm (Plate 12 B), setae abundant, dark brown, 1-5 septate, slightly curved, rigid, hardy, swollen at the base and tapering towards the apex. The average size of the setae was 179.76 x 6.63 µm and was within the range of 102.72-231.12 x 5.56-7.66 µm. Based on the characters, the isolate was identified as *C. capsici* (Table 13, 14).

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 13A). Conidiophores were hyaline and cylindrical. Average conidial size was 19.26 x 4.7 µm and were within the range of 17.12 - 21.14 x 4.28 - 5.35 µm. Acervuli bearing setae were round to elongated or irregular, brown and measured 158.36 µm in diameter and were within the range of 89.88 - 252.52 µm (Plate 13B). Setae abundant, brown to straight to slightly curved, 1-4 septate, swollen at the base and tapering towards the apex, average size of the setae was 124.12 x 6.42 µm and were within the range of 77.04 - 171.24 x 5.13-7.49 µm. From the cultural and conidial morphology, the isolate was identified as *C. gloeosporioides.*

4.4.1.3. Perfect Stage - Glomerella cingulata

The sexual stage *Glomerella cingulata* was obtained from three month old culture of *C. capsici*. The perithecia were dark pigmented, rounded with 250 µm diameter. Within the perithecia, club shaped, thin walled asci each of which contains eight

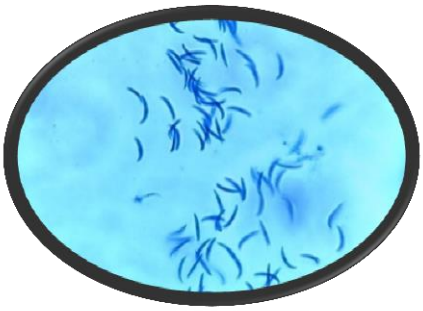


Plate 12 (A) Conidia of *C. capsici* (40 X)

Plate 12 (B) Acervuli with setae of *C. capsici* (400 X)

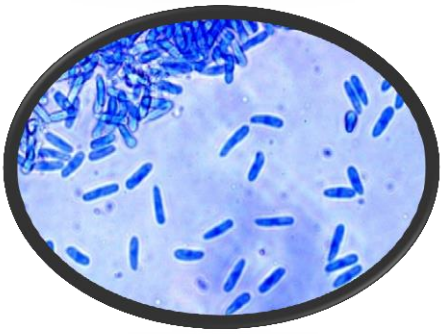


Plate 13 (A) Conidia of *C. gloeosporioides* (40 X)

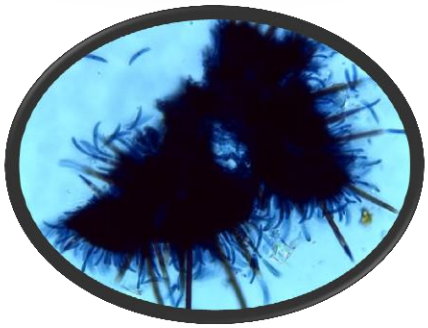


Plate 13 (B). Acervuli with setae of *C. gloeosporioides* (400 X)

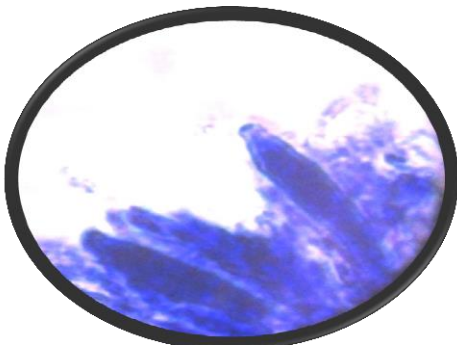


Plate 14 (A). Perfect stage of *C. capsici* – asci (40 X)

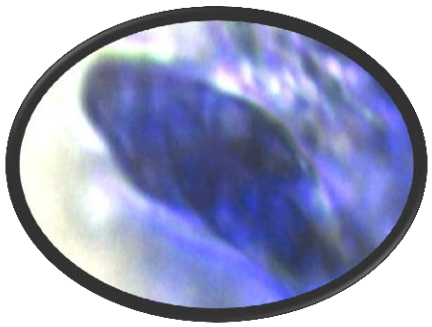


Plate 14 (B). Ascospores within asus

(40 X)

Table 13. Conidial and mycelial characters of different isolates of *Colletotrichum* spp.

Isolate	Conidial shape	Conidial measurements (µm)*		Mycelial width (µm)*
		Conidial length	Conidial breadth	
C1	Falcate	24.38	4.92	3.4
C2	Falcate	22.34	4.50	4.6
C3	Cylindrical with obtuse ends	18.9	4.82	3.00
C4	Cylindrical with obtuse ends	14.75	4.07	4.59
C5	Falcate	24.50	4.84	4.33
C6	Falcate	24.00	4.9	4.4
C7	Cylindrical with obtuse ends	16.26	4.68	3.8
C8	Cylindrical with obtuse ends	17.59	4.5	3.48
C9	Cylindrical with obtuse ends	18.64	4.72	3.78

*Mean of thirty replications

Table 14. Acervular characters of different isolates of *Colletotrichum* spp.

Isolate	Diameter of acervulus (µm)*	No. of setae/ acervulus
C1	152.8	55
C2	187.98	44
C3	122.9	22
C4	135.7	26
C5	150.90	56
C6	188.89	58
C7	145.79	22
C8	120.89	25
C9	139.87	27

*Mean of thirty replications

randomly arranged ascospores were observed (Plate 14A and 14B). The ascospores were aseptate, hyaline, fusiform and 15 µm in length and 4 µm in breadth.

4.5. ASSAY OF INFECTED SEEDS FOR SEED BORNE MYCOFLORA

Fruit rot affected chilli fruits were collected from different chilli growing fields of COA, Vellayani and were tested by standard blotter (Table 15) and standard agar plate method (Table 16).

4.5.1. Standard Blotter Test

The studies on the seed mycoflora of chilli by standard blotter method showed the presence of *C. capsici*, *C. gloeosporioides*, *Penicillium* sp., *Aspergillus* sp. and species of *Alternaria*. On seeds, white and black mycelial growth was started appearing on fifth day onwards. Gradually, the seeds germinated with roots over which the mass of fungal hyphae was visible (Plate 15). The results indicated the dominance of *C. capsici* (44.66 per cent) followed by *C. gloeosporioides* (12.00 per cent), *Alternaria* sp. (0.5 per cent). On seeds, the other saprophytic fungi recorded were *Penicillium* sp. (0.16 per cent) and *Aspergillus* sp. (0.16 per cent).

4.5.2. Standard Agar Plate Method

The important pathogenic fungi were also recorded by standard agar plate method. All the pathogens started growing within four days.The results of this study indicated the dominance of *C. capsici* followed by *C. gloeosporioides* (10 per cent) and *Alternaria* sp. (Plate 16). On seeds, the other saprophytic fungus recorded was species of *Penicillium*.

C. capsici was isolated most frequently both by the blotter and standard agar plate method suggesting that the pathogen could be present on the seed surface as well as inside the seed.

4.6. SURVIVAL OF THE PATHOGEN *C. CAPSICI* IN CROP DEBRIS

The survival ability of *C. capsici* on crop debris was studied under *in vitro* and *in vivo* conditions (Table 17). Under *in vitro* conditions *C. capsici* from the sample was

Table 15. Mycoflora isolated from chilli seeds from infected by standard blotter method

Sample No	Source of seed	Per cent seed infection					Total per cent of seed infection
		C. capsici	*C. gloeosporioides*	*Alternaria sp.*	*Penicillium sp.*	*Aspergillus sp.*	
1	Dept. of Olericulture 2014	16	12	-	1	2	31
2	Instructional farm 2014	34	10	-	4	4	50
3	Crop museum 2014	8	-	04	-	-	12
4	Dept. of Olericulture 2014	10.5	-	-	3	1	15
5	Crop museum 2014	6.2	2	-	-	-	8
6	Instructional farm 2014	45	5	-	-	-	50

Plate 15. Mycoflora isolated from chilli seeds by standard blotter method

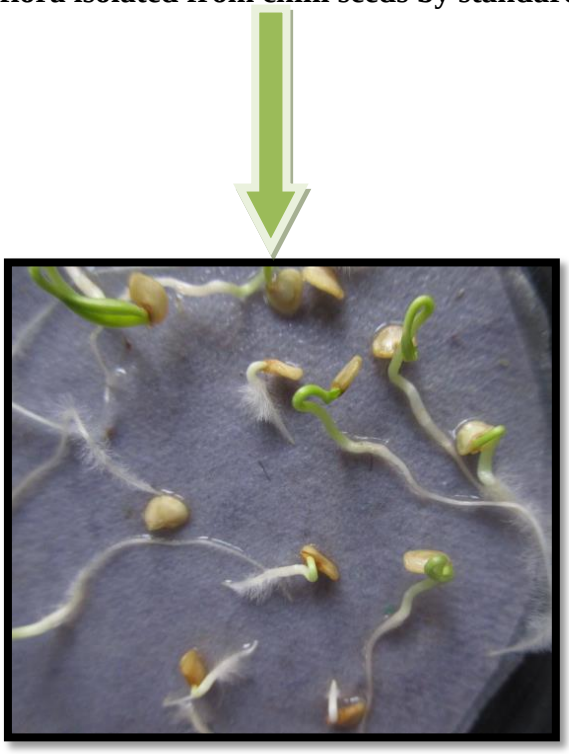


Plate 15 (A). Mycelial growth on seeds

Table 16. Mycoflora isolated from chilli seeds from infected fruits by standard agar plate method

Sample. No	Source and time of seed collection	Per cent seed infection*				Total per cent of seed infection
		C. capsici	*C.gloeosporioides*	*Alternaria*	*Penicillium*	
1	Dept. of Olericulture, 2014	13.33	4.33	1	2	20.66
2	Instructional farm, 2014	16	06	-	3	25
3	Crop museum, 2014	10.66	-	-	-	10.66
4	Dept. of Olericulture, 2014	15	3.66	-	-	18.66
5	Crop museum, 2014	08	-	-	3	11
6	Instructional farm, 2014	25	5	-	-	50

*Mean of four replications

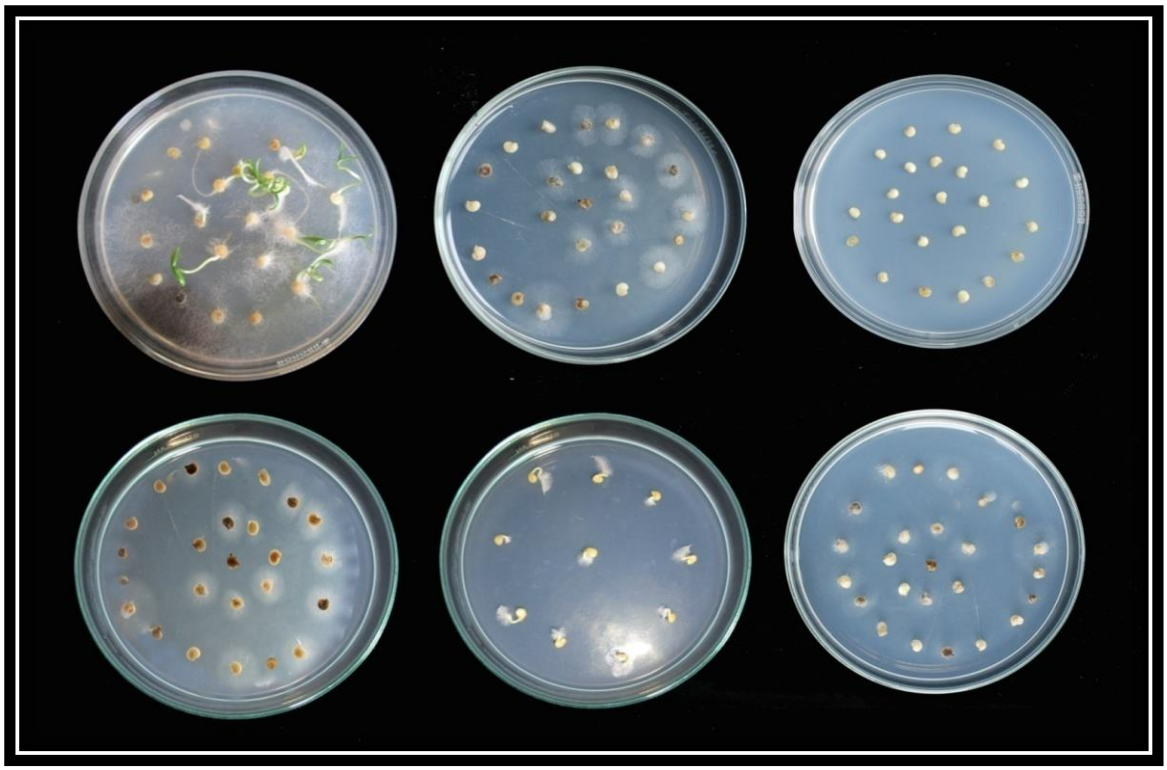
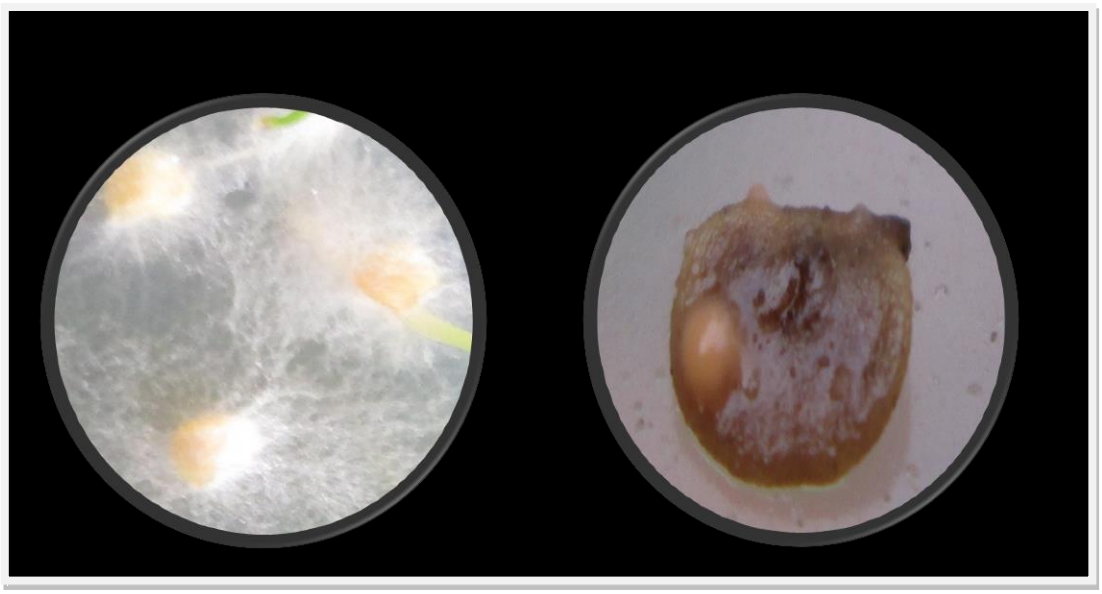


Plate 16. Mycoflora isolated from chilli seeds by standard agar plate method



16 (A) Mycelial growth and orange coloured spore mass production on seeds

recovered up to six weeks. After six weeks, no recovery of the pathogen was observed on the PDA after isolation from the infected fruit. Under *in vivo* conditions viable culture of *C. capsici* from the buried sample was recovered up to 11 weeks. The study showed that *C. capsici* survived for 77 days in infected fruits and leaves in the soil.

4.7. STUDIES ON THE HOST RANGE OF *C. CAPSICI* IN OTHER CROPS GROWN IN VEGETABLE FIELD AND COMMON WEEDS.

Attempts were made to isolate *C. capsici* from vegetable plants, other than chilli, growing in and around the area where infection was detected and exhibiting fruit rot or die back symptoms. Natural incidence of the *C. capsici* infection was observed in fruits of tomato (*Lycopersicon esculentum* Mill.) (Plate 17 A) and brinjal (*Solanum melongena* L.) (Plate 17 B). However, on artificial inoculation of *C. capsici*, brown lesions were observed on leaves of green gram (*Vigna mungo* L.) (Plate 18 A), brinjal (*Solanum melongena* L.) (Plate 18 B), sesame (*Sesamum indicum* L.*)* (Plate 18 C and 18 D), ivy gourd (*Coccinia indica* L.) (Plate 18 E), black gram (*Vigna radiata* L.) (Plate 18 F) and sweet potato (*Ipomoea batatas* L.) (Plate 18 G). Thus, these plants were confirmed as alternative hosts to the pathogen (Table 18).

Natural incidence of disease was observed on weeds *Richardia scabra* L. (Rough Mexican clover*)* and *Commelina benghalensis* L. (Tropical spiderwort) (Plate 19 A and 19 B, Table 19) as brown spots on leaves.

4.8. ISOLATION OF MICROFLORA AND IDENTIFICATION OF ANTAGONISTS

The saprophytic mycoflora was isolated from chilli fruits, rhizosphere and leaves from disease free chilli plants among the anthracnose infected chilli plants in the field (Plate 20 A- C). Similarly, bacterial genera were also isolated (Plate 21). The details of the fungi and bacteria isolated are given in the table 20.

Table 17. Survival of the pathogen *C. capsici* in crop debris

Weeks after incubation	Recovery of *C. capsici*	
	In vitro condition	*In vivo* condition
1	+	+
2	+	+
3	+	+
4	+	+
5	+	+
6	+	+
7	-	+
8	-	+
9	-	+
10	-	+
11	-	+
12	-	-

\+ Recovered

\- Not recovered

Table 18. Host range of *C. capsici*

Sl No.	Crop	Scientific name	Affected plant part	NI/AI	Disease reaction
1.	Tomato	*Lycopersicon esculentum* Mill.	Leaf , Fruit	NI	+ +
2.	Brinjal	*Solanum melongena* L.	Leaf , Fruit	NI	+ +
3.	Ivy gourd	*Coccinea indica* L.	Leaf	AI	+
4.	Green gram	*Vigna mungo* L.	Leaf	AI	+
5.	Sesamum	*Sesamum indicum* L.	Leaf	AI	+
6.	Black gram	*Vigna radiata* L.	Leaf	AI	+
7.	Sweet potato	*Ipomoea batatas* L.	Leaf	AI	+

NI- Natural infection
AI-Artificial inoculation
+ Infected
- Not infected

(A) Brinjal (B) Tomato

Plate 17. Symptoms produced by *C. capsici* on solanaceous crops under natural conditions

(A) Green gram (B) Brinjal

Plate 18. Symptoms produced by *C. capsici* by artificial inoculation on different crops

(C) Leaf Spot on sesame

(D) Lesion on sesame tender leaves

(E) Ivy gourd

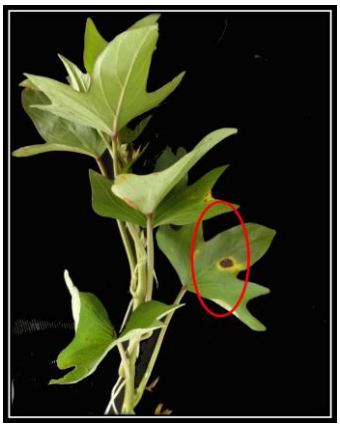
(F) Black gram

Plate 18. Symptoms produced by *C. capsici* by artificial inoculation on different crops contd.

Table 19. Response of common weeds of chilli field to *C. capsici*

Sl No.	Scientific name	Common name	Disease infection
1.	*Amaranthus viridis* L.	Slender Amaranth	-
2.	*Cynodon dactylon* L.	Dog's tooth grass	-
3.	*Cyperus rotundus* L.	Purple nut sedge	-
4.	*Cleome rutidospermum* L.	Fringed spider flower	-
6.	*Panicum repens* L.	Torpedo grass	-
7.	*Richardia scabra* L.	Rough Mexican clover	+
8.	*Commelina benghalensis* L.	Tropical spiderwort	+

+ Infected
- Not infected



(G) Sweet potato

Plate 18. Symptoms produced by *C. capsici* by artificial inoculation on different crops contd.

