SEED BIOPRIMING AND SPRAYING AT FRUIT SET FOR THE MANAGEMENT OF CHILLI ANTHRACNOSE CAUSED BY *Colletotrichum capsici* (Sydow) Butler and Bisby

by

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"Seed biopriming and spraying at fruit set for the management of chilli anthracnose caused by *Colletotrichum capsici* (Sydow) Butler and Bisby"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled **"Seed biopriming and spraying at fruit set for the management of chilli anthracnose caused by *Colletotrichum capsici* (Sydow) Butler and Bisby"** is a record of research work done independently by Ms. Athira P. V. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Per cent
µm	Micrometer
ANOVA	Analysis of variance
CD	Critical difference
cm	Centimeter
CRD	Completely Randomized Design
DAI	Days after inoculation
DTCP	Days taken for complete coverage of petridish
DTSA	Days taken for symptom appearence
et al.	And other co workers
Fig.	Figure
g	Gram
h	Hour
ha	Hectares
hpi	Hours post inoculation
KAU	Kerala Agricultural University
KB	King's B
kg	Kilogram
M	Molar
mg	Milligram

Min.	Minute
mL	Millilitre
mM	Millimolar
mm	Millimeter
N	Normality
NA	Nutrient agar
No.	Number
°C	Degree Celsius
PDA	Potato dextrose agar
PDI	Per cent disease index
SE (m) ±	Standard error of mean
SD	Standard deviation
Sl.	Serial
sp. or spp.	Species (Singular and plural)
viz.,	Namely

Introduction

1. INTRODUCTION

Chilli (*Capsicum annuum* L.), is a common spice cum vegetable crop used since ancient times for its pungency, flavour and colour. Chilli belongs to the genus *Capsicum* of the family *Solanaceae.* They are good sources of antioxidants such as vitamin-A, flavonoids, β-carotene, α-carotene, lutein, zeaxanthin, and cryptoxanthin. The extracts of chilli are widely used as an analgesic for arthritis, burns, headaches and neuralgia. They also boost immune system, lower the cholesterol level, reduce high blood pressure and increase the peripheral circulation (Geetha and Selvarani, 2017). In India chilli is cultivated throughout the year. Sahitya *et al.* (2014) reported that chilli could lessen the risk of cancer by preventing carcinogens binding to DNA and reduce the calorie intake by upsurging thermogenesis.

Chilli production is threatened by numerous biotic and abiotic factors. Among the biotic factors, the major ones are diseases caused by fungi, bacteria and viruses. The important diseases affecting chilli are damping off, fruit rot (anthracnose), die back, powdery mildew, bacterial leaf spot, cercospora leaf spot, alternaria rot, fusarium wilt, leaf curl and mosaic. Among these, anthracnose disease caused by *Colletotrichum capsici* (Syd.) Butler and Bisby is of great importance. The disease is both seed and air borne; and hinders seed germination and vigour (Asalmol *et al.,* 2001). *C. capsici* is a necrotrophic fungus belonging to Kingdom - Fungi, Division - Eumycota, Subdivision - Deuteromycotina, Class - Coelomycetes, Order - Melonconiales under family Melonconiaceae (Kirk *et al.*, 2008). *Colletotrichum* spp. stands eighth among the most important group of plant pathogenic fungi (Dean *et al.*, 2012). In India, anthracnose causes a yield loss of 8 to 60 per cent in chilli (Pooja and Simon, 2019).

Chemical fungicides can be used for the management of diseases in chilli. But problems like residual toxicity, resistance development, environmental pollution and several health hazards in humans and animals accompanies, if they

are used continuously and indiscriminately. In this context there is a need to formulate alternative eco-friendly approaches for disease management.

A promising way to maintain current level of agricultural production, while bringing down the use of chemical pesticides polluting the environment is biological control (Mishra *et al.*, 2011). Biocontrol agents are environmentally safe and their use has long lasting disease management capability. There are different modes of application of biocontrol agents to plants, and seed priming with biocontrol agents, commonly called as 'seed biopriming' is one of the emerging techniques.

Seed priming is the controlled hydration of seeds to the level that metabolic activities needed for germination could occur but prevent radicle emergence (Bisen *et al.*, 2015; Prasad *et al.*, 2016). Seed priming improves speed and uniformity of germination and establishment of seedlings, stress tolerance, yield and quality of crop (Mahmood *et al.*, 2016).

Seed biopriming is a seed treatment technique which integrates biological and physiological aspects of seed treatment. Biological aspect involves the inoculation of seeds with beneficial microorganisms to protect the seeds and physiological aspect involves controlled seed hydration. Seed biopriming protects the seeds from seed and soil borne pathogens, improves the speed and uniformity of germination and final stand of the crop. Biopriming allows the microbe to colonize the seeds prior to planting (Callan *et al.*, 1991; El-Mohamedy, 2004; Reddy, 2013; Bisen *et al.*, 2015; Ananthi, *et al.*, 2017). Biopriming improves the physiology of seeds resulting in enhanced vigour (Ghassemi *et al.*, 2008). Seed biopriming also ensures the availability of healthy and quality seeds with high germination percentage which in turn increases production. It also manages seed borne inoculum of pathogens.

Keeping in view of the above facts, the present work was undertaken to study the effect of seed biopriming and spraying biocontrol agents during fruit set

for the effective management of the anthracnose / fruit rot disease of chilli caused by *C. capsici*.

The main objective of the research was to study the seed borne nature of chilli anthracnose / fruit rot caused by *C. capsici* and the effect of seed biopriming and spraying during fruit set for its management.

Review of Literature

2. REVIEW OF LITERATURE

Chilli (*Capsicum annuum* L.) sometimes called as the 'wonder spice' is the fourth most important vegetable crop in the world. Chilli originated in Peru, South America and Mexico is considered to be the secondary centre of origin and diversity. It is grown in both the tropics and the sub tropics. Major chilli producing states in India are West Bengal, Orissa, Assam, Bihar and Gujarat. The active principle capsaicin ensures chilli its pungency. It has high consumptional, nutritional and cash value to farmers and consumers in the world.

In India, chilli is grown in an area of 0.797 million ha with a production of 1.389 million tonnes of dried and 0.679 lakh tonnes of green fruits (FAO, 2017). Chilli export from India is 43,490 MT which values around 21,75.11 million rupees. In Kerala chilli is grown in an area of 1860 ha with a production of 12,470 tonnes. Dry chilli production in Kerala is about 1600 MT which values about Rs. 89.0 million (GOI, 2017). India is the largest producer, consumer and exporter of chilli in the world and Andhra Pradesh ranks first in chilli production in India (Abarna *et al.*, 2019).

Among the major fungal diseases in chilli, anthracnose or ripe fruit rot caused by *C. capsici* was reported for the first time in India by Sydow in 1913 from Coimbatore of then Madras Presidency. It is a wide spread problem which affects the profitable cultivation and seed production of chilli. The disease can cause yield loss up to 50 per cent and most of the commercial chilli varieties are susceptible to *C. capsici*. The fungus infects both unripe and ripe fruits; and survives on seed as acervuli and micro sclerotia (Raj *et al.*, 2014).

Many species of *Colletotrichum* viz. *C. capsici*, *C. gloeosporioides*, *C. acutatum, C. fructicola, C. siamense and C. coccodes* were reported to cause anthracnose in chilli. Among them *C. capsici* and *C. gloeosporioides* were the dominant causal agents of anthracnose in India. Both these pathogens affect chilli fruits at green and red ripe stage (Mishra *et al.*, 2019).

2.1. COLLECTION OF CHILLI SEEDS AND ISOLATION OF SEED BORNE MICROFLORA.

Tripathi *et al.* (1984) isolated both externally and internally seed borne fungi from chilli seeds using the standard blotter and agar plate techniques. Three species of *Aspergillus* and *Rhizopus* were found associated externally with the seeds. Four species of *Aspergillus*, *Penicillium* sp., *Alternaria alternata*, *Curvularia lunata*, and *Fusarium oxysporum* were found to be both externally and internally seed borne. *Colletotrichum* sp. was found internally only.

George (1992) collected chilli seed samples from two locations in Kerala *viz.,* Vellayani and Vellanikkara; and isolated both externally and internally seed borne fungi. *Aspergillus flavus, Chaetomium globosum, Curvularia clavata, Penicillium purpurogenum and Rhizopus oryzae* were present externally and *Penicillium pinophilum* was present both externally and internally. Culture filtrates of *P. pinophilum* caused 95 per cent inhibition in germination of seeds while it was 65.45 and 62.56 per cent for *C. clavata* and *A. flavus* respectively.

Mridha and Siddique (1989) obtained *Curvularia* sp., *Fusarium* sp., *Alternaria* sp. and *C. capsici* from chilli seeds. Hashmi (1989) recorded the presence of three species of *Fusarium* along with *C. capsici* and *A. alternata* in seeds of chilli. Padaganur and Naik (1991) reported *Fusarium* sp., *Alternaria* sp., *A. flavus* and *C. capsici* in chilli seeds collected from the disease affected fruits. Presence of *C. capsici, Cercospora sp., Alternaria sp., Penicillium sp.* and *Aspergillus sp.* were detected in the seed samples collected from eight districts of Northern Karnataka (Hemannavar, 2008). *C. capsici, A. niger, A. flavus, Fusarium* sp., *Rhizopus* sp. and *Penicillium* sp. were detected both externally and internally in seeds of popular chilli varieties collected from Regional Horticulture Research Station (RHRS), Navsari (Chauhan *et al.*, 2018).

Sitara and Hasan (2011) isolated 38 species of fungi belonging to 19 genera from chilli seeds using blotter method. *Aspergillus* spp. were the dominant fungi. Seven species of *Aspergillus*, four species of *Alternaria* and *Fusarium*, two

species of *Phoma* and *Myrothecium*, three species of *Chaetomium*, *Curvularia* and *Drechslera*, and other fungi *viz., C. capsici*, *Phomopsis* sp., *Phyllosticta* sp., *Cladosporium* sp., *Rhizopus* sp., *Rhizoctonia solani*, *Helminthosporium* sp., *Nigrospora* sp., *Cercospora* sp., *Cephaliospora* sp. and *Trichoderma* sp. were obtained from the seeds.

Fungi belonging to Anamorphs, Oomycota and Zygomycota were obtained from seeds extracted from naturally infected chilli fruits of Benue State, Nigeria. The anamorphic fungi obtained were seven species of *Aspergillus*, three species of *Colletotrichum* including *C. capsici*, three species of *Fusarium*, two species each of *Penicillium*, *Botryotrichum*, and *Humicola* and other fungi *viz., R. solani*, *Phoma* sp., *Alternaria* sp., *Botrydiplodia* sp., *Bispora* sp., *Phomopsis* sp., *Peniconia* sp., *Coniothyrium* sp., *Fumago* sp., and *Septonema* sp. The oomycetous fungi included four species of *Pythium*. *Mucour* sp., *Rhizopus* sp. and *Syncephalastrum* sp. were the Zygomycetous fungi obtained from the seeds (Chigoziri and Ekefan, 2013).

2.2. PATHOGENICITY STUDIES

2.2.1. Survey, Collection and Isolation of the Pathogen

C. capsici, *C. gloeosporioides* and *C. piperatum* were the causal organisms for anthracnose and ripe fruit rot of chilli in India (Thind and Jhooty, 1990). *C. capsici* and *C. gloeosporioides* were found as the major fungal pathogens associated with anthracnose and fruit rot of chilli in Kerala (Golda, 2010).

From the samples of chilli fruit rot collected from five locations in Jammu and Kashmir, three distinct isolates of *C. capsici* along with *F. moniliforme*, *F. pallidoroseum*, *F. oxysporum*, *A. alternata* and *A. flavus* were obtained (Parey *et al.*, 2013).

Vinaya *et al.* (2009) conducted a survey of the major diseases of chilli in Karnataka and found that *C. capsici* was the predominant fungi (71.24 %). A

survey was conducted in five major chilli growing blocks of Ghaziabad district of Uttar Pradesh during first week of July to November in 2016 and 2017. The disease started during July and the whole crop was damaged by November. The disease incidence varied from 3.6 to 23.0 per cent in plants and 4.5 to 26.0 per cent in fruits. Disease severity in fruits ranged from 1.0 to 4.7 per cent (Javed *et al.*, 2017). A survey was conducted in six different locations of Kerala *viz.,* Vellayani, Kumarakom, Thrissur, Pattambi, Ambalavayal and Padanakkad to study the incidence and severity of anthracnose and fruit rot of chilli. Isolates of *C. capsici* were obtained from the fruit rot samples collected from Vellayani, Kumarakom, Thrissur, Ambalavayal and Padanakkad. Isolate of *C. gloeosporioides* was obtained from Ambalavayal. The incidence of fruit rot in the survey locations ranged from 20-80 per cent and disease severity ranged from 23-54 per cent (Anjana, 2018). A survey was undertaken during Kharif 2017-2018 in the chilli growing areas of Banaskantha district of Gujarat and severity of anthracnose ranged from 40.22-59.48 per cent (Katediya *et al.*, 2019).

Anthracnose of chilli is caused by a variety of *Colletotrichum* species *viz., C. acutatum, C. coccodes, C. gleosporioides, C. atramentarium, C. dematium* and *C. capsici. C. capsici* was obtained from chilli fruits showing anthracnose symptoms collected from Cuddalore and Krishnagiri districts of Karnataka (Abarna *et al.*, 2019).

2.2.2. Symptomatology of Anthracnose and Fruit Rot Caused by *C. capsici* in Chilli

Chilli anthracnose caused by *C. capsici* occurred in three phases. The first phase was the seedling blight or damping off which was predominant in the nursery, followed by leaf spotting and die back seen at different stages of growth. The third phase was the fruit rot in which the ripened fruits are affected. The fruit rot stage caused the maximum damage and reduced the market value (Hemannavar, 2008).

C. capsici infected the leaves, stems and flowers of chilli. The damage was more severe on mature ripe fruits and sometimes infection was also seen on green fruits. The symptoms initially appeared as small, elliptical or oblong straw coloured, slightly sunken lesions on the surface of mature chilli fruits. Subsequently black acervuli got arranged in concentric rings giving a target board appearance. Naturally infected seeds by *C. capsici* showed numerous acervuli on the seed surface. The pathogen was both externally and internally seed borne (Meon and Nik, 1988; Javed *et al.*, 2017).

C. capsici infected the leaves, branches and fruits of chilli. Small circular spots were seen on the leaves and defoliation occurred on severe infection. Die back symptom was also observed as the infection of the growing tips lead to necrosis of branches which progressed backwards and later resulted in destruction of the whole plant The diseased area was spotted with large number of acervuli (Gupta, 2016). The disease even continued upto drying and storage of chilli fruits (Singh *et al.* 2017). Kaur *et al.* (2018) observed that the most prominent phase was fruit rot based on a survey conducted in chilli growing areas of Punjab during July-September 2014. Die back was seen in few places only.

Anthracnose and fruit rot of chilli was more predominant in rainy season and high humidity favoured the disease. The symptoms observed under natural conditions included leaf blight, twig necrosis and dieback. The leaf blight symptom was seen as dry rotting of the leaf lamina starting from the edges towards the centre surrounded by yellow halo. Brown necrotic lesions with black acervuli were seen on the stems. Fruit rot symptoms started as sunken water soaked lesions that later turned to straw or dark brown elliptical lesions and gradually caused severe rotting of fruit. Thick mass of black acervuli were seen on the rotted area. Later the fruits got deformed, dried and turned black. The seeds were also infected and had white mycelial growth initially and completely turned dark brown or black later with acervuli on the surface (Anjana, 2018). Mridha and Siddique (1989) opined that there is a positive correlation between fruit infection and seed infection in case of *C. capsici*.

2.2.3. Morphological and Cultural Characterization of *C. capsici*

Christopher *et al.* (2013) studied the cultural characters of 20 isolates of *C. capsici* collected from chilli growing areas of Tamil Nadu using PDA medium. The fastest growing isolate showed full growth in petri plate on 8th day of incubation. The maximum radial growth observed at 8th day was 9 cm followed by 8.8 cm. The least was 3.0 cm. Three isolates exhibited flat growth with concentric rings and two produced raised colonies without any concentric rings. Four isolates had profuse sporulation, seven isolates sporulated moderately and two isolates did not sporulate on PDA.

Masoodi *et al.* (2013) collected 20 isolates of *C. capsici* from three districts of Kashmir valley *viz.*, Srinagar, Budgam and Pulwama; and studied the cultural and morphological variability. The colour of mycelia showed a range of variation like white, grey, brown, dull white, dull grey, light brown, light smokey grey and grey. The colonies were either fluffy or suppressed with margins either regular or irregular. The radial growth after 7 days ranged from 3.20 to 6.75 cm. The average conidial length ranged from 19.70 to 33.60 µm and breadth ranged from 2.23 to 4.86 µm. The shapes of the conidia were either fusiform or falcate. The length and breadth of setae ranged from 65.0 to 194.6 µm and 4.4 to 6.6 µm respectively. The acervuli were seen either submerged and scattered or raised and scattered or raised and in concentric rings.

Anjana (2018) studied the morphological and cultural variation of five different isolates of *C. capsici* collected from different districts of Kerala. The mycelium in general was hyaline, slender and sepatate. The colour of the mycelium on the upper side of the culture was white, white to cream, white later turning to grey or white to off-white while the reverse side was white, yellowish white, creamy to brown, creamy white or creamy to dark brown. All the cultures in PDA showed sparse growth with concentric zonations and regular or irregular margins. The diameter of the mycelial growth ranged from 7.53 to 8.43 cm at 7th day of growth on PDA. The diameter of the acervuli varied from 127.40 to 183.28

μm. The average number of setae per acervuli ranged from 18 – 47 and length ranged from 72.50 to 110.41 μm. The length and width of appressoria ranged from 8.78 to 12.68 μm and 5.67 to 7.29 μm respectively.

Twenty isolates of *C. capsici* were obtained in a survey conducted in chilli growing areas of Punjab during July-September 2014. The morphological and cultural characters were studied. Three types of colony growth *viz.*, fluffy, less fluffy and appressed were observed. The colour of the colonies were ash gray, cloudy gray, smoky gray, white, platinum, black, black eel or gray goose. Acervuli were either arranged in rings or scattered. Colony margins were either regular or irregular. The colony diameter ranged from 74 to 85 mm after 10 days of incubation. Four isolates were relatively fast growing with growth rate of 8.0 to 8.5 mm / day. The slowest growing isolate had a growth rate of 6.9 mm / day. The length of the conidia ranged from 14-28 μm and breadth ranged from 2-4 μm (Kaur *et al.*, 2018).

2.2.4. Pathogenicity Testing and Screening of the Isolates of *C. capsici.*

Twenty isolates of *C. capsici* were obtained from chilli growing areas of Tamil Nadu and virulence testing was carried out. The most virulent isolate showed Percent Disease Index (PDI) of 69.90 for fruit rot and PDI of 63.20 for leaf infection while the PDI were 3.80 and 5.20 respectively for the least virulent isolate (Christopher *et al.*, 2013). Fruit rot lesion size after 8 days of incubation ranged from 7.10 to 16.00 mm for different isolates of *C. capsici* on inoculating detached semi ripe fruits of chilli with conidial suspension (10^7 spores / ml) (Parey *et al.*, 2013).

Chacko (2015) studied the variability amongst different isolates of *C. capsici* by mycelial bit inoculation in chilli variety Vellayani Athulya. The days taken for the appearance of symptom varied from 3 - 6. The size of the lesions varied from 1.17 - 4.34 cm in unripe fruits and 1.60 - 5.00 cm in ripe fruits. Anjana (2018) studied the pathogenicity and comparative virulence of five isolates of *C. capsici* collected from Kerala by artificial inoculation using 5 mm

mycelial discs on detached leaves and fruits of chilli var. Vellayani Athulya. The lesion produced on leaves ranged from 0.56 to 2.66 cm in length at 5th day of inoculation. The lesions produced by the most and least virulent isolates were of sizes 2.23 cm and 0.83 cm on tender fruits, 0.63 cm and 1.73 cm on mature fruits and 1.70 cm and 2.83 cm on ripe red fruits respectively.

The pathogenic variability of seven isolates of *C. capsici* collected from Mymensingh district of Bangladesh was studied by Muhtarima *et al.* (2018). The areas infected by the most virulent and least virulent isolates were 74.00 per cent and 46.66 per cent on mature green fruits, and 74.99 per cent and 36.66 per cent on ripe red fruits respectively.

2.3. *In vitro* EVALUATION OF BIOCONTROL AGENTS AGAINST *C. capsici*

2.3.1. *In vitro* Evaluation by Dual Culture Technique

Biological control by biocontrol agents involved different mechanisms such as competition for nutrients and space, antibiosis, parasitism, cell wall degradation by lytic enzymes and induced systemic resistance (Singh, 2013).

Trichoderma spp. grew over fungal plant pathogens and caused hyphal coiling, hyphal abnormalities and lysis of hyphae (Malathi, 1996; Anand and Bhaskaran, 2009). *Trichoderma* spp. exhibited antagonistic effects against plant pathogens by a number of mechanisms such as mycoparasitism, production of lytic enzymes such as chitinases, proteases, β-1,3 and β-1,4 glucanases, competition and induction of host resistance (Almeida *et al.*, 2007; Ekefan *et al.*, 2009). *P. fluorescens* inhibited the growth of fungal pathogens by the secretion of chitinolytic enzymes and antibiotics such as pyoluteorin, pyrrolnitrin, phenazine-1-carboxylic acid and 2, 4-diacetyl phloroglucinol (Anees *et al.*, 2019).

Bacillus species colonized plants, promoted growth through the production of phytohormones, induced the synthesis of defence enzymes such as peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL),

catalase (CAT) and super oxide dismutase (SOD) which helped in systemic resistance against plant pathogens (Elanchezhiyan *et al.*, 2018).

Piriformospora indica is a fungal root endophyte of a range of crops which promoted plant growth and provided resistance against fungal pathogens including those that affected the roots and foliage (Serfling *et al.*, 2007; Johnson *et al.*, 2014; Anith *et al.*, 2015).

In vitro study by Anand and Bhaskaran (2009) revealed that an isolate of *T. viride* inhibited the mycelial growth of *C. capsici* by 63.7 per cent and an isolate of *P. fluorescens* inhibited the mycelial growth by 70.8 per cent. Mishra *et al.* (2011) observed that *T. viride* strains Tr.8, Tr.3, Tr.14 and Tr.12 inhibited the mycelial growth of *C. capsici* by 70.14, 66.25, 62.50 and 61.25 per cent respectively in dual culture. Jagtap *et al.* (2013) studied the antagonistic potential of *T. viride* and *P. fluorescens* against *C. capsici* following dual culture technique. *T. viride* inhibited the mycelial growth of *C. capsici* by 52.32 per cent while *P. fluorescens* showed an inhibition of 42.18 per cent. Lokhande *et al.* (2019) reported that *T. viride* and *P. fluorescens* inhibited the mycelial growth of *C. capsici* by 74.22 and 57.56 per cent respectively.

T. viride, *P. fluorescens* and *B. subtilis* inhibited the mycelial growth of *C. gloeosporioides* by 63.34%, 67.42% and 56.86% respectively (Ngullie *et al.*, 2010). *In vitro* study by Bal and Behera (2012) revealed that *P. flourescens, T. viride* and *B. subtilis* inhibited mycelial growth of *C. capsici* by 66.40%, 47.54% and 33.59% with mycelial growth of 13.66 mm, 21.33 mm and 27.00 mm respectively.

B. subtilis isolate AB10 inhibited the mycelial growth of *C. acutatum* by 54.90 per cent (Lamsal *et al.*, 2012). Ashwini and Srividya (2014) reported that *B. subtilis* inhibited the *in vitro* mycelial growth of *C. gloeosporioides* by 57 per cent. Rajkumar *et al.* (2018) studied the antagonistic effect of 30 isolates of *B. subtilis* against *C. capsici* showing inhibition percentages ranging from 11.98 to 63.42 per cent. Abarna *et al.* (2019) studied the antagonistic effect of five

isolates of *B. subtilis* against *C. capsici* and the percentage inhibition of mycelial growth ranged from 60.37 to 73.33 per cent. *Bacillus* sp. strain BSp.3/aM inhibited the mycelial growth of *C. capsici* by 26 per cent (Jayapala *et al.*, 2019).

B. pumilus strain BETL13 inhibited *in vitro* mycelial growth of *C. capsici* by 40 per cent (Amaresan *et al.*, 2012). *B. amyloliquefaciens* strain CNU114001 reduced mycelial growth of *C. acutatum* by 62.74 per cent and *C. orbiculare* by 70.91 per cent *in vitro* (Seung *et al.*, 2013). Han *et al.* (2015) studied the antagonistic activity of *B. amyloliquefaciens* strain LB01 and *B. pumilus* strain LB15 against *C. acutataum* and *C. gloeosporioides*. LB01 showed 60.15 per cent inhibition against *C. acutatum* and 58.12 per cent against *C. gloeosporioides*. LB15 inhibited the two pathogens by less than 40 per cent. *B. amyloliquefaciens* strain Y1 produced an inhibition zone of 11 mm against *C. gloeosporioides* with an inhibition percentage of 69.79 per cent (Jamal *et al.*, 2015). *B. amyloliquefaciens* inhibited the mycelial growth of *C. truncatum* by 13.8 per cent (Gowtham *et al.*, 2018).

P. indica showed antagonistic activity against lentil wilt pathogen *Fusarium oxysporum* f. sp. *lentis* in dual culture. The hyphae of *P. indica* coiled around the hyphae of pathogen and later penetrated into it (Dolatabadi *et al.*, 2012).

2.3.2. *In vitro* assays on Detatched Plant Parts

T. viride successfully controlled stem-end rot caused by *Botryodiplodia theobromae* in harvested mango (Kota *et al.*, 2006). *T. harzianum* when applied to harvested apple and subsequent challenge inoculation with *R. solnani* resulted in reduction of lesion size by 32.4 per cent (Batta, 2007). Citrus fruits dipped in a suspension of *P. fluorescens* and *T. atroviride* (1×10^9 cfu ml^{-1}) for two minutes and subsequent pathogen inoculation reduced citrus rot caused by *P. digitatum* by 36.6 and 65.6 per cent respectively (Panebianco *et al.*, 2015).

B. subtilis isolates PMB-123 and PMB-183 reduced the lesion length of anthracnose caused by *C. capsici* in postharvest fruit bioassay of chilli by 61.69

and 55.41 per cent respectively. *B. pumilus* reduced the lesion size by 50.29 per cent (Ramanujam *et al.*, 2012). *B. amyloliquefaciens* strain PPCB004 reduced the disease incidence of anthracnose caused by *C. gloeosporioides* in postharvest citrus fruits from 76.70 to 31.70 per cent (58.67 % reduction over control) (Arrebola *et al.*, 2010a).

Disease suppression assay of *B. amyloliquefaciens* strain LB01 and *B. pumilus* strain LB15 against *C. acutataum* was carried out on detached chilli fruits. The fruits were sprayed with a suspension of the biocontrol agents (10^8 cfu / ml) and inoculated with spore suspension of the pathogen (3.50×10^4 conidia / ml) after 24 h. These were compared to the fungicide pyraclostrobin. LB01 sprayed fruits showed only 5.30 per cent of fruit area infection with a control efficiency of 94.00 per cent which was comparable with the fungicide treatment (2.80 % fruit infection with 96.80 % control efficiency). In the case of LB15 treated fruits, 45.90 per cent of area was infected with a control efficiency of 48.10 per cent. The area infected in control fruit was 88.60 per cent (Han *et al.*, 2015).

2.3.3. Changes in Peroxidase and Polyphenol Oxidase Activities on Application of Antagonists

Peroxidase (PO) and polyphenol oxidase (PPO) are defense related oxido-reductive enzymes and are involved in the formation of physical barriers against pathogens such as strengthening of cell walls and production of lignin. They also induce production of reactive oxygen species which act as anti-microbials and aids in host defence (Ramamoorthy *et al.*, 2002; Do *et al.*, 2003). Increased activities of defense related enzymes like PO, PPO, phenylalanine ammonia lyase (PAL), lipoxygenase, chitinase and total phenol contents are essential components in local and systemic resistance in plants (Radjacommare *et al.*, 2004).

PO is involved in wound healing and is a useful marker of plant development, physiology, infection and stress. Increased activity of PO contributes to disease resistance in infected plants (Welinder, 1992;

Vidhyasekaran *et al.*, 1997). PPO oxidizes phenolic compounds to more toxic quinones which invade pathogenic microorganisms (Vinale *et al.*, 2005). PPO is involved in terminal oxidation of diseased plant tissues which aids in disease resistance (Jisha *et al.*, 2018). Treatment of black gram seeds with *T. viride* @ 4g / kg against dry root rot caused by *Macrophomina phaseolina* increased the PO activity (change in absorbance/min/g units) from 0.5 at 0^{th} day to 0.9 at 5^{th} day of inoculation while the changes were 0.6 to 0.9 units in inoculated and 0.3 to 0.5 units in uninoculated controls. The changes in PPO activities were 0.6 to 0.8, 0.5 to 0.7 and 0.4 to 0.7 units in treated, inoculated and uninoculated controls respectively (Christopher *et al.*, 2007).

Chistopher *et al.* (2014) studied the changes in PO and PPO activities in chilli leaf samples after seed treatment with *P. fluorescens* and *B. subtilis* each @ 10g/kg along with prophylactic spraying @ 0.2 per cent at 25 and 75 DAT against fruit rot of chilli caused by *C. capsici*. Treatment with *P. fluorescens* increased the activity from 8.4 units on the day of inoculation to 11.5 units on 7^{th} day and *B. subtilis* increased the activity from 9.5 units to 13.6 units. The activity in inoculated control increased from 6.5 units to 9 units. In healthy plants, the activity remained almost constant throughout the experiment (6.2 to 6.4 units).

Jayapala *et al.* (2019) studied the effect of seed biopriming with *Bacillus* sp. strain BSp.3/aM and subsequent challenge inoculation with *C. capsici* on the activities of PO and PPO on twenty day old chilli seedlings. PO activity in bioprimed + pathogen treated seedlings was the highest (6.49 units) compared to pathogen treated alone (4.97 units) and bioprimed alone (4.25 units) and these were higher than control (3.53 units). PPO activity was also the highest for bioprimed + pathogen treated seedlings (5.81 units). The maximum activity was observed 24 h after pathogen inoculation and thereafter decreased and maintained a stable level but still it was higher than the initial level.

Chilli seedlings treated with *B. amyloliquefaciens* and challenge inoculated with *C. truncatum* exhibited one to two fold increase in PO and PPO activities

compared to those inoculated with pathogen alone. There was a constant increase in activities from 0 hpi (hours post inoculation) to 72 hpi. PO activity reached a maximum of 42.43 units at 72 hpi for *B. amyloliquefaciens* treated + challenge inoculated seedlings compared to 17 units in untreated inoculated control. PPO activity increased from 10.76 units (3 hpi) o 58.86 units (72 hpi) in treated + challenge inoculated seedlings (Gowtham *et al.*, 2018).

P. indica colonized tobacco plants after inoculation with *Phytophthora parasitica* var. *nicotiana* found 1.5 and 2.26 fold increase in PO and PPO activities for two and four days respectively (Bing *et al.*, 2015).

2.4. SEED BIOPRIMING

Seed biopriming is a seed treatment technique which integrates biological and physiological aspects of seed treatment. Biological aspect involves the inoculation of seeds with beneficial microorganisms to protect the seeds and physiological aspect involves controlled seed hydration. Seed biopriming protects the seeds from seed and soil borne pathogens, improves the speed and uniformity of germination and final stand of the crop. (Callan *et al.*, 1991; El-Mohamedy, 2004; Reddy, 2013; Bisen *et al.*, 2015; Ananthi, *et al.*, 2017). When the seeds are bioprimed, the antagonist could proliferate inside the seed ten-folds than the attacking pathogen (Callan *et al.*, 1990). Biopriming provides a complimentary environment for the bioinoculants to proliferate on the seed surface by improving nutrient uptake from seed exudates and increasing initial moisture content of the seeds (Wright *et al.*, 2003).

2.4.1. Standardization of Duration of Seed Priming

Among the different durations (3, 6, 9 and 12 h) of biopriming with humic acid (10 %) tested in chilli, 9 h was found to be the best in improving the speed of germination, germination percentage, seedling length, dry weight and vigour index (Ananthi *et al.*, 2017). Different durations (3, 6, 9 and 12 h) and concentrations (40, 60 and 80 %) of biopriming with *T. viride* and *P. fluorescens*

were tested in chilli and found that biopriming with *T. viride* at 60 per cent for 3 h and *P. fluorescens* at 60 per cent for 12 h gave the highest values for rate of germination, germination percentage, root length, shoot length, biomass production and vigour index (Ananthi *et al.*, 2014a). Seed biopriming of chilli with liquid *Azospirillum* 10 per cent for 9 h or liquid phosphobacteria 15 per cent for 9 h were found to be the best among the different concentrations (10, 15 and 20 %) and durations (3, 6, 9 and 12 h) tested (Ananthi *et al.*, 2014b).