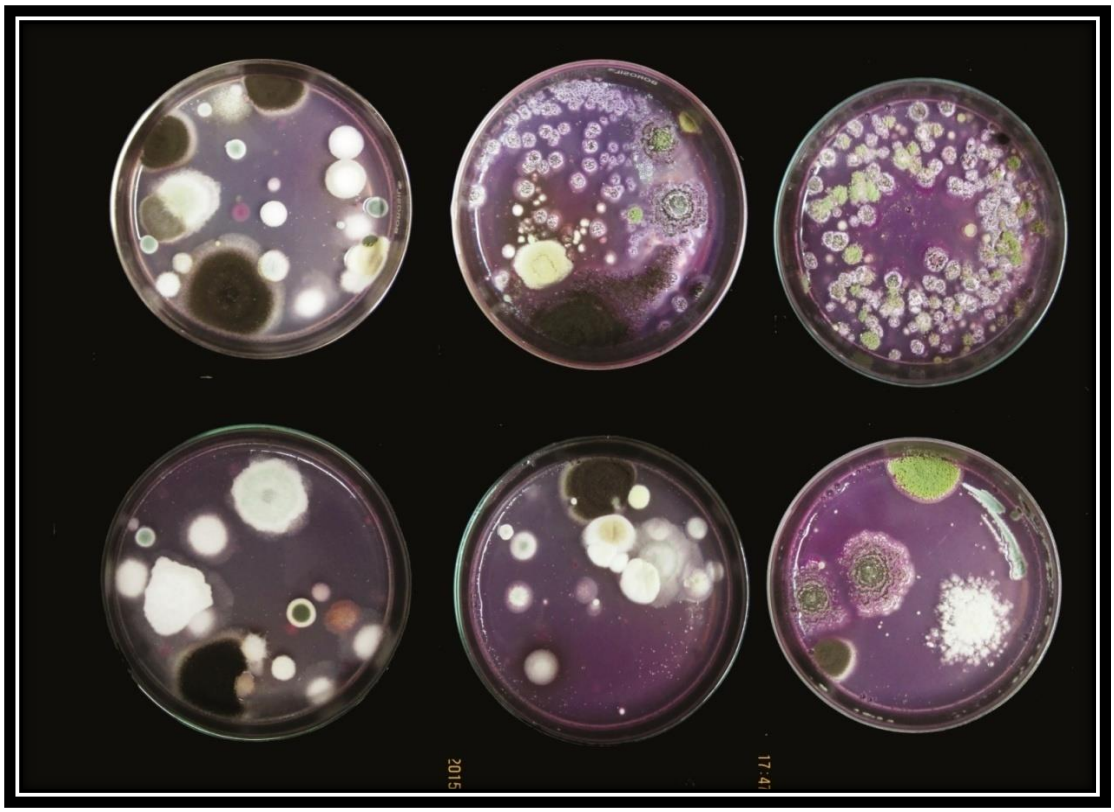
(A) Rough Mexican clover

(B) Tropical spiderwort

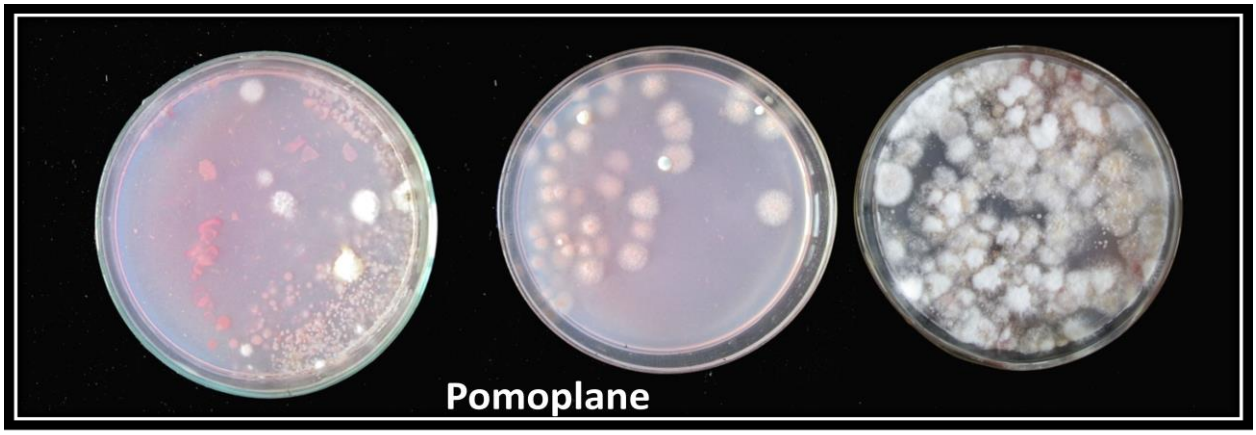
Plate 19. Symptoms produced by *C.capsici* on weeds under natural conditions

Table 20. List of predominant fungi and bacteria obtained from rhizosphere, phyllosphere and pomoplane of chilli plants

Source	Fungi		Bacteria	
	Predominant genera	No. of isolates	Predominant genera	No. of isolates
Phyllosphere	*Aspergillus*	2	*Bacillus*	1
	Penicillium	3		
	Cercospora	1		
Rhizosphere	*Trichoderma*	2	*Pseudomonas*	1
	Aspergillus	5	*Bacillus*	1
	Penicillium	4		
Pomoplane	*Aspergillus*	2	*Bacillus*	1
	Penicillium	3		



(A) Rhizosphere fungi



(B) Pomoplane fungi

Plate 20. Mycoflora isolated by serial dilution technique

(D) Phyllosphere fungi

Plate 20. Mycoflora isolated by serial dilution technique contd.

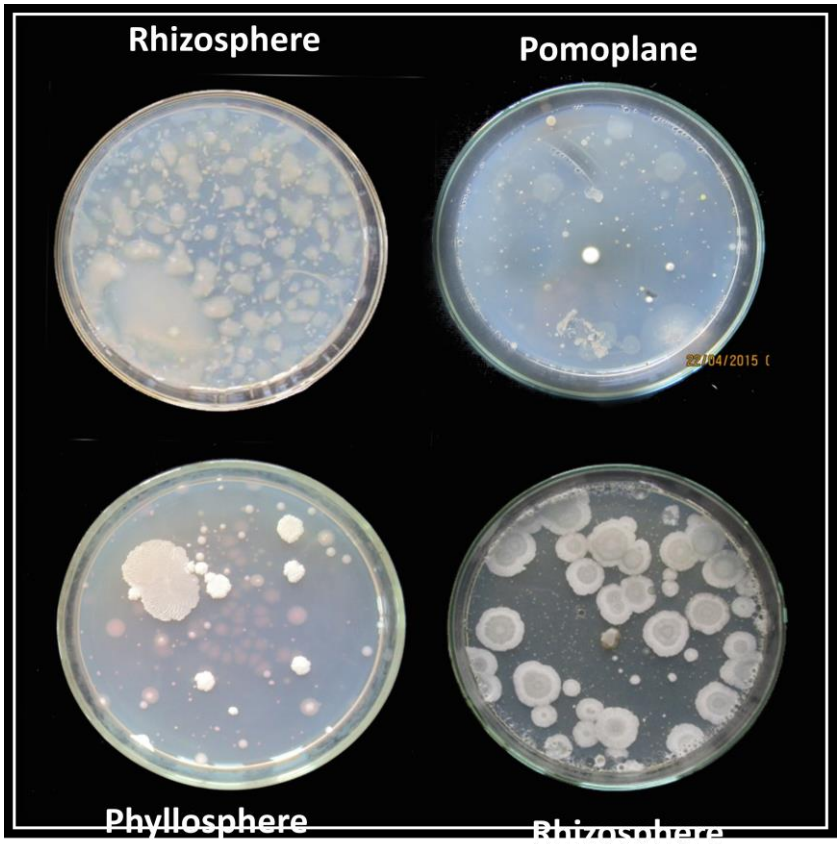


Plate 21. Bacteria isolated by serial dilution technique from rhizosphere, pomoplane and phyllosphere

4.8.1. Isolation of Saprophytes from Phyllosphere and Pomoplane Region of Chilli Plant

Fungi belonging to three different genera and a bacterium were isolated from the phyllosphere and pomoplane region of the disease-free chilli plants among the fruit rot infected chilli plants in the field.

4.8.2. Isolation of Saprophytes from Rhizosphere Region of Chilli Plant

Six fungi and two bacteria were isolated from the rhizosphere of the disease-free chilli plants among the fruit rot infected chilli plants in the field. The efficacy of these was tested using dual culture technique.

4.9. *IN VITRO* SCREENING OF THE FUNGAL ISOLATES AGAINST *C. CAPSICI*

In the primary evaluation, *Penicillium* sp. and *Trichoderma* sp. showed antagonistic action against *C.capsici*. For further confirmation these two fungal isolates were screened again by dual culture technique on PDA (Table 21). Based on the colony and conidial characters, the isolate of *Trichoderma* was identified to be *Trichoderma viride* and *Penicillium* sp. as *Penicillium citrinum. P.citrinum* gave an inhibition of 54.73 per cent (Plate 22) of the mycelial growth of the pathogen, whereas *T.viride* showed a radial mycelial inhibition of 68.40 per cent (Plate 23) with overgrowth against *C.capsici*. Hence, *P.citrinum* and *T.viride* were selected for evaluating the efficiency of disease suppression under pot culture and were morphologically characterised for species level identification.

4.10. IDENTIFICATION AND MORPHOLOGICAL CHARACTERIZATION OF SELECTED FUNGAL ANTAGONISTS

The *Trichoderma* sp. was identified as *T.viride* based on the cultural (Plate 24A) morphological studies (Plate 24B) of the fungus. The fungus was fast growing, initially in the culture the colony appeared white in colour which later turns to green mycelium. The conidiophores were sub-hyaline, measured 30-300 µm in length and 2.5-4.7 µm in diam. and the phialides were 17.58 µm length

Table 21. *In vitro* antagonism of saprophytic fungi against *C. capsici*

Fungal antagonists	Per cent mycelial inhibition of *C. capsici*
Penicillium citrinum	54.73 (47.72)[b]
Trichoderma viride	68.40 (55.79)[a]
Aspergillus sp.	43.04 (41.00)[c]
Control	0.00 (0.00)[d]
CD (0.05)	0.69

Table 22. *In vitro* antagonism of saprophytic bacteria against *Colletotrichum capsici*

Bacterial antagonists	Per cent mycelial inhibition of *C. capsici*
Pseudomonas fluorescens	73.33
Bacillus	88.88

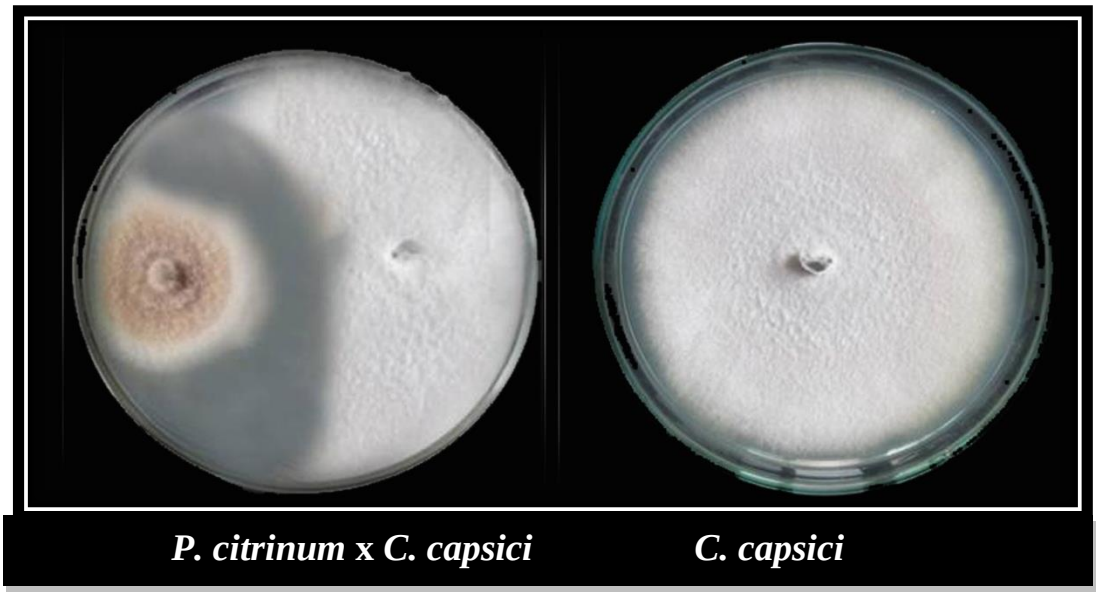


Plate 22. ***In vitro*** **suppression of mycelial growth of** ***C. capsici*** **by** ***P. citrinum***

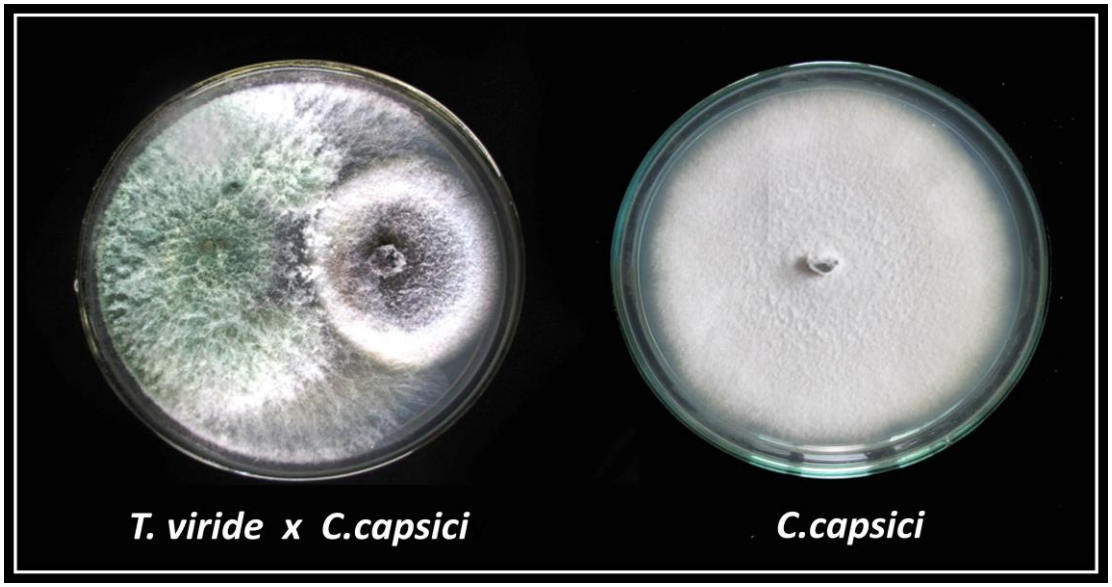


Plate 23. ***In vitro*** **suppression of mycelial growth of** ***C. capsici*** **by** ***Trichoderma viride***

(A) Colony of *Trichoderma viride*

(B) Trichoderma viride- Conidiophore (400X)

Plate 24. ***Trichoderma viride***

(A) Colony of *Penicillium citrinum*

(C) Penicillium citrinum - Conidiophore (400X)

Plate 25. ***Penicillium citrinum***

The *Penicillium* sp. was identified as *P. citrinum* based on the morphological and conidial characters (Plate 25 A and 25 B). The fungi had a yellow to brown colony; conidiophores terminating in verticl of metulae, phialides were ampulliform and produced spheroidal, smooth conidia, 2.5-3.4 µm. The conidiophores arise separately from submerged hyphae were sub-hyaline, measured 150 µm in length and metulae 16-30 x 3 µm and the phialides were 17.58 µm length which were in confirmation with the descriptions of *P. citrinum* by Gilman (1957).

4.11. *IN VITRO* SCREENING OF THE BACTERIAL ISOLATES AGAINST *C. CAPSICI* IN DUAL CULTURE TECHNIQUE.

Bacillus sp. inhibited the growth of *C. capsici* by 88.80 per cent (Plate 26) whereas the other gram negative bacteria *P. fluorescens* provided 73.33 per cent growth inhibition of the pathogen (Table 22) (Plate 27).

4.11.1. Identification of Bacterial Isolates

Two isolates of bacteria obtained were named as isolate 1, isolate 2 (Plate 28 A-28 B). The gram staining technique results revealed that isolate one was gram positive while isolate two was gram negative.

The two bacterial isolates were then transferred to nutrient agar media and found that both the bacteria grow rapidly in nutrient agar medium. When streaked on King's B medium, the bacterial isolate two grew rapidly and the colonies were fluorescent when observed under UV Transilluminator GeNei ^{TM} (Plate 28 C). It was identified as *P. fluorescens* based on the biochemical analysis. The colonies of bacterial isolate one showed an arborescent growth pattern on the nutrient agar medium, and smear prepared from 48 h old colonies revealed endospores when examined with oil immersion objective (100 X) of compound microscope after endospore staining with malachite green. Hence, the isolate one was identified as *Bacillus* sp.

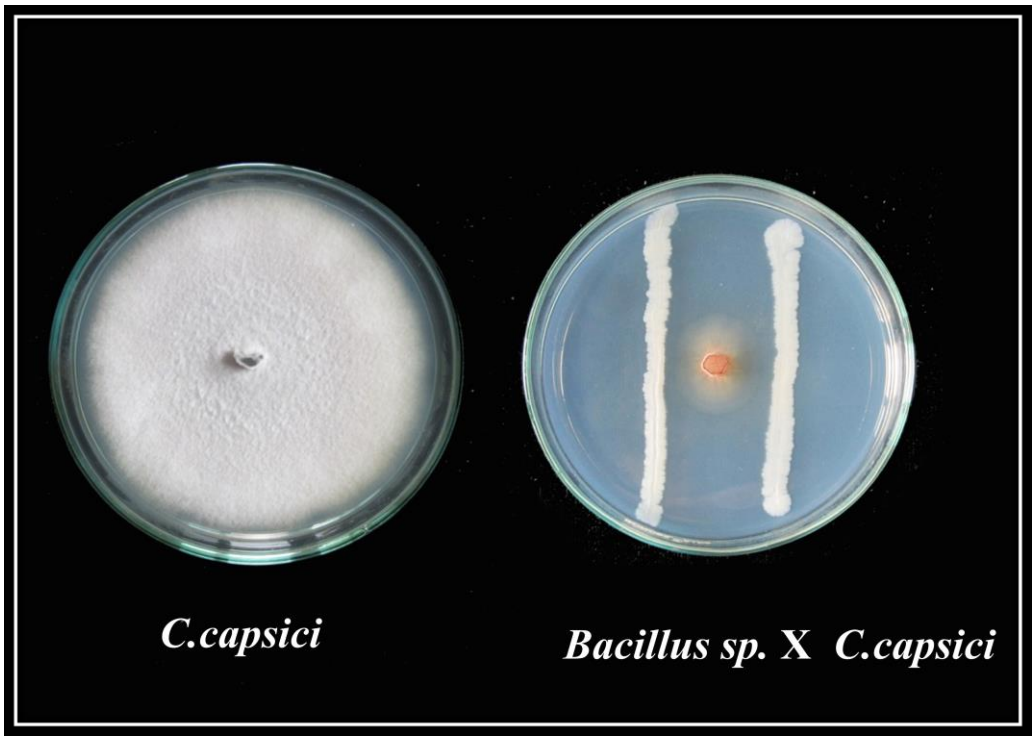


Plate 26. ***In vitro*** **suppression of mycelial growth of** ***C. capsici*** **by** ***Bacillus*** **sp.**

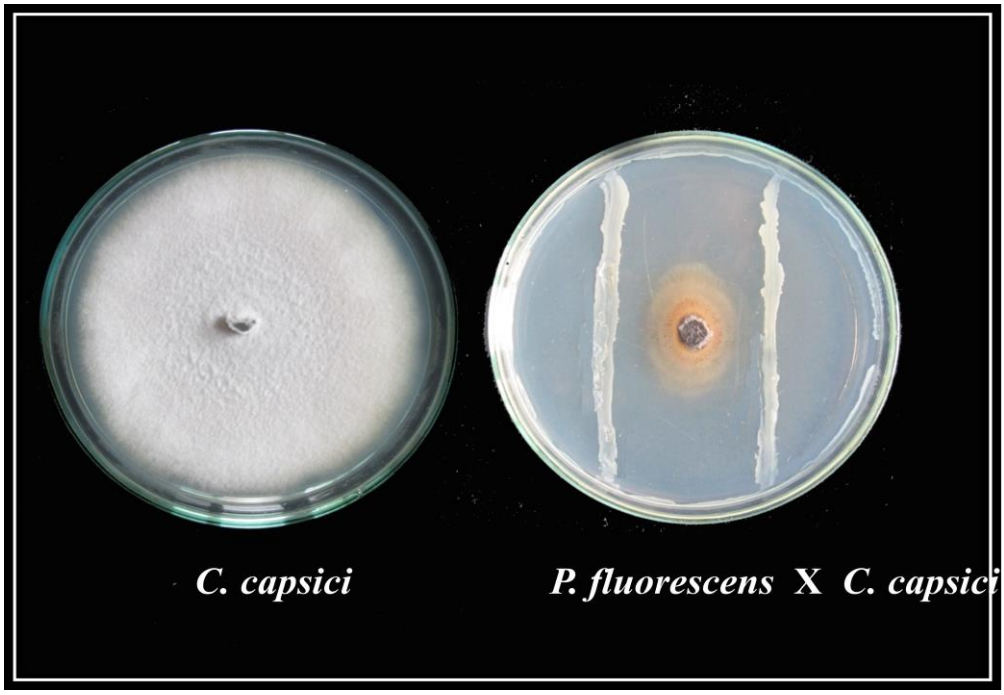


Plate 27. ***In vitro*** **suppression of mycelial growth of** ***C. capsici*** **by P. fluorescens**

4.12. *IN VITRO* EVALUATION OF CHEMICAL FUNGICIDES AGAINST *C. CAPSICI*

The *in vitro* inhibition of *C. capsici* by fungicides was done by using poisoned food technique in PDA. Seven commercially available fungicides were used for the *in vitro* study (Table 23) (Plate 29 A-29 F).