2.4.2. *In vitro* effect of seed biopriming on germination and biometric characters of seedlings

Biopriming of seeds with different biological control agents for varying concentration and time may influence the germination and other growth characters of seedlings (Reddy, 2013). Dhanalakshmi (2013) reported that biopriming chilli seeds with *T. viride* at 60 per cent concentration for 6 h and *P. fluorescens* at 80 per cent concentration for 3 h respectively enhanced speed of germination by 27 per cent and 35 per cent, germination percentage by 35 per cent, root length by 53 per cent and 35 per cent, shoot length by 91 per cent and 76 per cent, dry matter production by 167 per cent and 101 per cent, Seedling Vigour Index - I (SVI – I) by 119 per cent and 80 per cent and SVI – II by 271 per cent and 236 per cent over non-primed seeds.

Seed treatment with *T. harzianum*, *P. fluorescens*, *B. subtilis* and carbendazim at 10 g / kg increased the vigour of chilli seedlings by 122.43, 152.93, 122.25 and 128.15 per cent respectively and decreased the percentage infection of seedling rot and damping off by 61.25, 65.32, 59.43 and 71.61 respectively (Machenahalli *et al.*, 2014).

Ilyas *et al.* (2015) performed biopriming of chilli seeds for a duration of 24 h. The infection of *C. capsici* on seeds was reduced by 79, 76 and 65 per cent respectively on priming with *Bacillus* sp., *T. harzianum* and *P. fluorescens*. Percentage germination and vigor index for the treatments (*Bacillus* sp. – 79 %

and 72 %; *T. harzianum* – 78 % and 81 %, *P. fluorescens* – 72 % and 67 % respectively) were higher compared to untreated control (71 % and 56 %).

Sathya *et al.* (2016) studied the effect of seed biopriming in chilli with *B. amyloliquefaciens* (6% liquid formulation for 12 h) and *P. fluorescens* (8 % liquid formulation for 12 h) and compared it with dry seed treatment (10 g / kg of seed) and metalaxyl (1 g / kg of seed). Between papers method was followed and observations were taken on the 14th day after sowing. Biopriming with *B. amyloliquefaciens* recorded highest population of biocontrol agent on seed wash (20×10^8 cfu / ml), maximum speed of germination (6.6), germination percentage (89), root length (10.8 cm), shoot length (4.5 cm), vigour index (1362) and dry matter production (26.4 mg / 10 seedlings) followed by biopriming with *P. fluorescens* with respective values 17×10^8 cfu / ml, 6.6, 88 per cent, 10.5 cm, 4.5 cm, 1320 and 25.9 mg / 10 seedlings for the above parameters compared to dry seed, metalaxyl and without priming treatments.

Chauhan and Patel (2017a) studied the effect of seed biopriming on percent germination and percent infection of chilli seeds. The germination percentages on biopriming with *P. fluorescens* (86.7), *T. viride* (84.4) and *B. subtilis* (74.5) were higher than that of hydropriming (70.9) and control (69.9). The percentage seed infection were lower on biopriming with *P. fluorescens* (21.3), *T. viride* (23.1) and *B. subtilis* (30.3) compared to hydropriming (32.6) and control (33.2). Seed biopriming of chilli cultivar Ac - 615 with *T. viride* and *P. fluorescens* for 2 h resulted in germination percentage of 84.25 and 86, shoot length of 4.27 cm and 4.01 cm, root length of 8.56 cm and 8.26 cm, seedling length of 12.83 cm and 12.27 cm and VI 1 of 1081 and 1057 respectively (Rai and Behera, 2019).

Chilli seeds soaked in a suspension of *P. fluorescens* strain Pf1 and *B. subtilis* for 12 h showed higher germination percentages (94.16 and 96) and vigour indices (1390.28 and 1404.96) over control (germination-58.01 and vigour index - 464.14) (Bharathi *et al.*, 2004). *B. pumilus* strain INR7 and *B. subtilis* strain IN937b respectively in pearl millet enhanced the vigour index by 64 and 38

per cent and germination percentage by 10 (Raj *et al.*, 2003). Biopriming chilli seeds with *B. pumilus* strain BETL13 @ 10^8 cfu/ml for 1 h increased the primary root length of seedlings by 42.13 per cent, wet weight by three times and dry weight by 2.5 times over non primed seedlings (Amaresan *et al.*, 2012).

Seed biopriming with *Bacillus* sp. strain BSp.3/aM for 6 h in chilli increased the mean shoot length by 2.27 per cent (5.28 cm in control to 5.40 cm in treatment), the mean root length by 5.12 per cent (8.20 to 8.62 %), SVI-I by 8.36 per cent (1268 to 1374) and germination percentage by 4.26 (94 % to 98 %) (Jayapala *et al.*, 2019). Chilli seeds treated with *B. subtilis* recorded 100 per cent germination compared to 90 per cent in the control (Ashwini and Srividya, 2014). Seed biopriming with *B. amyloliquefaciens* resulted in maximum seed germination (84.75 %) and seedling vigour (1423.8) in chilli compared to control with 76.75 per cent germination and SVI - I of 721.45. (Gowtham *et al.*, 2018).

Priming of uniformly rooted tissue culture plantlets of *Boswellia serrata* Roxb. with the endophytic fungus *P. indica* increased the height of the plantlet, number of leaves per plantlet, number of roots per plantlet, root length, fresh weight and dry weight compared to control (Suthar and Purohit, 2012). Hydropriming refers to soaking of seeds in water before sowing which hydrates the seeds to moisture of 25-30 per cent (Pill and Necker, 2001). Hydropriming of chilli seeds for 12 h recorded 81 per cent germination and SVI - I of 1004 (Ananthi, *et al.*, 2017).

2.5. *In vivo* EVALUATION OF SEED BIOPRIMING AND SPRAYING OF BIOCONTROL AGENTS

2.5.1. Effect of Seed Biopriming on Plant Growth, Yield and Control of Plant Pathogens

Seed biopriming with *Pseudomonas* spp. increased the number of branches and 1000 grain weight in safflower (Sharifi, 2012). Biopriming chilli seeds with *P. fluorescens* strain PG01 and *B. polymixa* strain BG25 resulted in higher number

of primary branches per plant, root length and number of fruits per plant than seed treatment with Dithane M-45 and untreated healthy seeds. The incidence of anthracnose was lower for biopriming (12 % for both the treatments) compared to Dithane M-45 (60 %) and control (62 %) (Ilyas *et al.*, 2015). Chauhan and Patel (2017b) observed that PDI of anthracnose or fruit rot caused by *C. capsici* in chilli at 30 DAS was reduced by seed biopriming with *P. fluorescens* (2.6 %), *T. viride* (6.2 %) and *B. subtilis* (7.2 %) compared to hydropriming (13.4 %) and control (18.4 %).

Seed biopriming with *B. pumilus* strain INR7 and *B. subtilis* strain IN937b in pearl millet enhanced the yield by 36 and 33 per cent respectively (Raj *et al.*, 2003). *B. subtlils* isolate AB17 increased the plant height of chilli by 39, root length by 40.44, shoot fresh weight by 42 and root biomass by 47 percentages (Lamsal *et al.*, 2012). Seed biopriming with *B. amyloliquefaciens* in chilli resulted in significant protection of 71 per cent against anthracnose caused by *C. truncatum* and also the maximum plant height (18.32 cm), shoot fresh weight (3.52 g), dry weight (1.53 g) and number of leaves per plant (15.25) at 30 DAS (Gowtham *et al.*, 2018). Biopriming chilli seeds with *Bacillus* sp. strain BSp.3/AM for 6 h reduced the incidence of anthracnose by 20 per cent under greenhouse conditions (Jayapala *et al.*, 2019).

El-Mohamedy *et al.* (2006) reported that biopriming cowpea seeds with *T. harzianum* reduced the root rot caused by *M. phaseolina*, *F. solani* and *R. solani* at pre-emergence (56.3 – 64 % reduction) and at post-emergence stages (57.1 - 64% reduction). El-Mohamedy and Abd El-Baky (2008) reported that seed biopriming in pea with *T. harzianum*, *B. subtilis* and *P. fluorescens* recorded superior control of root rot caused by *F. solani*, *R. solani* and *S. rolfsi* over fungicide seed treatment and the reduction ranged from 72.7 - 84.5, 72.2 - 82.9 and 67.6 - 80.0 per cent after 15, 45 and 60 days of sowing respectively. Meena *et al.* (2012) studied the effect of seed biopriming with *T. harzianum* and *P. fluorescens* (2×10^9 cfu / ml for 24 h) in sorghum against anthracnose caused by *C. graminicola* under field conditions and observed a reduction in disease

severity, increase in plant height and yield by 28.10, 28.20 and 6.59 per cent respectively for *T. harzianum* by 22.40, 15.90 and 4.81 per cent respectively for *P. fluorescens*. The fungal root endophyte *P. indica* colonizes the roots of many plants and provides protection against diseases and abiotic stress and also eventually higher yield. A study revealed that *P. indica* protected barley from *F. graminearum* root rot (Deshmukh and Kogel, 2007).

2.5.2. Effect of Spraying of Biocontrol agents on the Plant Pathogens

Spraying of biocontrol agents to the foliage during crop growth may suppress plant diseases in various crops (Huang *et al.*, 2000). Anand and Bhaskaran (2009) studied the effect of spraying of *T. viride* and *P. fluorescens* (2 % talc based formulations) against fruit rot of chilli caused by *C. capsici*. Two sprays were given, 1st at fruit set stage and the 2nd, 20 days after fruit set. Disease incidence and severity were measured 15 days after the 2nd spray. *T. viride* reduced the disease incidence by 47.5 per cent (DI - 37.52 %) and severity by 44.22 per cent (PDI - 40.76) while *P. fluorescens* reduced the incidence by 53.4 per cent (DI - 33.32 %) and severity by 60.26 per cent (PDI - 35.70). These were compared to carbendazim (0.1 %) spray with incidence of 14.65 per cent (79.5% reduction) severity of 18.05 per cent (139.78 % reduction). Two sprays with biocontrol agents were found to be more effective than one spray.

Two spray treatments with *T. harzianum* and *P. fluorescens* each at 2×10^9 cfu / ml reduced the sorghum anthracnose caused by *C. graminicola* by 33 and 24.8 per cent respectively under field conditions (Meena *et al.*, 2012). Three sprayings of *T. viride* ($5X10^6$ cfu / g) and *P. fluorescens* (2×10^8 cfu / g) against anthracnose in chilli caused by *C. capsici* reduced the disease incidence by 31.39 and 24.31 per cent and PDI by 35.93 and 29.66 respectively compared to the control. The yield was increased by 46.13 per cent in case of spraying with *T. viride* and 26.94 per cent in case of *P. fluorescens* (Kamble *et al.*, 2015). Three sprayings of *T. viride* (2 %) reduced the average PDI of anthracnose of chilli caused by *C. capsici* by 52.97 (PDI – 19.84) and *P. fluorescens* reduced the

disease by 41.73 per cent (PDI – 24.58) over control (PDI – 42.19) (Lokhande *et al.*, 2019).

Kilian *et al.* (2000) reported an increase in yield of potato by spraying the leaves with *B. subtilis* strain FZB24. Ji *et al.* (2018) reported that *B. amyloliquefaciens* strain CNU114001 reduced *Sclerotinia sclerotiorum* rot on tomato plants by 52 per cent over control. Spray treatment of *B. amyloliquefaciens* strain S13-3 ($1.5x10^8$ cfu/ml) reduced the severity of anthracnose of strawberry caused by *C. gloeosporioides* (Yamamoto *et al.*, 2014). Three sprays of carbendazim (0.1 %) at 20 days interval 10 days after inoculation (DAI) reduced chilli anthracnose caused by *C. capsici* by 55.68 per cent and increased the yield by 46.07 per cent over control (Gopinath *et al.*, 2006).

2.5.3. Effect of Seed Biopriming and Spraying of Biocontrol Agents on Plant Growth, Yield and Control of Plant Pathogen

Seed biopriming (@ 2×10^9 cfu / ml for 24 h) followed by two spraying treatments with *T. harzianum* and *P. fluorescens* in sorghum reduced anthracnose caused by *C. graminicola* by 43.9 and 30.3 per cent respectively under field conditions (Meena *et al.*, 2012). Seeds of bhendi bioprimed with *P. fluorescens* (60 %) for 12 h followed by foliar spray @ 2g / L at 30 and 45 DAS improved the plant growth and development and increased the seed yield by 49 per cent (Mariselvam, 2012). Seed treatment, seedling dip, soil and foliar application of *P. fluorescens* and *B. subtilis* reduced the fruit rot of chilli caused by *C. capsici* in field by 59.25 and 57.84 per cent with PDI 9.38 and 9.70 respectively. The reduction obtained on treatment with carbendazim was only 51.82 per cent (PDI-11.09) over control (PDI-23.02) (Bharathi *et al.*, 2004).

Christopher *et al.* (2014) studied the effect of seed treatment @ 10g / kg followed by prophylactic spraying of *B. subtilis* (0.2 %) 25 and 75 DAT on chilli under greenhouse conditions. Incidence of fruit rot was decreased by 28.97 per cent (64.9 % in control to 46.1 % in treatment), plant height was increased by 68.89 per cent (58.5 cm to 98.8 cm), number of fruits per plant was increased by

126.57 per cent (42 to 96) and fruit yield (g / plant) was increased by 118.92 per cent (160.6g to 351.6g). *B. subtilis* treatment was found better than the chemical check mancozeb (disease incidence - 46.9 %, plant height - 94.2 cm, 95 fruits per plant and fruit yield of 348 g / plant).

Amaresan *et al.* (2014) studied the effect of seed treatment and foliar application of talc based formulations of *B. subtilis* strain BECL11 and *B. amyloliquefaciens* strain BECR2 on the yield and incidence of anthracnose in chilli and compared it with seed treatment of thiram (2.5 g / kg seed) and foliar spray of fytolan (copper oxychloride) (2.5 g / L) as the chemical check. The PDI of anthracnose on leaves was 31.33 for BECL11 and 28.00 for BECR2 while the PDI for chemical check was 14.67 and control was 28.67. Considerable reduction (66.67 %) in anthracnose of fruits treated with BECL11 (PDI - 8.00) compared to control (PDI - 24.00) was observed and was lower than the chemical check (PDI - 12.00). BECR2 reduced the anthracnose on fruits by 44.46 per cent (PDI - 13.33). The yield was also increased by the treatment BECL11 (7.72 t / ha) and BECR2 (7.05 t / ha) compared to control (5.15 t / ha).

Materials and Methods

3. MATERIALS AND METHODS

The present study entitled 'Seed biopriming and spraying at fruit set for the management of chilli anthracnose caused by *Colletotrichum capsici* (Sydow.) Butler and Bisby' was carried out at Department of Plant Pathology, College of Agriculture, Vellayani and Coconut Research Station, Balaramapuram during the period 2017-2019.

3.1. COLLECTION OF CHILLI SEEDS AND ISOLATION OF SEED BORNE MICROFLORA

3.1.1. Collection of Chilli Seeds

Chilli seed samples were collected from five agro-ecological zones of Kerala viz. Northern, Central, Southern, Special Problem Area and High Range zones. A minimum of three sets of seed samples were collected from each zone.

3.1.2. Determination of Percentage of Infected Seeds

The percentage of infected seeds were determined by standard moist blotter method (ISTA, 1985). Three layers of sterilized moistened filter paper discs were placed at the base of sterile petriplates (9 cm in diameter). Twenty five chilli seeds were kept equidistantly on each petriplate and were covered with lids. Four replications were maintained for each treatment and 100 seeds were observed for each replication. The plates were incubated at room temperature (28±2°C) for seven days and observations were taken. Seeds showing fungal growth and black or brown dicolouration were considered infected and the infection percentage was calculated.

3.1.3. Isolation of Seed Borne Microflora.

3.1.3.1. Isolation of Externally and Internally Seed Borne Microflora

The method developed by Suryanarayana and Bhombe (1961) was used for the study. About 100 seeds were selected at random from each seed sample.

Externally seed borne fungi and bacteria were isolated by placing the seeds without surface sterilization on PDA (Appendix I) and Nutrient Agar (NA) (Appendix I) media respectively and then incubated at room temperature. About 10 seeds were placed in each petriplate. For the isolation of internally seed borne microflora, the seeds were surface sterilized using 0.1 per cent mercuric chloride for one min followed by three washings in sterile distilled water. These seeds were then placed on PDA for the isolation of fungi and on NA for the isolation of bacteria @ 10 seeds per plate and incubated at room temperature.

The petriplates were examined periodically for 10 days. When fungal growth was initiated, small bits were transferred into PDA slants and pure cultured. Bacterial cultures obtained were transferred to NA slants. The characteristics of the fungi were studied by slide culture technique. Permanent slides were prepared from the cultures and observed under microscope. Width of mycelia, conidial shape and size, and presence of fruiting bodies if any were noted. The bacterial isolates obtained were quadrant streaked on NA plates and individual colony characters were studied. Each bacterial isolate was Gram stained and observed under microscope.

3.2. PATHOGENICITY STUDIES

3.2.1. Survey and Collection of Disease Samples

Chilli fields in five different locations of Kerala were surveyed and fruits showing symptoms of anthracnose were collected. The survey locations were College of Agriculture (COA) Vellayani, Regional Agricultural Research Station (RARS) Kumarakom, College of Horticulture (COH) Thrissur, RARS Ambalavayal and College of Agriculture Padanakkad. From each field, 10 plants were randomly selected and the number of infected and healthy fruits were counted. The incidence of fruit rot was calculated by using the formula given by Machenahalli (2014). About 100 plants were observed in each location for assessing the disease incidence.

$$\text{Per cent disease incidence} = \frac{\text{Number of infected fruits}}{\text{Total number of fruits observed}} \times 100$$

The severity of the disease was determined by calculating the Percent Disease Index (PDI). A standard score chart was used for this purpose and scoring was done based on a 0-4 disease scale given by Vishwakarma and Sitaramaiah (1986) (Table 1, Plate 1). Accordingly different scores were assigned to fruits based on the percentage fruit area infected. PDI was calculated using the formula given by Mc Kinney (1923). Ten plants were selected randomly at each location and 10 fruits were observed in each selected plant for calculating the PDI.

$$\text{PDI} = \frac{\text{Sum of all individual disease ratings}}{\text{Total number of fruits observed} \times \text{Maximum disease grade}} \times 100$$

Table 1. Score chart for assessing severity of chilli fruit rot caused by *C. capsici*

Grade	Fruit area infected
0	Healthy
1	1-5%
2	6-25%
3	26-50%
4	51-100%

3.2.2. Isolation of the Pathogen

Microscopic slides were prepared from tissue scrapings of infected chilli fruits and the presence of *C. capsici* was confirmed. Such fruits were selected for the isolation of the pathogen. Small pieces of diseased tissue along with healthy portions were cut and surface sterilised in 0.1 per cent mercuric chloride solution for 30 seconds and washed thrice in sterile distilled water. These bits were then dried using sterile tissue paper and transferred to PDA and incubated at room

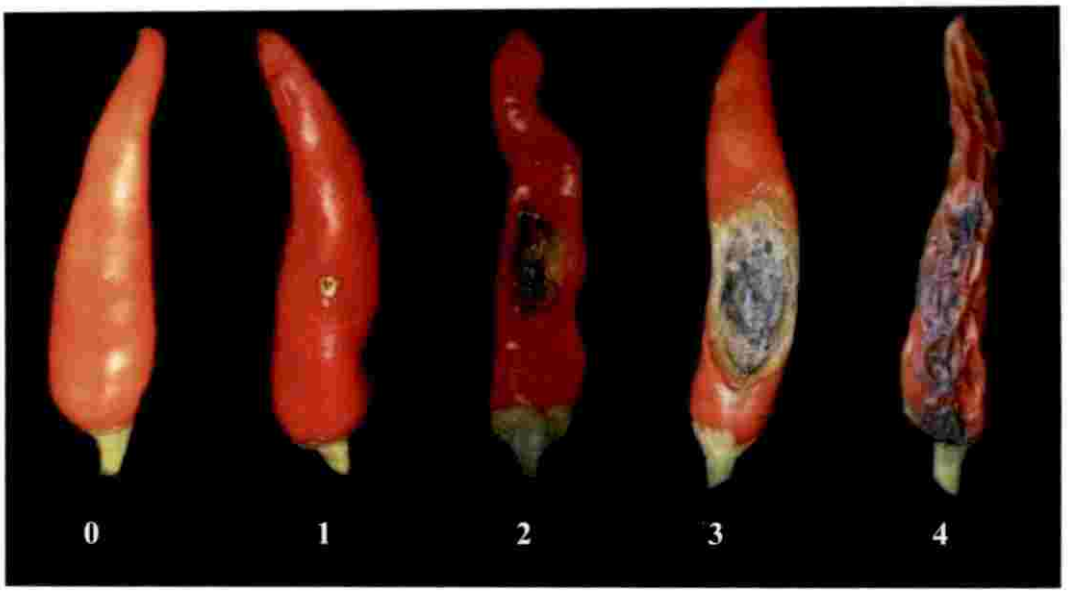

Plate 1. Score chart for assessing the severity of fruit rot disease in chilli caused by *C. capsici*

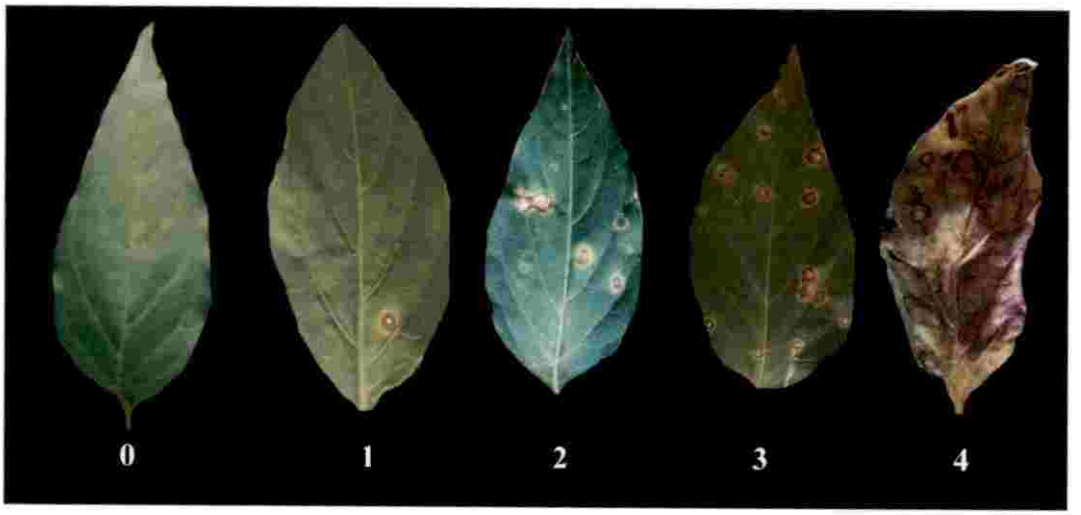

Plate 2. Score chart for assessing the severity of anthracnose in leaves of chilli caused by *C. capsici*

temperature for three days. Chilli seeds showing infection of *C. capsici* were also taken for isolation. After incubation fungal bits obtained were transferred to PDA slants and also to another set of petriplates. Later these cultures were pure cultured by single spore isolation.

3.2.3. Proving the Pathogenicity of the Isolates of *C. capsici*

Pathogenicity of all the five isolates obtained were proved using the disc inoculation method developed by Sander and Kortsen (2003). Detached fruits of susceptible chilli variety 'Vellayani Athulya' were surface sterilized with 70 per cent ethanol and air dried for 5 min to remove the alcohol. Pinpricks were made on the surface of the fruits and a five mm mycelial disc of the pathogen was placed on the injured area. Fruits pinpricked but without pathogen served as control. The inoculated fruits were kept for incubation at room temperature in petriplates provided with moist tissue paper at the base and moist cotton in the lid which provided the required humidity. The fruits were examined at definite intervals for symptom development and progress. After the symptom was developed, re-isolation of pathogen was done. The cultures thus obtained were compared with originally isolated cultures and were maintained for further studies.

3.2.4. Single Spore Isolation and Maintenance of Pure Culture

Single spore isolation technique (Sharma *et al.*, 2005) was followed to obtain pure culture of the isolates. Spore masses of cultures obtained in petriplates were picked up and transferred to sterile distilled water in testubes and shaken well to form uniform spore suspension. From this 0.1 mL was transferred to petriplates containing solidified water agar (2 %) and spread evenly with a spreader. These plates were observed under microscope, single spores were located and marked with a marker pen. The plates were then kept for incubation under room temperature. The fungal growth at marked portions were scooped out and transferred to PDA slants. These purified cultures were periodically subcultured at monthly intervals.

3.2.5. Characterization of the Isolates of *C. capsici*.

The morphological variation among the *C. capsici* isolates obtained were determined by studying the cultural and conidial characteristics (appearance of the colony, colour and size of mycelium, size of acervuli, and shape and size of spores).

3.2.5.1. Cultural Characterization

Five mm mycelial discs of seven day old cultures of the isolates of *C. capsici* were transferred to sterile petriplates containing solidified PDA medium. The petriplates were incubated at room temperature (28±2°C) for two weeks. Three replications were maintained for each culture. Diameter of the mycelial growth of cultures were measured at 3rd, 5th, 7th, 10th and 15th day of incubation (Roy *et al.*, 2013). Observations were taken on appearance of mycelia, radial growth, colour, zonation and sporulation of the cultures.

3.2.5.2. Morphological and Conidial Characteristics

Observations on microscopic characters were done through slide culture technique. For this, slide culture units were prepared and autoclaved. A slide culture unit consisted of blotter papers placed at the lid and bottom, a microscopic slide, coverslips and two broken glass rods. After autoclaving, the unit was taken inside the laminar air flow chamber and the blotter papers were moistened with sterile water. The slide was placed above the blotter paper supported by the broken glass rods. A 10 mm x 10 mm bit of water agar was placed above the glass slide. The four edges of the agar bit was inoculated with mycelia and spores of the pathogen isolates. Then the cover slip was placed over the agar bit (Cai *et al.*, 2009). The unit was incubated at room temperature and observations were taken 24 - 48 h after incubation. The mycelial and appressorial characters were studied by this method.

Conidial characteristics included shape and size of conidia, number and length of setae and diameter of acervulus. Cultures of *C. capsici* isolates on PDA medium grown for 15-20 days were used for the study. After sporulation of the cultures, the conidia were harvested and transferred to microscopic slides containing one to two drops of lactophenol cotton blue stain (Appendix II). The slides were then observed under compound microscope with ZEN camera and the length and width of 30 conidia were measured. Similarly acervuli and setae were selected and observations on size of acervuli and length of setae were made for ten numbers each.

3.2.6. Virulence testing of the *C. capsici* Isolates

Fruits of chilli variety 'Vellayani Athulya' were collected and washed with tap water, wiped with 70 per cent ethanol and air dried. The fruits were separated into five sets and each set was inoculated with a different isolate of *C. capsici*. Culture discs were used for the inoculation. The experiment was repeated for fruits at three different growth stages *viz.,* immature green fruits, mature green fruits and ripe red fruits. The fruits were incubated in a moist chamber at room temperature and observations on symptom development and lesion length were taken on 3rd, 5th, 7th, 10th and 15th day after inoculation.

3.3. *In vitro* SCREENING OF SELECTED BIOCONTROL AGENTS AGAINST *C. capsici*

The biocontrol agents for the screening *viz*., *T. viride* (KAU), *P. fluorescens* PN 026 (KAU), *B. subtilis* VLY 62, *B. pumilus* VLY 17 and *B. amyloliquefaciens* VLY 24 were obtained from the Department of Microbiology, College of Agriculture, Vellayani. *P. indica* culture was obtained from Department of Plant Pathology, College of Agriculture, Vellayani.

Dual culture technique was adopted for the *in vitro* screening of biocontrol agents against *C. capsici*. The fungal biocontrol agents *viz*., *T. viride* and *P. indica* were screened against *C. capsici* according to the methodology given by Mishra *et*

al. (2011). A five mm culture disc of the pathogen was placed 2.5 cm away from the periphery of the petriplates containing solidified PDA medium. After three days a five mm disc of *T. viride* culture was placed opposite to the pathogen 2.5 cm away from periphery. For screening *P. indica* against *C. capsici* initially five mm culture disc of *P. indica* was placed 2.5 cm away from the periphery of the petriplate containing PDA and *C. capsici* was placed three days later. Control plates containing five mm disc of pathogen placed at the centre were maintained for both the biocontrol agents. Three replications were maintained for both the treatments.

The antagonistic property of the four bacterial antagonists *viz., P. fluorescens*, *B. subtilis*, *B. pumilus* and *B. amyloliquefaciens* were screened against *C. capsici* by the methodology described by Nguille *et al.* (2010). Both PDA and NA media were prepared and autoclaved. Equal volume of both the media were poured into a conical flask and mixed thoroughly. These were then transferred to sterile petriplates and allowed to solidify. A five mm plug of the pathogen was placed at the centre of each petriplate and allowed to grow. After three days the bacterial biocontrol agent was streaked on both sides of the pathogen, the two streaks being parallel to each other. Three replications were maintained for each treatment. A control plate containing pathogen alone was also maintained. The plates were incubated at room temperature. Observations were taken when the pathogen completely covered the control plate. The inhibition zone formed between the pathogen and streaked bioagent was measured. The percentage inhibition of mycelia growth of pathogen was also calculated.

Percentage suppression of mycelia growth of the pathogen over control was determined using the formula given by Vincent (1927) as follows:

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

Where,

I = Per cent inhibition or suppression of mycelia growth

C = Diameter of pathogen growth in control plate

T = Diameter of pathogen growth in treatment plate

3.4. *In vitro* ASSAYS ON FRUITS

The biocontrol agents used for the fruit assay were *T. viride* (KAU isolate), *P. fluorescens* PN 026 (KAU isolate), *B. subtilis* VLY 62, *B. pumilus* VLY 17, *B. amyloliquefaciens* VLY 24 and *P. indica.*

3.4.1. Preparation of Spore Suspension of Biocontrol Agents

T. viride spore suspension was prepared by flooding the surface of five day old culture in PDA plates with sterile water and scraping the surface with a bent glass rod. The suspension was filtered through sterile muslin cloth and the spore count was determined using haemocytometer and was adjusted to 10^8 spores / ml by adding sterile water (Bankole and Adebanjo, 1996). The same procedure was repeated for preparation of spore suspension of *P. indica.*

P. fluorescens and *Bacillus* strains were heavily cross streaked respectively on King's B (Appendix I) and NA agar plates. The plates were incubated for 48 h at room temperature. Then the plates were drenched with 10 ml of sterile distilled water and the bacterial cells were suspended in it by using a sterile glass spreader. The suspension was collected in sterile conical flasks. The Optical Density (OD) value was adjusted to 0.6 at 660 nm using sterile distilled water so that the suspension contained approximately 10^8 cfu ml^{-1} (Athira, 2018).

3.4.2. *In vitro* Screening of Biocontrol Agents against *C. capsici* on Detached Chilli Fruits

Healthy chilli fruits of uniform maturity and size belonging to variety Vellayani Athulya were collected. The fruits were washed first with tap water, then with sterile water, wiped with 70 per cent ethanol and air dried. These were then dipped in the prepared suspensions of respective fungal and bacterial

biocontrol agents for 15 min, taken out and allowed to shade dry. After drying pinpricks were given on the surface of fruits using a sterile needle and five mm culture disc of the pathogen was placed over the injured area (Batta, 2007). The inoculated fruits were kept for incubation at room temperature in Petriplates provided with moist tissue paper at the base and moist cotton in the lid which provided the required humidity. Fruits not dipped in bioagents but inoculated with pathogen served as control. The fruits were examined at 3rd, 5th, 7th, 10th and 15th day after inoculation for symptom development and lesion progress. Three replications were maintained and twenty fruits were taken for each replication.

3.4.3. Estimation of Peroxidise (PO) and Polyphenol Oxidase (PPO) activity in Chilli Fruits

Healthy chilli fruits of uniform maturity and size belonging to variety Vellayani Athulya were collected. The fruits were washed first with tap water, then with sterile water, wiped with 70 per cent ethanol and air dried. These were then dipped in the prepared suspension of respective fungal and bacterial biocontrol agents for 15 min, taken out and allowed to shade dry. These fruits were subjected to PO and PPO enzyme anaysis after 0, 24, 48 and 72 h to study the effect of biocontrol agents on the enzyme activity. The same procedure was repeated on another set of fruits. After drying, pinpricks were given on the surface of fruits using a sterile needle and five mm culture disc of the pathogen was placed over the injured area. These fruits were then subjected to PO and PPO enzyme analysis at 0, 24, 48 and 72 h after inoculation of pathogen to study the combined effect of biocontrol agents and pathogen on the enzyme activity. Untreated fruits served as control for both the experiments.

3.4.3.1. Estimation of PO Enzyme Activity

PO enzyme activity was estimated according to the spectrophotometric method developed by Srivastava (1987). One gram of fruit sample was ground with five ml of 0.1 M sodium phosphate buffer (pH 6.5) (Appendix III) in a pre-chilled pestle and mortar. A pinch of polyvinyl pyrrolidone (PVP) was added to

the mixture while grinding. It was filtered through cheese cloth and the filtrate was taken in 1.5 ml eppendorf tubes and centrifuged at 5000 rpm for 15 min at 4°C. The supernatant obtained after centrifugation is the enzyme extract. The reaction mixture contained one ml of 0.05 M pyrogallol and 50 µl of enzyme extract. One ml of 1 per cent hydrogen peroxide (H_2O_2) was added to initiate the reaction. One ml of 0.05 M pyrogallol mixed with one ml of 1 per cent H_2O_2 served as blank. After the blank was set in spectrophotometer, the reaction mixture was added to sample cuvettes and changes in absorbance at 420 nm was measured every 30 seconds for three min. PO activity ($mg^{-1}g^{-1}min^{-1}$) was calculated using the formula given below.

$$\text{PO activity} = \frac{\text{Difference in absorbance in one minute}}{50} \times 5000$$

3.4.3.2. Estimation of PPO Enzyme Activity

PPO enzyme activity in the detatched chilli fruits after biocontrol spray but before and after pathogen inoculation was estimated according to the spectrophotometric method developed by Mayer *et al.* (1966). One gram of fruit sample was ground with five ml of 0.1 M sodium phosphate buffer (pH 6.5) in a pre-chilled pestle and mortar. A pinch of polyvinyl pyrrolidone (PVP) was added to the mixture while grinding. It was filtered through cheese cloth and the filtrate was taken in 1.5 ml eppendorf tubes and centrifuged at 5000 rpm for 15 min at 4°C. The supernatant obtained after centrifugation is the enzyme extract. The reaction mixture contained one ml of 0.1 M sodium phosphate buffer and 50 µl of enzyme extract. One ml of 0.01 M pyrocatechol was added to initiate the reaction. One ml of 0.1 M sodium phosphate buffer mixed with one ml of 0.01 M pyrocatechol served as blank. After the blank was set in spectrophotometer the reaction mixture was added to sample cuvettes and changes in absorbance at 495 nm was measured every 30 seconds for three min. PPO activity ($mg^{-1}g^{-1}min^{-1}$) was calculated using the formula given below.

$$\text{PPO activity} = \frac{\text{Difference in absorbance in one minute}}{50} \times 5000$$

3.5. STANDARDISATION OF BIOPRIMING TECHNIQUES

3.5.1. Standardisation of Seed Soaking Time

Chilli seeds of variety Vellayani Athulya were collected from Depatment of Vegetable Science, College of Agriculture, Vellayani.

3.5.1.1. Biopriming of Seeds

Biopriming of chilli seeds was done according to the method described by El-Mougy and Abdel-Kader (2008). Chilli seeds were surface sterilized with 0.1 per cent mercuric chloride solution for one minute and washed with three washings of sterile distilled water. Suspensions of fungal and bacterial biocontrol agents were prepared according to procedure as mentioned in section 3.4.1. Carboxy methyl cellulose (CMC) was added @ 0.1g 10 ml^{-1} of the suspension as an adhesive. For the chemical check 0.1 per cent of carbendazim was prepared by mixing 0.2 g of the fungicide Bavistin (Carbendazim 50 WP) in 100 ml water. For hydropriming the seeds were soaked in sterile distilled water. Chilli seeds were soaked in the respective treatments for different time durations *viz*., 20 min, 1 h, 2 h, 4 h, 8 h and 16 h. After soaking for the specified time, the seeds were taken out and air dried. Seeds without priming served as control. The germination percentage and biometric parameters of the seedlings were determined using the paper towel method.

3.5.1.2. Calculation of Germination Percentage of Seeds

The germination test was carried out following between paper method (ISTA, 1985). Fifty seeds were placed equidistantly between two sheets of the germination paper soaked in water, rolled and tagged and kept for incubation under room temperature. Three replications were maintained for each treatment. Observations on germination percentage and time taken for germination were

taken from 7th day of sowing and continued up to 14th day. The germination percentage was calculated using the formula:

$$\text{Germination \%} = \frac{\text{Number of normal seedlings produced}}{\text{Total number of seeds used}} \times 100$$

3.5.1.3. Observations on Root Length, Shoot Length, Seedling Length, Seedling Dry Weight and Seedling Vigour Index.

Primed seeds (10 numbers) were selected from each treatment and were placed equidistantly between two sheets of the germination paper soaked in water, rolled, tagged and kept for incubation under room temperature. Three replications were maintained for each treatment. Observations on root length, shoot length and seedling length were taken on the 14th day after sowing. Seedling dry weight was obtained by keeping in hot air oven kept at 60°C for 24 hours. Seedling Vigour Index (SVI) was calculated based the formula given by Abdul-Baki and Anderson (1973).

SVI – I = Germination % × Length of seedlings in cm

SVI – II = Germination % × Seedling dry weight in g.

3.6. EFFECT OF SEED BIOPRIMING AND SPRAYING AT FRUIT SET ON CHILLI UNDER POT CULTURE STUDIES

3.6.1. *In vivo* Effect of Seed Biopriming on Chilli

3.6.1.1. Preparation of Inoculum of C. capsici

Potato Dextrose Broth (PDB) was prepared in 250 ml conical flasks and autoclaved. The flasks were then inoculated with five mm culture discs of *C. capsici* and incubated at room temperature. After the complete covering of mycelia and with enough sporulation obtained in the conical flasks, the conidial

Chilli seeds

Suspension of biocontrol agent

Chilli seeds soaked in suspension of biocontrol agent

Seeds placed equidistantly in germination paper

Germination paper rolled and tied after the seeds are placed

Rolled germination paper labelled, soaked in water and kept for incubation

Germinated chilli seedlings - 14 days after incubation

Plate 3. Different steps in standardisation of soaking time of chilli seeds in suspension of biocontrol agents for biopriming

masses were collected and dispersed in sterile water. The concentration of the conidia in water was determined using a haemocytometer and adjusted to 10^6 conidia ml^{-1} using sterile water.

3.6.1.2. Pot Culture Experiment

Seeds of chilli variety Vellayani Athulya were bioprimed according to the method described in 3.5.1.1. using the best three biocontrol agents obtained from the *in vitro* studies. The treatments were as follows:

T1 – *T. viride* (KAU)
T2 – *B. subtilis* VLY 62
T3 – *B. amyloliquefaciens* VLY 24
T4 – Chemical check (Carbendazim 0.1%)
T5 – Inoculated control
T6 – Uninoculated control

The pot culture experiment was conducted at Coconut Research Station, Balaramapuram during March – July 2019 to find out the effect of seed biopriming on the management of anthracnose and fruit rot caused by *C. capsici* and yield and growth parameters of chilli. The experiment was laid out in completely randomised design (CRD) with six treatments and four replications. The crop was raised as irrigated. Manuring and other intercultural operations were done according to package of practices of Kerala Agricultural University.

The conidial suspension of *C. capsici* prepared was sprayed after fruit set to the mature fruits. Disease severity in fruits were scored based on a 0 - 4 scale given by Vishwakarma and Sitaramaiah (1986) and PDI was calculated at 5^{th}, 10^{th} and 15^{th} day after inoculation. The disease severity score on leaves was calculated based on the score chart given by Inglis *et al.* (1988) (Table 2, Plate 2) and PDI was calculated on 5^{th}, 10^{th} and 15^{th} day after inoculation. Plant height was measured at 40, 80 and 120 days after sowing (DAS). Days to flowering was calculated when 50 per cent of the plants in a treatment completed flowering.

Branches per plant was taken 120 DAS. Three harvests of chilli fruits were taken and observations on number of fruits per plant, fruit yield per plant, seeds per fruit, 100 seed weight and seed yield per plant were calculated. The incidence of major diseases and pests during the crop was also observed.

Table 2. Score chart for assessing the severity of anthracnose in leaves of chilli caused by *C. capsici*

Scale	Leaf area infected
0	No disease
1	1-10%
2	11-25%
3	26-50%
4	> 50%
5	Defoliation

3.6.2. *In vivo* Effect of Seed Biopriming and Spraying During Fruit set on Chilli

Pot culture experiment was repeated as in 3.6.1.2. In addition to seed biopriming, the plants were sprayed twice with the suspension of biocontrol agents first when 50 per cent of the plants have set fruits and the second, 15 days after the first spray. Pathogen inoculation was done 48 h after second spray. Observations on PDI of fruits and leaves were taken on 5th, 10th and 15th day after pathogen inoculation. Other observations were taken as same as in 3.6.1.2.

3.7. STATISTICAL ANALYSIS

The data obtained from the experiments were subjected to analysis of variance (ANOVA). The critical difference (CD) was calculated at 5 per cent level of significance and used for the comparison of difference between the treatment

means. Standard error mean and standard deviation of observations were also determined. Statistical analysis of the data was done using both WASP 2.0 and OPSTAT softwares.

Results

4. RESULTS

The present study entitled "Seed biopriming and spraying at fruit set for the management of chilli anthracnose caused by *Colletotrichum capsici* (Sydow.) Butler and Bisby" was undertaken during 2017-2019 at Department of Plant Pathology, College of Agriculture, Vellayani, Thiruvananthapuram and Coconut Research Station, Balaramapuram, Thiruvananthapuram with the objective to study the seed borne nature of chilli anthracnose/fruit rot and the effect of seed biopriming and spraying during fruit set for its management.

4.1. COLLECTION OF CHILLI SEEDS AND ISOLATION OF SEED BORNE MICROFLORA

4.1.1. Collection of Chilli Seeds

Chilli seed samples were collected from five agro-ecological zones of Kerala *viz.,* Northern, Central, Southern, Special Problem Area and High Range zones. A total of 20 sets of seed samples were obtained. The details about the zone of collection, district, location and variety of seeds collected are presented in table 3.

4.1.2. Percentage of Infected Seeds

Most of the chilli seed samples collected were either infected by fungi, bacteria or both. The percentage of seed infection is presented in table 3. The highest percentage of infection was noticed in seed sample of a local cultivar collected from Parassala (43.33 %) followed by seeds of local cultivars collected from Kayamkulam (38.85 %) and Kattappana (38.45 %). The lowest per cent of infection was found in seed sample of Ujwala variety collected from Vellayani (6.62 %).

Table 3. Details of location, variety and percentage of infection of chilli seeds collected from five different agro-ecological zones of Kerala.

Agro-ecological zone	District	Location	Chilli variety	Infection (%)
Northern zone	Kasaragod	Padannakkad	Ujwala	13.34
	Kannur	Kottiyoor	Local variety	28.50
		Payyannur	Local variety	18.51
	Kozhikode	Perambra	Local variety	31.11
High range zone	Wayanad	Ambalavayal	Anugraha	13.44
		Mananthavadi	Local variety	41.36
	Idukki	Kattappana	Local variety	38.45
Central zone	Thrissur	Vellanikkara	Anugraha	11.11
		Pananchery	Local variety	31.11
	Palakkad	Kozhinjampara	Local variety	23.45
	Ernakulam	Aluva	Local variety	13.34
Special problem zone	Kottayam	Kumarakom	Ujwala	13.34
			Local variety	16.44
	Alappuzha	Kayamkulam	Local variety	38.85
Southern zone	Thiruvanan-thapuram	Vellayani	Vellayani Athulya	13.22
			Ujwala	6.62
			Samrudhi	11.11
			Vellayani Thejus	7.81
		Balaramapuram	Ujwala	10.03
		Parassala	Local variety	43.33

4.1.3. Isolation of Seed Borne Microflora

Both externally and internally seed borne fungi and bacteria were isolated from the seeds. Fifteen different isolates of fungi were obtained from the seeds. Among them six belonged to *Aspergillus* spp., three belonged to *Penicillium* spp. Remaining fungi were *Pestalotia* sp., *Curvularia* sp., *Alternaria* sp., *Mucor* sp., *Fusarium* sp. and *C. capsici*. *Aspergillus* spp. and *Penicillium* spp. were found to be the major fungal species frequently associated with the seed samples. *Aspergillus* spp., *Penicillium* spp., *Alternaria* sp., *Fusarium* sp. and *C. capsici* were both externally and internally seed borne. *Pestalotia* sp. and *Curvularia* sp. were externally seed borne and *Mucor* sp. was internally seed borne. Majority of the bacteria isolated from the seeds were externally seed borne except three which were both externally and internally seed borne.

The cultural characters of the fungi were studied by growing them in PDA medium. The upper and rear side view, and colour of the mycelia of the cultures were noted. The morphological characters of the fungi were studied by making microscopic slides from the cultures. The width of mycelium and size and shape of spores were studied. The seed borne nature of the fungi were also noted during the isolation. The details are presented in tables 4a and 4b and plates 4a to 4e.

Eighteen different types of bacteria were also isolated from the seed samples and named from B1 to B18. The shape, colony characters, appearance, pigmentation, texture, seed borne nature and gram reaction of the bacteria were studied and presented in table 5a and 5b and plates 5a to 5c.

4.2. PATHOGENICITY STUDIES

4.2.1. Survey and Collection of Disease Samples

A survey was conducted during June - November 2018 in five different locations of Kerala *viz.*, Vellayani in Thiruvananthapuram, Kumarakom in Kottayam, Vellanikkara in Thrissur, Ambalavayal in Wayanad and Padanakkad in

Table 4a. Characterisation and nature of seed borne fungi isolated from chilli seeds collected from five agro-ecological zones of Kerala

Sl. No.	Fungi isolated from seeds	Appearance		Colour of mycelium	Width of mycelium (µm)*	Spore shape	Spore size (µm)*	Seed borne nature
		Upper side view	Rear side view					
1.	*Aspergillus* sp. (A1)	Light green	White	White	2.46±0.10	Spherical	4.94±1.24	ESB and ISB
2.	*Aspergillus* sp. (A2)	Black	White	White	1.75±0.09	Spherical	5.37±0.10	ESB and ISB
3.	*Aspergillus* sp. (A3)	Green	Yellow	White	2.60±0.09	Spherical	3.88±0.09	ESB and ISB
4.	*Aspergillus* sp. (A4)	Yellow	Light yellow	White	2.01±0.07	Spherical	3.57±0.11	ESB and ISB
5.	*Aspergillus* sp. (A5)	Brown	White	White	1.30±0.14	Spherical	3.20±0.17	ESB
6.	*Aspergillus* sp. (A6)	Orange	Light brown	White	1.37±0.06	Spherical	2.83±0.11	ESB
7.	*Penicillium* sp. (P1)	Olive green	Yellow	White	3.35±0.11	Spherical	2.58±0.12	ESB
8.	*Penicillium* sp. (P2)	Light brown	Dark brown	White	2.78±0.12	Spherical	4.74±0.13	ESB
9.	*Penicillium* sp. (P3)	Dark green	Yellow	White	1.61±0.06	Spherical	3.56±0.04	ESB and ISB

ESB – Externally seed borne, ISB – Internally seed borne

*Mean of five observations

Table 4b. Characterisation and nature of seed borne fungi isolated from chilli seeds collected from five agro-ecological zones of Kerala

Sl. No.	Fungi isolated from seeds	Appearance		Colour of mycelium	Width of mycelium (µm)*	Spore shape	Spore size (µm)*	Seed borne nature
		Upside view	Rear view					
10.	*Pestalotia* sp.	White with black spore mass	Off white	White	1.32±0.07	Cylindrical	19.01±0.19 X 6.23±0.10	ESB
11.	*Curvularia* sp.	White	Off white	White later turns to grey	2.60±0.13	Curved	22.49±0.14 X 9.53±0.14	ESB
12.	*Alternaria* sp.	Dark grey with concentric zonations	Light black	Grey	3.73±0.23	Club shaped	30.47±0.24 X 12.09±0.10	ESB and ISB
13.	*Mucor* sp.	White with black spore heads	White	White	4.02±0.16	Spherical	4.57±0.55	ISB
14.	*Fusarium* sp.	Slightly pink	Light pink	White turns to slightly pink	2.73±0.15	Sickle shaped	16.21±0.18 X 3.16±0.14	ESB and ISB
15.	*C. capsici*	White	Off white with concentric zonations	White	2.49±0.51	Sickle shaped	19.55±1.43 X 2.72±0.58	ESB and ISB

ESB – Externally seed borne, ISB – Internally seed borne

*Mean of five observations

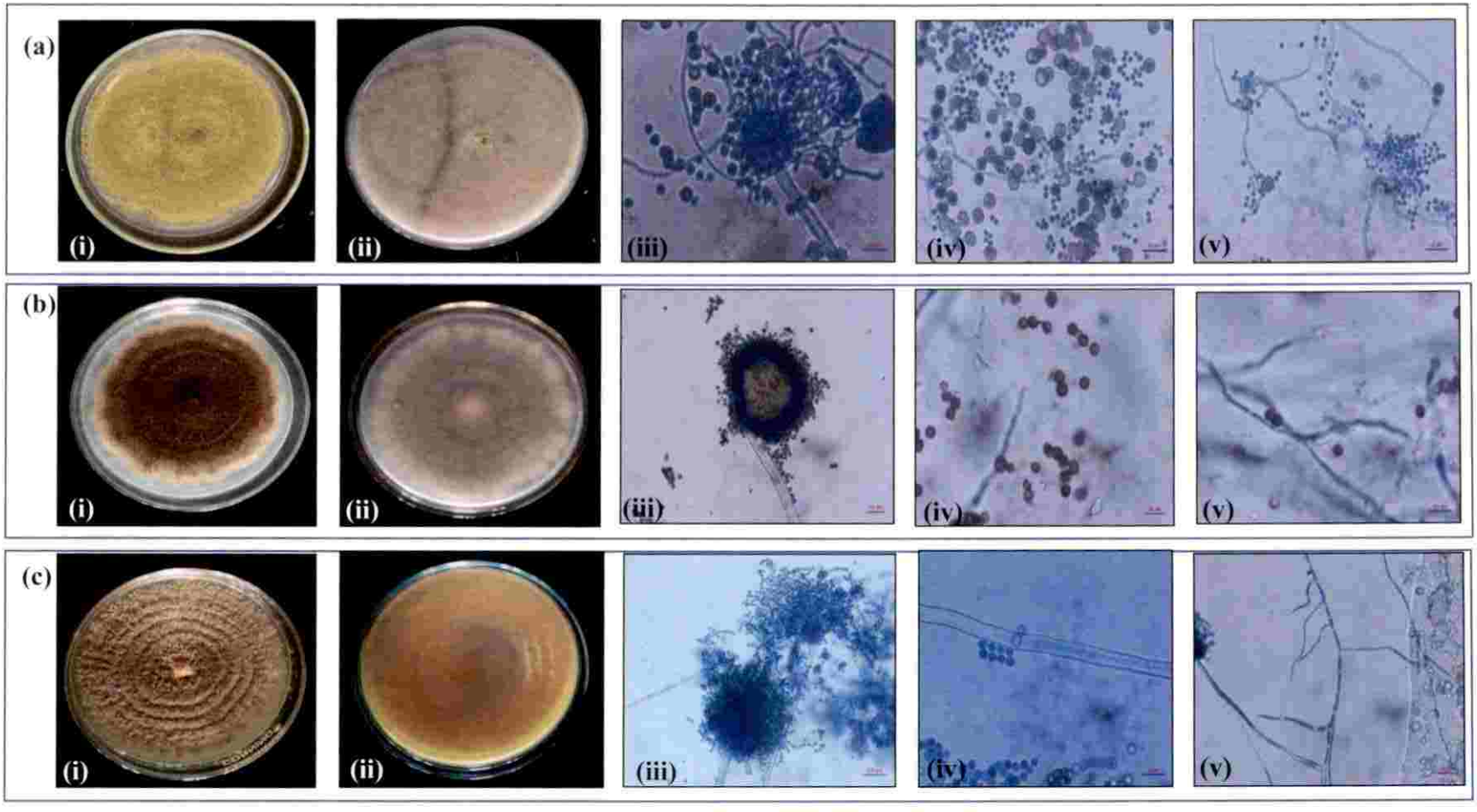

Plate 4a. Morphological and cultural characters of seed borne fungi isolated from chilli seeds collected from five agro-ecological zones of Kerala (a) *Aspergillus* sp. Isolate - A1, (b) *Aspergillus* sp. Isolate - A2 and (c) *Aspergillus* sp. Isolate - A3 from chilli seeds. (i) Upper side view, (ii) Rear side view, (iii) Conidiophore (400X), (iv) Conidia (1000X) and (v) Mycelia (1000X)

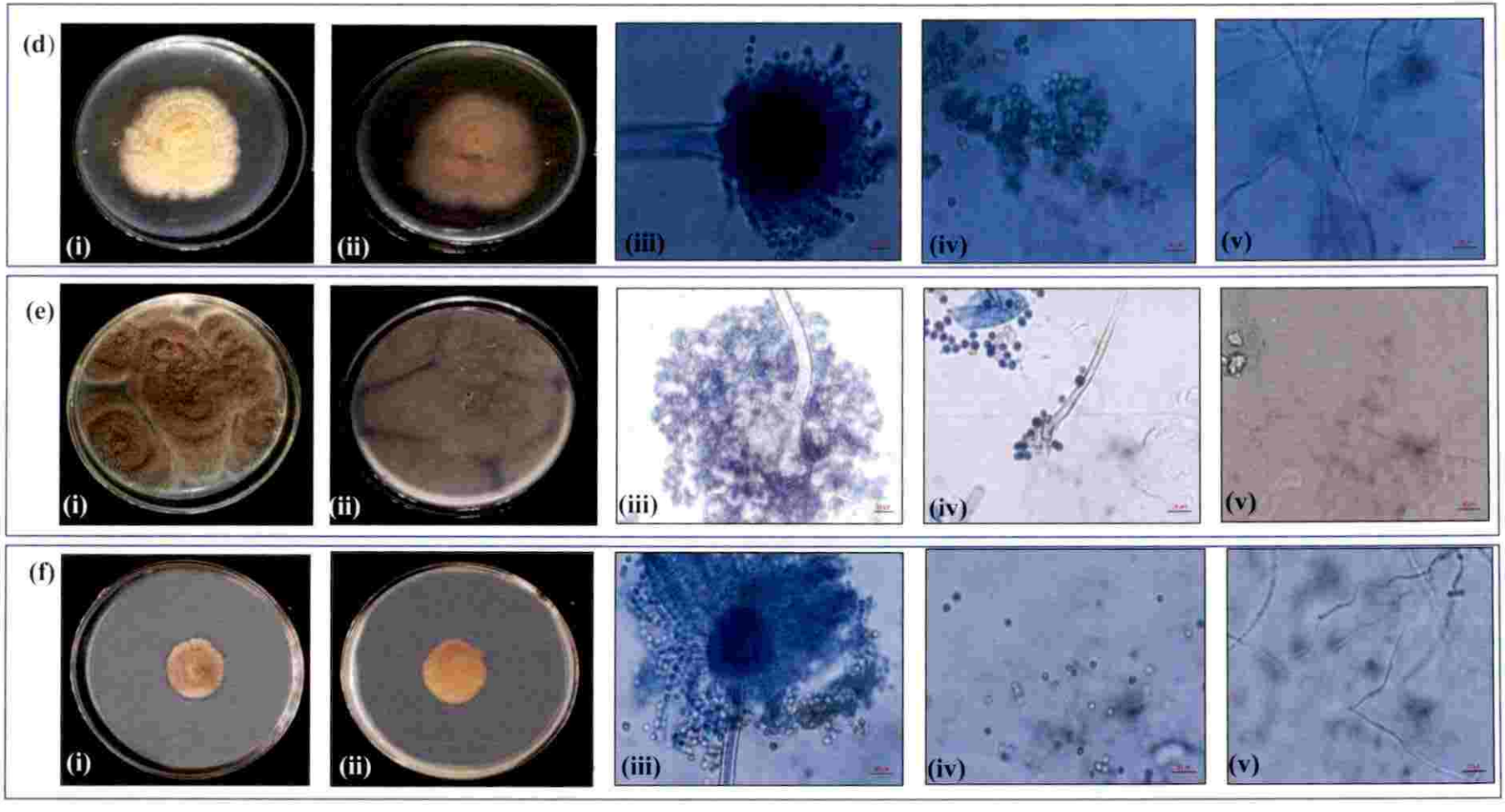

Plate 4b. Morphological and cultural characters of seed borne fungi isolated from chilli seeds collected from five agro-ecological zones of Kerala (d) *Aspergillus* sp. Isolate - A4, (e) *Aspergillus* sp. Isolate - A5 and (f) *Aspergillus* sp. Isolate - A6 from chilli seeds. (i) Upper side view, (ii) Rear side view, (iii) Conidiophore (400X), (iv) Conidia (1000X) and (v) Mycelia (1000X)

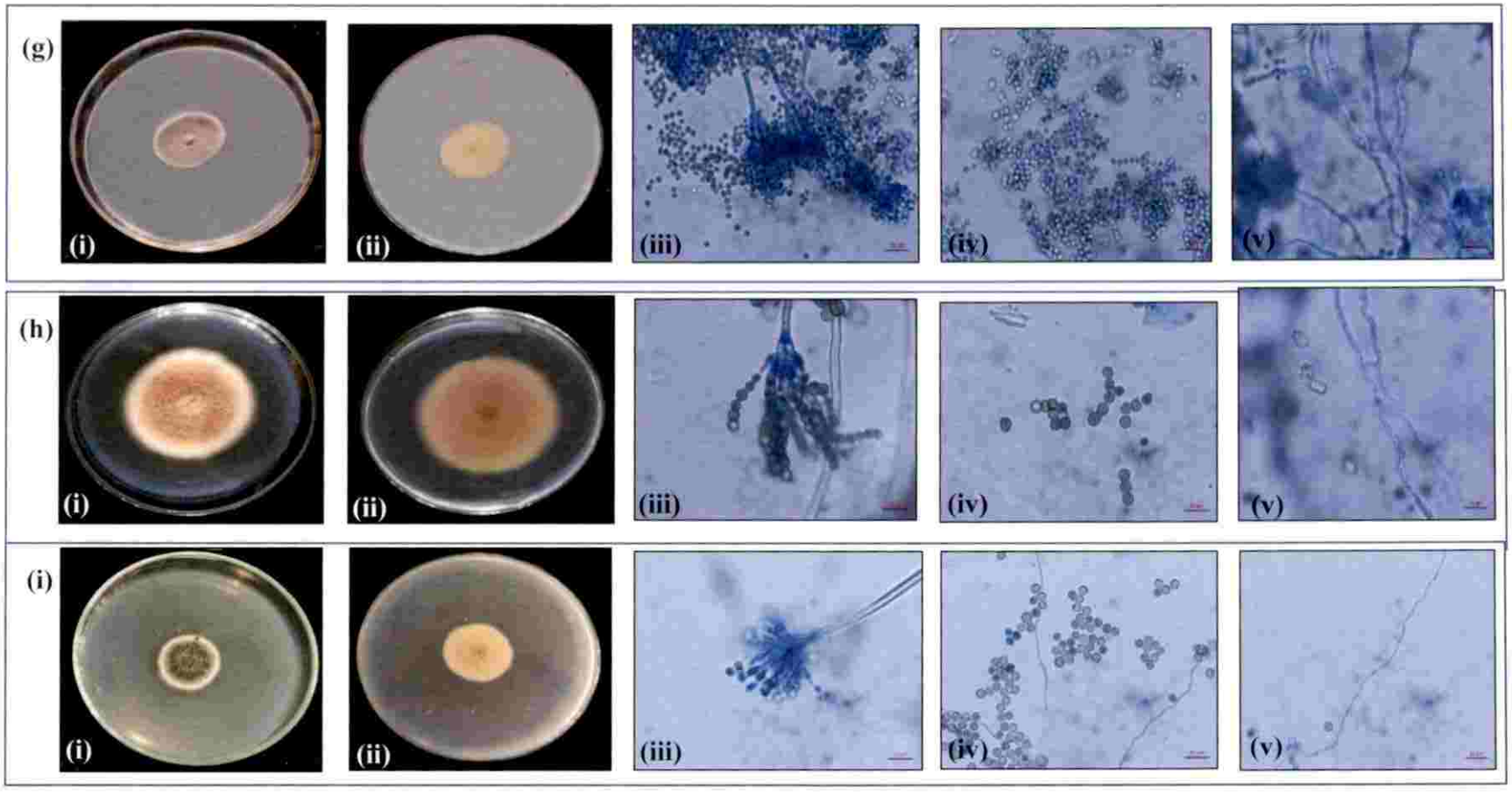

Plate 4c. Morphological and cultural characters of seed borne fungi isolated from chilli seeds collected from five agro-ecological zones of Kerala (g) *Penicillium* sp. Isolate - P1, (h) *Penicillium* sp. Isolate - P2 and (i) *Penicillium* sp. Isolate - P3 from chilli seeds. (i) Upper side view, (ii) Rear side view, (iii) Conidiophore (400 X), (iv) Conidia (1000X) and (v) Mycelia (1000X)

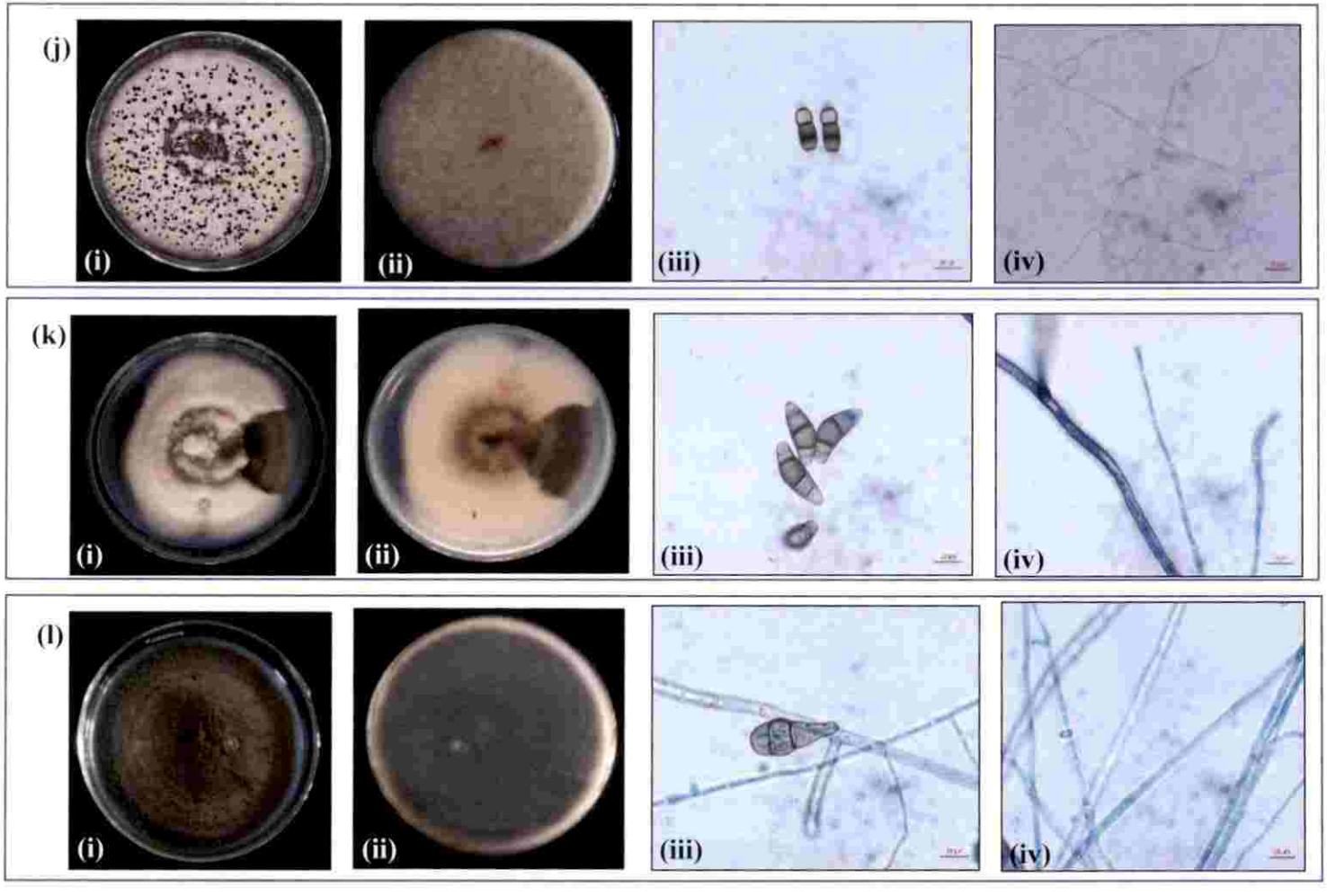

Plate 4d. Morphological and cultural characters of seed borne fungi isolated from five agro-ecological zones of Kerala (j) *Pestalotia* sp., (k) *Curvularia* sp. and (l) *Alternaria* sp. isolated from chilli seeds. (i) Upper side view, (ii) Rear side view, (iii) Conidia (1000 X) and (iv) Mycelia (1000X)