In vitro assay of fungicides with poisoned food technique revealed that at the recommended dose, propiconazole (0.1 per cent), difenoconazole (0.05 per cent) and mancozeb (0.2 per cent) completely inhibited (100 per cent) the mycelial growth of *C. capsici* and was significantly better than all the other fungicides. This was followed by azoxystrobin (0.1 per cent) where there was 97.53 per cent inhibition to the growth of the pathogen. The fungicide captan+hexaconazole at 0.1 per cent recorded 85.10 per cent inhibition. This was followed by carbendazim (0.05 per cent) which recorded mycelial inhibition of 88.51 per cent. The least inhibition was recorded by chlorothalonil at 0.1 per cent.

At the lower dose than recommended field concentration, propiconazole (0.05 per cent), difenoconazole (0.01 per cent) provided cent per cent inhibition to the growth of the pathogen. The next best fungicide which showed good inhibition (88.74 per cent) to the mycelial growth of the pathogen was mancozeb at 0.1 per cent. This was followed by carbendazim (0.05 per cent) which recorded 51.24 per cent suppression to the growth of *C. capsici*. The fungicide captan+hexaconazole at 0.05 per cent recorded 45.53 per cent and azoxystrobin (0.05 per cent) exhibited 5.44 per cent mycelial inhibition of mycelial growth of the pathogen. The least inhibition was recorded by chlorothalonil at 0.1 per cent.

At the higher dose than recommended field concentration, propiconazole (0.15 per cent), difenoconazole (0.1 per cent), mancozeb (0.3 per cent) and azoxystrobin (0.15 per cent) completely inhibited (100 per cent) the mycelial growth of *C. capsici* and was significantly better than all other fungicides. This was followed by carbendazim (0.1 per cent) which recorded 97.52 per cent suppression to the

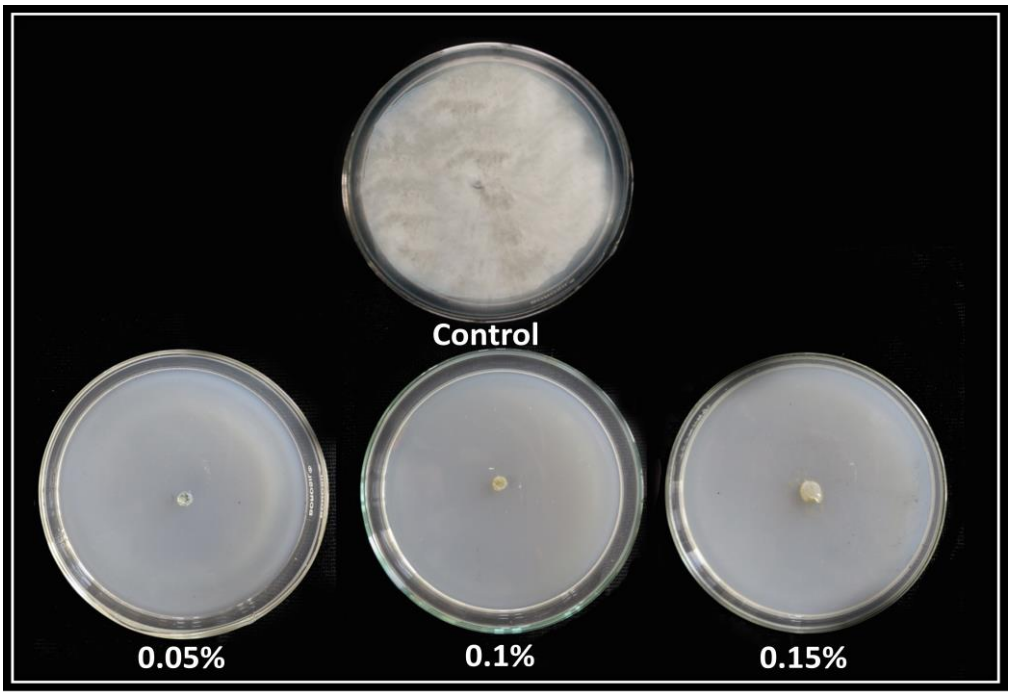
Table 23. *In vitro* evaluation of fungicides against *C. capsici*

Treatment	Fungicide	Percentage mycelial inhibition of *C. capsici* (cm)*		
		Lower dose	Field dose	Higher dose
T1	Propiconazole	100 (90.00)[a]	100 (90.00)[a]	100 (90.00)[a]
T2	Difenoconazole	100 (90.00)[a]	100 (90.00)[a]	100 (90.00)[a]
T3	Chlorothalonil	0.00 (0.00)[f]	0.00 (0.00)[e]	0.00 (0.00)[d]
T4	Azoxystrobin	5.44 (13.49)[e]	97.53 (80.97)[b]	100 (90.00)[a]
T5	Captan + hexaconazole	45.53 (42.44)[d]	88.51 (70.19)[c]	78.58 (93.07)[c]
T6	Carbendazim	51.24 (45.71)[c]	86.92 (68.80)[d]	97.52 (96.28)[b]
T7	Mancozeb	88.74 (70.39)[b]	100 (90.00)[a]	100 (90.00)[a]
Control	Control	0.00 (0.00)[f]	0.00 (0.00)[e]	0.00 (0.00)[d]
	CD (0.05)	0.13	1.35	0.28

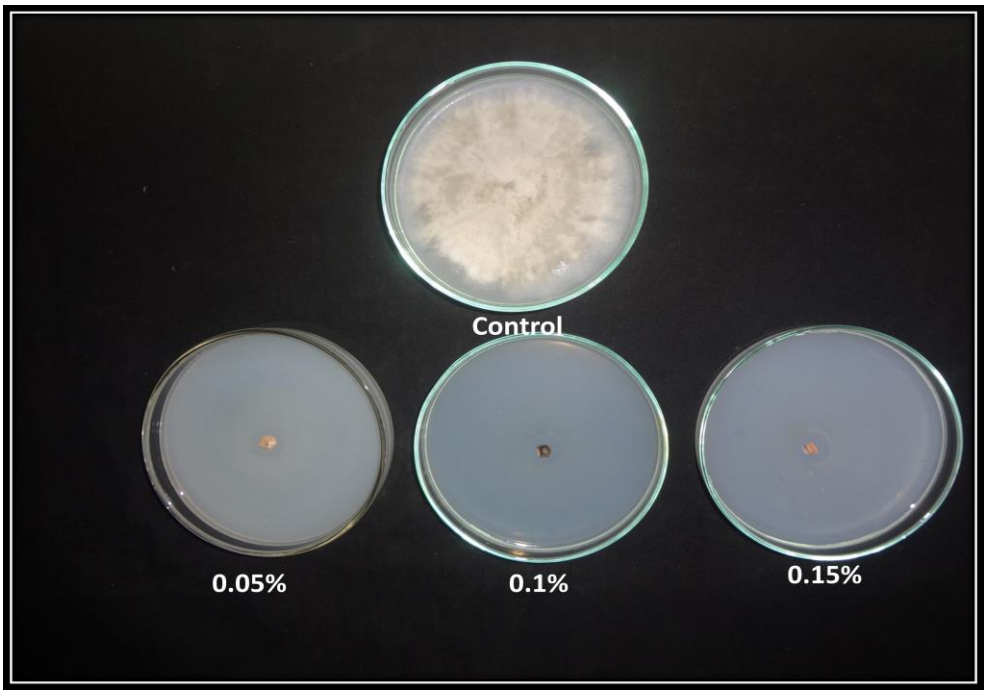
Values in parenthesis are arcsine transformed

*Mean of four replications

**Figures followed by same letter do not differ significantly according to one way ANOVA at P=0.05

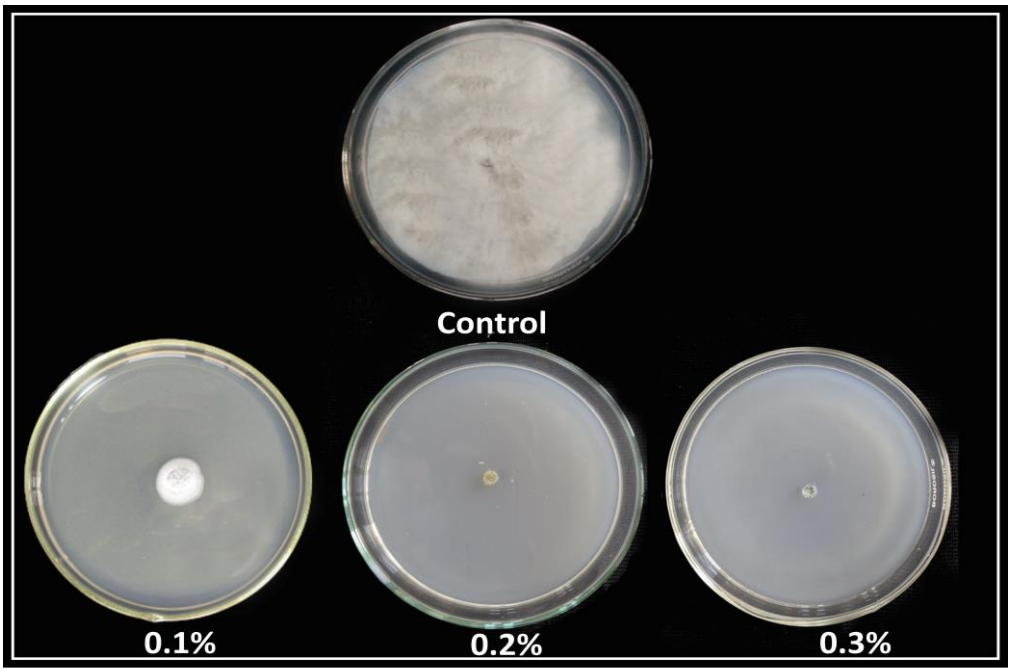


(A) Effect of different levels of difenoconazole on radial growth of *C. capsici*

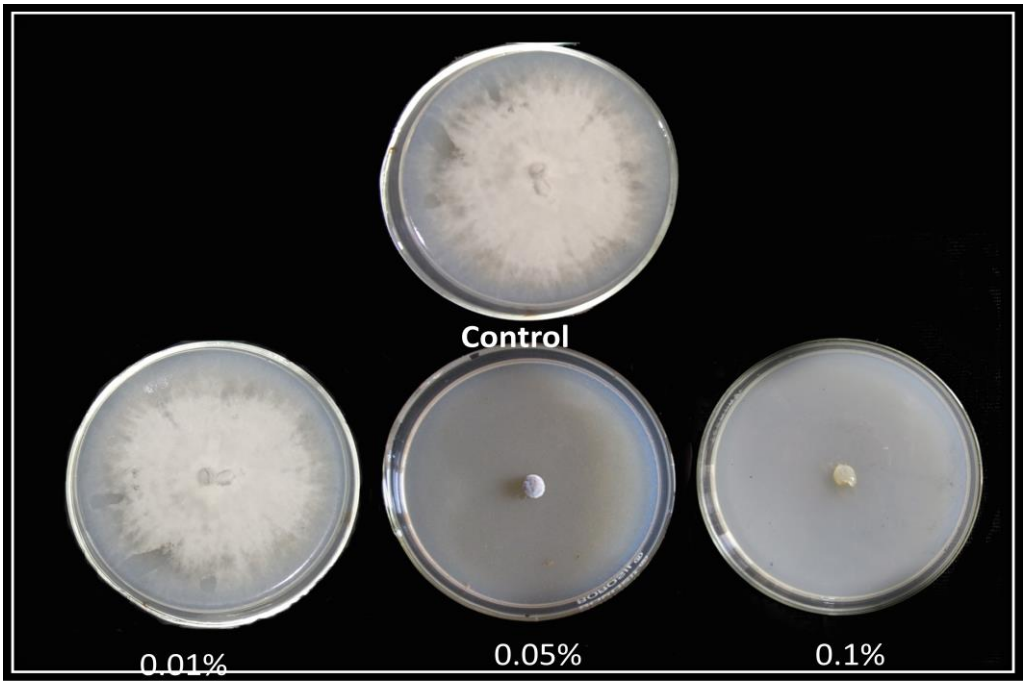


(B) Effect of different levels of propiconazole on radial growth of *C. capsici*

Plate 29. Effect of different fungicides on radial growth of *C. capsici*

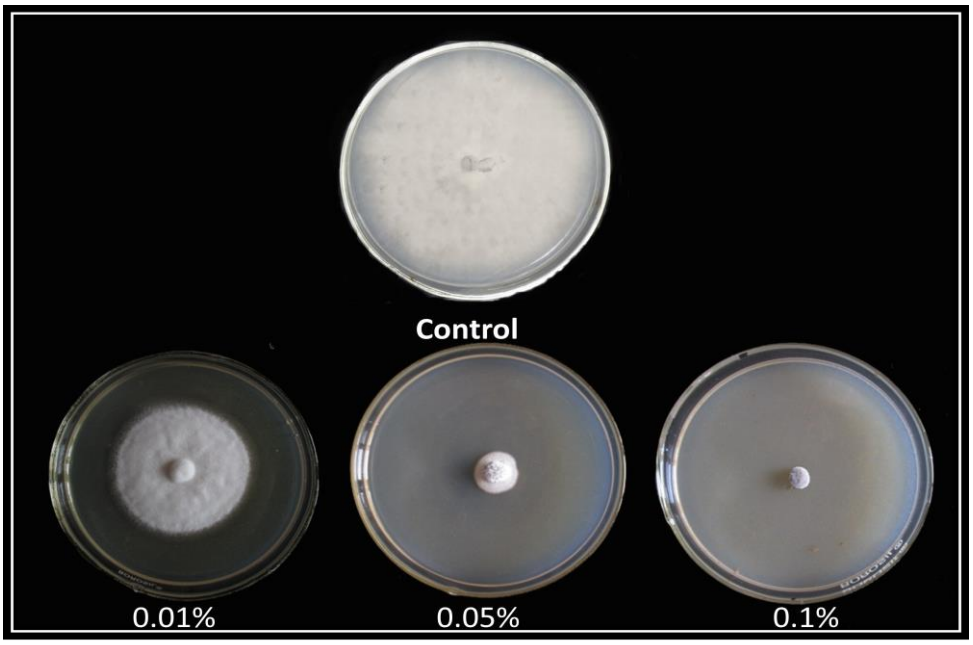


(C) Effect of different levels of mancozeb on radial growth of *C. capsici*

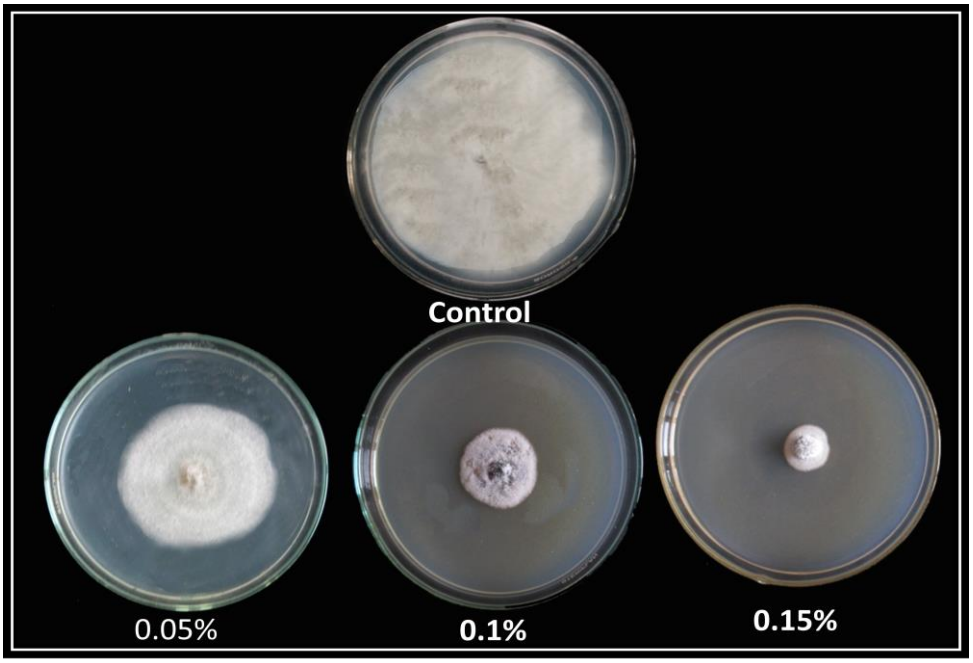


(D) Effect of different levels of azoxystrobin on radial growth of *C. capsici*

Plate 29. Effect of different fungicides on radial growth of *C. capsici* contd.

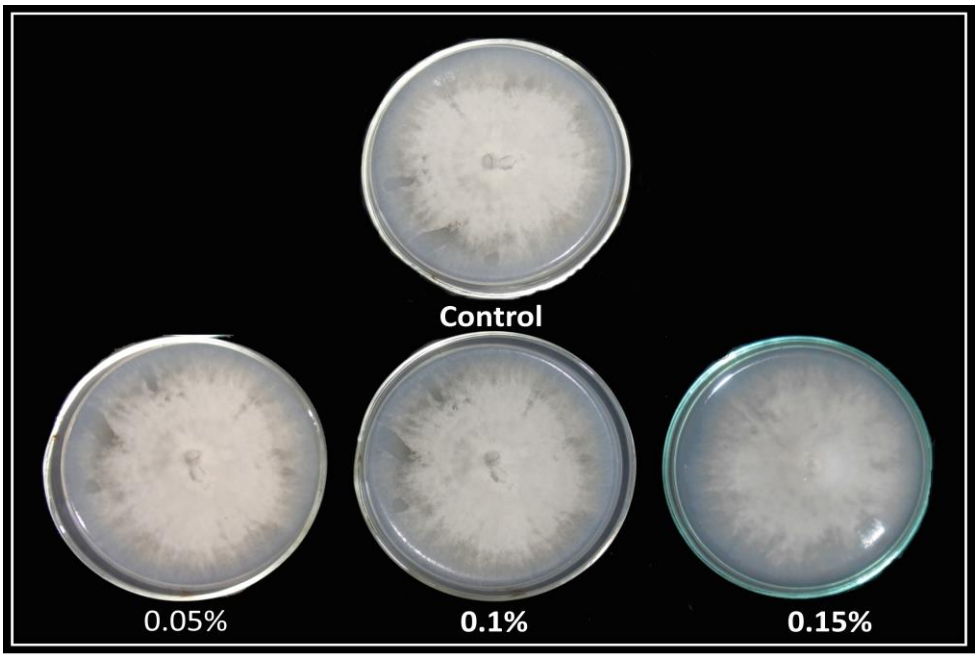


(E) Effect of different levels of carbendazim on radial growth of *C. capsici*



(F) Effect of different levels of captan+ hexaconazole on radial growth of *C. capsici*

Plate 29. Effect of different fungicides on radial growth of *C. capsici* contd.



(G) Effect of different levels of Chlorothalonil on radial growth of *C. capsici*

Plate 29. Effect of different fungicides on radial growth of *C. capsici* contd.

growth of *C. capsici*. The fungicide combination, captan+hexaconazole at 0.15 per cent recorded 78.58 per cent. The least inhibition was showed by chlorothalonil at 0.1 per cent.

4.13 *IN VITRO* EVALUATION OF ORGANIC PREPARATIONS AGAINST *C. CAPSICI*

The *in vitro* assay of organic preparations i.e. pachagavya, jeevamruth and fish amino acid against *C. capsici* was done by using poisoned food technique in PDA (Plate 30 A and 30 B) (Table 24).

The result of the experiment showed that, the recommended dose of panchagavya (2.5 per cent) gave complete inhibition (100 per cent) of the growth of the pathogen was significantly better than all other organic preparations. This was followed by jeevamruth (2.5 per cent) which provided 36.40 per cent inhibition of the mycelial growth. The least inhibition was recorded by fish amino acid at 2.5 per cent (10.60 per cent).

A similar trend was followed at 5 per cent concentration of all the organic preparations tested. Panchagavya showed complete inhibition (100 per cent) to radial mycelial growth of *C. capsici.* The next best organic preparation was jeevamruth (75.45 per cent) which showed better inhibition of the mycelial growth of the pathogen. The least inhibition was recorded by fish amino acid (11.13 per cent).

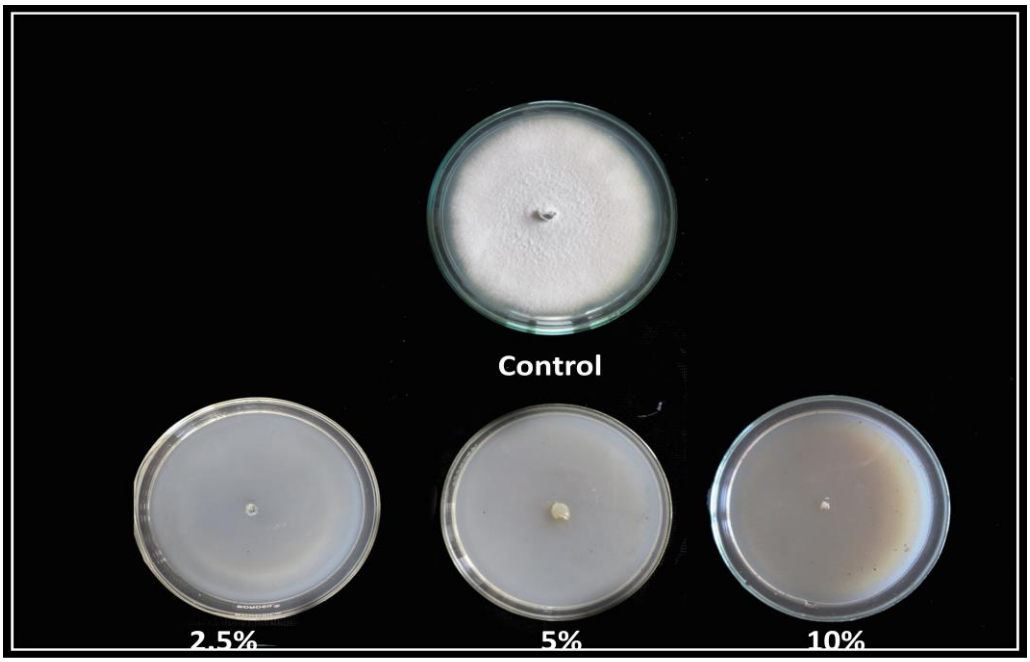
At 10 per cent concentration of all the organic preparations tested, the highest inhibition of the radial growth of the pathogen was provided by panchagavya (100 per cent) and was followed by jeevamruth (96.39 per cent). The least inhibition was recorded by fish amino acid (15.84 per cent).

Table 24. *In vitro* evaluation of organic preparations against *C. capsici*

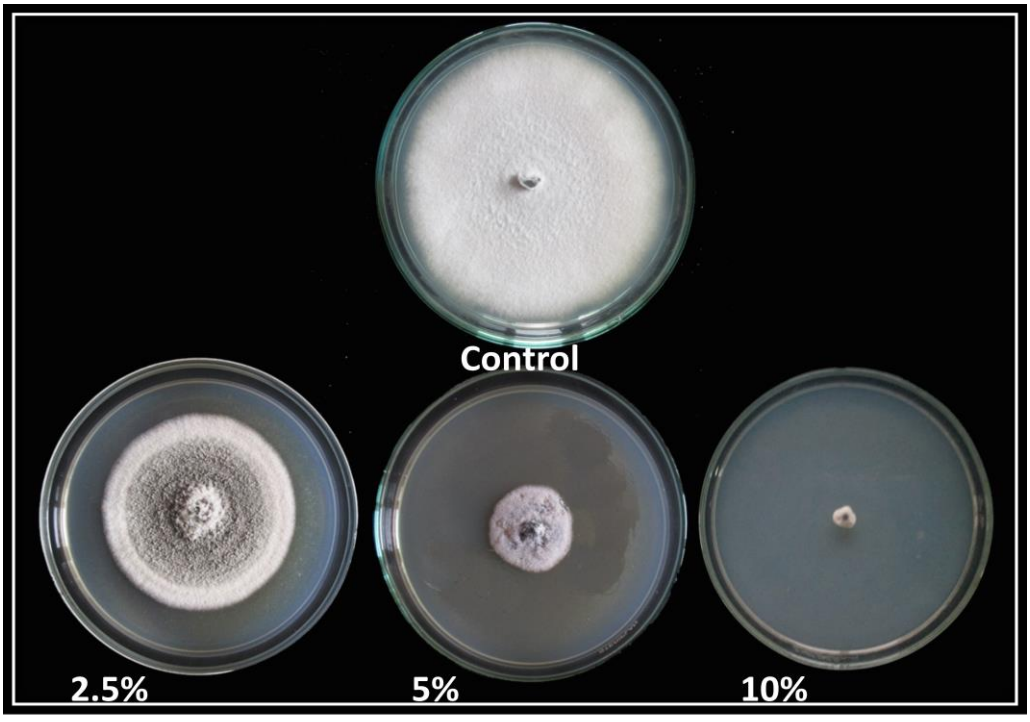
Organic preparations	Per cent mycelial inhibition *		
	2.5%	5%	10%
Panchagavya	100.00 (90.00)[a]	100.00 (90.00)[a]	100.00(90.00)[a]
Fish amino acid	10.60 (3.38)[c]	11.13 (19.50)[c]	15.84 (23.45)[c]
Jeevamruth	36.40 (35.22)[b]	75.45 (60.30)[b]	96.39 (79.05)[b]
CD (0.05)	0.16	1.37	0.73

Values in parenthesis are arcsine transformed

*Mean of four replications



(A) Effect of panchagavya on radial growth of *C. capsici*



(B) Effect of jeevamruth on radial growth of *C. capsici*

Plate 30. Effect of different organic preparations on radial growth of *C. capsici*

4.10. *IN VITRO* EVALUATION OF DIFFERENT AGENTS FOR THE CONTROL OF DISEASE ON SEEDLINGS.

The two fungal and bacterial antagonists obtained during the study, *viz Penicillium citrinum* and *T. viride*, the fungal endophyte *P. indica*, *P. fluorescens* and *Bacillus* sp. were evaluated for the disease suppression at seedling stage along with the KAU released biocontrol agents *P. fluorescens* and *T. harzianum* and seven commercially available fungicides such as propiconazole, difenoconazole, mancozeb, carbendazim, captan + hexaconazole, azoxystrobin and chlorothalonil.

The per cent seedling mortality due to *C. capsici* was lowest for the seeds treated with the isolated bacterium *Bacillus* sp. (10.46 per cent) followed by KAU released talc based biocontrol agent *P. fluorescens* (15.36 per cent) and was found to be the best for suppressing the seed borne pathogen *C. capsici*. The seeds treated with *T. viride* showed 20.46 per cent seedling mortality. The KAU released biocontrol agents *P. fluorescens* and *T. harzianum* recorded 25.23 per cent and 25.59 per cent seedling mortality, respectively and were also on par. The highest seedling mortality (45.36 per cent) was showed by *P. indica* treated seeds (Table 25).

The results of the seedling assay with bio agents revealed that the bio agents had significant effect on seedling vigour index over untreated. The seedling vigour index of seeds treated with *P. fluorescens* (10370.17) was highest which was on par with the bio agents; *T. viride* (10000), KAU released talc based biocontrol agent *P. fluorescens* (8625.23), *P. citrinum* (8437.33), KAU talc based formulation *of Trichoderma* (8250.29). This was followed by *Bacillus* treated seedlings with a vigour index of 8064. *Piriformospora indica* treated seedlings had lowest seedling vigour index among bio agent treated seeds (5885.47) (Plate 31 A).

The results of the seedling assay with fungicides revealed that seeds treated with propiconazole (0.1 per cent) exhibited lowest percentage seedling mortality (10.37 per cent) followed by difenoconazole at 0.1 per cent (12.44 per cent). The

Table 25. Seedling assay of different bio agents for the control of the infection on seedlings

Treatment	Bio agents	Percentage mortality	Seedling vigour index
T1	*Penicillium citrinum*	32.16 (34.55)^{c}	8437.33^{ab}
T2	*Trichoderma viride*	20.46 (26.89)^{e}	10000^{ab}
T3	*Bacillus sp.*	10.46 (18.87)^{g}	8064.47^{b}
T4	*Pseudomonas fluorescens*	15.36 (23.07)^{f}	10370.17^{a}
T5	*Piriformospora indica*	45.36 (42.34)^{b}	5885.47^{c}
T6	KAU talc based formulation of *Pseudomonas fluorescens* (2%)	25.23 (30.15)^{d}	8625.23^{ab}
T7	KAU talc based formulation of *Trichoderma* (2%)	25.59 (30.39)^{d}	8250.29^{ab}
T8	Control	55.46 (48.13)^{a}	3870.49^{c}
	CD (0.05)	0.43	3016.90

*Mean of four replications

Values in parenthesis are arcsine transformed

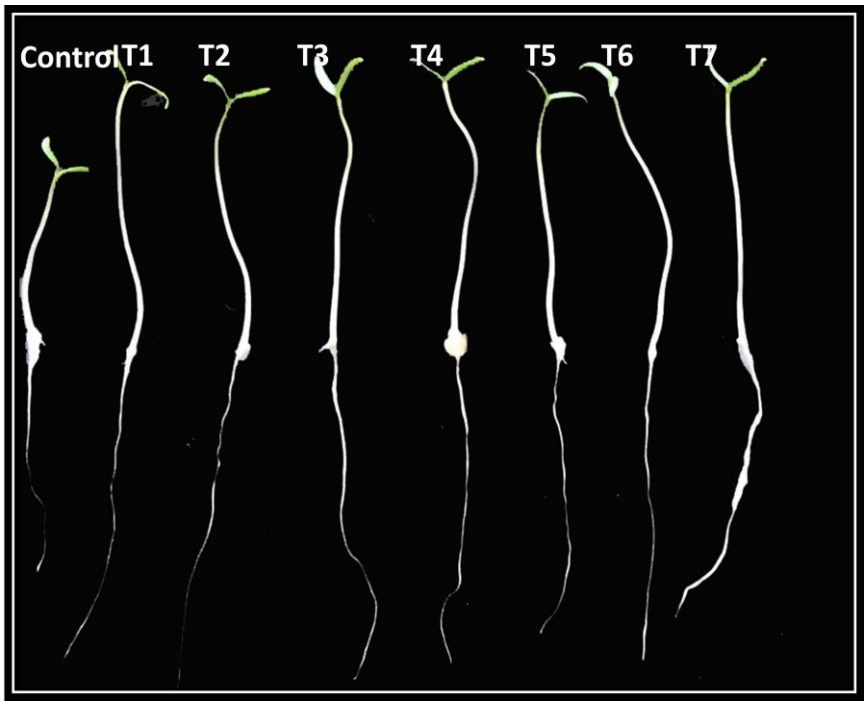


Plate 31 (A). Effect of different bioagents on growth of *C. capsici* inoculated

Plate 31 (B) . Effect of different fungicides on growth of *C. capsici* inoculated

seeds treated with carbendazim (0.05 per cent) showed 15.49 per cent seedling mortality. The next best fungicide which provided lowest percentage seedling mortality (16.57) was azoxystrobin at 0.1 per cent. The fungicide captan + hexaconazole at 0.1 per cent recorded 19.13 per cent mortality. The seedlings treated with mancozeb (0.2 per cent) exhibited 32.62 per cent mortality. The highest seedling mortality (45.49 per cent) was showed by chlorothalonil (0.1 per cent) (Table 26).

The seedling assay with fungicides showed that seedling vigour index of seeds treated with propiconazole (0.1 per cent) was the highest (13660). This was followed by difenoconazole at 0.1 per cent and azoxystrobin at 0.1 per cent which provided seedling vigour index of 11834 and 10450 and were significantly different. The fungicide carbendazim (0.05 per cent) provided 8245.46 vigour to the treated seedlings. This was followed by captan + hexaconazole (0.1 per cent) and mancozeb (0.2 per cent) and was significantly superior over the untreated seedlings (3870). Among the fungicides, the seeds treated with chlorothalonil (0.1 per cent) had the seedlings with lowest vigour (4840) (Plate 31 B).

4. 17. *IN VIVO* MANAGEMENT OF FRUIT ROT OF CHILLI CAUSED BY *C. CAPSICI*

The effect of selected antagonists, organic preparations and chemicals on the suppression of the fruit rot of chilli was studied by pot culture experiment (Plate 32) with 15 treatments. The treatments were applied as foliar spray on the plants at fortnightly intervals after creating disease pressure by artificial inoculation. The observations were taken at weekly intervals for three weeks. The per cent disease incidence, per cent disease index and percentage of suppression of disease were calculated based on the standard procedures.

Table 26. Seedling assay of different fungicides for the control of the infection on seedlings

Treatment	Chemical fungicide	Dose (%)	Per cent mortality	Seedling vigour index
T1	Difenoconazole	0.05	12.44 (20.65)^{g}	11834^{b}
T2	Propiconazole	0.1	10.37 (18.78)^{h}	13660^{a}
T3	Azoxystrobin	0.1	16.57 (24.02)^{e}	10450^{c}
T4	Chlorothalonil	0.1	45.49 (42.41)^{b}	4840^{d}
T5	Mancozeb	0.2	32.62 (34.83)^{c}	6473.6^{f}
T6	Carbendazim	0.05	15.49 (23.18)^{f}	8245.46^{d}
T7	Captan +Hexaconazole	0.1	19.13 (25.93)^{d}	7777.4^{e}
T8	Control		55.46 (48.13)^{a}	3870^{h}
CD (0.05)			0.53	0.58

*Mean of four replications

Values in parenthesis are arcsine transformed

Plate 32. General view of pot culture experiment

4. 17.1. Preparation of Pathogen Inoculum and Foliar Application

Conidial suspension of *C. capsici* containing 10^6 conidia ml^{-1} prepared in 0.5 per cent peptone water was sprayed on chilli plants 20 days after fruit formation. The promising treatments selected from the *in vitro* seedling assay including two organic preparations, panchagavya and jeevamruth; two talc based formulation of biocontrol agents, *P. fluorescens* and *Trichoderma* sp. released by KAU, isolated biocontrol agents ie., two bacteria and *Penicillium sp*. and six commercially available fungicides including propiconazole, difenoconazole, mancozeb, carbendazim, captan + hexaconazole, azoxystrobin, were tested under pot culture. All the treatments were repeated at fortnightly intervals.

4. 17.2. Disease Incidence

The per cent disease incidence (Table 27) was assessed based on the number of fruits infected by fruit rot pathogen and calculated by the formula followed by. Data on fruit rot incidence is given in table 15. There was minimum incidence (16.31 per cent) of fruit rot disease in the chilli plants treated with propiconazole (0.1 per cent) followed by difenoconazole (0.05 per cent) with an incidence of 19.64 per cent. This was on par with incidence level exhibited by biocontrol agent *Pseudomonas fluorescens* (21.32 per cent). The biocotrol agent *Bacillus* and the fungicide azoxystrobin (0.1 per cent) also showed reduced disease incidence to the tune of 24. 17 and 25.98 respectively. The effects of these were better than that of the fungicidal check; mancozeb and biocontrol checks; talc based formulation of *Pseudomonas fluorescens* and *Trichoderma* sp.

Among the organic preparations foliar spraying of panchagavya at 2.5 per cent caused reduction in disease incidence. The disease incidence in this treatment was only 31.76 per cent which was more than that of fungicidal check.

Table 27. Effect of different treatments on incidence of fruit rot in chilli

	Treatments	Per cent disease incidence
	Biocontrol agents	
T1	*Bacillus* sp.	24.14 (29.43)^{f}
T2	*Penicillium citrinum*	44.73 (41.98)^{b}
T3	*Trichoderma viride*	38.50 (38.35)^{c}
T4	*Pseudomonas fluorescens*	21.32 (27.50)^{g}
Organic preparations		
T5	Panchagavya	31.76 (34.30)^{d}
T6	Jeevamruth	43.66 (41.36)^{b}
	Chemical fungicides	
T7	Difenoconazole	19.64 (26.31)^{g}
T8	Propiconazole	16.31 (23.82)^{h}
T9	Azoxystrobin	25.98 (30.65)^{f}
T10	Captan +Hexaconazole	36.99 (37.46)^{c}
T11	Carbendazim	31.50(34.14)^{d}
T12	Talc based formulation of *Pseudomonas fluorescens*	38.17(38.15)^{c}
T13	Talc based formulation of *Trichoderma* sp.	28.66(32.37)^{e}
T14	Mancozeb	38.83(38.55)^{c}
T15	Inoculated control	67.00(54.94)^{a}
	CD (0.05)	1.49

*Mean of four replications

Values in parenthesis are arcsine transformed

However, the *Trichoderma viride* isolate recorded disease incidence of 38.50 per cent which was on par with the effect of positive check; talc based formulation of *Pseudomonas fluorescens* (38.17 per cent).

Application of carbendazim (0.05 per cent) and captan +hexaconazole (0.1 per cent) reduced the incidence of fruit rot in chilli which recorded 31.5 per cent and 36.99 per cent incidence where the effect of combinational fungicide on disease incidence was on par with positive check; talc based formulation of *P. fluorescens* (38.17 per cent) and fungicidal check; mancozeb (38.83 per cent).