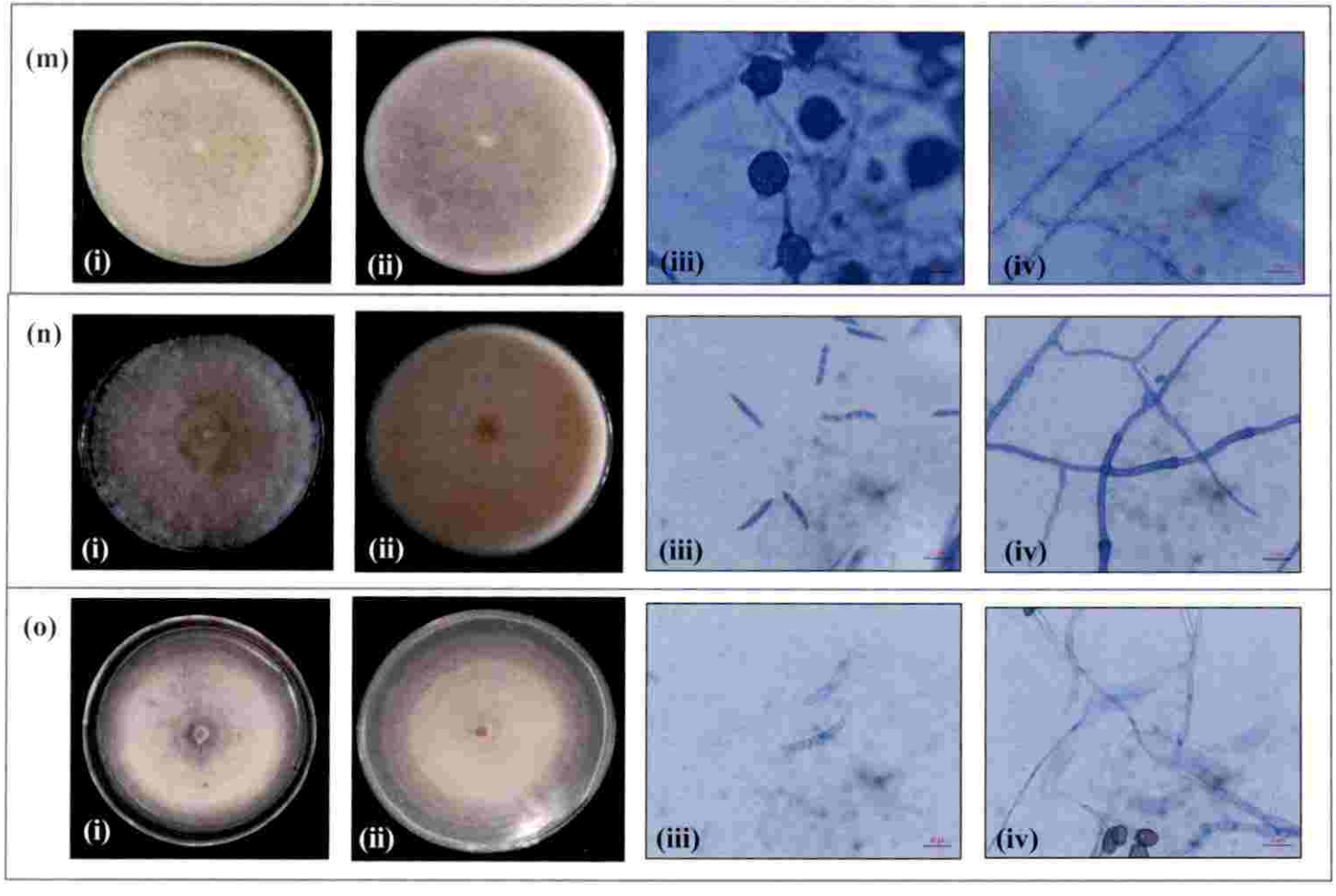

Plate 4e. Morphological and cultural characters of seed borne fungi isolated from five agro-ecological zones of Kerala (m) *Mucor* sp., (n) *Fusarium* sp. and (o) *C. capsici* isolated from chilli seeds. (i) Upper side view, (ii) Rear side view (iii), Conidia (1000X) and (iv)mycelia (1000X)

Table 5a. Characterisation and nature of seed borne bacterial isolates obtained from chilli seeds collected from five agro-ecological zones of Kerala

Sl. No.	Isolates	Shape	Colony characters			Appearance	Gram reaction	Pigmentation	Texture	Seed borne nature
			Form	Elevation	Margin					
1.	B-1	Coccus	Circular	Raised	Entire	Shiny	Negative	Non-Pigmented (cream)	Smooth	ESB
2.	B-2	Rod	Circular	Convex	Entire	Shiny	Positive	Cream	Smooth	ESB
3.	B-3	Bi - coccus	Circular	Raised	Erose	Shiny	Negative	Cream	Smooth	ESB
4.	B-4	Coccus	Irregular	Convex	Erose	Dull	Negative	Cream	Rough	ESB
5.	B-5	Long rods	Circular	Raised	Entire	Shiny	Negative	Cream	Smooth	ESB
6.	B-6	Rod	Circular	Raised	Entire	Shiny	Negative	Cream	Smooth	ESB
7.	B-7	Rod	Circular	Raised	Undulate	Dull	Positive	Cream	Rough	ESB and ISB
8.	B-8	Rod	Circular	Convex	Entire	Shiny	Negative	Cream	Smooth	ESB
9.	B-9	Coccus	Circular	Convex	Entire	Shiny	Negative	Yellow	Smooth	ESB

ESB – Externally seed borne, ISB – Internally seed borne

Table 5b. Characterisation and nature of seed borne bacterial isolates obtained from chilli seeds collected from five agro-ecological zones of Kerala

Sl. No.	Isolates	Shape	Colony characters			Appearance	Gram reaction	Pigmen-tation	Texture	Seed borne nature
			Form	Elevation	Margin					
10.	B-10	Rod	Circular	Convex	Entire	Shiny	Positive	Cream	Smooth	ESB
11.	B-11	Rod	Circular	Raised	Undulate	Dull	Positive	Cream	Smooth	ESB
12.	B-12	Coccus	Circular	Raised	Erose	Dull	Negative	Cream	Rough	ESB
13.	B-13	Rod	Filamentous	Flat	Filamentous	Shiny	Negative	Cream	Smooth	ESB
14.	B-14	Coccus	Circular	Convex	Entire	Shiny	Positive	Orange	Smooth	ESB
15.	B-15	Rod	Irregular	Convex	Erose	Dull	Positive	Cream	Rough	ESB and ISB
16.	B-16	Rod	Circular	Raised	Undulated	Dull	Negative	Cream	Rough	ESB
17.	B-17	Long rods	Cicular	Convex	Entire	Shiny	Negative	Cream	Smooth	ESB
18.	B-18	Coccus	Irregular	Raised	Lobate	Dull	Positive	Orange	Rough	ESB and ISB

ESB – Externally seed borne, ISB – Internally seed borne

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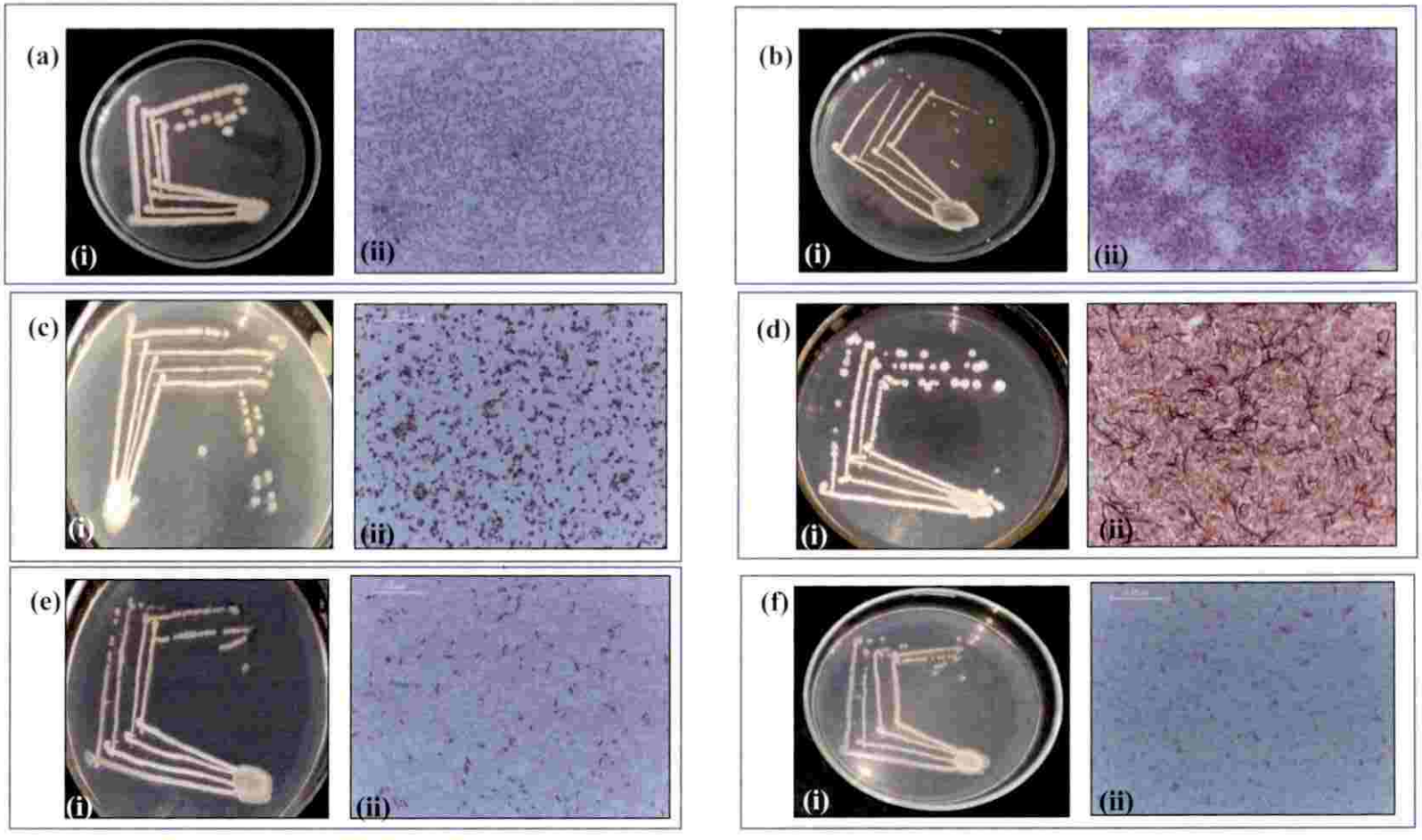

Plate 5a. Bacterial isolates obtained from chilli seeds collected from five agro-ecological zones of Kerala (a) B1, (b) B2, (c) B3, (d) B4, (e) B5 and (f) B6. (i) Quadrant streaked petriplates showing individual bacterial colonies and (ii) Gram reaction (1000X) of bacterial isolates

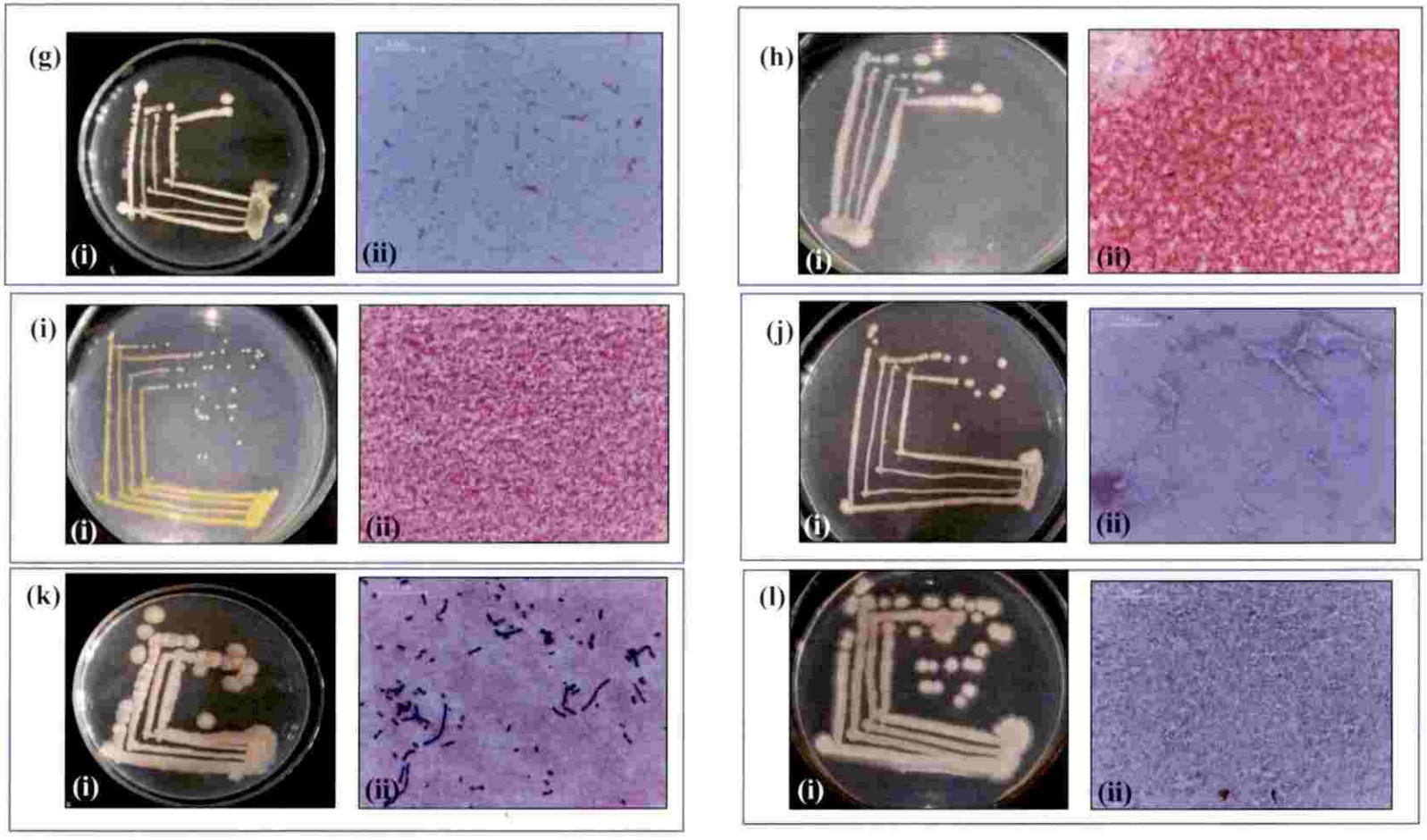

Plate 5b. Bacterial isolates obtained from chilli seeds collected from five agro-ecological zones of Kerala (g) B7, (h) B8, (i) B9, (j) B10, (k) B11 and (l) B12. (i) Quadrant streaked petriplates showing individual bacterial colonies and (ii) Gram reaction (1000X) of bacterial isolates

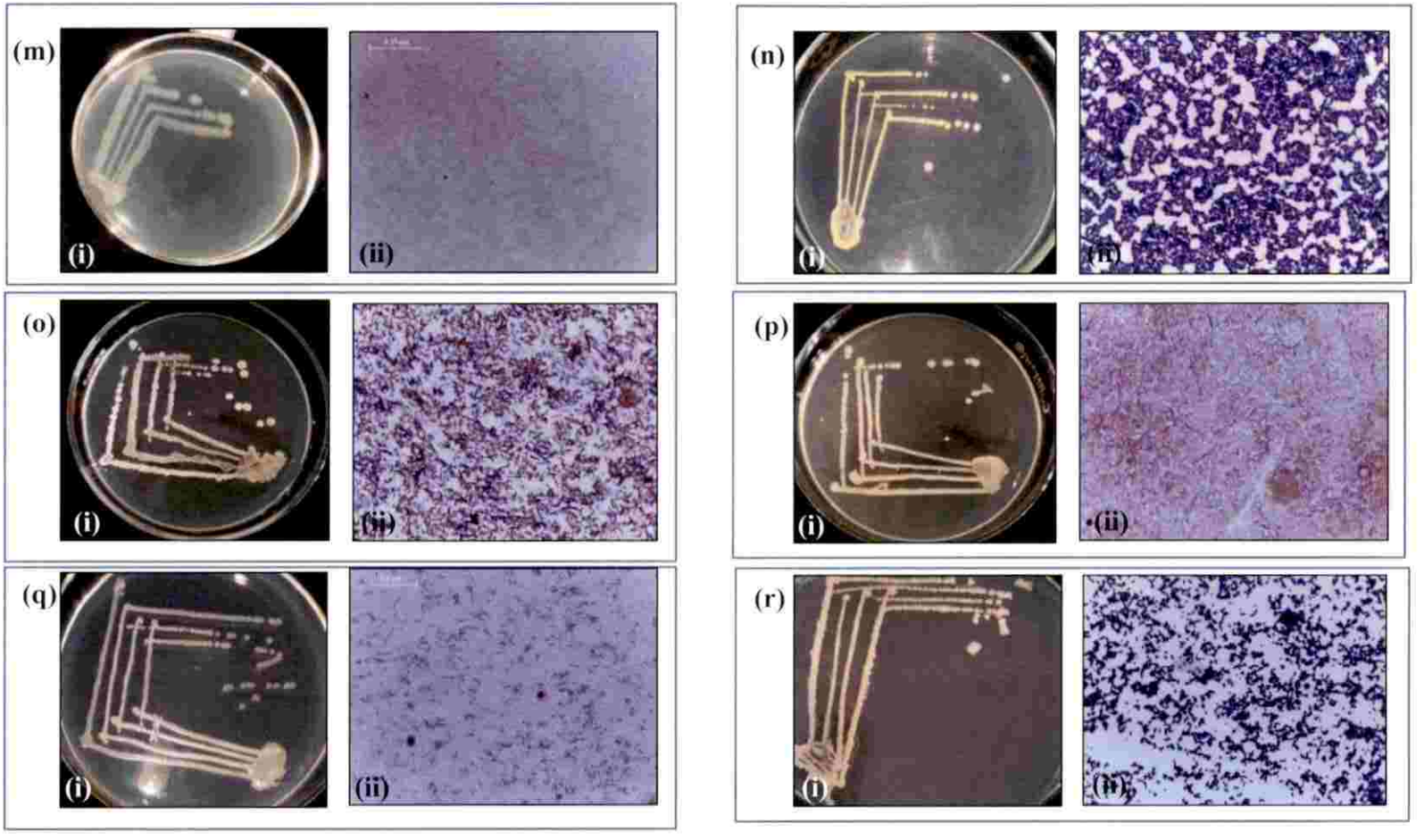

Plate 5c. Bacterial isolates obtained from chilli seeds collected from five agro-ecological zones of Kerala (m) B13, (n) B14, (o) B15, (p) B16, (q) B17 and (r) B18. (i) Quadrant streaked petriplates showing individual bacterial colonies and (ii) Gram reaction (1000X) of bacterial isolates

Kasargod (Table 7). Fruit rot samples were collected from each location. Weather data of the locations during the survey was also recorded (Table 6).

The disease incidence (DI) and severity of fruit rot were studied in each location. The symptoms of anthracnose / fruit rot were also noticed. The disease incidence varied from 20 to 75%. The highest disease incidence was observed in Vellanikkara (75 %) which was followed by Vellayani (65 %). The relative humidity recorded were also high in these locations *viz.*, 87 % in Vellanikkara and 86 % in Vellayani which favours the disease. There was not much variation in the average temperature of the five locations with values ranging from 26°C to 28°C. The least disease incidence was noted in Kumarakom (20 %). The PDI was also highest in Vellanikkara (67.25) followed by Vellayani (58.0). The least PDI was observed in Kumarakom (43.50) (Table 6 and 7.)

4.2.2. Symptomatology of the Disease under Natural Condition

The symptom on leaves were noticed as leaf spot which were brown necrotic lesions with concentric zonations surrounded by yellow halo. Apart from this, leaf blight symptom appeared as dry rotting from the edges of the leaves and moved towards the centre surrounded by yellow halo. Symptoms were also seen on the stem as brown necrotic lesions with black acervuli in it. The most damaging phase of the disease was fruit rot since they are economic part of chilli. The symptoms on fruit began as sunken water soaked lesions which later enlarged to straw - dark brown coloured elliptical lesions. These lesions later covered the entire fruit surface causing the entire fruit to rot. Black coloured acervuli were seen on the rotted area as dots arranged on concentric rings. Gradually the fruits got deformed, dried and turned black. The infection also spread to inner parts of the fruit and affected the seeds. Infected seeds initially had white mycelial growth. Slowly the seeds turned dark brown to black with black acervuli on the seed surface. At the advanced stage of the disease, complete drying of the whole plant was also observed (Plates 6, 7a and 7b).

Table 6. Weather data of surveyed locations during June 2018 - November 2018 in five agro-ecological zones of Kerala

Location	Average temperature (^{o}C)	Relative humidity (%)
Vellayani	27	86
Kumarakom	28	81
Vellanikkara	26	87
Ambalavayal	28	83
Padannakkad	27	80

Table 7. Incidence and severity of anthracnose / fruit rot of chilli observed at different survey locations in five agro-ecological zones of Kerala

Sl. No.	Location of collection	Zone representing the location	DI (%)	PDI (%)
1.	Vellayani, Thiruvananthapuram	Southern zone	65	58.00
2.	Kumarakom, Kottayam	Special problem area zone	20	43.50
3.	Vellanikkara, Thrissur	Central zone	75	67.25
4.	Ambalavayal, Wayanad	High altitude zone	50	55.00
5.	Padanakkad, Kasargod	Northern zone	40	40.00

DI - Disease Incidence, PDI - Percentage Disease Index

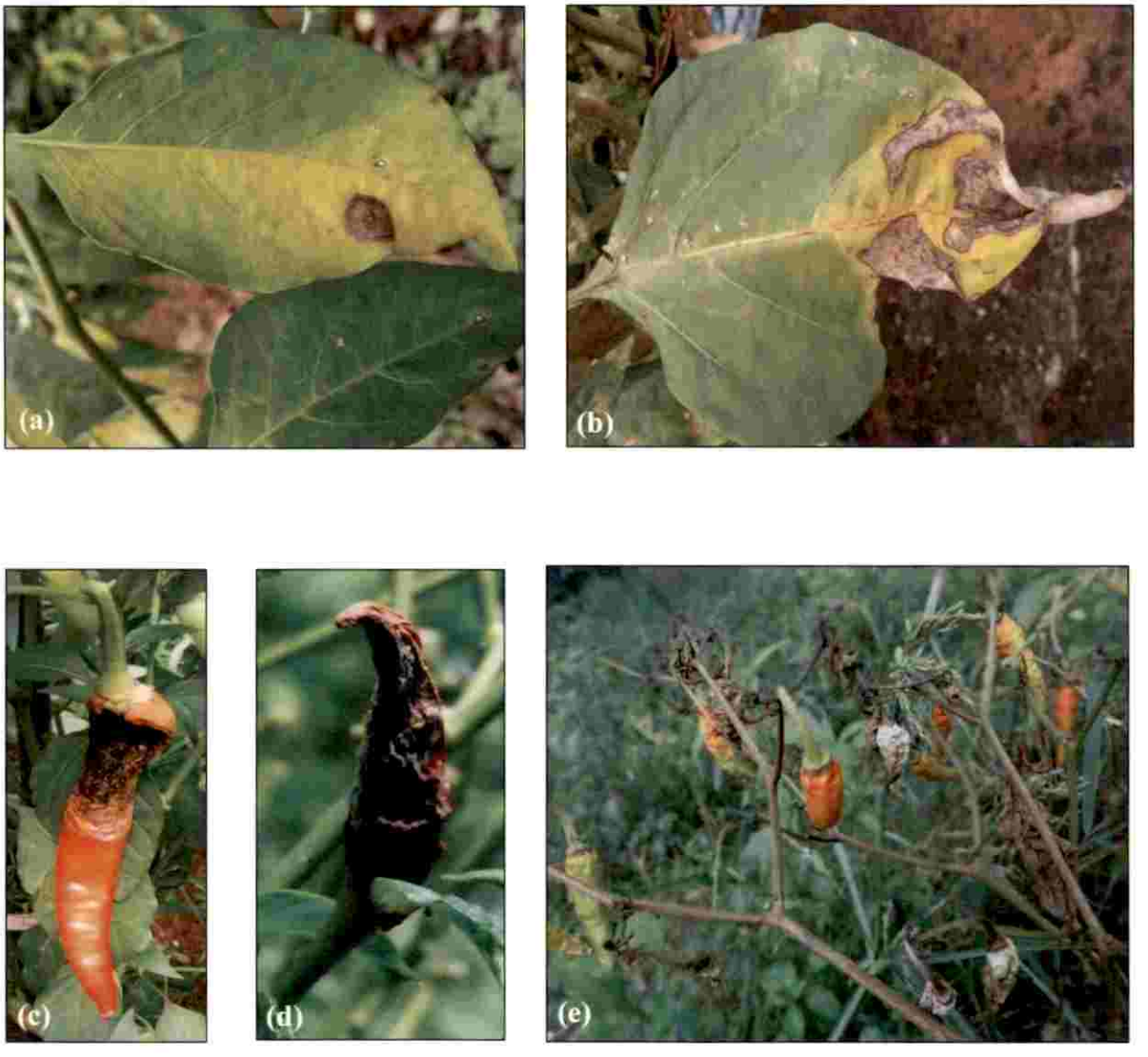

Plate 6. Common symptoms of anthracnose / fruit rot of chilli caused by *C. capsici* (a) leaf spot (b) leaf blight (c) fruit rot (d) mummified and deformed fruit (e) complete drying of plant

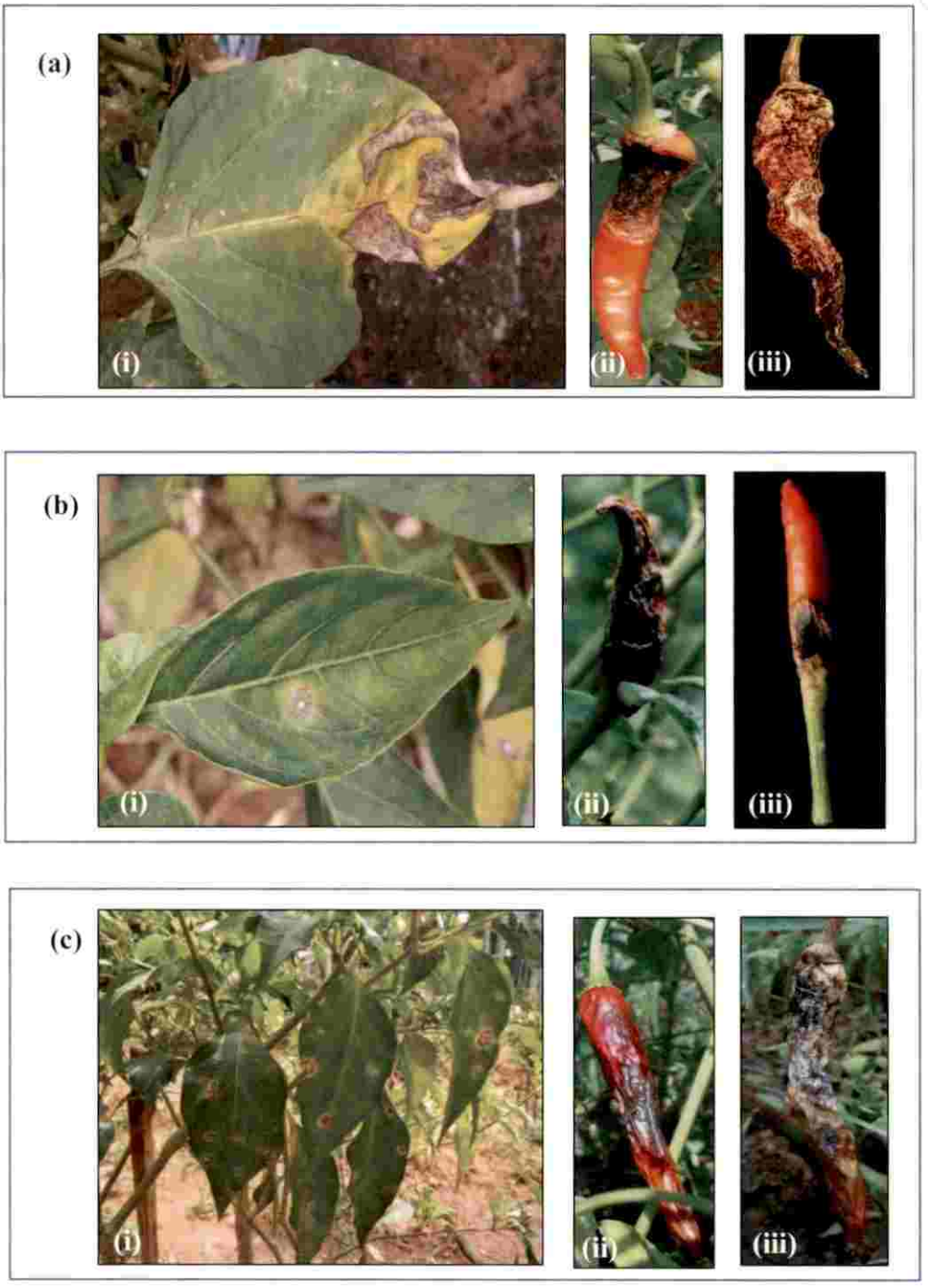

Plate 7a. Symptoms of anthracnose of chilli observed at different survey locations (a) Vellayani (Thiruvananthapuram) (b) Kumarakom (Kottayam) and (c) Thrissur - (i) Anthracnose on leaves (ii) and (iii) fruit rot symptoms

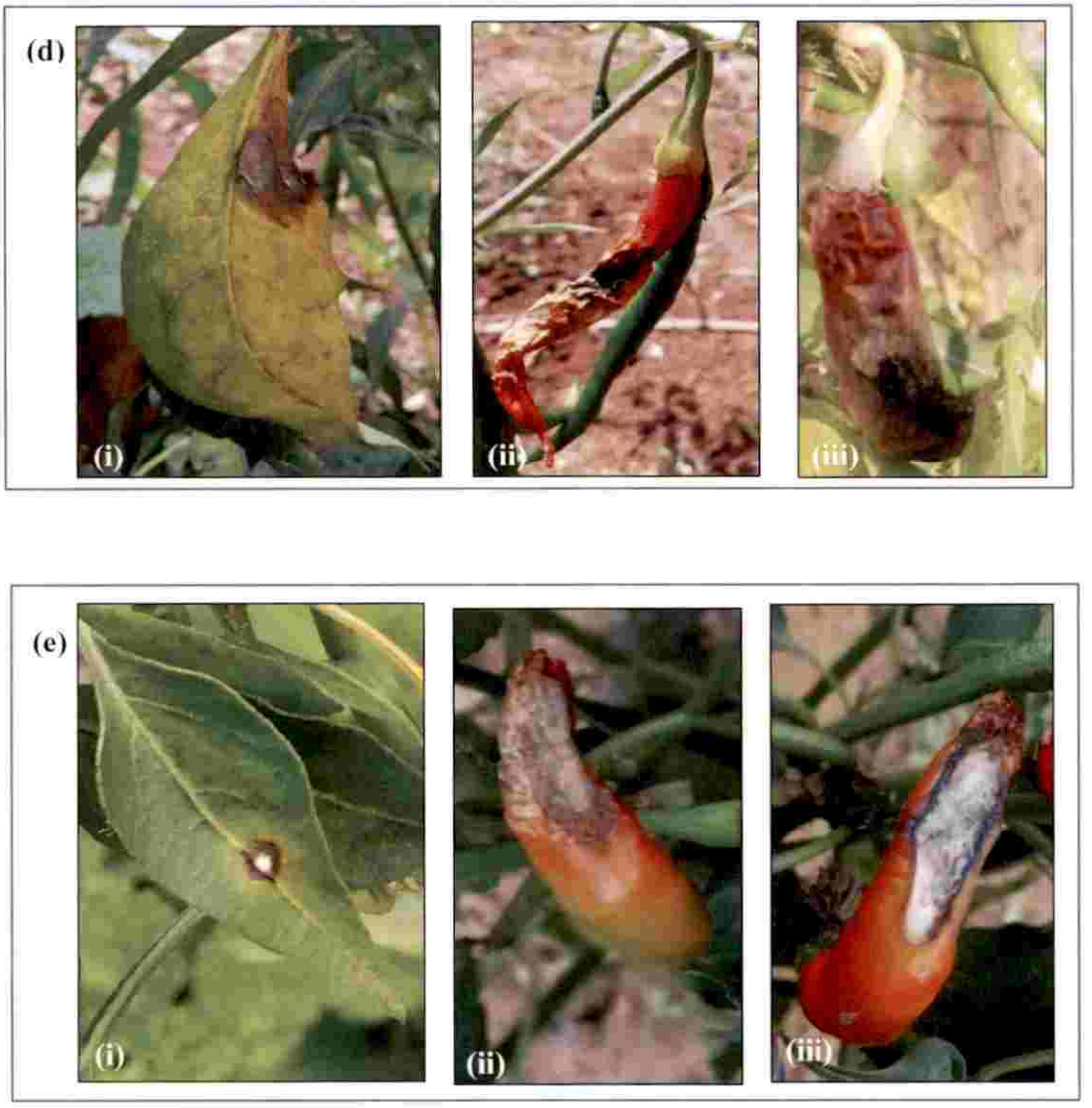

Plate 7b. Symptoms of anthracnose of chilli observed at different survey locations (d) Wayanad and (e) Kasaragod - (i) Anthracnose on leaves (ii) and (iii) fruit rot symptoms

4.2.3. Isolation of the Pathogen

The pathogen *C. capsici* was isolated from the collected specimen using standard isolation procedure. The medium used was PDA. A total of five isolates of *C. capsici* were obtained from the samples and were named as Isolate C1 (isolate from Vellayani), Isolate C2 (isolate from Kumarakom), Isolate C3 (isolate from Thrissur), Isolate C4 (isolate from Wayanad) and Isolate C5 (isolate from Kasargod) (Table 8 and Plate 9).