Foliar spraying of *P. citrinum* (10^8conidia/ml) and jeevamruth (2.5 per cent) caused reduction in fruit rot incidence as compared to inoculated control (67.00 per cent). Jeevamruth recorded 43.66 per cent wheras *P. citrinum* recorded 44.73 per cent incidence.

4. 17.3 Disease Intensity

The percent disease index was assessed (Plate 33 A-33 D) based on the damage caused by *C. capsici* and calculated by the formula followed by McKinney (1923). Data on percent disease index after first treatment application (three days after inoculation of the pathogen), after second treatment application (second week after inoculation of the pathogen) and after third treatment application (third week after inoculation of the pathogen) are given in table 28.

The per cent disease index after the first treatment application was lowest (26.88) for the chilli plants treated with the fungicide propiconazole (0.1 per cent) which provided maximum disease suppression (32.06 per cent). It was observed that the plants treated with difenoconazole (0.05 per cent) provided 30.80 per cent disease suppression where 27.38 PDI recorded. This was statistically on par with the effect of the isolated bacterium *Bacillus* sp. on fruit rot intensity with 28.75 PDI and provided 27.34 per cent disease suppression. The result also revealed that the organic

Table 28. Effect of different treatments on fruit rot disease in chilli

	Treatments	Per cent disease index			Per cent disease suppression		
		1St week	2ndweek	3rd week	1st week	2ndweek	3rd week
	BIOCONTROL AGENTS						
T1	*Bacillus* sp.	28.75 (32.42)^{h}	34.37 (35.89)^{i}	38.59 (38.41)^{k}	27.34	46.87	59.59
T2	*Penicillium citrinum*	36.61 (37.24)^{c}	39.48 (38.93)^{f}	46.59 (43.04)^{g}	7.44	35.02	51.21
T3	*Trichoderma viride*	33.27 (35.23)^{e}	37.45 (37.73)^{h}	43.30 (41.15)^{i}	15.92	38.36	54.65
T4	*Pseudomonas fluorescens*	35.30 (36.45)^{d}	39.42 (38.89)^{f}	45.58 (42.47)^{h}	11.20	35.12	52.27
	ORGANIC PREPARATIONS						
T5	Panchagavya	31.61 (34.21)^{g}	34.50 (35.97)^{i}	40.47 (39.50)^{j}	20.04	43.21	57.6
T6	Jeevamruth	38.63 (38.43)^{b}	45.83 (42.61)^{b}	53.70 (47.12)^{b}	2.37	24.57	43.76

Table 28 contd.

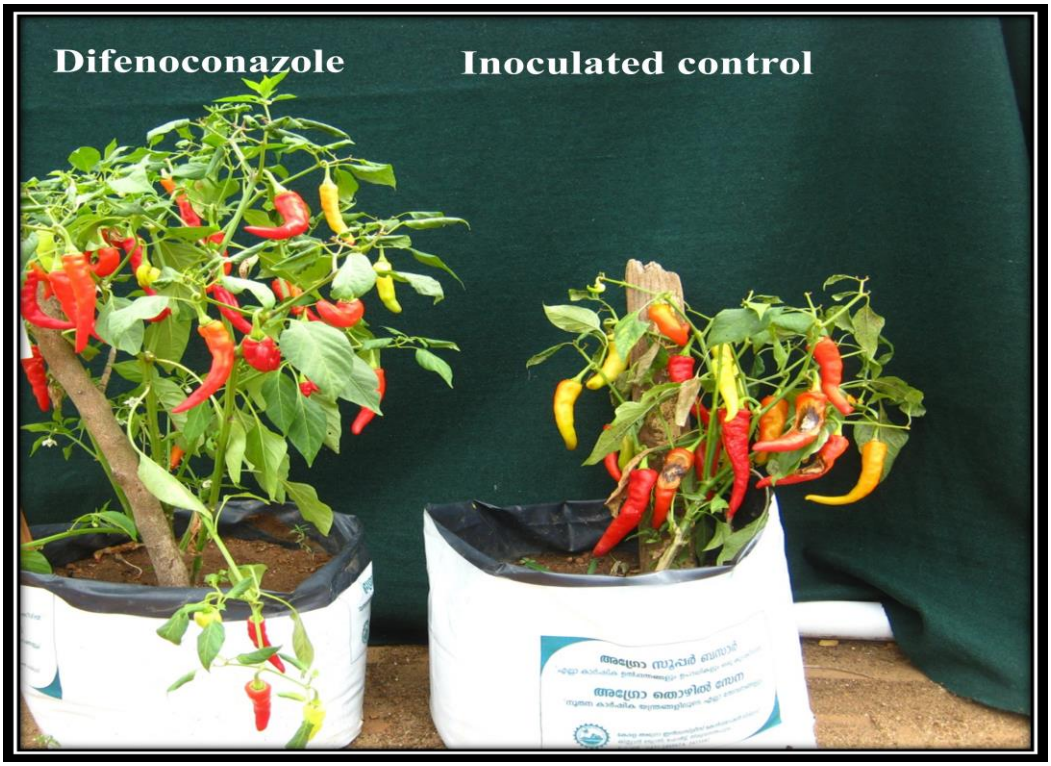
Table 28. Effect of different treatments on fruit rot disease in chilli contd.

	CHEMICAL FUNGICIDES						
T7	Difenoconazole	27.38 (31.55)^{i}	33.70 (35.49)^{j}	36.67 (37.27)^{l}	30.80	44.5	61.60
T8	Propiconazole	26.88 (31.23)^{j}	30.6 (33.64)^{k}	35.47 (36.55)^{l}	32.06	49.6	62.85
T9	Azoxystrobin	32.44 (34.72)^{f}	40.57 (39.56)^{e}	48.70 (44.26)^{e}	18.01	33.22	49.00
T10	Captan +Hexaconazole	35.32 (36.46)^{d}	42.46 (40.67)^{d}	48.10 (43.91)^{f}	10	30.11	49.6
T11	Carbendazim	32.87 (34.98)^{e}	38.57 (38.39)^{g}	45.27 (42.28)^{h}	16.9	36.5	52.29
	POSITIVE CHECKS						
T12	Talc based formulation of *P. fluorescens*	36.36 (37.08)^{c}	42.30 (40.57)^{d}	50.40 (45.23)^{c}	6.29	30.38	47.22
T13	Talc based formulation of *Trichoderma* sp.	38.55 (38.38)^{b}	39.67 (39.04)^{f}	49.53 (44.73)^{d}	2.57	34.71	48.16
T14	Mancozeb	35.46 (36.55)^{d}	43.60 (41.32)^{c}	48.00 (43.85)^{f}	10.38	28.24	49.73
T15	Inoculated control	39.57 (38.98)^{a}	60.76 (45.43)^{a}	95.50 (56.60)^{a}			
CD (0.05)		0.25	0.22	0.28			

*Mean of four replications

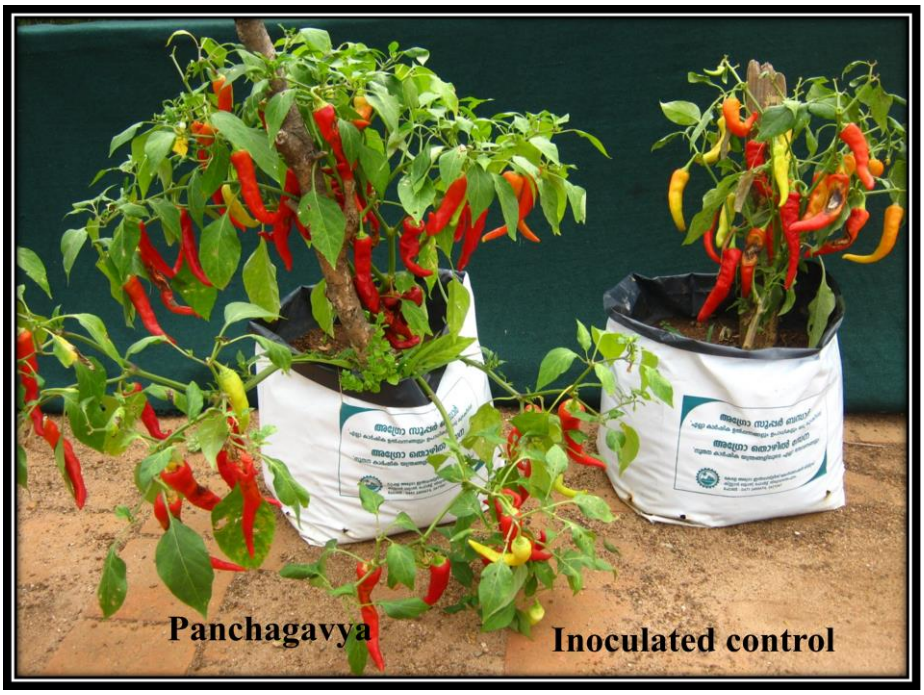
Values in parenthesis are arcsine transformed

(A) Plants treated with Propiconazole



(B) Plants treated with difenoconazole

Plate 33. Effect of different treatments on fruit rot disease suppression on chilli plants



(C) Plants treated with panchagavya

(D) Plants treated with mancozeb

Plate 33. Effect of different treatments on fruit rot disease suppression on chilli plants

preparation, panchagavya provided significant reduction in the disease severity (20.34 per cent) with 31.61 PDI compared to the inoculated control where 39.57 per cent PDI was observed. The foliar application of azoxystrobin (0.1 per cent) recorded 18.01 per cent disease suppression and 32.44 per cent disease intensity were superior than positive checks; KAU talc based formulation of *P. fluorescens*, KAU talc based formulation of *Trichoderma* sp. and mancozeb with 6.29, 2.57 and 10.38 per cent suppression, respectively. Among the different treatments tested, treatment T3 (*T. viride*) with fruit rot index of 33.27 was on par with treatment T11 (carbendazim 0.05 per cent) with PDI 32.87. The effect of combination fungicide; captan+hexaconazole (35.32 per cent) and the isolated bacterium; *P. fluorescens* (35.30 per cent) on disease intensity was on par with the positive check; mancozeb (35.46 per cent). The biocontrol agent *Penicillium citrinum* recorded 36.61 PDI and was on par with KAU talc based formulation of *P. fluorescens* (36.36 per cent). The other treatments including T6 (Jeevamruth 2.5 per cent) with 38.63 PDI and T 13; KAU talc based formulation of *Trichoderma* sp. (T13) 38.55 per cent were statistically on par.

The results of the second foliar spray also revealed that the disease severity was lowest (30.60 per cent) for the chilli plants treated with the fungicide; propiconazole (0.1 per cent) which provided maximum disease suppression (49.60 per cent). It was observed that the plants treated with difenoconazole (0.05 per cent) provided 44.50 per cent disease suppression where 33.70 PDI was recorded. Among the biocontrol agents; *Bacillus* sp. provided maximum disease suppression (46.87 per cent) with 34.37 PDI was on par with the effect of panchagavya on disease severity (34.50 per cent). The foliar application of conidial suspension of *T. viride* recorded 38.36 per cent disease suppression and 37.45 per cent disease intensity were superior to positive checks; KAU talc based formulation of *P. fluorescens*, KAU talc based formulation of *Trichoderma* sp. and mancozeb with 30.38 per cent, 34.71 per cent and 28.24 per cent suppression, respectively. This was followed by the treatment T11 with 38.57 PDI where carbendazim (0.05 per cent) was given as second foliar

spraying. Among the biocontrol agents, *P. citrinum, T. viride* and KAU talc based formulation of *Trichoderma* sp. recorded disease index of 39.48, 39.42 and 39.62 respectively. The treatments were on par and recorded a disease reduction of 35.02, 35.12 and 34.71 respectively. The foliar application of azoxystrobin (0.1 per cent) recorded 40.57 per cent disease suppression and 33.52 per cent disease intensity and was superior to positive checks. Among the different treatments tested, treatment T10 (Captan + Hexaconazole) with fruit rot index of 42.46 per cent was on par with treatment T12 (Talc based formulation of *P. fluorescens*) with 42.30 PDI. It was observed that the plants treated with jeevamruth (2.5 per cent) provided only 24.57 per cent disease suppression where PDI recorded was 45.83.

The effect of different treatments on fruit rot severity was recorded after the third foliar spray. The results showed that the treatments T8 (propiconazole 0.1 per cent) and T7 (difenoconazole 0.05 per cent) recorded the lowest disease index of 35.47 per cent and 36.47 per cent, respectively and were statistically on par. These treatments recorded a percent disease reduction of 62.85 and 61.50 respectively and were significantly better than all other treatments. The treatment T1 also recorded lower disease index of 38.59 per cent and suppressed the disease (59.59 per cent). This was followed by panchagavya (2.5 per cent) with 40.47 PDI and *T. viride* with 43.30 PDI. These were significantly different and were superior over the positive checks; KAU talc based formulation of *P. fluorescens* (50.40 per cent), KAU talc based formulation of *Trichoderma* sp. (49.53 per cent) and mancozeb (48.00 per cent). The treatments T4 and T11, where *P. fluorescens* and carbendazim applied as a third foliar spray recorded the disease index of 45.58 and 45.27 respectively and were on par and the treatments recorded disease reduction of 52.27 and 52.29, per cent respectively. It was observed that the plants treated with *P. citrinum* provided 51.21 per cent disease suppression where PDI recorded was 46.59. Among the different treatments tested, treatment T10 (Captan + Hexaconazole) with fruit rot index of 48.10 per cent was on par with treatment T 17 (Mancozeb 0.2 per cent). It was

observed that the plants treated with azoxystrobin (0.1 per cent) provided only 49.00 per cent disease suppression where PDI recorded was 48.70. The highest disease score (53.70 per cent) and the least disease reduction (43.76 per cent) among different treatments were recorded in the plants treated with jeevamruth.

4. 17.4 Effect of treatments on biometric observations

Treatments involving panchagavya and jeevamruth showed maximum influence on shoot length. The maximum shoot length of 49.98 cm was obtained for panchagavya followed by 45.67 cm in jeevamruth, 43.76 cm in *Trichoderma*, 40 in *Pseudomonas*. Bavistin and mancozeb applied plants recorded a shoot length of 38.67cm whereas azoxystrobin recorded 39.98. Propiconazole recorded shoot length of 40.08cm followed by difenoconazole with 37.89 cm in control plants the shoot length recorded was the least with 29.85 cm.

The maximum root length, 24.99 was obtained for panchagavya followed by Trichoderma ie., 22 cm. The plants treated with jeevamruth recorded 24.26 cm root length.

On comparison of the average fruit yield per plant obtained from each treatments, it was found that the maximum yield was obtained from the treatment panchagavya.

When the average plant fresh weight of each treatment was compared; it was found that maximum plant fresh weight was obtained with treatment panchagavya which was on par with jeevamruth and propiconazole. A similar trend was noticed with respect to average plant dry weight

When the average number of leaves per plant of each treatment was compared; it was found that maximum numbers of leaves were observed with the treatment panchagavya which was significantly superior over all other treatments followed the organic preparation jeevamruth and biocontrol agent trichoderma which was on par with all other treatments (table 29).

Table 29. Effect of different treatments on growth and yield parameters of chilli

Treatment	Fruit yield per plant(g)	No. of fruits per plant	Plant fresh weight (g)	Plant dry weight (g)	Root length (cm)	Shoot length (cm)	No. of leaves per plant
Bacillus sp.	211.5^{h}	21.5	133.1^{cd}	49.39	23.25	35.65	121
Penicillium citrinum	200.98^{i}	20	150^{d}	50	21.5	33.50	124
Trichoderma viride	230^{g}	23.31	127.4^{cd}	44.99	25.75	37.37	130
Pseudomonas fluorescens	201.5^{i}	20.25	109^{ef}	34.91	20.10	35.97	126
Panchagavya	390^{a}	19.87	153.7^{a}	36.05	22.92	40.69	125
Jeevamruth	340^{b}	19.44	136.04^{c}	53.8	17.60	35.45	85
Difenoconazole	338.8^{b}	19.65	111.7^{e}	34.84	16.07	35.71	77.25
Propiconazole	300^{c}	22.45	147.89^{b}	55.67	24.26	43.76	111
Azoxystrobin	299.84^{c}	19.07	103.67^{ef}	36.01	22.78	40	123.
Captan +Hexaconazole	200^{i}	20.44	136.04^{c}	51.87	24.48	40.12	114.5
Carbendazim	190^{j}	18.23	150.76^{j}	55.04	15.00	34.78	70.78
Talc based *P. fluorescens*	288^{d}	20.9	117^{e}	58.78	24.99	49.98	136
Talc based *Trichoderma* sp.	295^{f}	23.44	136.4^{d}	49.09	22	45.67	127
Mancozeb	250^{f}	19	99.99^{f}	39	18.90	29.99^{h}	75
Inoculated control	190.5^{j}	19.2	100.2^{f}	40	20.8	34.99	119.55
CD (0.05)	4.49	NS	9.947	NS	NS	NS	NS

Discussion

5. DISCUSSION

Chilli (*Capsicum annuum* L.) is one of the major spice cum vegetable crop used all over the world. India is the largest grower, consumer and exporter of chilli, currently exporting dry chilli and chilli products to over 90 countries around the world (Singal, 1999). Chilli cultivation is mostly concentrated in the southern states Andhra Pradesh, Karnataka, Maharashtra, Orissa and Tamil Nadu occupying nearly 75 per cent of the total area under this crop in India. Anthracnose or fruit rot of chillies is one of the major constraints affecting the quality of fruits leading to significant yield losses. Taking into account the diversity, pathogenic variability, host range and fungicide resistance outbreak of the fruit rot pathogen, management practices solely depending on any single method of control may not be adequate for effective suppression of the disease. In this context, the present study on "Integrated management of anthracnose in chilli (*Capsicum annuum* L.)" was undertaken to investigate the efficacy of some biocontrol agents, organic preparations and chemical fungicides for the management of anthracnose of chilli and to develop an integrated management package.

Nine isolates of the pathogenic fungi were obtained from infected fruits or leaves collected from different chilli growing fields of College of Agriculture, Vellayani during August, September and October months of 2013. Among these, five isolates were identified as *Colletotrichum capsici* whereas the other four isolates were identified as *Colletotrichum gloeosporioides* and confirmed as the causal agents of the fruit rot disease in chilli by conducting the Koch's postulate. The first report of chilli anthracnose in India was from Coimbatore in the Madras Presidency of British India (Sydow, 1913) and *Colletotrichum capsici* (Syd.) Butler and Bisby was reported as the causal organism. Kim *et al.* (1989) also reported the pathogenic fungi *C. capsici* and *C. gloeosporioides* as the causal agents of chilli anthracnose in the tropical Asia while Sangchote *et al.* (1998) identified these fungi as the major causal agents of chilli anthracnose in Thailand. The findings of Ramachandran *et al.* (2007)

from Karnataka and Andhra Pradesh in India that *C. capsici* was the most predominant species causing fruit rot disease is in consonance with the present study. Anamika (2014) also reported that under field conditions, chilli anthracnose was more due to *C. capsici* on green fruits. In the present investigation under Kerala climatic conditions *C. capsici* was found to be the most prevalent fungal pathogen causing chilli anthracnose during August to October months and similar observations has been made by Golda (2010) from Kerala that *C. capsici* was prevalent during the months of August to January.

During August to October months an average rainfall of 10 .15 cm, relative humidity 89.70 per cent (RH) and 29℃ temperature was prevailing in the premises of College of Agriculture, Vellayani and during this period, *C. capsici* was frequently isolated from the infected samples. These findings are in confirmity with the reports of Golda (2010) who mentioned that an average maximum temperature of 30.2˚C was favourable for the occurrence of *C. capsici*. Mehrotra and Aggarwal (2003) reported that continuous rain and high humidity incite the *C. capsici* infection causing fruit rot of chilli during the end of September and begining of October. The present study showed that a rainfall of 10 .15 cm, 29^0 C temperature was the favourable climatic conditions for the growth of *C. capsici*. Misra and Mahmood (1960) observed that, maximum chilli fruit rot disease development occured at 28˚C and 95.7 per cent RH. They also obtained correlation of severe yield losses with rainy weather because the spores get washed and splashed to other fruits resulting in infections.

Chilli anthracnose is a disease that can affect fruits, leaves, and pedicel. At severe stage it affects the entire plant. Moreover, during transit and storage, this disease also causes severe damage to chilli fruits. Three major characteristic symptoms were observed during the present study and they include leaf spot, die back, and fruit rot. Singh (2000) and Mistry *et al*. (2008) also observed these three characteristic symptoms as in the present investigation from India.

In the present study also, it was observed that the disease was most damaging on mature ripe fruits but symptoms were occasionally observed on green fruits as well. The first indication of the disease was the appearance of small, elliptical or oblong 1-2 mm dia., straw-coloured, slightly sunken lesions on the surface of the mature fruit which gradually spread in the direction of the long axis (four to five cm dia.), thus becoming more or less elliptical. This was followed by the development of numerous black acervuli bearing pale buff to salmon spore mass occur scattered or arranged in concentric rings, giving a target board appearance. When a diseased fruit was cut open, the lower surface of the skin was covered with minute, spherical, black stromatic masses of the fungus. Other workers have also recorded similar symptoms wherein the anthracnose caused by *C. capsici* on chilli fruits was more severe on mature, ripe fruits. According to Mesta *et al.* (2007), in *Capsicum* spp. red fruits recorded (50 per cent) more fruit infection than in green fruits. Roberts *et al.,* (2001) reported that the disease is more likely to develop faster on mature fruits as observed in the present study. The reason for the increased severity on mature ripe fruits has been attributed to the higher wax content on the green fruit surface when compared with the ripe mature fruits (Chuowdhury, 1957) while Azad (1991) found that phenolic compounds which offer resistance to plant pathogens reduced in levels with increasing fruit maturity in chilli.

Golda (2010) described the fruit rot of chilli caused by *C. capsici* as small (2-5 mm dia.), brown sunken lesions on the fruit surface which later turned necrotic. These lesions spread in the direction of 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 mass. This is in confirmation with the results of the present study. As observed in the present study, Than *et al*. (2008) also observed that the lower surface of the fruit skin is covered with minute, spherical, black stromatic masses or sclerotia of the fungus and a mat of fungal

hyphae covered the seeds. Such seeds turned rusty in colour and affected fruits were deformed. However, in the present study, sclerotia of the fungi were not observed.

Rodeva *et al.* (2009) also observed that often lesions increased progressively in number on the fruit, coalesced and quickly covered most of the fruit surface which is similar to the findings in the present study.

As observed in the present study, Ngullie *et al*. (2010) also observed that the symptoms of the fruit rot disease appeared mostly on ripe fruits. The infected tissue formed a depression and the fruit shrinks. The spots on the tissue measure 20-40 mm in dia.

Studies on pathogenicity and comparative virulence of the isolates of *Colletotrichum* spp. isolates causing fruit rot of chilli were carried out following Koch's postulates. The pathogenicity of *Colletotrichum* spp. was proven by inoculating the pathogen on fruits resulting in lesions on fruits. Ratanacherdchai *et al.* (2010) also had proven pathogenicity of *Colletotrichum* spp. causing fruit rot of chilli by inoculating on fruits and observed a maximum lesion size of 2.95 cm on long cayenne fruits inoculated with *C. capsici*. In the present study, the inoculated fruits produced water soaked lesion on fruits at 3-6 days after inoculation. The time taken for production of symptom varied between different isolates. The isolates C2, C3, C4, C7, C8 and C9 took only three days for the initiation of symptoms while the isolate C6 took maximum time of six days for symptom expression. Similar results regarding the variation in symptom expression between isolates were observed by Montri (2008), where she observed that the chilli fruits inoculated with *C. capsici* developed initial symptoms at about 3 days after inoculation and the least aggressive isolate produced symptoms at nine DAI.

The infection resulted in progression of lesion size with time and the lesion size varied with different isolates based on which virulence rating of the different isolates was done. Among the nine isolates, the isolate C2 showed the largest lesion

of 4.34 cm on unripe fruit and 5 cm on ripe fruit was rated as the most virulent isolate. Ratanacherdchai *et al.* (2010) also rated the virulence of *Colletotrichum* spp. on chilli fruits based on lesion size and grouped 24 isolates into three categories: two isolates in low virulent group, 17 in the moderately virulent group and 5 in the high virulent group. In the present study, among the nine isolates, the isolate C9 did not produce any symptom on inoculating on susceptible chilli fruits. Similar non pathogenic isolates of *Colletotrichum* spp. on chilli were also observed by Montri (2008). The incompaitable reaction of the isolate C9 could be attributed to the resistance factor in the host parasite interaction which has been reported by Oanh *et al*. (2004) who found that *C. capsici* (isolate 12) did not produce symptoms on chilli variety Mae Ping.

The pathogens causing anthracnose or fruit rot of chilli were isolated and the pure culture of the isolates was obtained by hyphal tip culture followed by single spore isolation. The identification of *Colletotrichum* spp. was done based on the spore morphology, colony and other taxonomic details of the fungus described in "*Colletotrichum:* Biology, pathology and control" by Bailey and Jeger (1992).

In the present investigation, the isolates of *Colletotrichum* spp. showed variability in their morphological attributes. The colonies of *Colletotrichum capsici* isolates C1, C2, C3, C5 and C6 had suppressed cottony mycelial growth with regular margin and took 5-7 days for completing the mycelial growth in 9cm dia. petri plate. The colour of these isolates varied from white to grey whereas the isolate C3 had a brownish tinge. The surface mycelium of the isolate C2 was uniform whereas concentric ring pattern was observed in C1, C3, C5 and C6. Reverse side of the colony were brownish grey to black. All the isolates of *C. capsici* showed sporulation on PDA media after 10 DAI and produced salmon pink coloured spore mass whereas the no. of days taken for spore mass production ranged from 10-20 days. The morphological characteristics of the isolates C1, C2, C3, C5 and C6 such as conidia, acervuli and setae were observed. All the isolates had falcate type of conidia. The

average conidial size of the isolates ranged from 22.34 - 24.50 x 4.5 - 4.86 µm. The size of the acervuli ranged from 135.70 - 188.89 µm and the no. of setae per acervuli ranged from 44 - 56.

Guldekar *et al*. (2009) have classified ten monoconidial isolates of *C. capsici*; chilli anthracnose pathogen on the basis of colony colour and conidial characters. They have also reported major variations after micrometrical observations on dimensions of conidia, acervuli and setae. The results of the present study were also in agreement with the depiction of morphological variability among the isolates of *C. capsici (*Syd.*)* Butler and Bisby by Masoodi *et al*. (2013). According to him, the *Colletotrichum capsici* isolate colour varied from light to dark grey with whitish or brownish tinge. In general, the colonies had suppressed cottony growth or fluffy mycelial growth with regular to irregular margin. Surface mycelial growth was with V- shaped or concentric or uniform pattern. The average conidial size ranged from 19.70 - 33.60 x 2.23 - 4.86 µm. and acervuli dia. ranged from 60-350 µm and the no. of setae ranged up to 60 and these results are in conformity with the results of the study. Akhtar and Singh (2007) have also reported the difference in the radial mycelial growth of *C. capsici* isolates on PDA. As in the present study, Maiello (1988) observed the initiation of acervuli of *C. capsici* on 12^{th} day and the initiation of fructification after three days of plating.

In the present study, the colonies of *Colletotrichum gloeosporioides* isolates C4, C7 and C8 had greyish white to dark grey colour. Aerial mycelium had cottony, tufty growth and produced salmon pink coloured or creamy white spore mass at the centre of the colony and the no. of days taken for spore mass production ranged from 10-20. Reverse side of the colony were unevenly white to grey or darker especially with age. The isolate C5 had a distinct olivaceous grey zone alternated with rosy buff zone was observed on the reverse side of the colony. The morphological characteristics of the isolates C4, C7 and C8 such as conidia, acervuli and setae were observed under Motic BA 2.10 compound microscope under 400 X objective

magnification. All the isolates had cylindrical conidia with obtuse ends. The average conidial size of the isolates ranged from 14.75-18.9 x 4.07-4.82 µm. The size of the acervuli ranged from 120.89- 145.79 µm and the number of setae per acervuli ranged from 22-27.

These results are in agreement with the findings of Golda (2010) who observed that the colonies of *C. gloeosporioides* (Penz.) Penz. and Sacc. appeared white in colour which gradually turned dark greyish white as it grew older and the growth pattern was evenly felty with salmon pink conidial pustules at the centre of the colony. Diurnal zonations were not 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 was observed. Yee and Sariah (1993) reported that *C. gloeosporioides* (Penz.) Penz. and Sacc. produced cylindrical conidia with 4-24 x 2-6 µm and and acervuli dia. ranged from 60-250 µm and the no. of setae per acervuli ranged up to 60 which is in agreement with the observations in the present study.

Hence based on the morphological characterization as well as the comparison with the standard keys, the isolates of *Colletotrichum* spp. were identified as *C. gloeosporioides* (Penz.) Penz. and Sacc. and *C. capsici (*Syd.*)* Butler and Bisby. Thus the study revealed that variability did exist in the morphology characters as well as pathogenicity of the isolates of *Colletotrichum* spp.

Akhtar and Singh (2007), Sangdee *et al.* (2011), Christopher *et al.* (2013) and Masoodi *et al.* (2013) had also observed variability in morphology as well as pathogenicity among *Colletotrichum* spp. isolates causing anthracnose of chilli.

The identification of the most virulent isolates of *C. capsici* (C2) and *C. gloeosporioides* (C5) were confirmed by NFCCI, Agharkar Research Institute, Pune as *C. capsici (*Syd.*)* Butler and Bisby and *C.gloeosporioides* (Penz.) Penz. and Sacc.

The two pathogens were deposited at NFCCI with accession no. NFCCI-3411 (C2) & NFCCI- 3412 (C5), respectively.

In the present investigation, the seed borne nature of the *Colletotrichum* spp. was studied by standard agar plate method and standard blotter technique. The studies with respect to the seed mycoflora of chilli by standard blotter method revealed the presence of *C. capsici, C. gloeosporioides*, *Penicillium* sp., *Aspergillus* sp. and species of *Alternaria*. The results indicated that the *C. capsici* (44.66 per cent) was the dominant fungus followed by *C. gloeosporioides* (12.00 per cent) and *Alternaria* sp. (0.5 per cent). The results of the standard blotter technique also indicated the dominance of *Colletotrichum capsici* (54.58 per cent) followed by *Colletotrichum gloeosporioides* (10 per cent) and *Alternaria* sp.

The results of this study revealed that seed-borne infection by *Colletotrichum* spp. appeared to be common in seed samples collected from affected chilli fields. Similar observations were made by Nayaka *et al*. (2009)

As in the present study, Meon and Nik (1988) also obtained *C. capsici* and *C. gloeosporioides* by blotter and agar plate method. Solanki *et al.* (1974) also reported the presence of *C. capsici*, *Aspergillus niger*, *A. flavus* and *Alternaria alternata* from chilli seed samples by both the techniques. Chigoziri and Ekefan (2013) also isolated *C. capsici* with the highest frequency of 54.75 per cent, *A. flavus* with 19.50 per cent and *Penicillium corylophilum* with 0.25 per cent from infected chilli seed samples in Nigeria.

C. capsici was isolated most frequently both by the blotter and standard agar plate method suggesting that the pathogen could be present on the seed surface as well as inside the seed and hence *C. capsici* was confirmed to survive on seeds and spread as externally and internally seedborne infection. Similar findings were reported by Grover and Bansod (1970) and Rout and Rath (1972) on chilli seeds by blotter and agar plate technique. The internally seed borne nature of the anthracnose

pathogen, *C. capsici* has been clearly indicated in the study conducted by Meon and Nik (1988). Pernezny *et al*. (2003) also reported that *Colletotrichum* species can survive in and on chilli seeds.

The present study under *in vivo* conditions revealed that from buried sample viable culture of *C. capsici* was recovered up to 11 weeks. The study showed that *C. capsici* survived for 77 days in the infected fruits and leaves in the soil. Under *in vitro* conditions *C. capsici* was recovered up to six weeks from the infected sample. After six weeks, no recovery of the pathogen was observed on the PDA after isolation from the infected fruit. Dasagupta (1989) reported that the survival of *C. capsici* (Syd.) Butler and Bisby. causing anthracnose of betelvine is possible through competitive saprophytic ability. Wilson *et al.* (1992) also isolated *C. acutatum* up to third month from infected strawberry fruits. Eastburn and Gubler (1992) also isolated *Colletotrichum truncatum* after 90 days from the green gram crop debris retained under natural field conditions which are in agreement with the results obtained in the study. The survival of *C. capsici* in soil, infected residues and weeds has been earlier documented by Saxena *et al.* (1982).

In the present investigation, the host range studies revealed that natural incidence of *C. capsici* infection was observed on fruits of tomato (*Lycopersicon esculentum* Mill.) and brinjal (*Solanum melongena* L.). On tomato and brinjal brown to black sunken, circular lesions were observed which gradually enlarged and caused fruit rot. However, on artificial inoculation of *C. capsici,* brown lesions were observed on leaves of green gram (*Vigna radiata* L.), ivy gourd (*Coccinea indica* L.), sesame (*Sesamum indicum* L.*)*, black gram (*Vigna mungo* L.) and sweet potato (*Ipomoea batatas* L.). Thus, these plants were confirmed as alternative hosts to the fruit rot pathogen.

Meua (1971) reported sesame anthracnose (*Colletotrichum* sp.) from Kanpur with 10-12 per cent disease index and observed oval water soaked lesions on leaf and

leaf axis as characteristic of this disease which was also noticed in the present study. Mordue (1971) also recorded *C. capsici* infection on young fruits of brinjal from Africa, Asia, America and Australia. Hadden (1989) have reported the occurrence of *C. capsici* on tomato causing fruit rot symptom. Beniwal *et al.* (1985) observed the occurrence of anthracnose caused by *C. capsici* on green gram (*Vigna radiate)* cultivars T44 and Jawahar 45 in the field. Pandey, (2006) described the symptoms produced by *C. capsici* on partially matured to red ripened tomato fruits as large, water-soaked, depressed, circular, blackish brown to black lesions which was similar to the findings of the present study. Davis *et al.* (2005) reported *Colletotrichum* sp. as the major leaf spot pathogen of ivy gourd.

During the present study, native fungi isolated from phyllosphere, pomoplane and rhizosphere of chilli were tested for their inhibitory effect on the pathogen. The phyllosphere of disease free chilli plants in the the fruit rot affected field harboured three predominant fungal genera; *Aspergillus* sp., *Penicillium* sp.and *Cercospora* sp. whereas the pomoplane of chilli harboured *Aspergillus* sp. and *Penicillium* sp. Similarly, the rhizosphere of the chilli plants hrarboured an additional fungus, *Trichoderma* sp. Based on the conidial morphology and other taxonomic criteria, the isolate of *Trichoderma* was identified to be *T. viride* and *Penicillium* sp. as *Penicillium citrinum.* The bacteria from phyllosphere, pomoplane and rhizosphere of chilli were also isolated.

Bacillus sp. was isolated from phyllosphere and pomoplane while *Pseudomonas* sp. was isolated from rhizosphere. The bacterial antagonists obtained were identified by morphological characters, gram staining, catalase test, fluorescence test (*P. fluorescens)* and endospore staining (*Bacillus* sp.). Forty-four isolates of phylloplane/pomoplane bacteria and 45 isolates of endophytic bacteria from leaf/fruit tissues of chillies were isolated from 45 leaf samples and nine fruit samples (Basha *et al.*, 2010).

In the present investigation, the dual culture technique revealed that *Trichoderma viride* showed a radial mycelial inhibition of 68.40 per cent with overgrowth against *C. capsici* whereas *Penicillium citrinum* inhibited the mycelial growth of the pathogen by 54.73 per cent. Jeyalakshmi *et al.* (1998) reported that *Trichoderma viride, T. harzianum* and *T. koningii (*Oudem) inhibited mycelial growth of *C. capsici* by 51.7, 56.6 and 42.5 per cent respectively. Mandeep *et al.* (2006) recorded that *T. viride* reduced the mycelia growth of *C. capsici* by 52.5 per cent followed by *T. virens* (38.12 per cent). Tiwari *et al.* (2008) observed that *T. viride* provided 50 per cent inhibition of mycelial growth of *C. capsici.* Basha *et al.* (2010) screened the pomoplane and phyllosphere fungi and among these *P. citrinum* recorded 47.9 per cent inhibition of the radial growth of *C. capsici*. Ngullie *et al.* (2010) recorded 63.34 per cent inhibition of the mycelial growth of *C. capsici* by *T. viride.* Barhate *et al.* (2012) tested the antagonistic effect of five isolates of *Trichoderma* and reported that *T. viride* inhibited the mycelial growth of *C. capsici* by 56.66 per cent. Tembhare *et al.* (2012) isolated the phyllosphere fungi (*Penicillium, Aspergillus, Curvularia, Cladosporium*) of chilli and observed that *Penicillium* sp. inhibited the radial growth of *C. capsici* by 25.20 per cent.

In the present study, *Bacillus sp*. inhibited the growth of *C. capsici* by 88.80 per cent whereas the other gram –ve bacterium *P. fluorescens* showed 73.33% growth inhibition of the pathogen. Hegde *et al.* (2001) reported that *P. fluorescens* showed antagonistic activity against *C. capsici* recording growth inhibition of 54.38 per cent. Tiwari *et al.* (2008) tested the antagonistic effect of ten isolates of *P. fluorescens* and found that the per cent mycelial inhibition varied between 28.2-38.8 per cent. Chandramani *et al.* (2013) reported that *P. aeruginosa* WS-1 demonstrated 53.21 per cent inhibition of *C. capsici* under *in vitro.* Linu and Jisha (2013) tested the *in vitro* antagonistic effect of two isolates of *P. fluorescens* and revealed that both the isolates showed more than 70 per cent of inhibition of *C. capsici. P. fluorescens* suppresses the foliar pathogen by inducing systemic resistance (Wei *et al.,* 1944). Murhopper *et*

al. (1998) also reported that the lipopolysacharides present in the *P. fluorescens* cell wall act as signal molecules and elicit various defense compounds including salicylic acid responsible for induction of systemic resistance for control of plant pathogens. Ngullie *et al.* (2010) recorded 56.86 per cent inhibition of the mycelial growth of *C. capsici* by *Bacillus subtilis* whereas *T. viride* recorded 67.42 per cent inhibition.

Seven fungicides were evaluated at three concentrations under *in vitro* conditions to study their effect on growth of *C. capsici* (Figure1). The fungicide propiconazole (0.05, 0.1 and 0.15 per cent) and difenoconazole (0.01, 0.05 and 0.1 per cent) showed complete inhibition to the radial mycelial growth of the pathogen at all the concentrations tested. The results of the present study are in accordance with the findings of Gopinath *et al.* (2006) who tested seven concentrations of the fungicides propiconazole and difenoconazole and results indicate that the complete inhibition of mycelial growth of *C. capsici* was observed at 50 µg ml^{-1} for all the chemicals tested.

In the present study, mancozeb @ 0.1 per cent recorded 88.74 per cent inhibition to the mycelial growth of *C. capsici* whereas at 0.2 per cent and 0.3 per cent completely inhibited (100 per cent) the mycelial growth of *C. capsici.* Kasyap *et al.* (2008) also observed that mancozeb at 0.3 per cent completely inhibited the mycelial growth of *C. capsici.*

Azoxystrobin (0.15 per cent) completely inhibited the mycelial growth of *C. capsici* and 0.1 per cent recorded 97.53 per cent inhibition to the growth of the pathogen where as at 0.05 per cent exhibited only 5.44 per cent mycelial inhibition. Ahiladevi *et al.* (2014) reported that azoxystrobin (0.12 per cent) drastically reduced the mycelial growth of *C. capsici* on chilli (83.27 per cent).