4.2.4. Proving the Pathogenicity of the Isolates of *C. capsici*

The isolates obtained were separately inoculated to detached healthy fruits of chilli variety Vellayani Athulya. All the isolates produced water soaked symptoms within two days of inoculation which enlarged further. On the advanced stage, orange coloured spore masses were also produced on the fruit surface. This confirmed the pathogenicity of the isolates. Re-isolation of the pathogen was done from the fruits and the cultures thus obtained were compared with the initial cultures and were found to be the same. Thus the Koch's postulates were proved (Plate 8).

4.2.5. Single Spore Isolation and Maintenance of Pure Culture

All the five isolates obtained were purified through single spore isolation technique and were sub-cultured on PDA slants and stored in refrigerator at $15^{O}C$ and room temperature ($28\pm2^{O}C$). These cultures were periodically sub-cultured and used for further studies. Virulence of the isolates were maintained by inoculating them to healthy host and subsequent re-isolation.

4.2.6. Characterization of the Isolates of *C. capsici*.

4.2.6.1. Cultural Characterization

The cultural characters of the isolates were studied by observing the growth pattern in PDA medium. It was found that mycelial growth of all the five isolates

Plate 8. Symptoms observed on chilli fruits var. Vellayani Athulya seven days after inoculation of *C. capsici* isolates (a) C1, (b) C2, (c) C3, (d) C4 and (e) C5

exhibited concentric zonations with the presence of acervuli. The colour of the mycelium of isolate C1 was white in the upper side of petriplate which later turned to off white. The reverse side was white to off white. The margin of the isolate was regular. Isolate C2 was white on the upper side, yellow to dark brown on the lower side and with regular margin. The concentric zonations of the isolate was prominent on the reverse side. The isolate C3 was white which later turned to off white with white to cream reverse side. The margin was found to be irregular. The isolate C4 and C5 were white in the upper side which later turned to grey. The reverse side of the isolate C4 was cream and it had regular margin. The reverse side of the isolate C5 was white to cream and it exhibited an irregular margin (Table 8 and Plate 9).

The mycelial growth of the five isolates in PDA was studied and the radial growth was recorded at 3rd, 5th, 7th, 10th and 15th day of incubation. It was found that the isolate C1 from Vellayani was the fastest growing isolate and showed the highest mycelial growth for all the days of observation and recorded a radial growth of 8.80 cm on 7th day of incubation. This was followed by the isolate C2 from Kumarakom with radial growth of 8.61 cm on 7th day. The slowest growing one was the isolate C5 from Kasargod with a mycelial growth of 7.95 cm on 7th day of incubation (Table 9).

4.2.6.2. Morphological Characterization

The morphology of the five isolates such as the mycelial, conidial, acervular and appressorial characters were studied through the slide culture technique. The mycelia of the *C. capsici* isolates were hyaline and septate. The average width of the mycelia of isolates ranged from 2.19 µm (for isolate C3) to 3.32 µm (for isolate C4). The conidia were sickle shaped with an oil globule at the centre. The average length of the conidia varied from 19.55 µm (for isolate C1) to 24.85 µm (for isolate C4) and the average width of the conidia varied from 2.72 µm (for isolate C1) to 3.42 µm (for isolate C5). The fungus also produced asexual fruiting bodies known as acervuli which were open structures, saucer shaped and lined by

Table 8. Cultural characters of *C. capsici* isolates obtained from five agro-ecological zones of Kerala in PDA medium

Isolates	Appearance	Colour of mycelia		Margin
		Upper side	Reverse side	
Isolate C1 (Vellayani)	Concentric zonation with acervuli	White to off white	White to cream	Regular
Isolate C2 (Kumarakom)		White	Yellow to dark brown	Regular
Isolate C3 (Thrissur)		White to off white	White to cream	Irregular
Isolate C4 (Wayanad)		White later turns to grey	Cream	Regular
Isolate C5 (Kasargod)		White later turns to grey	White to cream	Irregular

Table 9. Radial growth of *C. capsici* isolates obtained from five agro-ecological zones of Kerala in PDA medium

Isolate	Radial growth (cm)*				
	3rd day	5th day	7th day	10th day	15th day
C1	3.29±0.03[a]	5.89±0.04[a]	8.80±0.12[a]	9	9
C2	3.23±0.05[a]	5.66±0.06[b]	8.61±0.08[ab]	9	9
C3	3.06±0.07[b]	5.43±0.11[c]	8.46±0.02[b]	9	9
C4	2.91±0.02[b]	5.44±0.08[c]	8.48±0.05[b]	9	9
C5	3.03±0.03[b]	4.93±0.05[d]	7.95±0.03[c]	9	9
SEm±	0.04	0.07	0.07		
CD(0.05)	0.13	0.21	0.21		

*Mean ± standard deviation of three replications

Values followed by same superscripts in a column are not significantly different at 5% level of significance

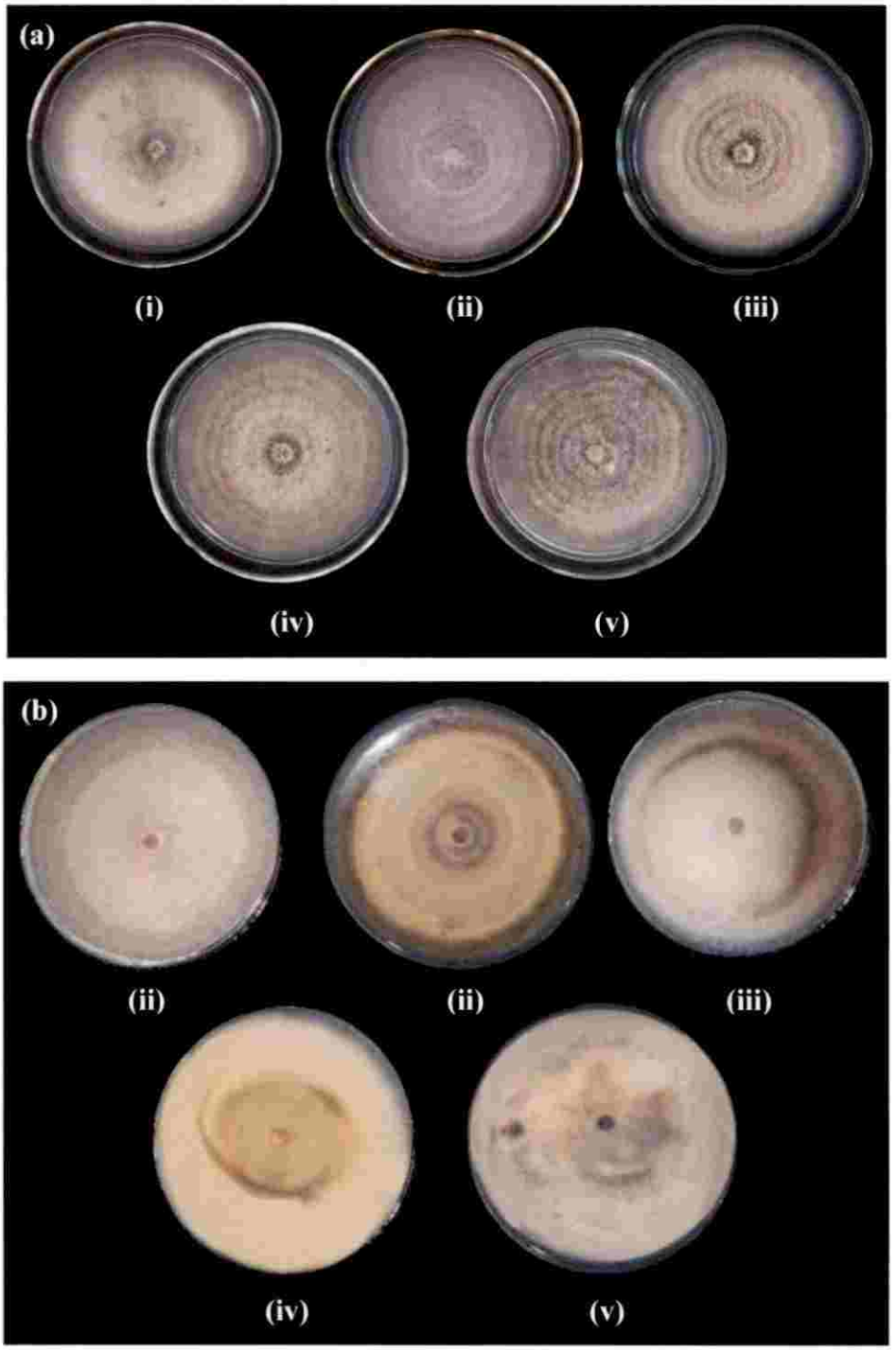

Plate 9. Growth of *C. capsici* isolates obtained from five different locations of Kerala in PDA medium. (a) Upper side view (b) Reverse side view of isolates (i) C1 (Vellayani) (ii) C2 (Kumarakom) (iii) C3 (Thrissur) (iv) C4 (Wayanad) and (v) C5 (Kasaragod)

numerous setae on the periphery. The acervular characters of the isolates were also studied. The average diameter of the acervulus ranged from 121.23 µm (for isolate C4) to 147.03 µm (for isolate C2). The setae were dark brown, elongated, straight and pointed structures. The number of setae varied in each isolate from 13-24 (for isolate C5) to 35-56 (for isolate C2) (Table 10 and Plates 10, 11 and 12).

C. capsici produced appressoria which were dark brown elliptical structures and were used for penetrating the host tissue. The formation of the appressoria was observed in the slide culture studies and the dimensions were measured. The length of the appressoria varied from 11.71 µm (C3) to 17.17 (C5) and width varied from 6.26 µm (C2) to 8.56 µm (C5) (Table 11 and Plate 13).

4.2.7. Screening of Virulent Isolate of *C. capsici*

Screening of the virulent isolate was done by artificial inoculation of *C. capsici* isolates on tender, mature and ripe fruits of chilli (var. Vellayani Athulya). The most virulent isolate was identified based on the size of the lesion produced by it on chilli fruits. The days taken for symptom appearance and PDI of each isolate were also recorded.

4.2.7.1. Screening by Inoculating on Tender Fruits

The tender fruits of chilli variety Vellayani Athulya on artificial inoculation produced characteristic fruit rot symptoms. The symptoms started as brown water soaked lesions which later spread to entire fruit surface. Black pin head sized acervuli were also seen on the fruit surface (Plate 14). The isolates C1 and C3 produced symptoms within 24 h of inoculation while the rest of the isolates took 48 h for producing symptom. On comparing the lesion progression on successive days, the isolate C1 recorded the maximum lesion size. On 3rd day of inoculation isolate C1 gave a lesion size of 1.50 cm which was followed by the isolate C3 (lesion size of 1.29 cm). Isolate C4 gave the least lesion size (0.33 cm). On 5th day the lesion size produced by isolate C1 was 3.06 cm followed by isolate C3 (2.36

Table 10. Morphological characters of *C. capsici* isolates from five agro-ecological zones of Kerala

Isolate	Size (width) of mycelia (µm)*	Conidial characters			Size (diameter) of acervulus (µm)*	Number of setae in acervuli*
		Shape	Length (µm)**	Width (µm)**		
C1	2.49±0.51	Sickle shape with oil globule at centre	19.55±1.43	2.72±0.58	138.52±5.28	21-39
C2	2.92±0.47	Sickle shape with oil globule at centre	24.05±2.13	2.94±0.40	147.03±5.28	35-56
C3	2.19±0.51	Sickle shape with oil globule at centre	21.78±1.85	2.91±0.38	128.98±9.05	15-30
C4	3.32±0.67	Sickle shape with oil globule at centre	24.85±1.71	3.04±0.44	121.23±7.58	18-33
C5	2.92±0.46	Sickle shape with oil globule at centre	23.56±1.60	3.42±0.55	127.46±9.56	13-24

*Values are mean ± standard deviation of 10 observations

**Values are mean ± standard deviation of 30 observations

Table 11. Length and width of appressoria of *C. capsici* isolates from five agro-ecological zones of Kerala

Isolates	Appressoria length (µm)*	Appressoria width (µm)*
C1	15.16±2.13	6.63±0.54
C2	15.66±1.96	6.26±0.62
C3	11.71±1.51	6.39±0.85
C4	12.42±2.14	6.44±0.83
C5	17.17±2.51	8.56±1.36

*Values are mean ± standard deviation of 10 observations

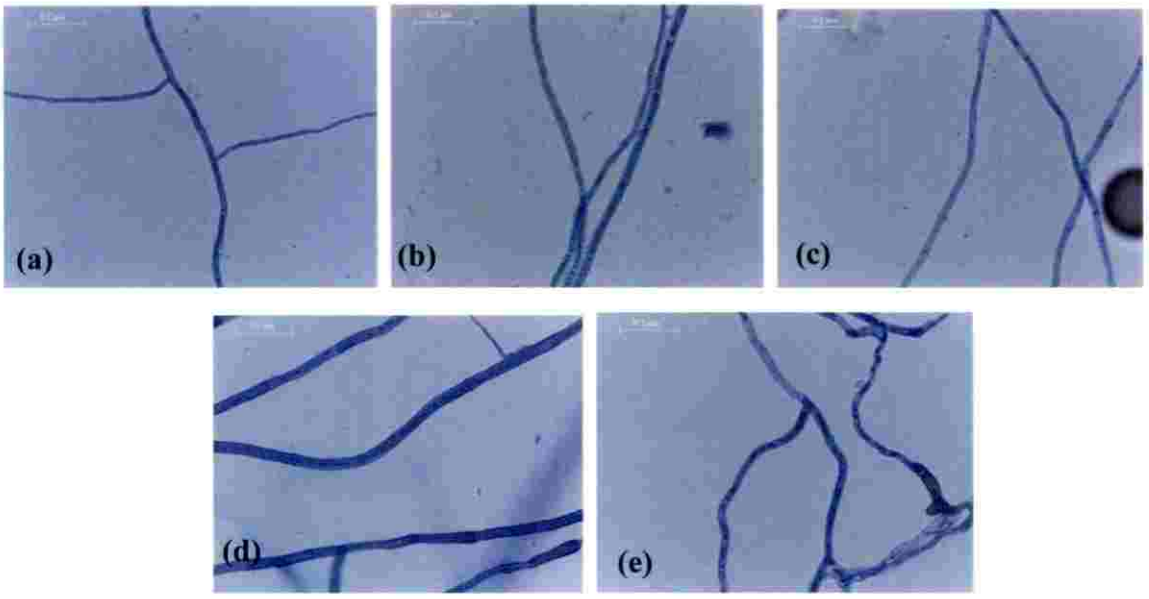

Plate 10. Microscopic images of the mycelia of the *C. capsici* isolates (a) C1, (b) C2, (c) C3, (d) C4 and (e) C5 at 1000X magnification

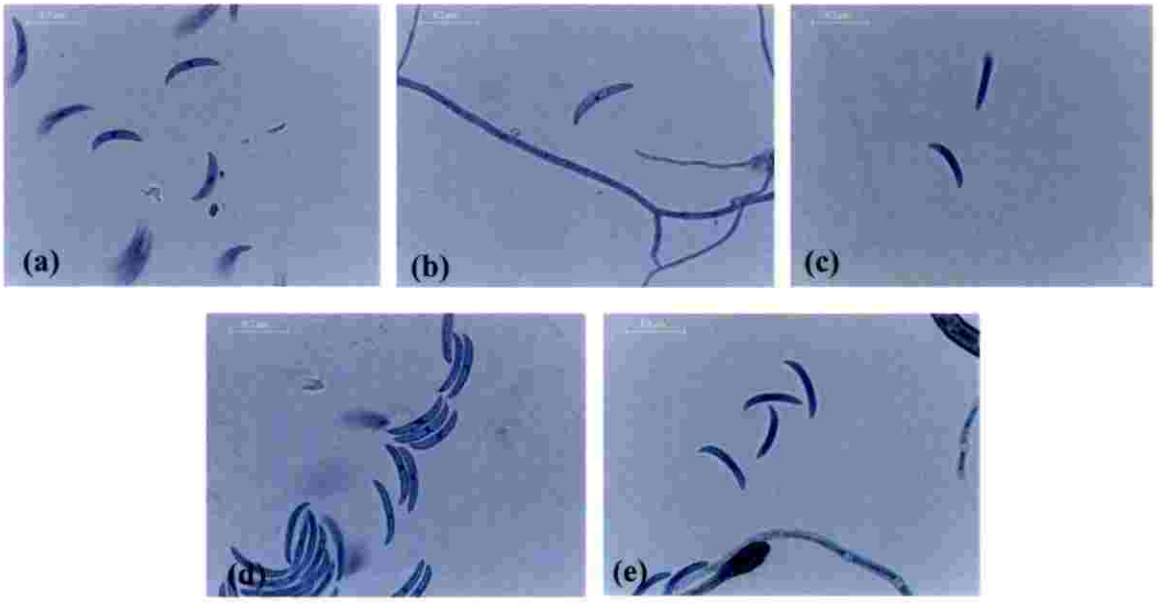

Plate 11. Microscopic images of the conidia of the *C. capsici* isolates (a) C1, (b) C2, (c) C3, (d) C4 and (e) C5 at 1000X magnification

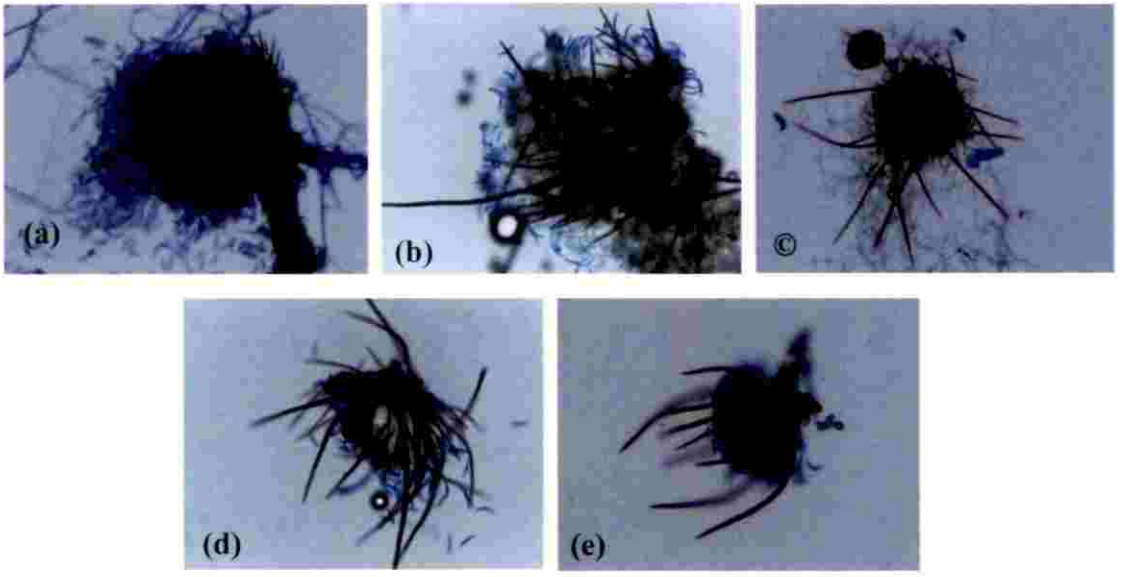

Plate 12. Microscopic image of the acervuli of the *C. capsici* isolates (a) C1, (b) C2, (c) C3, (d) C4 and (e) C5 at 400X magnification

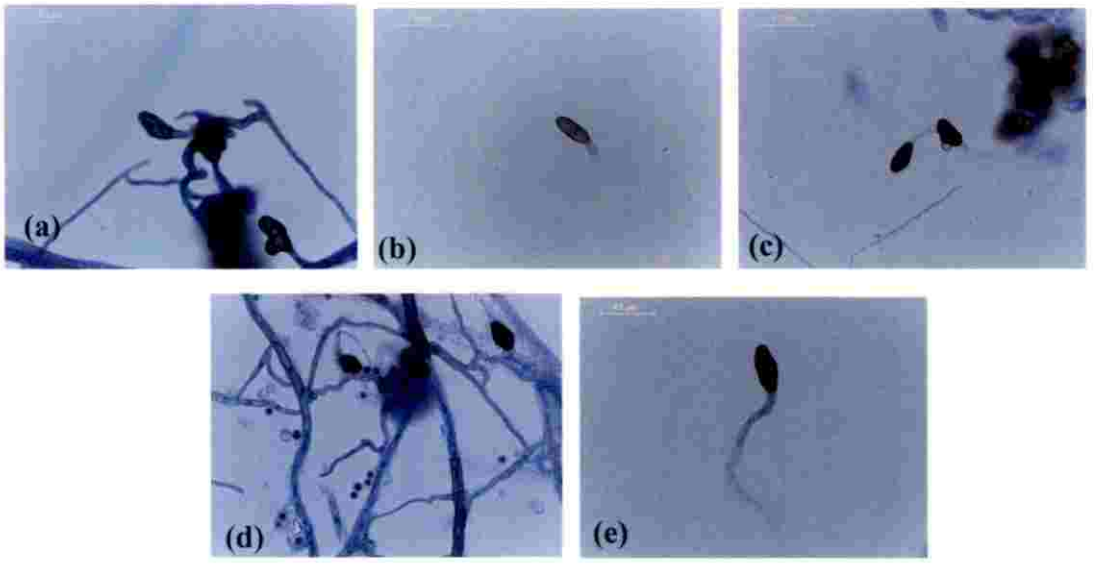

Plate 13. Microscopic image of the appressoria of the *C. capsici* isolates (a) C1, (b) C2, (c) C3, (d) C4 and (e) C5 at 1000X magnification

cm). On 7th day of inoculation, isolate C1 produced a lesion size of 3.81cm followed by isolate C3 (2.83 cm). The least lesion size was recorded for isolate C2 (0.61 cm). After 7th day some of the fruits got completely rotten. The PDI at 7th day of inoculation was highest for isolate C1 (100%) followed by isolate C3 (95%). The least PDI was shown by isolate C4 (25%). Thus the C1 isolate was identified as the most virulent one as it produced the maximum lesion size and PDI (Table 12, Plate 14).

4.2.7.2. Screening by Inoculating on Mature Fruits

The mature fruits of chilli variety Vellayani Athulya when artificially inoculated with *C. capsici* produced brown water soaked lesions which later spread to entire fruit surface with black pin head sized acervuli (Plate 15). The isolates C1 and C3 produced symptoms within 24 h of inoculation while C2 and C5 took 48 h for producing symptom. The isolate C4 produced symptom only on 3rd day of inoculation. On comparing the lesion progression on successive days, the C1 isolate showed the maximum lesion size. On 3rd day of inoculation the C1 isolate gave a lesion size of 1.52 cm which was followed by the C3 isolate (lesion size of 1.32 cm). The C4 isolate from Wayand gave the least lesion size (0.35 cm). On 5th day the lesion size produced by isolate C1 was 3.23 cm followed by isolate C3 (2.67 cm). On 7th day of inoculation, isolate C1 produced a lesion size of 4.02 cm followed by isolate C3 (3.17 cm). The least was shown by isolate C2 (0.43 cm). After 7th day some of the fruits got completely rotten. The PDI at 7th day of inoculation was highest for isolate C1 (95 %) followed by isolate C3 (85 %). The least PDI was recorded for isolate C4 (20 %). The above results also proved that isolate C1 was the most virulent one (Table 13, Plate 15).

4.2.7.3. Screening by Inoculating on Ripe Fruits

The ripe red fruits of chilli variety Vellayani Athulya when artificially inoculated with *C. capsici* produced brown water soaked lesions which later spread to entire fruit surface with black pin head sized acervuli. White mycelial growth was also seen over the fruit surface (Plate 15). The isolates C1 and C3

Table 12. Lesion size progression and percentage disease index of *C. capsici* isolates on artificial inoculation on tender fruits of chilli var. Vellayani Athulya

Isolate	DTSA	Lesion size (cm)*					PDI at 5th day (%)
		3rd day	5th day	7th day	10th day	15th day	
C1	1	1.5±0.05[a]	3.06±0.11[a]	3.81±0.07[a]	Complete rotting		100
C2	2	0.34±0.05[d]	0.47±0.04[d]	0.61±0.07[d]	0.79±0.06	Rotting	40
C3	1	1.29±0.05[b]	2.36±0.35[b]	2.83±0.08[b]	Complete rotting		80
C4	2	0.33±0.02[d]	0.49±0.06[d]	0.63±0.07[d]	0.81±0.07	Rotting	60
C5	2	0.80±0.06[c]	1.54±0.15[c]	2.1±0.08[c]	2.49±0.45	Rotting	60
SEm±		0.05	0.18	0.08			
CD(0.05)		0.14	0.52	0.22			

*Mean ± SD of five replications

Values followed by same superscripts in a column are not significantly different at 5% level of significance

DTSA: Days taken for symptom appearance

Table 13. Lesion size progression and percentage disease index of *C. capsici* isolates on artificial inoculation on mature fruits of chilli var. Vellayani Athulya

Isolate	DTSA	Lesion size (cm)*					PDI at 7th day (%)
		3rd day	5th day	7th day	10th day	15th day	
C1	1	1.52±0.01[a]	3.23±0.08[a]	4.02±0.06[a]	Complete rotting		95
C2	2	0.38±0.03[d]	0.40±0.03[d]	0.43±0.02[e]	0.55±0.04	Rotting	25
C3	1	1.32±0.05[b]	2.67±0.13[b]	3.17±0.10[b]	Complete rotting		85
C4	3	0.35±0.03[d]	0.43±0.07[d]	0.70±0.06[d]	0.88±0.10	Rotting	20
C5	2	1±0.10[c]	1.82±0.16[c]	2.43±0.10[c]	3.58±0.86	Rotting	45
SEm±		0.06	0.10	0.07			
CD(0.05)		0.17	0.30	0.22			

*Mean ± SD of five replications

Values followed by same superscripts in a column are not significantly different at 5% level of significance

DTSA: Days taken for symptom appearance

Plate 14. Symptom development seven days after inoculation of *C. capsici* isolates on tender fruits of chilli var. Vellayani Athulya. The fruits were inoculated with isolates (a) C1, (b) C2, (c) C3, (d) C4, (e) C5 and (f) control

Plate 15. Symptom development seven days after inoculation of *C. capsici* isolates on mature fruits of chilli var. Vellayani Athulya. The fruits were inoculated with isolates (a) C1, (b) C2, (c) C3, (d) C4, (e) C5 and (f) control

produced symptoms within 24 h of inoculation. Isolate C5 took 48 h for producing symptom. The isolates C2 and C4 produced symptom only at 3rd day of inoculation. On comparing the lesion progression on successive days, isolate C1 showed the maximum lesion size. On 3rd day of inoculation isolate C1 produced a lesion size of 0.40 cm which was followed by isolate C3 (0.36 cm). Isolate C4 gave the least lesion size (0.24 cm). On 5th day the lesion size produced by isolate C1 was 0.88 cm followed by isolate C3 (0.70 cm). On 7th day of inoculation, isolate C1 produced a lesion size of 1.94 cm followed by isolate C3 (1.46 cm). On 10th day of inoculation isolate C1 produced a lesion size of 2.80 cm followed by isolate C3 (1.96 cm). The least lesion size was produced by isolate C4 (0.48 cm). After 10th day some of the fruits got completely rotten. The PDI at 10th day of inoculation was highest for isolates C1 and C3 (100 %). The least PDI was recorded for isolate C4 (30 %). The above results also proved isolate C1 as the most virulent one (Table 14, Plate 16).

Screening of the five isolates of *C. capsici* revealed that the isolate C1 from Vellayani took the minimum days for symptom production, produced the maximum lesion size on chilli fruits and recorded the maximum PDI. Thus it was identified as the most virulent isolate and was selected for further studies.

4.3. *In vitro* SCREENING OF SELECTED BIOCONTROL AGENTS AGAINST *C. capsici*

4.3.1. Percentage Inhibition of Mycelial Growth of *C. capsici*

Dual culture assay revealed that the maximum percentage inhibition of mycelial growth of *C. capsici* was recorded by *B. amyloliquefaciens* VLY 24 (inhibition of 62.96 %) followed by *B. subtilis* VLY 62 (56.30 %) and *T. viride* (51.85 %). The mycelial growth inhibition recorded by *P. indica* was 46.67 per cent and *B. pumilus* VLY 17 was 45.19 per cent. *P. fluorescens* PN 026 did not show inhibition of mycelial growth of *C. capsici* (Table 15, Plate 17).