In the present study, carbendazim at 0.01, 0.05 and 0.1 per cent recorded mycelial inhibition of 51.24, 88.51 and 97.52 per cent, respectively. Raju *et al.* (2007) tested seven concentrations of carbendazim with twenty one isolates of *C. capsici*

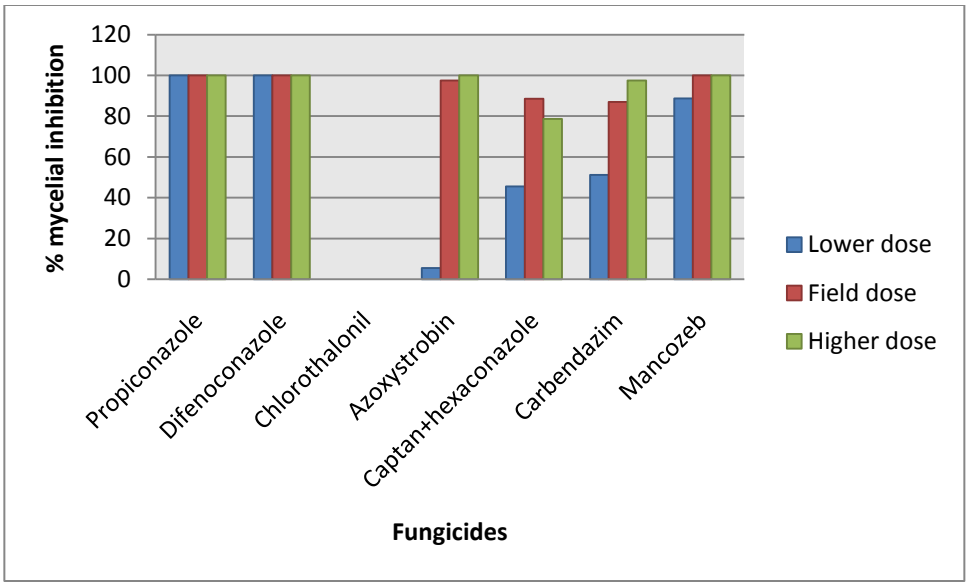


Figure 1. Effect of different fungicides on mycelial growth of *C. capsici*

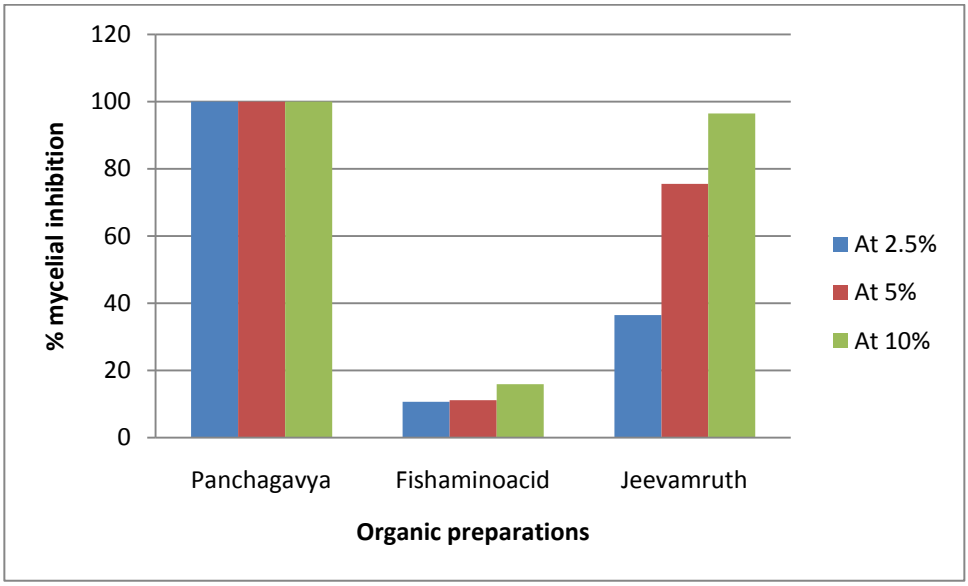


Figure 2. Effect of different organic preparations on mycelial growth of *C. capsici*

from chilli and recorded that the maximum inhibition was observed at 1000 µgml^{-1} and the inhibition varied from 3.3-100%. Ngullie *et al.* (2010) reported that carbendazim at 0.1% provided 83.4% inhibition of mycelial growth of *C. capsici.*

According to the present study, captan+hexaconazole @ 0.05, 0.1 and 0.15 per cent recorded 45.53, 78.58 and 85.1 per cent mycelial inhibition of the pathogen tested. Mina *et al.* (2009) reported that hexaconazole (0.1 per cent) were effective to inhibit the growth of the pathogen.

The result of the *in vitro* assay of organic preparations (Figure 2) for their activity in suppressing the growth of *C. capsici* revealed that, the recommended dose of panchagavya (2.5 per cent) gave complete inhibition (100 per cent) of the growth of the pathogen and was significantly better than all other organic preparations which was followed by jeevamruth (2.5 per cent) where there was 36.40 per cent inhibition to the growth. The least inhibition was recorded by fish aminoacid at 2.5 per cent (10.60 per cent). Reena (2011) reported that panchagavya at 10 and 20 per cent provided 5.6 per cent mycelial inhibition of *C. capsici*. A combination of neem oil and panchagavya at 10 and 20 per cent inhibited the mycelial growth of *C. capsici* 60.55 and 67.7 per cent, respectively.

The results of the seedling assay with bio agents (Figure 3, 4) revealed that the percentage seedling mortality due to *C. capsici* was lowest for the seeds treated with the isolated bacterium *Bacillus* sp. (10.46 per cent) followed by KAU released talc based biocontrol agent *P. fluorescens* (15.36 per cent) and was found to be the best for suppressing the seed borne pathogen *C. capsici*. The seeds treated with *Trichoderma viride* showed 20.46 per cent seedling mortality**.** The KAU released biocontrol agents *P. fluorescens* and *T. harzianum* recorded 25.23 per cent and 25.59 per cent seedling mortality, respectively and were also on par.

Suthin Raj *et al.* (2009) also studied the mortality rate of the seeds treated with biocontrol agents and the study revealed that percent mortality was lowest for

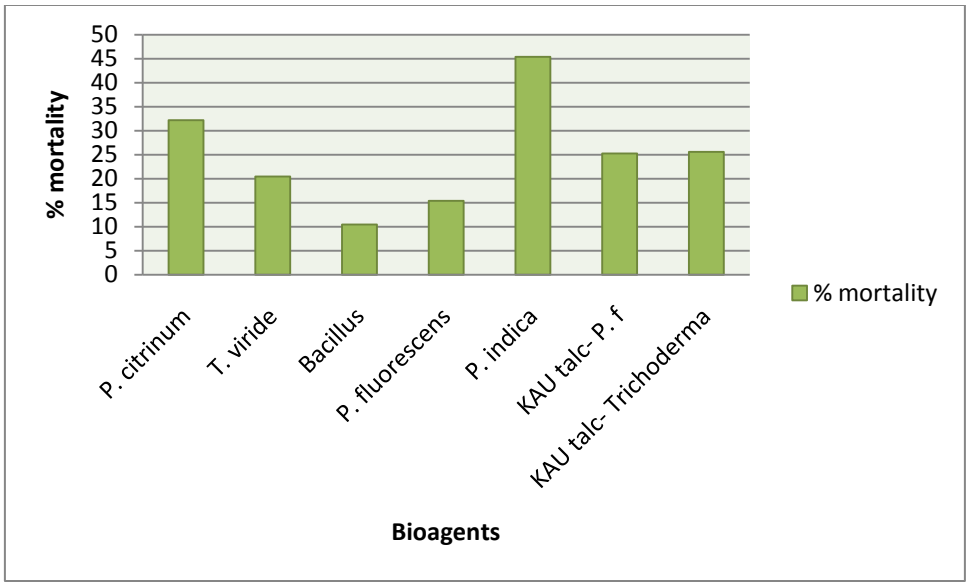


Figure 3. Effect of different bioagents on per cent mortality of *C. capsici* inoculated seedlings

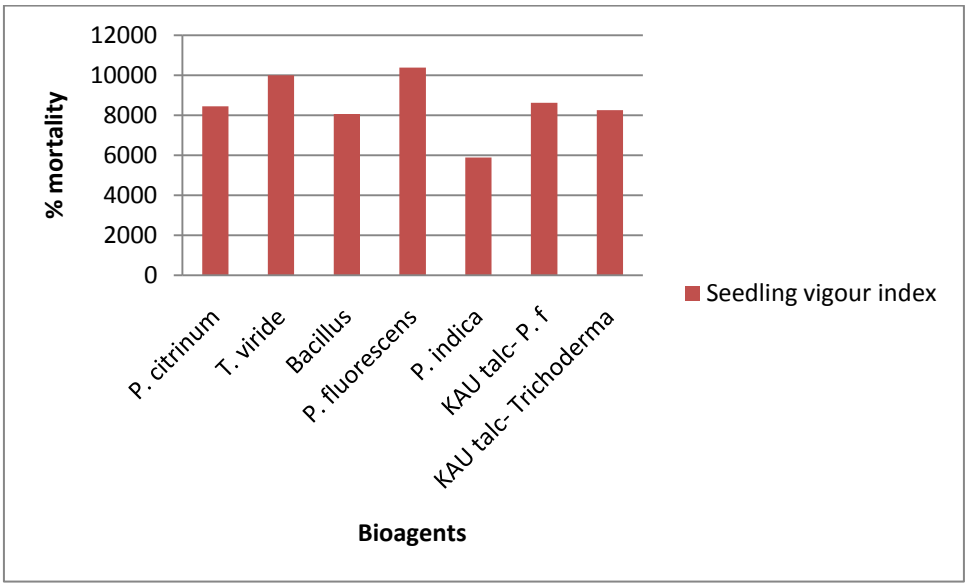


Figure 4. Effect of different bioagents on seedling vigour index of *C. capsici* inoculated seedlings

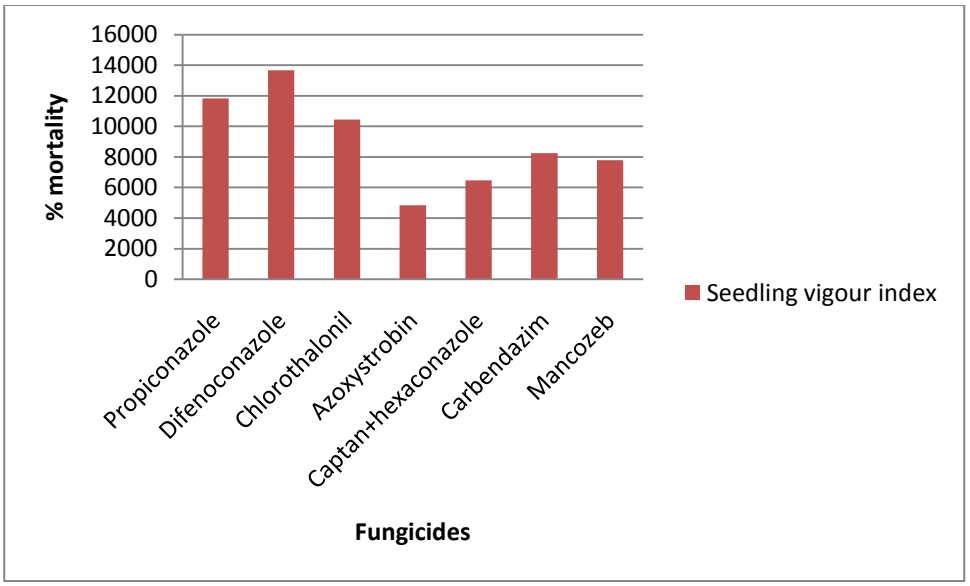


Figure 5. Effect of different fungicides on per cent mortality of *C. capsici* inoculated seedlings

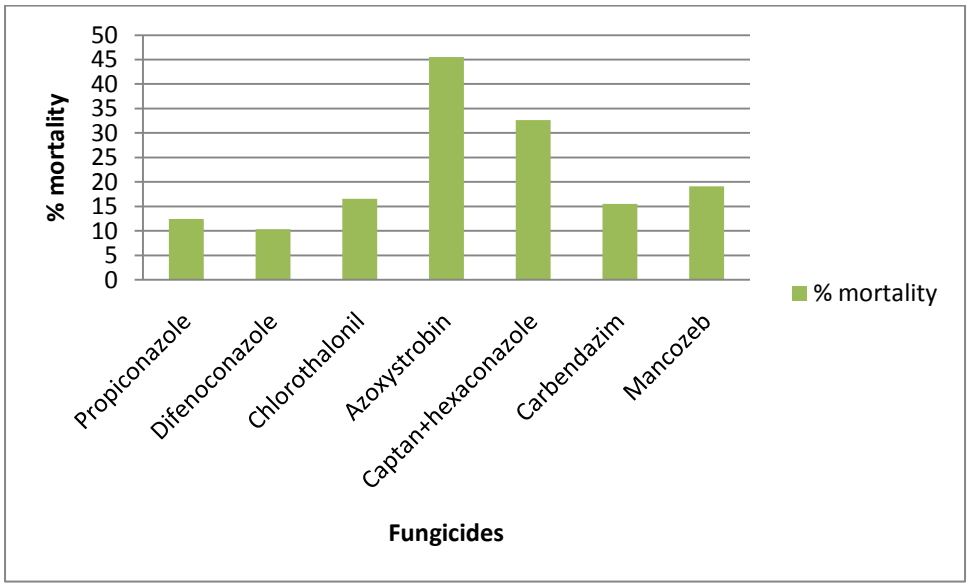


Figure 6. Effect of different fungicides on seedling vigour index of *C. capsici* inoculated seedlings

the seeds treated with pure cultures of *P. fluorescens* (18.4 per cent) *and T. harzianum* (19.9 per cent) and the formulation of *P. fluorescens* (20 per cent). Usharani (2006) has observed that the shoot and root length, seed germination and seedling vigour was best in the carbendazim treated seeds followed by *Trichoderma* plus *Pseudomonas fluorescens*.

The results of the seedling assay with fungicides (Figure 5, 6) revealed that seeds treated with propiconazole (0.1 per cent) exhibited lowest percentage seedling mortality (10.37 per cent) followed by difenoconazole at 0.1 per cent (12.44 per cent). The seeds treated with carbendazim (0.05 per cent) showed 15.49 per cent seedling mortality. The next best fungicide which provided lowest percentage seedling mortality (16.57) was azoxystrobin at 0.1 per cent. The fungicide captan + hexaconazole at 0.1 per cent recorded 19.13 per cent mortality. The seedlings treated with mancozeb (0.2 per cent) exhibited 32.62 per cent mortality.

Suthin Raj *et al.* (2009) also studied the mortality rate of the chilli seeds treated with fungicides and the study indicates that the seeds treated with captan (2g kg^{-1}) and carbendazim (2g kg^{-1}) exhibited lowest percent mortality 27.3 and 28.5 per cent.

Based on the results of the *in vitro* seedling assay for the management of *C. capsici* infection and seedling vigour, the treatments were selected for *in vivo* management study. In pot culture experiments (Figure 7, 8), the treatment where propiconazole (0.1 per cent) and difenoconazole (0.05 per cent) was applied as foliar spraying at fortnightly intervals resulted in lowest disease index of 35.47 per cent and 36.47 per cent respectively and was statistically on par. These treatments recorded a percent disease reduction of 62.85 and 61.50 respectively and were significantly better than all other treatments. Gopinath *et al*. (2006) reported that foliar spraying of propiconazole (0.1 per cent) and difenoconazole (0.05 per cent) at 10 days interval caused a dramatic reduction of chilli anthracnose disease incidence by 70 per cent and

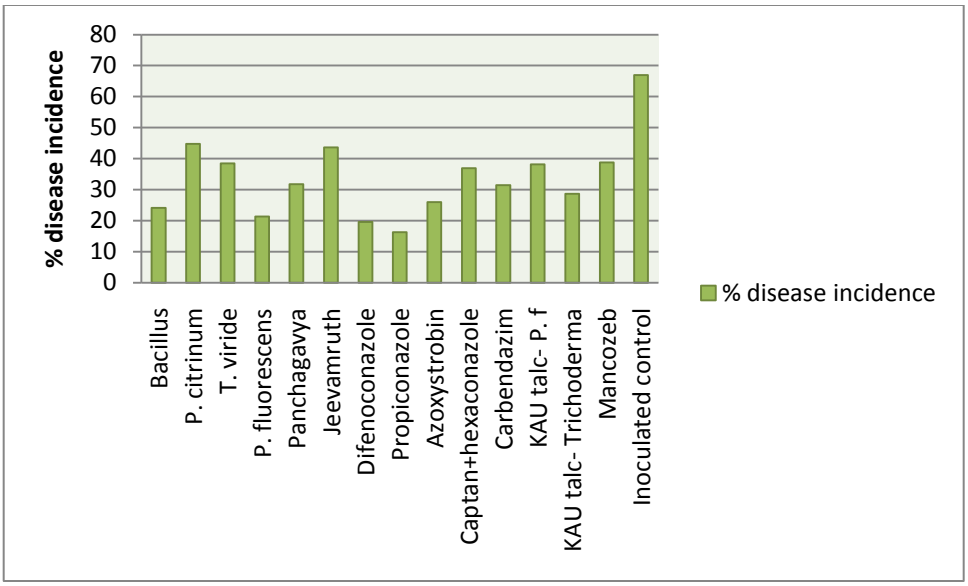


Figure 7. Effect of different treatments on per cent fruit rot disease incidence

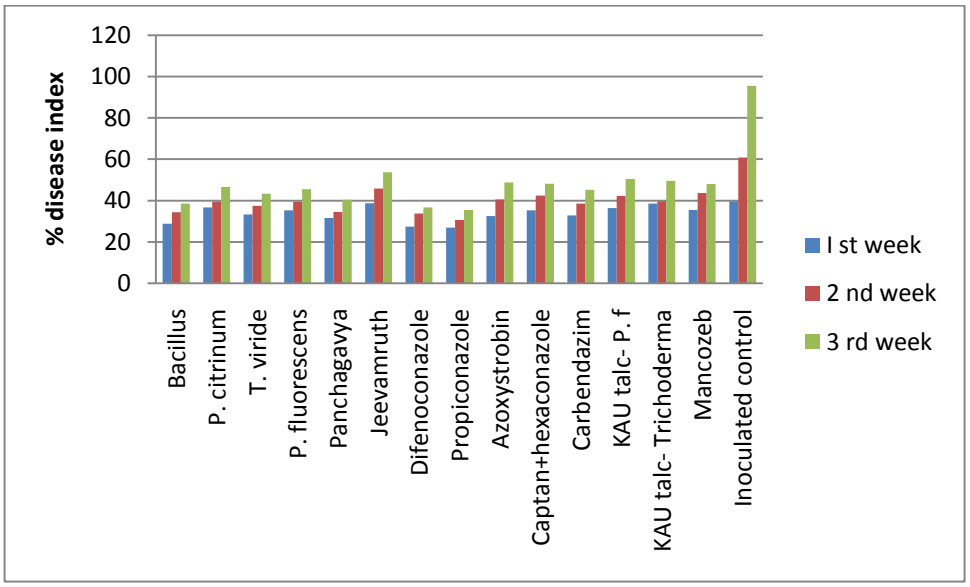


Figure 8. Effect of different treatments on fruit rot disease index

58 per cent respectively. Propiconazole acted by the demethylation of C-14 during ergosterol biosynthesis, and leading to accumulation of C-14 methyl sterols. The biosynthesis of these ergo sterols are critical to the formation of cell walls of fungi. The lack of normal sterol production slows or stops the growth of the fungus, effectively preventing further infection and/or invasion of host tissues (Uesugi, 1998).