Table 14. Lesion size progression and percentage disease index of *C. capsici* isolates on artificial inoculation on ripe fruits of chilli var. Vellayani Athulya

Isolate	DTSA	Lesion size (cm)*					PDI at 10th day (%)
		3rd day	5th day	7th day	10th day	15th day	
C1	1	0.40±0.03^{a}	0.88±0.06^{a}	1.94±0.05^{a}	2.80±0.08^{a}	Rotting	100
C2	3	0.26±0.02^{bc}	0.54±0.02^{c}	0.66±0.05^{d}	0.78±0.04^{d}	Rotting	35
C3	1	0.36±0.05^{ab}	0.70±0.03^{b}	1.46±0.05^{b}	1.96±0.05^{b}	Rotting	100
C4	3	0.24±0.02^{c}	0.32±0.02^{d}	0.42±0.04^{e}	0.48±0.04^{e}	Rotting	30
C5	2	0.34±0.04^{abc}	0.64±0.04^{bc}	1.02±0.04^{c}	1.42±0.04^{c}	Rotting	70
SEm±		0.04	0.04	0.05	0.05		
CD(0.05)		0.11	0.11	0.14	0.16		

DTSA: Days taken for symptom appearance

*Mean ± SD of five replications

Values followed by same superscripts in a column are not significantly different at 5% level of significance

Table 15. Percentage inhibition of mycelial growth of *C. capsici* by biocontrol agents in dual culture assay

Treatments	Percentage inhibition of mycelial growth (%)
T. viride	51.85 (46.06)[c]
P. fluorescens	0.00 (0.28)[e]
B. subtilis	56.30 (48.62)[b]
B. pumilus	45.19 (42.24)[d]
B. amyloliquefaciens	62.96 (52.51)[a]
P. indica	46.67 (43.09)[d]
Pathogen alone (Control)	0.00 (0.28)[e]
SEm±	0.65
CD(0.05)	1.98

Values are mean ± standard deviation of three replications

Values followed by same superscripts are not significantly different at 5 % level of significance

Values in parenthesis are arcsine transformed values

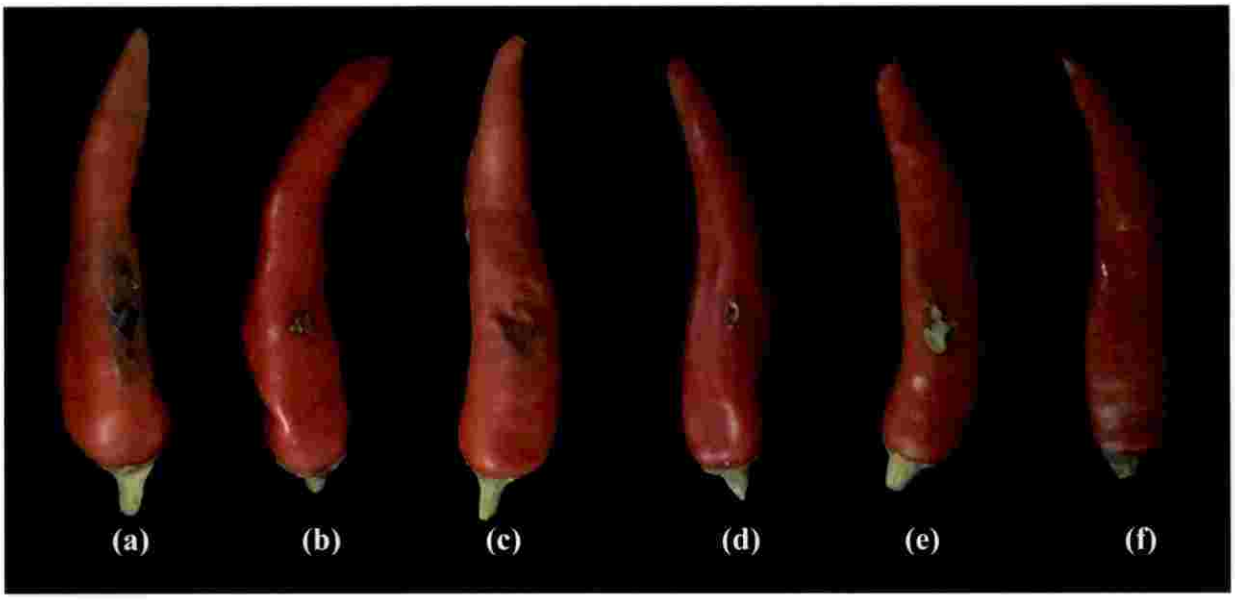

Plate 16. Symptom development ten days after inoculation of *C. capsici* isolates on ripe fruits of chilli var. Vellayani Athulya. The fruits were inoculated with *C. capsici* isolates (a) C1, (b) C2, (c) C3, (d) C4, (e) C5 and (f) control

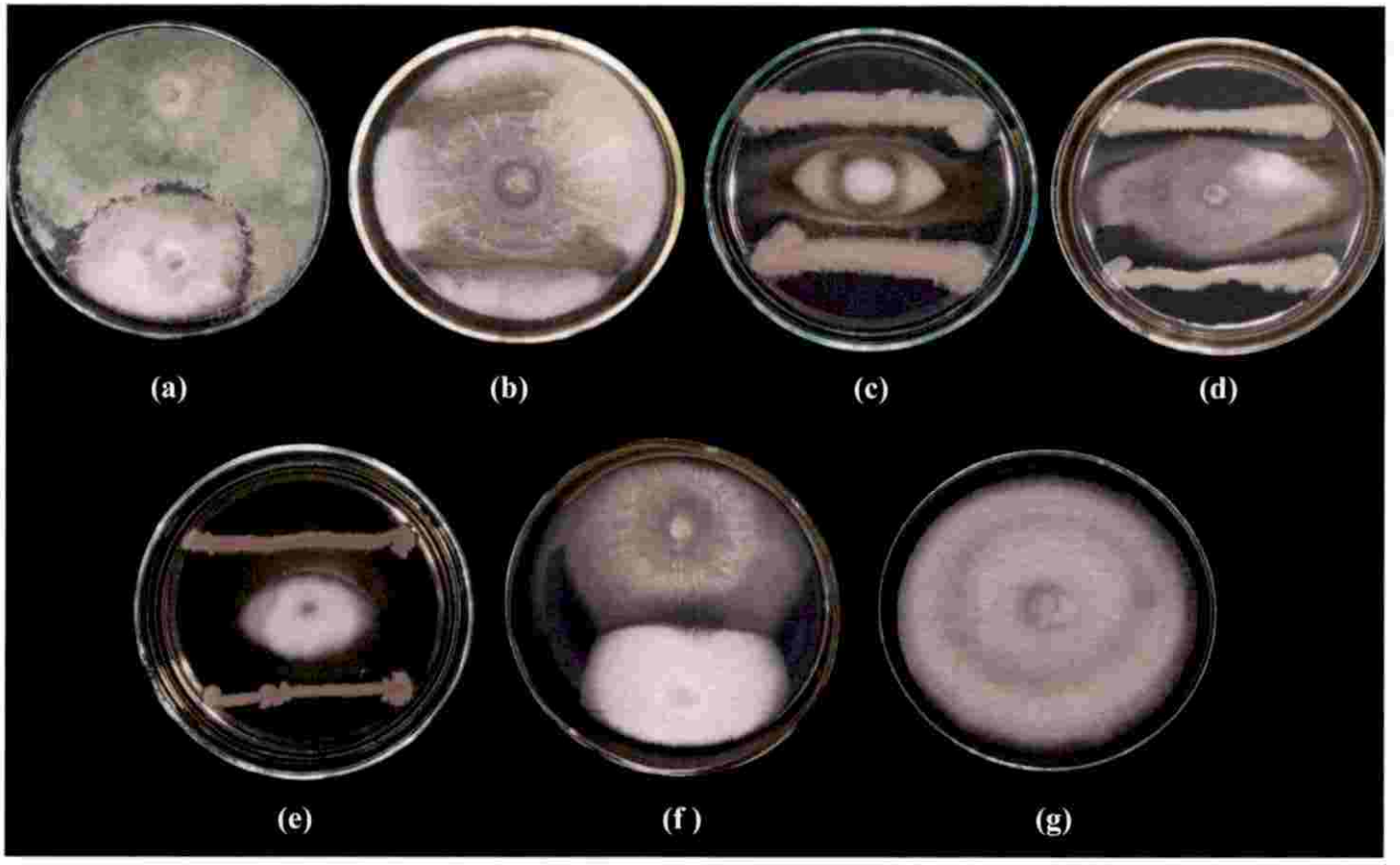

Plate 17. Inhibition of mycelial growth of *C. capsici* in dual culture by different biocontrol agents on 8th day of dual culturing (a) *T. viride* (b) *P. fluorescens* (c) *B. subtilis* (d) *B. pumilus* (e) *B. amyloliquefaciens* and (f) *P. indica* (g) *C. capsici* control

4.3.2. Zone of Inhibition Formed by Bacterial Bioagents in Dual Culture Assay

The clear zone formed between the pathogen and the bacterial bioagent was measured. It was found that the highest inhibition zone was produced in case of *B. amyloliquefaciens* (0.53 cm) followed by *B. subtilis* (0.35 cm) and *B. pumilus* (0.30 cm). No inhibition zone was produced by *P. fluorescens*. *C. capsici* grew over *P. fluorescens* (Table 16, Plate 17). The best three treatments obtained from the dual culture assay were *B. amyloliquefaciens, B. subtilis* and *T. viride*.

4.4. *In vitro* ASSAYS ON FRUITS

4.4.1. *In vitro* Screening of Biocontrol Agents against *C. capsici* on Detached Chilli Fruits

In vitro assays on fruits revealed that treatment with *B. amyloliquefaciens* was most effective in reducing fruit rot which gave the least lesion size on successive days of observation. On 3rd day of inoculation fruits treated with *B. amyloliquefaciens* recorded the lowest lesion length of 0.39 cm followed by those treated with *B. subtilis* (lesion size of 0.52 cm) and *T. viride* (0.56 cm). The maximum lesion size was recorded for fruits treated with *P. fluorescens* (0.95 cm). The lesion sizes on 5th day were 1.16 cm for the treatment *B. amyloliquefaciens*, 1.30 cm for *B. subtilis* and 1.76 cm for *T. viride*. On 7th day of inoculation the lesion sizes were 1.89 cm, 2.45 cm and 2.47 cm respectively for *B. amyloliquefaciens*, *B. subtilis* and *T. viride*. On 10th day a lesion size of 2.71 cm was observed for *B. amyloliquefaciens* treated fruits (a reduction in lesion size of 55.64 % over control). This was followed by treatment with *B. subtilis* with lesion size of 2.97 cm (51.39 % reduction) and *T. viride* with lesion size of 3.81 cm (37.64 % reduction). The least effective was the treatment with *P. fluorescens* which gave a lesion size of 5.18 cm with only 15.22 per cent reduction over control (Table 17, Plate 18).

Table 16. Zone of inhibition formed by bacterial biocontrol agents against *C. capsici* in dual culture assay

Treatments	Zone of inhibition (cm)
P. fluorescens	0.00 (0.71)^{c}
B. subtilis	0.35 (0.92)^{b}
B. pumilus	0.30 (0.89)^{b}
B. amyloliquefaciens	0.53 (1.02)^{a}
Pathogen alone (Control)	0.00 (0.71)^{c}
SEm±	0.01
CD(0.05)	0.05

Values are mean ± standard deviation of three replications

Values followed by same superscripts are not significantly different at 5% level of significance

Values in parenthesis are square root transformed values

Table 17. Number of lesions produced, lesion progression and percentage reduction of *C. capsici* infection on chilli fruits var. Vellayani Athulya treated with biocontrol agents

Treatment	No. of lesions on fruits	Lesion size (cm)*					Reduction over control on 10th day (%)
		3rd day	5th day	7th day	10th day	15th day	
T.viride	1	0.56±0.02^{d}	1.76±0.01^{c}	2.47±0.02^{d}	3.81±0.04^{e}	Complete rotting	37.64
P. fluorescens	1	0.95±0.04^{b}	2.09±0.06^{b}	3.07±0.08^{b}	5.18±0.06^{b}	Complete rotting	15.22
B. subtilis	1	0.52±0.02^{d}	1.30±0.07^{d}	2.45±0.04^{d}	2.97±0.09^{f}	Complete rotting	51.39
B. pumilus	1	0.68±0.01^{c}	1.86±0.02^{c}	2.59±0.10^{cd}	4.97±0.06^{c}	Complete rotting	18.65
B. amyloliquefaciens	1	0.39±0.01^{e}	1.16±0.02^{e}	1.89±0.03^{e}	2.71±0.05^{g}	Complete rotting	55.64
P. indica	1	0.67±0.03^{c}	1.82±0.06^{c}	2.66±0.07^{c}	4.03±0.07^{d}	Complete rotting	34.04
Pathogen alone (control)	1	1.32±0.02^{a}	2.83±0.02^{a}	3.82±0.04^{a}	6.11±0.10^{a}	Complete rotting	
SEm±		0.02	0.04	0.06	0.07		
CD(0.05)		0.06	0.13	0.19	0.21		

*Mean ± standard deviation of three replications

Values followed by same superscripts in a column are not significantly different at 5% level of significance

Plate 18. Lesions formed on chilli fruits var. Vellayani Athulya (10 DAI) after treatment with biocontrol agents and challenge inoculation with *C. capsici*. Fruits are treated with (a) *T. viride* (b) *P. fluorescens* (c) *B. subtilis* (d) *B. pumilus* (e) *B. amyloliquefaciens* and (f) *P. indica* (g) *C. capsici* control.

4.4.2. Estimation of Peroxidase Enzyme Activity

PO activity after treatment with biocontrol agents but before inoculation with pathogen was found to be increasing upto 48 h after treatment and was found decreasing thereafter for all the treatments. PO activity before inoculation with pathogen was found to be highest in chilli fruits treated with *B. amyloliquefaciens*. The activity was 8.81 mg g^{-1} min^{-1} on the day of inoculation which increased to 16.11 mg g^{-1} min^{-1} after 24 h and 23.70 mg g^{-1} min^{-1} after 48 h. The activity was found to be decreasing after 72 h (18.31 mg g^{-1} min^{-1}). This was followed by *B. subtilis* where the activity after 0, 24, 48 and 72 h of treatment were 6.52, 10.60, 23.06 and 19.14 mg g^{-1} min^{-1} respectively and *T. viride* (activity of 4.86, 8.32, 13.82 and 10.86 mg g^{-1} min^{-1} respectively after 0, 24, 48 and 72 h). The least activity was shown by fruits treated with *P. fluorescens* (activity of 2.21, 4.54, 10.30 and 7.99 mg g^{-1} min^{-1} respectively after 0, 24, 48 and 72 h) (Table 18).

PO enzyme activity after treatment with biocontrol agents and inoculation with pathogen were found higher than the activity before pathogen inoculation and was found to be increasing even after 48 h of inoculation for all the treatments. The enzyme activity was found to be the highest in chilli fruits treated with *B. amyloliquefaciens* where the activity after 0, 24, 48 and 72 h of treatment were 10.61, 22.03, 32.94 and 41.23 mg g^{-1} min^{-1} respectively followed by *B. subtilis* (activity of 7.58, 16.12, 27.51 and 36.55 mg g^{-1} min^{-1} respectively) and *T. viride* (activity of 6.44, 15.31, 24.32 and 33.11 mg g^{-1} min^{-1} respectively). *P. fluorescens* treated fruits exhibited the least activity (activity of 3.43, 10.17, 17.59 and 24.59 mg g^{-1} min^{-1} respectively after 0, 24, 48 and 72 h) (Table 19).

4.4.3. Estimation of Polyphenol Oxidase Enzyme Activity

PPO enzyme activity after treatment with biocontrol agents but before inoculation with pathogen was found to be increasing upto 48 h of inoculation and was decreasing thereafter for all the treatments. Polyphenol oxidase enzyme

Table 18. Peroxidase enzyme activity after treatment with biocontrol agents and before inoculation with *C. capsici* in detached chilli fruits var. Vellayani Athulya

Treatments	Peroxidase enzyme activity (mg g^{-1} min^{-1})			
	Hours after treatment			
	0h	24h	48h	72h
T.viride	4.86±0.03^{d}	8.32±0.06^{d}	13.82±0.12^{c}	10.86±0.10^{c}
P. fluorescens	2.21±0.04^{g}	4.54±0.05^{g}	10.30±0.14^{f}	7.99±0.12^{d}
B. subtilis	6.52±0.05^{b}	10.60±0.22^{b}	23.06±0.08^{b}	19.14±0.10^{a}
B. pumilus	5.29±0.05^{c}	9.31±0.06^{c}	13.41±0.11^{d}	6.91±0.07^{f}
B. amyloliquefaciens	8.81±0.08^{a}	16.11±0.07^{a}	23.70±0.13^{a}	18.31±0.13^{b}
P. indica	3.02±0.06^{e}	7.99±0.03^{e}	12.59±0.16^{e}	5.95±0.04^{g}
Uninoculated control	2.03±0.04^{f}	3.66±0.09^{f}	4.96±0.09^{g}	3.42±0.06^{h}
SEm±	0.05	0.09	0.11	0.09
CD(0.05)	0.15	0.29	0.34	0.28

Values are mean ± standard deviation of three replications

Values followed by same superscripts in a column are not significantly different at 5% level of significance

Table 19. Peroxidase enzyme activity after treatment with biocontrol agents and inoculation with *C. capsici* in detached chilli fruits var. Vellayani Athulya

Treatments	Peroxidase enzyme activity (mg g^{-1} min^{-1})			
	Hours after inoculation			
	0h	24h	48h	72h
T. viride + *C. capsici*	6.44±0.07^{c}	15.31±0.06^{c}	24.32±0.12^{d}	33.11±0.08^{c}
P. fluorescens + *C. capsici*	3.43±0.05^{e}	10.17±0.04^{f}	17.59±0.04^{f}	24.59±0.02^{f}
B. subtilis + *C. capsici*	7.58±0.03^{b}	16.12±0.05^{b}	27.51±0.06^{b}	36.55±0.06^{b}
B. pumilus + *C. capsici*	5.23±0.07^{d}	14.94±0.04^{d}	25.65±0.05^{c}	32.27±0.09^{d}
B. amyloliquefaciens + *C. capsici*	10.61±0.04^{a}	22.03±0.06^{a}	32.94±0.08^{a}	41.23±0.04^{a}
P. indica + *C. capsici*	5.23±0.05^{d}	13.55±0.06^{e}	22.52±0.06^{e}	29.59±0.02^{e}
C. capsici alone	2.43±0.04^{f}	5.68±0.09^{g}	9.69±0.09^{g}	7.42±0.06^{g}
Uninoculated control	2.03±0.04^{f}	3.66±0.09^{h}	4.96±0.09^{h}	3.82±0.06^{h}
SEm±	0.05	0.06	0.08	0.06
CD(0.05)	0.16	0.18	0.23	0.18

Values are mean ± standard deviation of three replications

Values followed by same superscripts in a column are not significantly different at 5% level of significance

activity before inoculation with pathogen was found to be highest in chilli fruits treated with *B. amyloliquefaciens*. The activity was 0.34, 0.68, 0.99 and 0.73 mg g^{-1} min^{-1} after 0, 24, 48 and 72 h of treatment. This was followed by *B. subtilis* (activity of 0.28, 0.57, 0.95 and 0.65 mg g^{-1} min^{-1} respectively) and *T. viride* (activity of 0.35, 0.55, 0.89 and 0.68 mg g^{-1} min^{-1} respectively). The least activity was observed for fruits treated with *P. fluorescens* (activity of 0.36, 0.42, 0.62 and 0.53 mg g^{-1} min^{-1} respectively after 0, 24, 48 and 72h of treatment) (Table 20).

Polyphenol oxidase enzyme activity after treatment with biocontrol agents and inoculation with pathogen were higher than the activity before pathogen inoculation and was found to be increasing upto 48 h of inoculation and then found decreasing thereafter for all the treatments. The enzyme activity was found to be highest in chilli fruits treated with *B. amyloliquefaciens* where the activity for 0, 24, 48 and 72 h were 1.06, 1.97, 2.66 and 1.24 mg g^{-1} min^{-1} respectively followed by *B. subtilis* (activity of 0.88, 1.43, 2.27 and 1.13 mg g^{-1} min^{-1} respectively) and *T. viride* (activity of 0.79, 1.32, 2.08 and 1.16 mg g^{-1} min^{-1}respectively). *P. fluorescens* treated fruits exhibited the least activity (activity of 0.42, 0.78, 1.19 and 0.64 mg g^{-1} min^{-1} respectively after 0, 24, 48 and 72 h of inoculation) (Table 21).

The best three treatments obtained from the fruit assay were *B. amyloliquefaciens* VLY 24, *B. subtilis* VLY 62 and *T. viride* (KAU isolate). These were also the best treatments obtained in dual culture assay. These three bioagents were selected for further studies.

4.5. STANDARDISATION OF BIOPRIMING TECHNIQUES

4.5.1. Standardisation of Seed Soaking Time

Chilli seeds were bioprimed with biocontrol agents *viz.*, *T. viride* (KAU isolate), *P. fluorescens* PN 026 (KAU isolate), *B. subtilis* VLY 62, *B. amyloliquefaciens* VLY 24, *B. pumilus* VLY 17 and *P. indica*. Seeds were also primed with carbendazim (0.1 %) and hydroprimed. After priming, the seeds were

Table 20. Polyphenol oxidase enzyme activity after treatment with biocontrol agents and before inoculation with *C. capsici* in detached chilli fruits var. Vellayani Athulya

Treatments	Polyphenol oxidase enzyme activity (mg g^{-1} min^{-1})			
	Hours after treatment			
	0h	24h	48h	72h
T. viride(KAU)	0.35 ± 0.01^{a}	0.55 ± 0.01^{bc}	0.89 ± 0.01^{c}	0.68 ± 0.01^{b}
P. fluorescens (KAU)	0.36 ± 0.02^{a}	0.42 ± 0.01^{d}	0.62 ± 0.01^{f}	0.53 ± 0.01^{e}
B. subtilis	0.28 ± 0.01^{b}	0.57 ± 0.02^{bc}	0.95 ± 0.01^{b}	0.65 ± 0.01^{c}
B. pumilus	0.34 ± 0.01^{a}	0.52 ± 0.01^{c}	0.85 ± 0.01^{d}	0.54 ± 0.01^{de}
B. amyloliquefaciens	0.34 ± 0.01^{a}	0.68 ± 0.01^{a}	0.99 ± 0.01^{a}	0.73 ± 0.01^{a}
P. indica	0.26 ± 0.01^{bc}	0.53 ± 0.02^{bc}	0.75 ± 0.02^{e}	0.65 ± 0.01^{c}
Uninoculated control	0.23 ± 0.01^{c}	0.25 ± 0.01^{e}	0.33 ± 0.01^{f}	0.27 ± 0.01^{f}
SEm±	0.01	0.01	0.01	0.01
CD(0.05)	0.03	0.04	0.04	0.03

Values are mean ± standard deviation of three replications

Values followed by same superscripts in a column are not significantly different at 5% level of significance

Table 21. Polyphenol oxidase enzyme activity after treatment with biocontrol agents and inoculation with *C. capsici* in detached chilli fruits var. Vellayani Athulya

Treatments	Polyphenol oxidase enzyme activity (mg g^{-1} min^{-1})			
	Hours after inoculation			
	0 h	24 h	48 h	72 h
T. viride + *C. capsici*	0.79±0.01^{c}	1.32±0.06^{c}	2.08±0.04^{b}	1.16±0.02^{b}
P. fluorescens + *C. capsici*	0.42±0.01^{f}	0.78±0.01^{e}	1.19±0.12^{d}	0.64±0.01^{d}
B. subtilis + *C. capsici*	0.88±0.01^{b}	1.43±0.01^{b}	2.27±0.08^{b}	1.13±0.01^{b}
B. pumilus + *C. capsici*	0.73±0.01^{d}	1.28±0.01^{c}	2.08±0.04^{b}	1.06±0.01^{c}
B. amyloliquefaciens + *C. capsici*	1.06±0.02^{a}	1.97±0.02^{a}	2.66±0.05^{a}	1.24±0.01^{a}
P. indica + *C. capsici*	0.64±0.01^{e}	1.14±0.01^{d}	1.66±0.03^{c}	1.04±0.01^{c}
C. capsici alone	0.23±0.01^{g}	0.45±0.01^{f}	0.63±0.01^{e}	0.57±0.01^{e}
Uninoculated control	0.23±0.01^{g}	0.25±0.01^{g}	0.33±0.01^{f}	0.27±0.01^{f}
SEm±	0.01	0.02	0.06	0.01
CD(0.05)	0.04	0.07	0.19	0.04

Values are mean ± standard deviation of three replications

Values followed by same superscripts in a column are not significantly different at 5% level of significance

incubated and subjected to germination test. Biometric characters of the seedlings were also recorded. The germination of seeds started from 7th day of incubation and continued upto 14th day. Effect of different duration of biopriming were studied.

The observations on the effect of seed biopriming on germination percentage of chilli are given in table 22. The observations on biometric characters of chilli seedlings *viz.*, shoot length (Table 23), root length (Table 24), seedling length (Table 25), dry weight (Table 26), SVI – I (Table 27) and SVI – II (Table 28) were also recorded.

The highest germination percentage, shoot length, root length, seedling length, seedling dry weight, SVI I and SVI II were observed in the case of seeds soaked for 1 h in case of *T. viride* and 4 h for the other treatments. It was also observed that there was an increase in the values of the biometric characters when the soaking time was increased from 20 minutes to 1 hour. But a slight decrease was recorded for 2 h. Thereafter it gradually increased and reached the maximum value at four hours of priming. After 4 h also a decrease was observed till 16h except in some cases where a decrease at 8 h was followed by an increase at 16 h (Tables 22 to 28).

Comparing the treatments for the best soaking time (1 h for *T. viride* and 4 h for other treatments *viz. P. fluorescens, B. subtilis, B. amyloliquefaciens, B. pumilus* and *P. indica*), seed priming with carbendazim (0.1 %) recorded the highest values for seed germination percentage (94.52 %), root length (9.17 cm), seedling length (13.05 cm) and SVI - I (1233.50) and stood second in the case of seedling dry weight (2.88 mg) and SVI - II (0.27). This was followed by priming with *P. indica* for germination percentage (93.84), seedling length (12.88 cm) and SVI - I (1209.04) and priming with *P. fluorescens* for root length (8.97 cm). The highest shoot length was recorded for priming with *T. viride* (4.33 cm) followed by *B. pumilus* (4.07 cm). The highest seedling dry weight was recorded in case of priming with *P. indica* (2.98 mg) and it also recorded the highest SVI - II (0.28).

The lowest germination percentage (82.22), root length (8.49 cm) and SVI - I (1054.49) were recorded for seed priming with *T. viride*. The lowest shoot length (3.42 cm) was observed in case of seed priming with *P. fluorescens*. Priming with *B. amyloliquefaciens* recorded the lowest seedling length (12.11 cm). The lowest seedling dry weight (2.07 mg) and SVI - II (0.19) were recorded for priming with *B. subtilis* (Tables 22 to 28).

4.6. EFFECT OF SEED BIOPRIMING AND SPRAYING AT FRUIT SET ON CHILLI UNDER POT CULTURE STUDIES

4.6.1. *In vivo* Effect of Seed Biopriming in Chilli

In vivo effect of seed biopriming on chilli was studied by conducting a pot culture experiment at Coconut Research Station, Balaramapuram during March - July 2019 (Plate 19). Bioprimed chilli seeds were used for raising the crop and observations on growth parameters, yield and incidence and severity of anthracnose / fruit rot were recorded. Days taken for 50 per cent of plants to set flower was found to be the same *ie.*, 51-52 DAS irrespective of the treatment (Table 29).

Plant height was measured at 40, 80 and 120 DAS. The plant heights were significantly increased by the biopriming treatments compared to the control. The maximum height at 40 and 80 DAS was observed for plants treated with *B. amyloliquefaciens ie.,* 38.38 cm and 77.50 cm respectively. This was higher than the chemical check carbendazim @ 0.1 per cent (plant height of 35.38 cm and 74.75 cm respectively at 40 and 80 DAS) and followed by *T. viride* (31.37 cm and 72.38 cm respectively). At 120 DAS maximum height was recorded for plants treated with *B. amyloliquefaciens* (105.63 cm) and this was on par with carbendazim (105.88 cm) and was followed by *T. viride* (100.13 cm). The least height at 40, 80 and 120 DAS was recorded for plants treated with *B. subtilis* which were 30.50, 61.75 and 96.88 cm respectively after 40, 80 and 120 DAS (Table 29).

Table 22. Effect of seed biopriming on germination percentage of chilli seeds var. Vellayani Athulya

Treatments (A)	Seed germination percentage (%)						
	Duration of soaking (B)						Mean A
	20 min	1 h	2 h	4 h	8 h	16 h	
T. viride	72.22	82.22	73.71	65.89	61.11	71.11	71.04
P. fluorescens	72.73	80.00	83.33	90.74	85.56	89.63	83.66
B. subtilis	75.93	81.11	77.40	91.11	82.22	85.78	82.26
B. pumilus	71.11	79.63	81.61	92.22	85.56	83.04	82.19
B. amyloliquefaciens	72.96	78.14	75.56	92.59	85.55	83.33	81.35
P. indica	76.30	81.39	86.38	93.84	88.15	90.47	86.09
Carbendazim (0.1 %)	80.73	86.30	77.03	94.52	91.26	81.63	85.24
Hydropriming	72.97	77.41	79.25	87.41	82.97	80.51	80.09
Control (Without priming)	72.96	72.96	72.96	72.96	72.96	72.96	72.96
Mean B	74.21	79.91	78.58	86.81	81.70	82.05	

Treatment effects	SEm (±)	CD (0.05)
A	0.37	1.03
B	0.30	0.84
AxB	0.90	2.53

Values are mean ± standard deviation of three replications

Table 23. Effect of seed biopriming on shoot length of chilli seedlings var. Vellayani Athulya

Treatments (A)	Shoot length of seedlings (cm)						
	Duration of soaking (B)						Mean A
	20 min	1 h	2 h	4 h	8 h	16 h	
T. viride	3.46±0.01	4.33±0.08	3.92±0.06	3.86±0.02	3.87±0.03	3.97±0.09	3.90
P. fluorescens	3.38±0.05	3.59±0.02	3.35±0.02	3.42±0.06	3.53±0.06	358±0.02	3.48
B. subtilis	3.27±0.01	3.53±0.05	3.24±0.05	3.61±0.04	3.40±0.03	3.20±0.03	3.38
B. pumilus	3.64±0.03	3.64±0.07	2.99±0.07	4.07±0.08	3.33±0.07	3.30±0.04	3.49
B. amyloliquefaciens	3.23±0.02	3.53±0.09	3.37±0.09	3.59±0.09	3.67±0.07	3.36±0.02	3.46
P. indica	3.43±0.03	3.90±0.02	3.85±0.02	4.19±0.02	3.71±0.01	3.90±0.11	3.83
Carbendazim (0.1%)	3.52±0.02	3.67±0.06	3.22±0.06	3.88±0.05	3.82±0.01	3.79±0.03	3.65
Hydropriming	3.71±0.01	3.80±0.03	3.68±0.03	3.95±0.07	3.19±0.01	3.51±0.04	3.64
Control (Without priming)	3.28±0.04	3.28±0.04	3.28±0.04	3.28±0.04	3.28±0.04	3.28±0.04	3.28
Mean B	3.44	3.70	3.43	3.76	3.53	3.54	
Treatment effects	SEm (±)	CD (0.05)					
A	0.02	0.05					
B	0.02	0.04					
AxB	0.05	0.13					

Values are mean ± standard deviation of three replications

Table 24. Effect of seed biopriming on root length of chilli seedlings var. Vellayani Athulya

Treatments (A)	Root length of seedlings (cm)						
	Duration of soaking (B)						Mean A
	20 min	1 h	2 h	4 h	8 h	16 h	
T. viride	8.24±0.05	8.49±0.02	7.74±0.03	7.96±0.02	7.98±0.04	8.23±0.07	8.11
P. fluorescens	7.41±0.02	8.23±0.06	8.11±0.05	8.97±0..03	8.56±0.06	8.26±0.03	8.26
B. subtilis	7.88±0.01	8.12±0.01	7.53±0.04	8.79±0.04	8.26±0.02	8.41±0.02	8.17
B. pumilus	8.19±0.04	7.71±0.04	8.24±0.08	8.50±0.04	8.49±0.03	8.84±0.03	8.33
B. amyloliquefaciens	6.79±0.06	8.15±0.03	7.45±0.05	8.51±0.06	8.18±0.04	8.36±0.05	7.91
P. indica	7.04±0.03	7.27±0.02	7.73±0.02	8.61±0.03	8.92±0.02	7.92±0.02	7.91
Carbendazim (0.1%)	8.82±0.05	8.92±0.06	9.10±0.10	9.17±0.02	9.02±0.03	8.96±0.05	9.00
Hydropriming	7.74±0.02	8.23±0.04	8.41±0.02	8.79±0.05	8.39±0.03	8.40±0.03	8.32
Control (Without priming)	7.21±0.02	7.21±0.02	7.21±0.02	7.21±0.02	7.21±0.02	7.21±0.02	7.21
Mean B	7.70	8.04	7.95	8.5	8.34	8.29	
Treatment effects	SEm (±)	CD (0.05)					
A	0.02	0.04					
B	0.01	0.04					
AxB	0.04	0.11					

Values are mean ± standard deviation of three replications

Table 25. Effect of seed biopriming on seedling length of chilli var. Vellayani Athulya

Treatments (A)	Length of seedlings (cm)						
	Duration of soaking (B)						Mean A
	20 min	1 h	2 h	4 h	8 h	16 h	
T. viride	11.69±0.05	12.83±0.06	11.66±0.04	11.82±0.03	11.86±0.04	12.20±0.10	12.01
P. fluorescens	10.79±0.06	11.81±0.04	11.46±0.03	12.39±0.04	12.09±0.04	11.84±0.04	11.73
B. subtilis	11.15±0.01	11.66±0.07	10.78±0.01	12.41±0.02	11.66±0.04	11.61±0.06	11.55
B. pumilus	11.83±0.06	11.34±0.04	11.17±0.15	12.59±0.05	11.84±0.08	12.24±0.06	11.84
B. amyloliquefaciens	10.02±0.06	11.69±0.04	10.82±0.06	12.11±0.06	11.85±0.09	11.76±0.07	11.37
P. indica	10.47±0.05	11.16±0.06	11.58±0.01	12.88±0.04	12.67±0.03	11.93±0.09	11.78
Carbendazim (0.1%)	12.34±0.05	12.59±0.04	12.31±0.13	13.05±0.03	12.85±0.03	12.76±0.06	12.65
Hydropriming	11.46±0.02	12.05±0.04	12.09±0.05	12.74±0.11	11.58±0.02	11.91±0.01	11.97
Control (Without priming)	10.49±0.02	10.49±0.02	10.49±0.02	10.49±0.02	10.49±0.02	10.49±0.02	10.49
Mean B	11.14	11.74	11.37	12.28	11.88	11.86	
Treatment effects	SEm (±)	CD (0.05)					
A	0.02	0.07					
B	0.02	0.05					
AxB	0.06	0.16					

Values are mean ± standard deviation of three replications

Table 26. Effect of seed biopriming on dry weight of chilli seedlings var. Vellayani Athulya

Treatments (A)	Seedling dry weight (mg)						
	Duration of soaking (B)						Mean A
	20 min	1 h	2 h	4 h	8 h	16 h	
T. viride	2.38±0.04	2.46±0.05	2.53±0.03	2.80±0.03	2.52±0.04	2.73±0.05	2.57
P. fluorescens	2.07±0.04	2.12±0.01	2.22±0.01	2.46±0.05	2.35±0.03	2.29±0.05	2.25
B. subtilis	2.11±0.06	2.01±0.05	2.14±0.03	2.07±0.04	2.14±0.01	2.13±0.04	2.10
B. pumilus	2.22±0.02	2.07±0.01	1.72±0.05	2.30±0.04	2.24±0.03	2.15±0.06	2.12
B. amyloliquefaciens	2.14±0.01	2.10±0.03	2.14±0.05	2.27±0.02	2.12±0.01	2.23±0.03	2.17
P. indica	1.56±0.05	2.61±0.01	2.74±0.05	2.98±0.02	2.89±0.03	2.76±0.07	2.59
Carbendazim (0.1%)	2.02±0.03	2.31±0.02	2.63±0.04	2.88±0.05	2.67±0.02	2.44±0.04	2.49
Hydropriming	2.23±0.02	2.44±0.05	2.23±0.04	2.80±0.11	2.37±0.04	2.40±0.01	2.41
Control (Without priming)	1.91±0.01	1.91±0.01	1.91±0.01	1.91±0.01	1.91±0.01	1.91±0.01	1.91
Mean B	2.07	2.22	2.25	2.50	2.36	2.34	
Treatment effects	SEm (±)	CD (0.05)					
A	0.02	0.04					
B	0.01	0.04					
AxB	0.04	0.11					

Values are mean ± SD of three replications

Table 27. Effect of seed biopriming on vigour index (SV - I) of chilli seedlings var. Vellayani Athulya

Treatments (A)	Seedling vigour index 1 (SVI - I)						
	Duration of soaking (B)						Mean A
	20 min	1 h	2 h	4 h	8 h	16 h	
T. viride	844.54±3.25	1054.49±4.88	859.23±3.29	778.574±1.99	724.65±2.77	867.87±7.07	854.89
P. fluorescens	784.98±4.06	945.07±3.51	954.83±2.47	1123.99±3.45	1034.74±3.40	1061.55±3.42	984.19
B. subtilis	846.35±0.98	945.44±5.48	834.15±1.15	1130.95±2.05	958.99±3.49	996.08±4.75	951.99
B. pumilus	841.33±4.42	903.25±2.71	911.61±12.18	1161.07±4.84	1012.96±6.53	1016.49±5.34	974.45
B. amyloliquefaciens	730.80±4.41	913.19±2.96	817.55±4.39	1120.90±5.17	1014.11±7.74	979.89±5.45	929.41
P. indica	798.57±4.08	908.15±4.97	1000.03±0.49	1209.04±4.12	1116.54±2.37	1079.65±8.69	1018.67
Carbendazim (0.1%)	996.42±3.88	1086.78±3.05	948.34±10.49	1233.50±2.86	1172.38±2.44	1041.75±5.03	1079.86
Hydropriming	835.99±1.25	932.59±3.16	958.44±2.71	1113.39±9.84	960.79±1.75	959.09±1.11	960.05
Control (Without priming)	765.36±3.41	765.36±3.41	765.36±3.41	765.36±3.41	765.36±3.41	765.36±3.41	765.36
Mean B	827.15	939.37	894.39	1070.75	973.39	974.19	
Treatment effects	SEm (±)	CD (0.05)					
A	4.87	13.66					
B	3.97	11.15					
AxB	11.92	33.45					

Values are mean ± standard deviation of three replications

Table 28. Effect of seed biopriming on vigour index (SVI - II) of chilli seedlings var. Vellayani Athulya

Treatments (A)	Seedling vigour index 2 (SVI - II)						
	Duration of soaking (B)						Mean A
	20 min	1 h	2 h	4 h	8 h	16 h	
T. viride	0.17±0.002	0.20±0.004	0.18±0.002	0.18±0.002	0.15±0.003	0.19±0.003	0.18
P. fluorescens	0.15±0.003	0.17±0.006	0.19±0.003	0.22±0.004	0.20±0.003	0.20±0.004	0.19
B. subtilis	0.16±0.004	0.16±0.004	0.17±0.002	0.19±0.003	0.18±0.004	0.18±0.003	0.17
B. pumilus	0.16±0.001	0.16±0.006	0.14±0.004	0.21±0.004	0.19±0.002	0.18±0.005	0.17
B. amyloliquefaciens	0.16±0.001	0.16±0.002	0.16±0.004	0.21±0.001	0.18±0.001	0.19±0.002	0.18
P. indica	0.12±0.004	0.21±0.005	0.24±0.004	0.28±0.002	0.25±0.003	0.25±0.007	0.23
Carbendazim (0.1%)	0.16±0.003	0.20±0.001	0.20±0.003	0.27±0.005	0.24±0.002	0.20±0.004	0.21
Hydropriming	0.16±0.001	0.19±0.004	0.18±0.003	0.24±0.009	0.20±0.003	0.19±0.001	0.19
Control (Without priming)	0.14±0.001	0.14±0.001	0.14±0.001	0.14±0.001	0.14±0.001	0.14±0.001	0.14
Mean B	0.15	0.18	0.18	0.22	0.19	0.19	
Treatment effects	SEm (±)	CD (0.05)					
A	0.001	0.005					
B	0.001	0.004					
AxB	0.004	0.011					

Values are mean ± standard deviation of three replications

The number of branches per plant at 120 DAS were significantly higher in the treated plants compared to the control. The maximum number of primary, secondary and tertiary branches were observed for plants treated with *T. viride* (6.25, 13.50 and 28.50 respectively) and this was higher than carbendazim (6.00, 11.75 and 25.25 respectively) and *B. subtilis* (5.25, 12.00 and 24.50 respectively). The number of primary, secondary and tertiary branches observed in case of plants treated with *B. amyloliquefaciens* were 5.00, 11.00 and 22.25 respectively (Table 29).

Biopriming also increased the number of fruits per plant, fruit yield per plant, hundred seed weight and seed yield per plant. The number of fruits per plant was maximum for plants treated with *T. viride* (72.75) followed by *B. subtilis* (67.00) which was on par with *B. amyloliquefaciens* (66.75). These were higher than that of plants treated with carbendazim (57.50). Fruit yield per plant was maximum for plants treated with *T. viride* (658.37 g) followed by *B. subtilis* (629.25 g) which was on par with *B. amyloliquefaciens* (627.80 g). These were higher than that of plants treated with carbendazim (529.03 g). There was no significant difference in the number of seeds per fruit among the treated plants and the control. Hundred seed weight was maximum for plants treated with *T. viride* (0.74 g) followed by *B. subtilis* (0.73 g) which was on par with *B. amyloliquefaciens* (0.71 g) and was higher than carbendazim (0.70 g). The seed yield was also the highest for plants treated with *T. viride* (23.38 g) which was on par with *B. subtilis* (20.49 g). This was followed by treatment with *B. amyloliquefaciens* (18.82 g) which was on par with carbendazim treatment (19.55 g) (Table 30).

The incidence and severity of fruit rot was calculated at 5^{th}, 10^{th} and 15^{th} days after inoculation of the pathogen. The disease incidence on all the treatments were 100 per cent since they were artificially inoculated. All the biopriming treatments were effective in reducing the severity of fruit rot disease. The disease severity at 5^{th} and 10^{th} day after inoculation were found to be the lowest for plants treated with *B. subtilis* with PDI values 19.07 and 25.00 respectively. This was

Table 29. Effect of seed biopriming on days to flowering, plant height and number of branches per plant of chilli var. Vellayani Athulya

Treatment	Days to flowering in 50% of plants	Plant height (cm)			Number of branches per plant (120 DAS*)		
		40 DAS	80 DAS	120 DAS	Primary	Secondary	Tertiary
T. viride (KAU)	51-52	31.37±0.89^{c}	72.38±0.55^{c}	100.13±0.97^{b}	6.25±0.25^{a}	13.50±0.65^{a}	28.50±0.96^{a}
B. subtilis	51-52	30.50±0.64^{cd}	61.75±0.48^{d}	96.88±0.43^{c}	5.25±0.25^{bc}	12.00±0.41^{ab}	24.50±0.65^{bc}
B. amyloliquefaciens	51-52	38.38±0.55^{a}	77.50±0.46^{a}	105.63±0.24^{a}	5.00±0.00^{c}	11.00±0.41^{bc}	22.25±0.48^{c}
Carbendazim (0.1%)	51-52	35.38±0.24^{b}	74.75±0.32^{b}	105.88±0.32^{a}	6.00±0.41^{ab}	11.75±0.48^{b}	25.25±0.48^{b}
Inoculated control	51-52	29.00±0.71^{de}	59.03±0.79^{e}	94.38±0.55^{d}	4.75±0.25^{c}	9.75±0.48^{c}	19.75±0.75^{d}
Uninoculated control	51-52	28.50±0.65^{e}	60.13±0.83^{de}	94.63±0.24^{d}	4.75±0.25^{c}	9.75±0.75^{c}	19.75±1.11^{d}
SEm±		0.65	0.60	0.52	0.26	0.54	0.77
CD(0.05)		1.94	1.80	1.56	0.79	1.63	2.31

*DAS – days after sowing

Values are mean ± standard deviation of four replications

Values followed by same superscripts in a column are not significantly different at 5% level of significance

Table 30. Effect of seed biopriming on number of fruits per plant, fruit yield per plant, seeds per fruit, 100 seed weight and seed yield per plant of chilli var. Vellayani Athulya

Treatment	Number of fruits per plant	Fruit yield per plant (g)	Number of seeds per fruit	100 seed weight (g)	Seed yield per plant (g)
T.viride(KAU)	72.75±0.85[a]	658.37±6.77[a]	44.60±3.46	0.74±0.01[a]	23.38±1.79[a]
B. subtilis	67.00±1.08[b]	629.25±3.54[b]	47.35±6.12	0.73±0.01[ab]	20.49±2.67[a]
B. amyloliquefaciens	66.75±0.25[b]	627.8±3.53[b]	43.25±2.32	0.71±0.01[ab]	18.82±1.43[ab]
Carbendazim (0.1%)	57.50±1.26[c]	529.03±401[c]	49.10±5.49	0.70±0.01[b]	19.55±2.52[ab]
Inoculated control	48.25±1.11[d]	412.44±5.91[e]	42.03±3.23	0.62±0.02[c]	14.14±1.18[b]
Uninoculated control	47.75±1.55[d]	432.34±4.31[d]	42.60±3.74	0.62±0.02[c]	14.62±1.02[b]
SEm±	1.09	4.84	4.27	0.01	1.88
CD(0.05)	3.27	14.48	NS	0.04	5.62

Values are mean ± standard deviation of four replications

Values followed by same superscripts in a column are not significantly different at 5% level of significance

followed by treatment with *T. viride* (PDI - 22.19 and 28.13 respectively for 5th and 10th day after inoculation) and *B. amyloliquefaciens* (PDI – 24.69 and 29.69 respectively). These were found to be lower than treatment with carbendazim (PDI - 29.69 and 37.50 respectively). The disease severity at 15th day was the lowest for plants treated with *B. subtilis* (PDI – 37.19) followed by those treated with *T. viride* and *B. amyloliquefaciens* (both PDI - 40.00) and were lower than carbendazim treatment (PDI - 46.56) (Table 31).

The incidence and severity of anthracnose in leaves was calculated at 5th, 10th and 15th day after inoculation of the pathogen. The disease incidence was 100 per cent since all the treatments were artificially inoculated. All the biopriming treatments were effective in reducing the severity of anthracnose. The disease severity at 5th day was the lowest for plants treated with *B. subtilis* (PDI – 2.70) followed by *T. viride* (PDI - 3.50) and *B. amyloliquefaciens* (PDI – 3.80). The disease severity at 10th day was the lowest for plants treated with *T. viride* (PDI – 6.90) which was on par with *B. subtilis* (PDI – 7.00) and was followed by *B. amyloliquefaciens* (PDI – 7.90). The lowest disease severity at 15th day was recorded for treatment with *B. subtilis* (PDI – 10.60) followed by *T. viride* (PDI – 11.30) which was on par with *B. amyloliquefaciens* (PDI – 11.90). The disease severity of the treated plants at 5th, 10th and 15th days were lower than that of the chemical check carbendazim with PDI 5.60, 10.10 and 13.70 per cent respectively (Table 32).

Biopriming with *B. subtilis* (10^8 cfu/ml) was found effective for the management of the disease and it significantly increased plant growth and yield.

4.6.2. *In vivo* Effect of Seed Biopriming and spraying during fruit set on Chilli

In vivo effect of seed biopriming and spraying during fruit set on chilli was studied by conducting a pot culture experiment at Coconut Research Station, Balaramapuram during March – July 2019. Bioprimed chilli seeds were planted

Table 31. Effect of seed biopriming on incidence and severity of fruit rot in chilli var. Vellayani Athulya

Treatment	Disease Incidence (DI) (%)	Disease severity (PDI)			Per cent reduction over inoculated control at 15th day
		5th day	10th day	15th day	
T. viride (KAU)	100	22.19 (28.09)^{d}	28.13 (32.03)^{d}	40.00 (39.23)^{c}	24.26
B. subtilis	100	19.07 (25.88)^{e}	25.00 (29.99)^{e}	37.19 (37.57)^{d}	29.58
B. amyloliquefaciens	100	24.69 (29.79)^{c}	29.69 (33.01)^{c}	40.00 (39.23)^{c}	24.26
Carbendazim (0.1%)	100	29.69 (33.01)^{b}	37.50 (37.76)^{b}	46.56 (43.03)^{b}	11.83
Inoculated control	100	33.75 (35.52)^{a}	41.56 (40.14)^{a}	52.81 (46.61)^{a}	
Uninoculated control	100	0.00 (0.29)^{f}	0.00 (0.29)^{f}	4.63 (12.39)^{e}	
SEm±		0.42	0.29	0.37	
CD(0.05)		1.26	0.88	1.11	

Values are mean ± standard deviation of four replications

Values followed by same superscripts in a column are not significantly different at 5% level of significance

Values in parenthesis are arcsine transformed values

Table 32. Effect of seed biopriming on the incidence and severity of anthracnose in chilli var. Vellayani Athulya

Treatment	Disease incidence (DI) (%)	Percentage disease index (PDI) / Disease severity			Per cent reduction over inoculated control at 15th day
		5th day	10th day	15th day	
T. viride (KAU)	100	3.50 (10.77)^{c}	6.90 (15.23)^{d}	11.30 (19.64)^{cd}	37.22
B. subtilis	100	2.70 (9.44)^{d}	7.00 (15.34)^{d}	10.60 (18.99)^{d}	41.11
B. amyloliquefaciens	100	3.80 (11.24)^{c}	7.90 (16.32)^{c}	11.90 (20.18)^{cd}	33.89
Carbendazim (0.1%)	100	5.60 (13.68)^{b}	10.10 (18.53)^{b}	13.70 (21.72)^{b}	23.89
Inoculated control	100	7.80 (16.22)^{a}	13.20 (21.30)^{a}	18.00 (25.10)^{a}	
Uninoculated control	100	0.60 (4.38)^{e}	2.00 (8.11)^{e}	3.20 (10.29)^{e}	
SEm±		0.28	0.21	0.22	
CD(0.05)		0.84	0.64	0.66	

Values are mean ± standard deviation of four replications

Values followed by same superscripts in a column are not significantly different at 5% level of significance

Values in parenthesis are arcsine transformed values

Plate 19. General view of pot culture experiment for the evaluation of seed biopriming for the control of anthracnose / fruit rot of chilli caused by *C. capsici* as well as yield and quality of chilli var. Vellayani Athulya

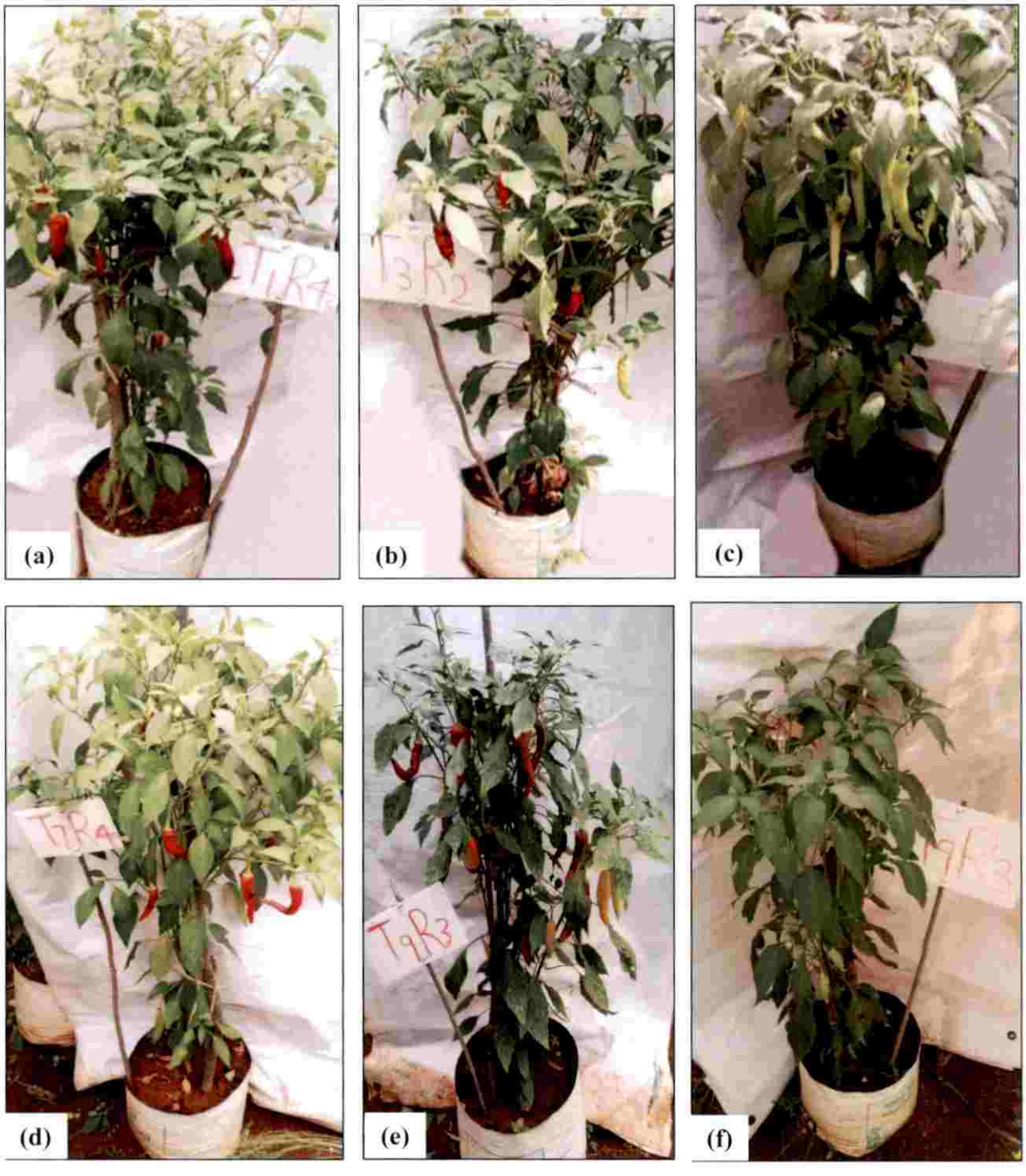

Plate 20. Effect of seed biopriming treatments for the management of anthracnose / fruit of chilli var. Vellayani Athulya (a) *T. viride* (b) *B. subtilis* (c) *B. amyloliquefaciens* (d) carbendazim (0.1%) (e) inoculated control and (f) uninoculated control

and biocontrol agent spray was given at when 50% of the plants set fruit and 15 days after the first spray and observations were taken. 129

The number of days taken for 50 per cent plants to set flower was 51-52 DAS irrespective of the treatments (Table 33). Plant height was measured at 40, 80 and 120 DAS. The maximum height at 40 DAS was recorded in case of plants treated with *B. amyloliquefaciens* (37.88 cm) followed by *B. subtilis* (31.75 cm) which was on par with *T. viride* (30.25 cm). At 80 DAS plant height was maximum for plants treated with *B. amyloliquefaciens* (75.88 cm) followed by *T. viride* (71.63 cm) and *B. subtilis* (61.50 cm). At 120 DAS maximum plant height was recorded for treatment with *B. amyloliquefaciens* (105.38 cm) and this was on par with carbendazim (105.50 cm) which was followed by *T. viride* (100.13 cm) and *B. subtilis* (96.75 cm) (Table 33).

The maximum number of primary, secondary and tertiary branches were observed in case of plants treated with *T. viride* (6.50, 14.00 and 28.00 respectively) and this was higher than those treated with carbendazim (5.75, 11.50 and 25.25 respectively). The number of primary, secondary and tertiary branches observed in plants bioprimed with *B. subtilis* were 5.50, 12.25 and 25 respectively and for *B. amyloliquefaciens* were 4.50, 11.00 and 21.50 respectively (Table 33).

The number of fruits per plant was maximum for plants treated with *T. viride* (72.50) followed by *B. subtilis* (66.25) which was on par with *B. amyloliquefaciens* (65.75). Fruit yield per plant was maximum for plants treated with *T. viride* (681.01 g) followed by *B. subtilis* (653.15 g) which was on par with *B. amyloliquefaciens* (651.11 g). These were higher than that of treatment with carbendazim (558.13 g). There was no significant difference in the number of seeds per fruit among the treatments and control. Hundred seed weight was maximum for plants treated with *T. viride* (0.75 g) followed by *B. subtilis* (0.72 g) which was on par with *B. amyloliquefaciens* (0.71 g) and was higher than carbendazim (0.70 g). The seed yield per plant was the highest for plants treated with *T. viride* (24.11 g) followed by *B. amyloliquefaciens* (22.73 g) which was on

Table 33. Effect of seed biopriming and spraying during fruit set on days to flowering, plant height and number of branches per plant of chilli var. Vellayani Athulya

Treatment	Days to flowering in 50% of plants	Plant height (cm)			Number of branches per plant		
		40 DAS	80 DAS	120 DAS	Primary	Secondary	Tertiary
T. viride (KAU)	51-52	30.25±0.48^{cd}	71.63±0.75^{c}	100.13±1.03^{b}	6.50±0.29^{a}	14.00±0.41^{a}	28.00±1.08^{a}
B. subtilis	51-52	31.75±0.47^{cd}	61.50±0.54^{d}	96.75±0.32^{c}	5.50±0.28^{bc}	12.25±0.48^{b}	25.00±0.41^{b}
B. amyloliquefaciens	51-52	37.88±0.43^{a}	75.88±0.43^{a}	105.38±0.32^{a}	4.50±0.28^{d}	11.00±0.41^{b}	21.50±0.65^{c}
Carbendazim (0.1%)	51-52	34.63±0.38^{b}	74.13±0.42^{b}	105.50±0.35^{a}	5.75±0.48^{ab}	11.50±0.65^{bc}	25.25±0.48^{b}
Inoculated control	51-52	29.13±0.83^{d}	59.75±0.72^{e}	94.38±0.43^{d}	4.50±0.29^{d}	9.50±0.65^{c}	19.50±0.96^{c}
Uninoculated control	51-52	29.13±0.97^{d}	59.63±0.55^{e}	94.13±0.24^{d}	4.75±0.25^{cd}	9.75±0.75^{c}	19.75±0.75^{c}
SEm±		0.63	0.58	0.52	0.32	0.57	0.76
CD(0.05)		1.89	1.75	1.56	0.97	1.71	2.27

Values are mean ± standard deviation of four replications

Values followed by same superscripts in a column are not significantly different at 5% level of significance

par with *B. subtilis* (21.58 g) and were higher than that of carbendazim (19.38 g) (Table 34). 131

The disease incidence and severity of fruit rot was calculated at 5th, 10th and 15th days after inoculation of the pathogen. The disease incidence on all the treatments was 100 per cent since they were artificially inoculated. All the biopriming treatments were effective in reducing the severity of fruit rot disease. The disease severity of fruit rot at 5th, 10th and 15th day of inoculation were found to be the lowest for plants treated with *B. amyloliquefaciens* (PDI – 13.13, 19.06 and 28.13 respectively for 5th, 10th and 15th day of inoculation) which were on par with the carbendazim treatment (PDI – 10.00, 17.81 and 29.38 respectively). Treatment with *T. viride* (PDI – 15.94, 22.19 and 32.50 respectively at 5th, 10th and 15th day after inoculation) was on par with *B. subtilis* treatment (PDI – 15.63, 21.25 and 31.25 respectively) (Table 35).

The disease incidence and severity of anthracnose in leaves was calculated at 5th, 10th and 15th day after inoculation of the pathogen. The disease incidence was 100% since all the treatments were artificially inoculated. All the treatments were effective in reducing the severity of anthracnose. The disease severity at 5th day was the lowest for plants treated with *B. amyloliquefaciens* (PDI – 1.00) which was on par with *B. subtilis* (PDI – 1.20) followed by *T. viride* (PDI - 1.90). The disease severity at 10th day was the lowest for plants treated with *B. amyloliquefaciens* (PDI – 3.60) which was on par with *B. subtilis* (PDI – 3.70) and was followed by *T. viride* (PDI - 4.60). The disease severity at 15th day was on par for all the three biocontrol agents with PDI values 8.50 for *T. viride*, 8.00 for *B. subtilis* and 8.10 for *B. amyloliquefaciens*. Plants treated with carbendazim recorded disease severity values of 0.60, 2.50 and 5.80 respectively at 5th, 10th and 15th day of inoculation (Table 36).

Other diseases and pests noted during the pot culture study were chilli leaf curl disease, chilli mite and red cotton bug.

Table 34. Effect of seed biopriming and spraying on number of fruits per plant, fruit yield per plant, seeds per fruit, 100 seed weight and seed yield per plant of chilli var. Vellayani Athulya

Treatment	Number of fruits per plant	Fruit yield per plant (g)	Number of seeds per fruit	100 seed weight (g)	Seed yield per plant (g)
T. viride (KAU)	72.50±0.65^{a}	681.01±4.99^{a}	46.15±3.93	0.75±0.01^{a}	24.11±1.78^{a}
B. subtilis	66.25±0.63^{b}	653.15±2.96^{b}	47.40±4.11	0.72±0.01^{ab}	21.58±2.17^{ab}
B. amyloliquefaciens	65.75±1.44^{b}	651.11±1.99^{b}	48.05±1.34	0.71±0.01^{ab}	22.73±0.99^{ab}
Carbendazim (0.1%)	58.00±0.91^{c}	558.13±2.91^{c}	45.00±3.95	0.70±0.01	19.38±1.79^{bc}
Inoculated control	48.50±1.04^{d}	414.63±3.62^{e}	43.90±1.75	0.61±0.02^{c}	15.39±1.10^{c}
Uninoculated control	48.25±1.50^{d}	435.61±3.58^{d}	43.72±1.99	0.61±0.02^{c}	15.62±0.92^{d}
SEm±	1.08	10.38	3.08	0.01	1.53
CD(0.05)	3.24	3.47	NS	0.04	4.59

Values are mean ± standard deviation of four replications

Values followed by same superscripts in a column are not significantly different at 5% level of significance

Table 35. Effect of seed biopriming and spraying on the incidence and severity of fruit rot in chilli var. Vellayani Athulya

Treatment	Disease Incidence (DI) (%)	Percentage Disease Index (PDI) / Disease severity			Percentage reduction over inoculated control at 15th day
		5th day	10th day	15th day	
T.viride(KAU)	100	15.94(23.52)[b]	22.19(28.1)[b]	32.50(34.75)[b]	38.17
B. subtilis	100	15.63(23.28)[b]	21.25(27.45)[b]	31.25(33.99)[b]	40.54
B. amyloliquefaciens	100	13.13(21.24)[c]	19.06(25.88)[c]	28.13(32.03)[c]	46.48
Carbendazim (0.1%)	100	10.00(18.42)[d]	17.81(24.96)[c]	29.38(32.82)[c]	44.10
Inoculated control	100	34.07(35.70)[a]	41.31(39.99)[a]	52.56(46.47)[a]	
Uninoculated control	100	0.00(0.29)[e]	0.00(0.29)[d]	4.86(12.74)[d]	
SEm±		0.35	0.36	0.27	
CD(0.05)		1.06	1.08	0.80	

Values are mean ± standard deviation of four replications

Values followed by same superscripts in a column are not significantly different at 5% level of significance

Values in parenthesis are arcsine transformed values

Table 36. Effect of seed biopriming and spraying on the incidence and severity of anthracnose in chilli var. Vellayani Athulya

Treatment	Disease Incidence (DI) (%)	Percentage Disease Index (PDI) / Disease severity			Percentage reduction over inoculated control at 15th day
		5th day	10th day	15th day	
T.viride(KAU)	100	1.90(7.89)[b]	4.60(12.38)[b]	8.50(16.95)[b]	52.78
B. subtilis	100	1.20(6.24)[c]	3.70(11.08)[c]	8.00(16.43)[b]	55.56
B. amyloliquefaciens	100	1.00(5.71)[c]	3.60(10.93)[c]	8.10(16.53)[b]	55.00
Carbendazim	100	0.60(4.38)[d]	2.50(9.08)[d]	5.80(13.93)[c]	67.78
Inoculated control	100	7.60(16.00)[a]	13.20(21.30)[a]	18.00(25.10)[a]	
Uninoculated control	100	0.60(4.38)[d]	2.00(8.11)[e]	3.20(10.30)[d]	
SEm±		0.38	0.27	0.22	
CD(0.05)		1.14	0.81	0.67	

Values are mean ± standard deviation of four replications

Values followed by same superscripts in a column are not significantly different at 5% level of significance

Values in parenthesis are arcsine transformed values

Plate 21. General view of pot culture experiment for the evaluation of seed biopriming and spraying during fruit set for the control of anthracnose / fruit rot of chilli caused by *C. capsici* as well as yield and quality of chilli var. Vellayani Athulya

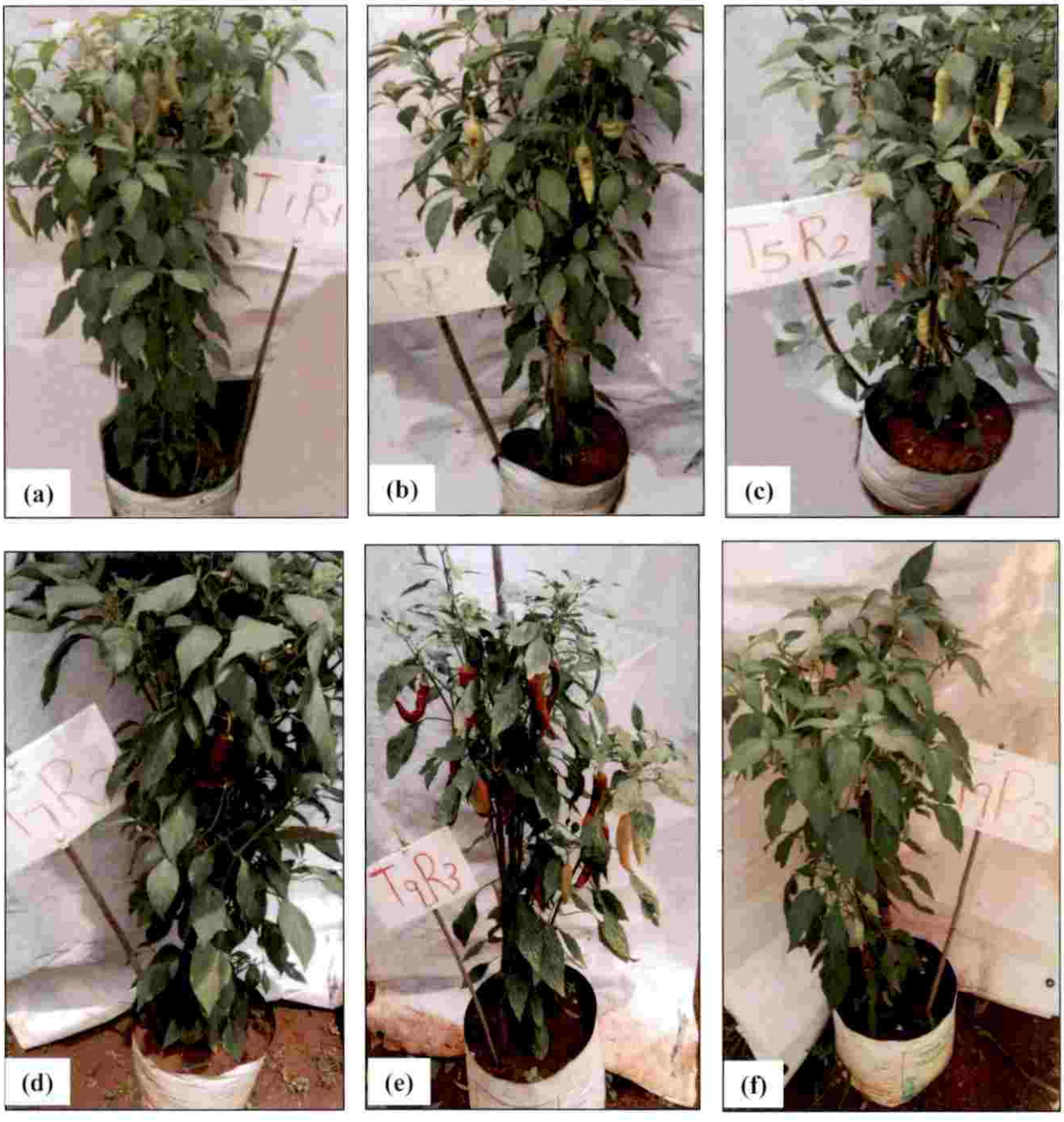

Plate 22. Effect of seed biopriming and spraying treatments for the management of anthracnose / fruit of chilli var. Vellayani Athulya (a) *T. viride* (b) *B. subtilis* (c) *B. amyloliquefaciens* (d) carbendazim (0.1%) (e) inoculated control (f) uninoculated control

Discussion

5. DISCUSSION

Chilli is an important cash crop grown worldwide. India is one of the leading producer and exporter of chilli in the world. Chilli contains high amount of vitamins, minerals, fibres and protein. It is also known to boost the immune system and lower the cholesterol levels. Anthracnose / fruit rot disease caused by *C. capsici* is an important disease of chilli which causes yield loss up to 50 per cent and affects the economic part *ie.*, the fruits. Chemical fungicides are widely used to curb the disease but are perilous to humans and the environment and also possess the threat of the pathogen developing resistance. Biological control is a promising eco-friendly alternative. The method of application of biocontrol agents to the plants is an important aspect to be considered. Seed biopriming is a biological and physiological method which primes the seeds with biocontrol agent thus protecting the seeds from pathogens and also improves the vigour. The present study aims to find the effect of seed biopriming as well as spraying with biocontrol agents on the control of anthracnose of chilli caused by *C. capsici*.