The treatment T1 (*Bacillus* sp.) also recorded lower disease index of 38.59 per cent and suppressed the disease (59.59 per cent). Lamsal *et al*. (2012) have reported 40 per cent reduction in incidence of anthracnose using different species of Bacillus as soil application. Ashwini *et al*. (2014) recorded 50 per cent reduction in the anthracnose disease due to the application of *Bacillus subtilis.* They also reported that the disease control ability of *B. subtilis* was due to its high ability for chilli root colonization.

Summary

6. SUMMARY

Anthracnose or fruit rot caused by *Colletotrichum capsici (*Syd.*)* Butler and Bisby and *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. is an important disease of chilli. The present study entitled "Integrated management of anthracnose in chilli (*Capsicum annuum* L.)" was undertaken at the College of Agriculture, Vellayani during 2013-2015 to study the pathogen causing fruit rot, symptomatology, seed borne nature, survival, and host range of fruit rot pathogen and to develop an integrated management strategy using effective biocontrol agents, organic preparations and fungicides compatible to manage the disease with an objective of evolving an integrated management practice. Chilli anthracnose is a disease that can affect fruits, leaves, and pedicel. At severe stage it affects the entire plant. Three major characteristic symptoms were observed during the present study and they include leaf spot, die back, and fruit rot. The first indication of the disease was the appearance of small, elliptical or oblong 1-2mm dia. sized, straw-coloured, slightly sunken lesions on the surface of the mature fruit which gradually spread in the direction of the long axis (four to five cm dia.), thus becoming more or less elliptical. This was followed by the development of numerous black acervuli bearing pale buff to salmon spore mass occur scattered or arranged in concentric rings. *Colletotrichum* sp. was isolated from the diseased fruits and leaves of chilli collected from College of Agriculture, Vellayani. The isolates were serially numbered from C1–C9. The colonies of *Colletotrichum capsici* isolates C1, C2, C3, C5 and C6 had suppressed cottony mycelial growth with regular margin and took 5-7 days for completing the mycelial growth in 9cm dia. petriplate. The colour of these isolates varied from white to grey whereas the isolate C3 had a brownish tinge. The average conidial size of the isolates ranged from 22.34-24.50x4.5-4.86 µm. The size of the acervuli ranged from 135.70-188.89 µm and the no. of setae per acervuli ranged from 44-56. The colonies of *Colletotrichum gloeosporioides* isolates C4, C7 and C8 had greyish white to dark grey colour. Aerial mycelium had even to cottony,

tufty growth and produced salmon pink coloured or creamy white spore mass at the centre of the colony. The average conidial size of the isolates ranged from 14.75-18.9 x 4.07-4.82 µm. The size of the acervuli ranged from 120.89- 145.79 µm and the no. of setae per acervuli ranged from 22-27.

Pathogenicity of the isolates was proven by following Koch's postulates and the most virulent isolate was selected based on the lesion size produced by each isolate after artificial inoculation on ripe and unripe chilli fruits. The inoculated fruits produced water soaked lesion on fruits at 3-6 days after inoculation. The time taken for production of symptom varied between different isolates. Among the nine isolates, the isolate C2 showed the largest lesion of 4.34 cm on unripe fruit and 5 cm on ripe fruit was rated as the most virulent isolate and used for further studies.

The identification of the most virulent isolates of *C. capsici* (C2) and *C. gloeosporioides* (C5) were confirmed by NFCCI, Agharkar Research Institute, Pune as *C. capsici (*Syd.*)* Butler and Bisby and *C.gloeosporioides* (Penz.) Penz. and Sacc. The two pathogens were deposited at NFCCI with accession nos. NFCCI-3411 (C2) & NFCCI- 3412 (C5), respectively.

The studies on the seed mycoflora of chilli by standard blotter method revealed the presence of *C. capsici*, *C. gloeosporioides*, *Penicillium* sp., *Aspergillus* sp. and species of *Alternaria*. The results indicated that the *C. capsici* (44.66 per cent) was the dominant fungus followed by *C. gloeosporioides* (12.00 per cent) and *Alternaria* sp. (0.5 per cent). The results of the standard blotter technique also indicated the dominance of *Colletotrichum capsici* (54.58 per cent) followed by *Colletotrichum gloeosporioides* (10 per cent) and *Alternaria* sp. *C. capsici* was confirmed to survive on seeds and spread as externally and internally seedborne infection.

The viable culture of *C. capsici* from the buried sample was recovered up to 11 weeks. The study showed that *C. capsici* survived for 77 days in the infected fruits

and leaves in the soil. Under *in vitro* conditions *C. capsici* could be isolated and recovered from the infected sample up to 6 weeks.

The natural incidence of *C. capsici* infection was observed on fruits of tomato (*Lycopersicon esculentum* Mill.) and brinjal (*Solanum melongena* L.). On tomato and brinjal brown to black sunken, circular lesions were observed which gradually enlarged and caused fruit rot. However, on artificial inoculation of *C. capsici*, brown lesions were observed on leaves of green gram (*Vigna radiata* L.), ivy gourd (*Coccinea indica* L.), sesame (*Sesamum indicum* L.*)*, black gram (*Vigna mungo* L.) and sweet potato (*Ipomoea batatas* L.). Natural incidence of disease was observed on weeds *Richardia scabra* L. *(*Rough Mexican clover*)* and *Commelina benghalensis* L. (Tropical spiderwort). Thus, these plants were confirmed as alternative hosts to the fruit rot pathogen.

The phyllosphere of disease free chilli plants in the the fruit rot affected field harboured three predominant fungal genera; *Aspergillus* sp.*, Penicillium* sp.and *Cercospora* sp. whereas the pomoplane of chilli harboured *Aspergillus* sp. and *Penicillium* sp. Similarly, the rhizosphere of the chilli plants harboured an additional fungus, *Trichoderma* sp. and *Penicillium citrinum* inhibited the mycelial growth of the pathogen by 54.73 per cent whereas *Trichoderma viride* showed a radial mycelial inhibition of 68.40 per cent with overgrowth against *C. capsici*. Two saprophytic bacteria; *Bacillus* sp. and fluorescent pseudomonads isolated from the rhizosphere, phyllosphere and pomoplane were found to be efficient in causing *in vitro* suppression of the mycelial growth of the pathogen by 88.80 per cent and 73.33 per cent, respectively.

Organic preparations like panchagavya, jeevamurth and fish amino acid were prepared and their effectiveness in suppression of mycelia growth of *C. capsici* under *in vitro* conditions was checked at different concentrations by poisoned food technique. The recommended dose of panchagavya (2.5 per cent) gave complete inhibition (100 per cent) of the growth of the pathogen and was significantly better

than all other organic preparations which was followed by jeevamruth (2.5 per cent) where there was 36.40 per cent inhibition to the growth.

Seven commercially available fungicides were used for the *in vitro* study. *In vitro* assay of fungicides with poisoned food technique revealed that at the recommended dose, propiconazole (0.1 per cent), difenoconazole (0.05 per cent) and mancozeb (0.2 per cent) completely inhibited (100 per cent) the mycelial growth of *C. capsici* and was significantly better than all the other fungicides. This was followed by azoxystrobin (0.1 per cent) where there was 97.53 per cent inhibition to the growth of the pathogen. The fungicide captan+hexaconazole at 0.1 per cent recorded 85.10 per cent inhibition. This was followed by carbendazim (0.05 per cent) which recorded mycelial inhibition of 88.51 per cent.

The two fungal antagonists obtained during the study, *viz Penicillium citrinum* and *Trichoderma viride*, the fungal endophyte *Piriformospora indica, P. fluorescens* and *Bacillus* sp. was evaluated for the disease suppression at seedling stage. The KAU released biocontrol agents *P. fluorescens* and *T. harzianum* and seven commercially available fungicides such as propiconazole, difenoconazole, mancozeb, carbendazm, captan + hexaconazole, azoxystrobin and chlorothalonil were assessed for the management of the disease on seedlings at the recommended concentration. Among seeds treated with biocontrol agents, the percentage seedling mortality due to *C. capsici* was lowest for the seeds treated with the isolated bacterium *Bacillus* sp. (10.46 per cent) followed by KAU released talc based biocontrol agent *P. fluorescens* (15.36 per cent) and was found to be the best for suppressing the seed borne pathogen *C. capsici*. Among the fungicides tested, the results of the seedling assay with fungicides revealed that seeds treated with propiconazole (0.1 per cent) exhibited lowest percentage seedling mortality (10.37 per cent) followed by difenoconazole at 0.1 per cent (12.44 per cent).

A pot culture experiment was conducted to evaluate the efficacy of organic preparations, biocontrol agents and fungicides for the management of fruit rot of

chilli revealed that that the plants treated with difenoconazole (0.05 per cent) provided 30.80 per cent disease suppression where 27.38 PDI recorded. This was statistically on par with the effect of the isolated bacterium *Bacillus* sp. on fruit rot intensity with 28.75 per cent PDI and provided 27.34 per cent disease suppression. The result also revealed that the organic preparation, panchagavya provided significant reduction in the disease severity (20.34 per cent) with 31.61 per cent PDI compared to the inoculated control where 39.57 per cent PDI was observed. The foliar application of azoxystrobin (0.1 per cent) recorded 18.01 per cent disease suppression and 32.44 per cent disease intensity were superior than positive checks; KAU talc based formulation of *P. fluorescens*, KAU talc based formulation of *Trichoderma* sp. and mancozeb with 6.29 per cent, 2.57 per cent and 10.38 per cent suppression, respectively.

Thus this study reveals that the integrated management of the anthracnose disease in chilli can be achieved by removing infected crop debris and excluding alternative hosts such as tomato, brinjal, green gram, black gram, sesamum, ivy gourd and sweet potato. The weed hosts such as *Richardia scabra* and *Commelina benghalensis* should be destroyed. Seed treatment with systemic fungicides like propiconazole (1 ml/Kg) or *Pseudomonas fluorescens* 10g/Kg prevent seed borne infection. Foliar spraying of propiconazole (1 ml/L) or difenoconazole (0.5 ml/L) or panchagavya(2.5 per cent at fortnightly interval after the onset of disease controls the disease.

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Integrated management of anthracnose in chilli (*Capsicum annuum* L.)

by

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Abstract of the thesis

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ABSTRACT

The study entitled "Integrated management of anthracnose in chilli (*Capsicum annuum* L.)" was conducted at Department of Plant Pathology, College of Agriculture, Vellayani, Thiruvanathapuram during 2013-2015 with the objective to evolve an integrated management package for anthracnose in chilli.

Nine isolates of the pathogen causing chilli anthracnose were isolated and based on morphological characters like colony colour, conidial shape and dimensions, the pathogens causing fruit rot disease of chilli were identified as *Colletotrichum capsici* (Syd.) Butler and Bisby and *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. which was further confirmed and deposited at Agharkar Research Institute, Pune with accession number NFCCI- 3411 & NFCCI- 3412, respectively. Pathogenicity was proven following Koch's postulates and virulence rating was done. From this the most virulent isolate C2, an isolate of *C. capsici* was selected for the further studies.

The results of standard blotter test and standard agar plate method revealed that *C. capsici* and *C.gloeosporiodes* infection in chilli is seed borne in nature. The results also indicated the dominance of *C. capsici* (44.66 per cent) followed by *C. gloeosporioides* (12.00 per cent), *Alternaria* sp. (0.50 per cent). Saprophytic fungi recorded were *Penicillium* sp. (0.16 per cent) and *Aspergillus* sp. (0.16 per cent).

The study showed that *C. capsici* survived for 77 days in the infected fruits and leaves in the soil and 42 days in the lab condition. The host range studies revealed that the solanaceous crops brinjal (*Solanum melongena* L.) and tomato (*Solanum lycopersicum* L.) at fruiting stage were found to be alternative hosts of *C. capsici* under natural conditions. However on artificial inoculation of *C. capsici*, brown lesions were observed on leaves of green gram (*Vigna radiata* L.), ivy gourd (*Coccinia indica* L.), sesame (*Sesamum indica* L.), black gram (*Vigna mungo* L.) and sweet potato (*Ipomoea batatus* L.). Natural incidence of disease was observed on

weeds *Richardia scabra* L. (Tropical girdlepod) and *Commelina benghalensis* L. (Tropical spiderwort).

The predominant micro-organisms isolated from chilli phyllosphere, pomoplane and rhizosphere *Penicillium citrinum*, *Trichoderma viride, Bacillus and Pseudomonas flourescens* exhibited 56.23 per cent, 64.45 per cent, 88.88 per cent and 73.33 per cent inhibition of the pathogen respectively under *in vitro* conditions.

In vitro assay of fungicides with poisoned food technique revealed that propiconazole (0.15 per cent), difenoconazole (0.05 per cent) and mancozeb (0.2 per cent) completely inhibited the mycelial growth of *C. capsici*. It was followed by azoxystrobin (0.15 per cent), captan+hexaconazole (0.1 per cent), carbendazim (0.1 per cent) with mycelial inhibition of 97.77 per cent, 88.30 per cent and 83.33 per cent respectively. No mycelial growth of the pathogen was observed in chlorothalonil (0.1 per cent) amended media. Among organic preparations, panchagavya provided complete inhibition of the growth of the pathogen at all the concentrations tested. Jeevamruth provided complete inhibition only at 10 per cent concentration, whereas, the amendment of media with fish amino acid did not affect the mycelial growth of the pathogen.

Chilli seedling assay of different bioagents for the control of disease on seedlings revealed that the percentage seedling mortality was lowest for the seeds treated with the biocontrol bacterium *Bacillus* sp. (10.46 per cent) isolated from phyllosphere and pomoplane followed by the KAU released biocontrol agent *Pseudomonas flourescens* (15.36 per cent). *Piriformospora indica* treated seeds exhibited 45.36 per cent mortality.Seedling assay with fungicides revealed that seeds treated with propiconazole (0.1 per cent) exhibited the lowest seedling mortality (10.37 per cent).

A pot culture study conducted with chilli variety Vellayani Athulya to evaluate the efficacy of organic preparations, biocontrol agents and fungicides and it revealed

that maximum disease suppression was provided by foliar spraying of 0.1 per cent propiconazole (62.85 per cent) and 0.05 per cent difenoconazole (61.06 per cent) at fortnightly intervals. Among the biocontrol agents, *Bacillus* sp. was found to be superior in suppressing the disease (59.59 per cent) and among organic preparations, panchagavya gave the maximum suppression of the disease (57.06 per cent).

Based on the results of the study, an integrated management package for anthracnose in chilli is proposed as follows: Seed treatment with systemic fungicides like propiconazole (1 ml kg^{-1}) or biocontrol agents like *Pseudomonas fluorescens* 10 g kg^{-1} prevent seed borne infection , removing infected crop debris and destroying alternative hosts such as tomato, brinjal, green gram, black gram, sesamum, ivy gourd and sweet potato and weed hosts such as *Richardia scabra* and *Commelina benghalensis* prevent disease incidence and foliar spraying of propiconazole (1 ml l^{-1}) or difenoconazole (0.5 ml l^{-1}) or panchagavya (2.5 ml l^{-1}) at fortnightly intervals after the onset of the disease controls the disease.



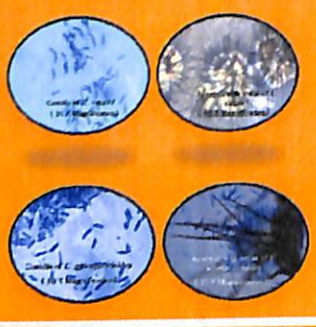



INTEGRATED MANAGEMENT OF ANTHRACNOSE IN CHILLI (*Capsicum annuum* L.)

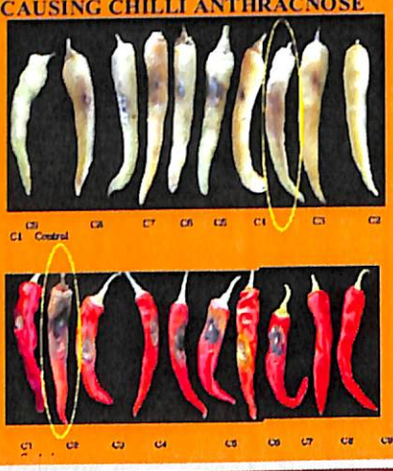
SYMPTOMATOLOGY

- Fruit rot- concentrically enlarged sunken spots on fruits covered with black acervuli.
- Leaf spot- brown necrotic spots.
- Die-back- Progressive death of twigs

CAUSAL AGENTS OF CHILLI ANTHRACNOSE



ISOLATES OF Colletotrichum spp. CAUSING CHILLI ANTHRACNOSE



ISOLATES OF Colletotrichum sp.

SURVIVAL OF *Colletotrichum capsici*

C. capsici survived for 77 days in infected fruits and leaves in the soil.

HOST RANGE OF *Colletotrichum capsici*


HOST RANGE OF *Colletotrichum capsici* on weeds

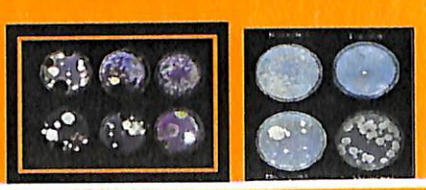

SEED BORNE NATURE OF *Colletotrichum capsici*

Colletotrichum capsici is both internally and externally seed-borne.

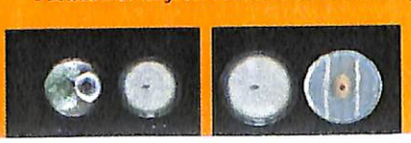
Standard blotter method- isolation of externally seed borne fungi

Standard agar plate method –isolation of internally seed borne fungi

MICROBIAL ANTAGONISTS ISOLATED FROM PHYLLOSPHERE, POMOPLANE AND RHIZOSPHERE OF CHILLI PLANT



MYCELIAL GROWTH INHIBITION OF Colletotrichum capsici BY *Trichoderma viride* *Pseudomonas fluorescens* and *Bacillus* sp.



EFFECT OF SOME FUNGICIDES ON MYCELIAL GROWTH OF *C. capsici*

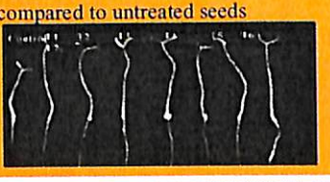
Fungicide	Per cent mycelia l inhibition atfField dose
Propiconazole	100 (90.00)[a]
Difenoconazole	100 (90.00)[a]
Chlorothalonil	0.00 (0.00)[e]
Azoxystrobin	97.53 (80.97)[b]
Captan + hexaconazole	88.51 (70.19)[c]
Carbendazim	86.92 (68.80)[d]
Mancozeb	100 (90.00)[a]
Control	0.00 (0.00)[e]
CD (0.05)	1.35

EFFECT OF ORGANIC PREPARATIONS ON MYCELIAL GROWTH OF *C. capsici*

Organic preparations	% mycelial inhibition at 5%
Panchagavya	100.00 (90.00)[a]
Fish amino acid	11.13 (19.50)[c]
Jeevamruth	75.45 (60.30)[b]
CD (0.05)	**1.37**

SEEDLING BIOASSAY WITH BIOAGENTS AND FUNGICIDES

- Seed treatment with bioagents *Trichoderma viride* and *Bacillus* sp. @ rate of 10^8 cfu g^{-1} reduced the seedling mortality (10.46 and 15.46% respectively) and increased the vigour (10000 & 8064) compared to untreated seeds
- Seed treatment with fungicides propoiconazole (0.1%) and difenoconazole (0.05%) reduced the seedling mortality (10.37 and 12.46% respectively) and increased the vigour (13660 & **11834**) compared to untreated seeds



POT CULTURE STUDIES

Foliar spraying of propiconazole (0.1%) and difenoconazzole (0.05%) at fortnightly intervals provided maximum anthracnose disease suppression 62.85 and 61.60%, respectively.

Foliar spraying of *Bacillus* sp. (10^8 cfu g^{-1}) at fortnightly intervals recorded maximum disease suppression (59.59%).

Foliar spraying of panchagavya (2.5%) provided maximum suppression of the disease (57.6%).