5.1. COLLECTION OF CHILLI SEEDS AND ISOLATION OF SEED BORNE MICROFLORA

5.1.1. Percentage of Infected Seeds

Twenty sets of seed samples were collected from five different agro-ecological zones of Kerala and the infection percentages were calculated using the standard blotter method. The infection percentages ranged from 6.62 to 43.33 per cent. Similarly, Hemannavar (2008) also recorded an infection percentage ranging from 8 to 52 per cent in chilli seed samples collected from Northern Karnataka region and opined that seeds may get infection through various sources. Pathogenic fungi may enter the seeds from the field as seed borne infection. Improper handling during harvesting, seed extraction, processing and improper storage can also lead to seed infection.

5.1.2. Isolation of Seed Borne Microflora

Externally and internally seed borne fungi and bacteria were isolated from the seeds. Six species of *Aspergillus*, three species of *Penicillium*, and other species of fungi such *Pestalotia*, *Curvularia*, *Alternaria*, *Mucor*, *Fusarium* and *C. capsici* were found associated with the seeds. *Aspergillus* spp. and *Penicillium* spp. were the major fungi associated with the seeds. *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp., *Fusarium* spp. and *C. capsici* were both externally and internally associated, *Pestalotia* spp. and *Curvularia* spp. were found to be externally seed borne only and *Mucor* spp. was internally seed borne. Eighteen different isolates of bacteria were also obtained from the seeds.

The results were in accordance with the findings of Tripathi *et al.* (1984) who observed that *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp., *Curvularia* spp. and *Fusarium* spp. were associated both externally and internally with chilli seeds. *Fusarium* spp., *Alternaria* spp., *Curvularia* spp. and *C. capsici* were isolated from seed samples of chilli (Hashmi, 1989; Mridha and Siddique, 1989; Padaganur and Naik, 1991). Sitara and Hasan (2011) reported that *Aspergillus* species was the dominant fungal species in chilli seed samples collected from Karachi region of Pakistan. Chigoziri and Ekefan (2013) isolated seven different species of *Aspergillus* from chilli seed samples collected from Benue State in Nigeria. *Mucor* species was also found in the seeds. Meon and Nick (1988) obtained *C. capsici* as both externally and internally seed borne and *Pestalotiopsis* species as internal infection from chilli seed samples collected from Universiti Pertanian Malaysia. Many bacteria were found associated with seeds. Some of the bacteria may enter inside the seeds and thus protect itself from surface sterilization procedures (Hayward, 1974).

From the present study it was revealed that storage fungi like *Aspergillus* spp. and *Penicillium* spp. were more prevalent in the seed samples than pathogenic fungi like *Colletotrichum* spp. and *Fusarium* spp. Hemannavar (2008) also noted a similar observation and opined that this may be due to the improper

handling of seeds during harvesting, extraction, threshing and unsafe storage. This could also be due to increase in storage period.

The major fungal species found associated with the seeds were *Aspergillus* spp. and *Penicillium* spp. George (1992) also reported a similar observation and stated that the occurrence of storage fungi like *Aspergillus* spp. and *Penicillium* spp. increased with increase in storage duration while the occurrence of field fungi like *Fusarium* spp. and *Curvularia* spp. decreased. This could be due to the innate ability of storage fungi to survive for longer periods. As the humidity increases the storage fungi in seeds also increases.

5.2. PATHOGENICITY STUDIES

5.2.1. Survey and Collection of Disease Samples

The survey conducted during June – November 2018 in five locations of Kerala *viz.*, Vellayani, Kumarakom, Thrissur, Wayanad and Kasargod revealed that anthracnose / fruit rot is an important disease affecting chilli and the pathogen *C. capsici* was mainly associated with the disease. The incidence of fruit rot ranged from 20 - 75 per cent (Figure 1) and the severity ranged from 43.50 - 67.25 per cent at the survey locations (Figure 2). The survey was conducted during June – November 2018. The average temperature during the survey ranged from 26 to 28°C and the relative humidity ranged from 81 to 87 per cent.

The occurence of *C. capsici* as the causal agent of anthracnose / fruit rot of chilli has been reported by several workers. *C. capsici*, *C. gloeosporioides* and *C. piperatum* are the causal organisms for anthracnose and ripe fruit of chilli in India (Thind and Jhooty, 1990). *C. capsici* and *C. gloeosporioides* were found as the major fungal pathogens associated with anthracnose and fruit rot of chilli in Kerala (Golda, 2010). A survey was conducted in six locations of Kerala *viz.*, Vellayani, Kumarakom, Thrissur, Pattambi, Ambalavayal and Padanakkad to study the incidence and severity of anthracnose and fruit rot of chilli and *C. capsici* was isolated from the diseased samples. The incidence of fruit rot

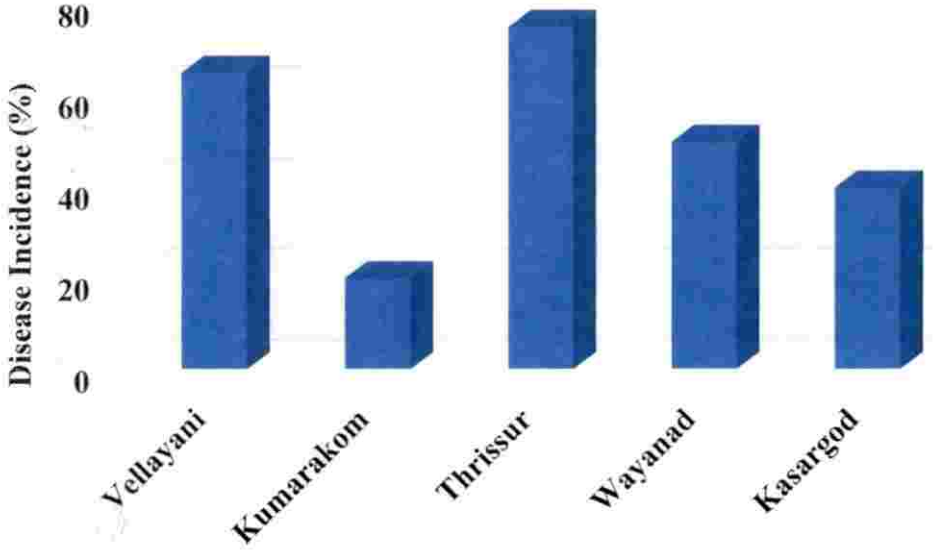

Figure 1. Incidence of fruit rot of chilli observed at five different agro-ecological zones of Kerala during the survey

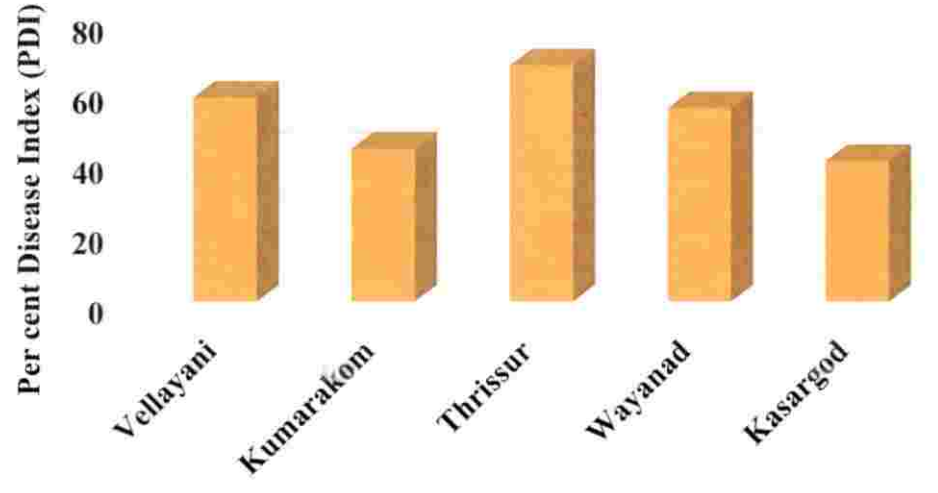

Figure 2. Severity of fruit rot of chilli observed at five different five different agro-ecological zones of Kerala during the survey

ranged from 20-80 per cent and disease severity ranged from 23 – 54 per cent at the different survey locations (Anjana, 2018). A survey was undertaken by Katediya *et al.* (2019) during Kharif 2017-2018 in the chilli growing areas of Banaskantha district of Gujarat. The PDI of anthracnose at survey locations ranged from 40.22 to 59.48.

The variation in incidence and severity of fruit rot observed at different locations could be due to the diversity in climatic conditions, variety cultivated, and virulence of the isolates (Anjana, 2018). The correlation between the weather condition and incidence of fruit rot can be observed the studies of Golda (2010) and Chacko (2015). They reported that chilli anthracnose in Kerala was prevalent during August – October with temperature of 29 -30^{0}C and relative humidity of 89.70 per cent.

5.2.2. Symptomatology of the Disease under Natural Condition

The symptoms observed on leaves were leaf spot and leaf blight which started off as dry rotting from the edges of the leaves and moved towards the centre, surrounded by yellow halo. Brown necrotic lesions with black acervuli were seen on the stem. The fruit rot symptom began as sunken water soaked lesion enlarging to straw or dark brown coloured elliptical lesion, later covering the entire fruit surface causing rot. Black coloured acervuli were seen on the rotted area as dots arranged in concentric rings. Fruits gradually got deformed, dried and turned black. The infection spread to seeds and such seeds initially had white mycelial growth and at advanced stage turned dark brown to black with acervuli on the surface. Severely infected plants dried up completely. Die back was not observed.

The anthracnose symptoms observed during the survey were similar to those observed by earlier workers and can be used as a diagnostic tool for early detection and management of the disease. *C. capsici* infected the leaves, stems and flowers of chilli, the most damaging being on mature ripe fruits. The initial symptom included the formation of small, elliptical or oblong straw coloured,

slightly sunken lesions on the surface of mature chilli fruits. Later black acervuli arranged in concentric rings giving a target board appearance was observed on the affected area. Naturally infected seeds had many acervuli over the surface. Pathogen was both externally and internally seed borne (Meon and Nik, 1988; Gupta, 2016). Anthracnose and fruit rot of chilli was more predominant in rainy season and high humidity favoured the disease. The symptoms observed under natural conditions included leaf blight, twig necrosis, dieback and fruit rot. Brown necrotic lesions with black acervuli were seen on the stem. The seeds were also infected and completely turned dark brown or black later on with acervuli on the surface (Anjana, 2018). Kaur *et al.* (2018) observed that the most prominent phase of chilli anthracnose was fruit rot and die back was seen in few places in a survey conducted in Punjab.

5.2.3. Morphological and Cultural Characterization of *C. capsici*

All the five isolates of *C. capsici* exhibited concentric zonations with acervuli. The cultures were white, off white or grey on the upper side and white, cream, yellow to dark brown on the reverse side. The margins were either regular or irregular. The radial mycelial growth at 7th day ranged from 7.95 to 8.80 cm (Figure 3). Microscopic observations revealed that mycelium was hyaline, septate and branched with width ranging from 2.19 to 3.32 µm. The conidia were sickle shaped with an oil globule at the centre with length ranging from 19.55 to 24.85 µm and the width ranging from 2.72 to 3.42 µm. Acervuli were black with diameters ranging from 121.23 to 147.03 µm. The number of setae in acervuli ranged from 13-56. Oval shaped brown coloured appressoria were observed in slide culture with length varying from 11.71 to 15.66 µm and the width varying from 6.26 to 8.56 µm.

The morphological and cultural characters of the *C. capsici* isolates observed were in accordance with the reports of many other workers. Five isolates of *C. capsici* were collected from different locations of Kerala. The colour of the mycelium ranged from white, white to cream, white later turning to grey or white

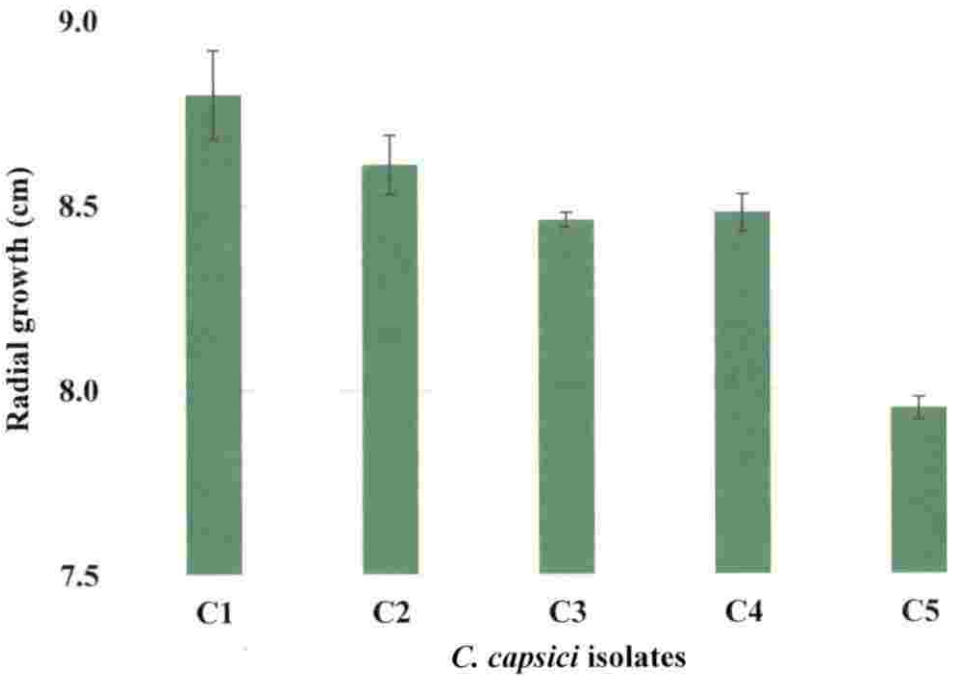

Figure 3. Radial mycelial growth of *C. capsici* isolates causing fruit of chilli seven days after growth in PDA medium. Error bars represent standard error of means of observed values.

to off-white on the upper side while the reverse side was white, yellowish white, creamy to brown, creamy white or creamy to dark brown. All the cultures had concentric zonations with acervuli with margins either regular or irregular. Radial mycelial growth ranged from 7.53 to 8.43 cm on 7th day of incubation. The mycelium was hyaline, slender and sepatate with width ranging 1.73 to 2.82 µm. The diameter of the acervuli varied from 127.40 to 183.28 µm. The average number of setae per acervuli ranged from 18 - 47 with length ranging from 72.50 to 110.41 µm. Length and width of appressoria ranged from 8.78 to 12.68 µm and 5.67 to 7.29 µm respectively (Anjana, 2018).

Masoodi *et al.* (2013) collected twenty different isolates of *C. capsici* from three districts of Kashmir valley. The cultures were white, grey, brown, dull white, dull grey, light brown, light smoky grey or grey in colour with regular or irregular margin. The acervuli were present as submerged and scattered or raised and scattered or raised and in concentric rings The average conidial length ranged from 19.70 to 33.60 µm and breadth ranged from 2.23 to 4.86 µm. The shape of the conidia were either fusiform or falcate. The length and breadth of setae ranged from 65.0 to 194.6 µm and 4.4 to 6.6 µm respectively.

The significant differences in the morphological and cultural characteristics suggests that there is variability in the pathogen *C. capsici* causing fruit rot of chilli in Kerala (Anjana, 2018).

5.2.4. Pathogenicity Testing and Virulence Rating of the Isolates of *C. capsici*

Pathogenicity of the isolates were studied following the Koch's postulates. All the isolates produced water soaked lesions on artificial inoculation to healthy chilli fruits. Re-isolation of the fungus produced cultures similar in morphology to the original cultures. Virulence testing was done by artificial inoculation on tender, mature and ripe fruits of chilli variety Vellayani Athulya. Characteristic fruit rot symptoms were observed and symptoms started as brown water soaked lesions which later enlarged and spread to entire fruit surface with black concentric zonations dotted with acervuli. The lesion sizes produced were 3.81

and 0.61cm on tender fruits (Figure 4), 4.02 and 0.43 cm on mature fruits (Figure 5) at 7th day of inoculation respectively and 2.80 and 0.48 cm at 10th day of inoculation on ripe fruits (Figure 6) respectively by the most virulent and least virulent isolates.

Anjana (2018) also studied the pathogenicity and comparative virulence of isolates of *C. capsici* by artificial inoculation on detached chilli fruits. The most virulent isolate produced a lesion length of 2.23 cm and the least virulent one produced a lesion size of 0.83 cm on tender fruits, 0.63 and 1.73 cm on mature fruits and 1.70 and 2.83 cm on ripe red fruits respectively at 7th day of inoculation. Chacko (2015) studied the variability among different isolates of *C. capsici* in chilli variety Vellayani Athulya and the size of the lesions varied from 1.17 to 4.34 cm in unripe fruits and 1.60 to 5.00 cm in ripe fruits.

The variying lesion sizes produced by the isolates under similar conditions confirms the pathogenic variability among the collected isolates. The results could be an indication of the presence of different pathotypes or races of *C. capsici* in different regions of Kerala.

5.3. *In vitro* SCREENING OF SELECTED BIOCONTROL AGENTS AGAINST *C. capsici*

Dual culture assay revealed that the maximum percentage suppression of mycelial growth of *C. capsici* was exhibited by *B. amyloliquefaciens* VLY 24 (62.96 %) followed by *B. subtilis* VLY 62 (56.30 %) and *T. viride* (51.85 %). The mycelial growth suppression given by *P. indica* was 46.67 per cent and *B. pumilus* VLY 17 was 45.19 per cent. *P. fluorescens* PN 026 did not show suppression of mycelial growth of *C. capsici* (Figure 7). Several other workers have also reported the *in vitro* inhibition of mycelial growth of *C. capsici* by the biocontrol agents mentioned above.

Jagtap *et al.* (2013) observed an inhibition percentage of 52.32 by *T. viride* against *C. capsici*. *T. viride* strains Tr8, Tr3, Tr14 and Tr12 inhibited the growth

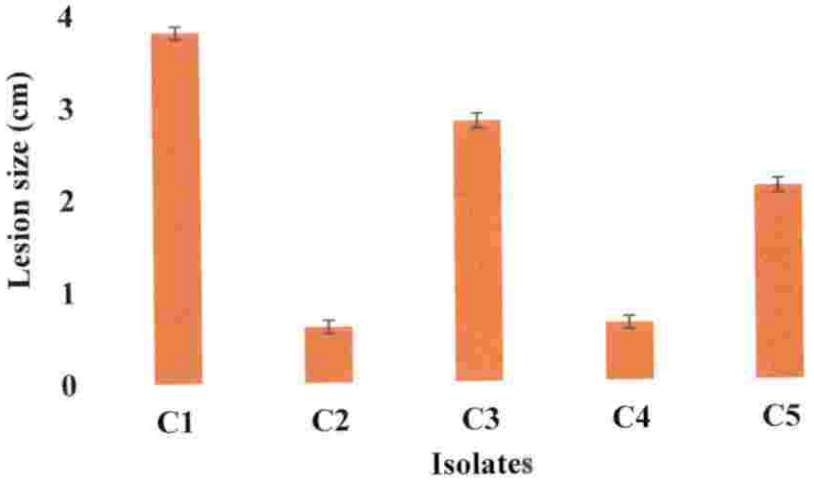

Figure 4. Lesion size produced by *C. capsici* isolates seven days after inoculation on tender fruits of chilli variety Vellayani Athulya; Error bars represent standard error of means of

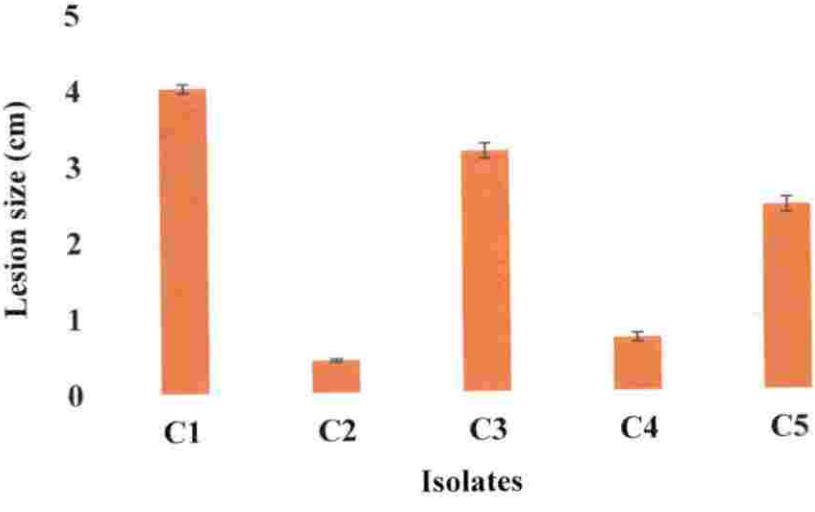

Figure 5. Lesion size produced by *C. capsici* isolates seven days after inoculation on mature fruits of chilli variety Vellayani Athulya; Error bars represent standard error of means of

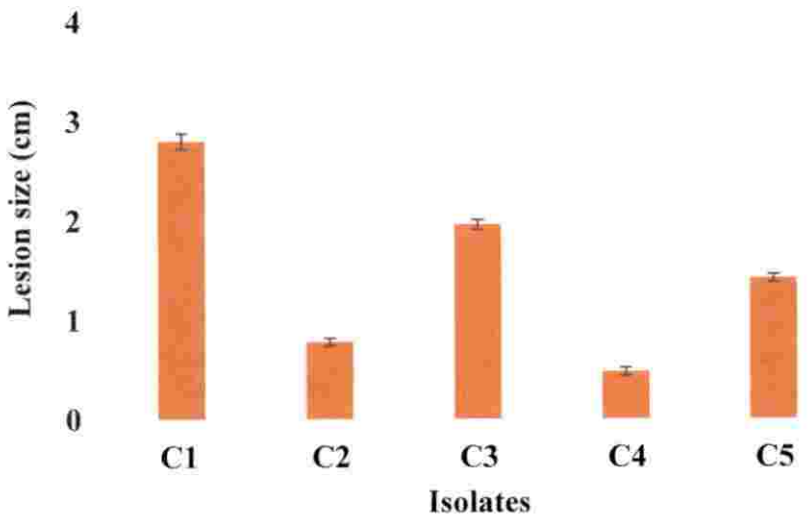

Figure 6. Lesion size produced by *C. capsici* isolates ten days after inoculation ripe fruits of chilli variety Vellayani Athulya; Error bars represent standard error of means of observed values.

of *C. capsici* by 70.14, 66.25, 62.50 and 61.25 per cent respectively in dual culture (Mishra *et al.*, 2011). In another *in vitro* study by Bal and Behera (2012) *T. viride* inhibited mycelial growth of *C. capsici* by 47.54 per cent. The antagonistic mechanism of *Trichoderma* spp. over fungal pathogens was studied by Anand and Bhaskaran (2009). *Trichoderma* grows over the fungal plant pathogens and causes hyphal coiling, hyphal abnormalities and lysis of hyphae.

The inability of *P. fluorescens* to check the growth of fungal pathogens was also reported by Kiran (2018) who observed a low inhibition percentage of 14.4 by *P. fluorescens* against *Alternaria brassicola* and opined that this could be due to the low competitive ability of the strain.

B. subtilis strain VLY 62 inhibited the mycelial growth of *C. capsici* by 56.30 per cent. Rajkumar *et al.* (2018) studied the antagonistic effect of 30 isolates of *B. subtilis* against *C. capsici* showing percentage inhibition ranging from 11.98 to 63.42. *B. subtilis* inhibited the growth of *C. gloeosporioides* by 57 per cent (Ashwini and Srividya, 2014) and 56.86 per cent (Ngullie *et al.*, 2010). *B. pumilus* VLY 17 inhibited *C. capsici* by 45.19 per cent and this was lower than other *Bacillus* species tested. This finding was similar to that of Amaresan *et al.* (2012) who observed that *B. pumilus* strain BETL13 recorded growth inhibition of *C. capsici* by 40 per cent *in vitro*. Han *et al.* (2015) observed that *B. pumilus* strain LB15 showed less than 40 per cent inhibition of the two pathogens *C. acutataum* and *C. gloeosporioides.*

The highest percentage inhibition of *C. capsici* among the antagonists was exhibited by *B. amyloliquefaciens* VLY 24 with inhibition percentage of 62.96 per cent. Several other workers also reported high inhibition percentages of *Colletotrichum* spp. by *B. amyloliquefaciens*. *B. amyloliquefaciens* strain CNU114001 reduced the rate of mycelial growth of *C. acutatum* by 62.74 per cent and *C. orbiculare* by 70.91 per cent *in vitro* (Seung *et al.* 2013). Han *et al.* (2015) reported that *B. amyloliquefaciens* strain LB01 inhibited *C. acutataum* and *C. gloeosporioides* by 60.15 and 58.12 per cent respectively. Jamal *et al.* (2015)

reported that *B. amyloliquefaciens* strain Y1 produced an inhibition zone of 11 mm against *C. gloeosporioides* with an inhibition percentage of 69.79.

Biocontrol by *Bacillus* spp. employs several mechanisms. *Bacillus* spp. produces lytic enzymes against pathogenic fungi (Amaresan *et al.*, 2014). *B. subtilis* and *B. amyloliquefaciens* were known to produce several antifungal lipopetides such as iturin, surfactin, fengycin which are known to inhibit the spore germination of fungi. Iturin A is a powerful antifungal lipopeptide which disrupts the cytoplasmic membrane of the fungus, creating transmembrane channels which leads to the release of vital ions such as K^+ from the fungal cells (Arrebola *et al.*, 2010a). Huang *et al.* (2012) studied the *in vitro* effect of *B. pumilus* strain N43 on *R. solani*. They found that N43 induced hyphal deformation, enlargement of cytoplasmic vacuoles and cytoplasmic leakage in *R. solani*.

P. indica suppressed the growth of *C. capsici* by 46.67 per cent. Dolatabadi *et al.* (2012) reported the antagonistic activity of *P. indica* against lentil wilt pathogen *Fusarium oxysporum* f. sp. *lentis* in dual culture. *P. indica* hyphae coiled around the hyphae of pathogen and later penetrated into it.

5.4. *In vitro* ASSAYS ON FRUITS

5.4.1. *In vitro* Screening of Biocontrol Agents against *C. capsici* on Detached Chilli Fruits

The lesion size of anthracnose in chilli fruits treated with biocontrol agents were significantly lower than untreated fruits. Fruits treated with *B. amyloliquefaciens* exhibited the lowest lesion size with highest reduction of 55.64 per cent over control. This was followed by *B. subtilis* (51.39 % reduction) and *T. viride* (37.64 % reduction). Reduction of lesion size by *P. indica* and *B. pumilus* were 34.04 and 18.65 per cent respectively. The least effective was *P. fluorescens* with only 15.22 per cent reduction over control (Figure 8).

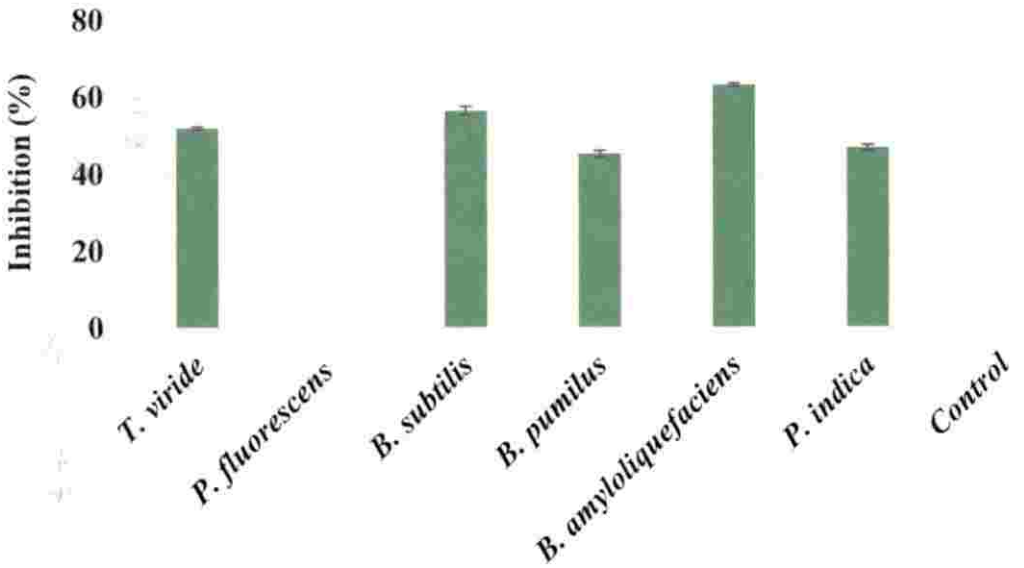

Figure 7. Percentage inhibition of mycelial growth of *C. capsici* by biocontrol agents in dual culture assay; Error bars represent standard error of means of observed values.

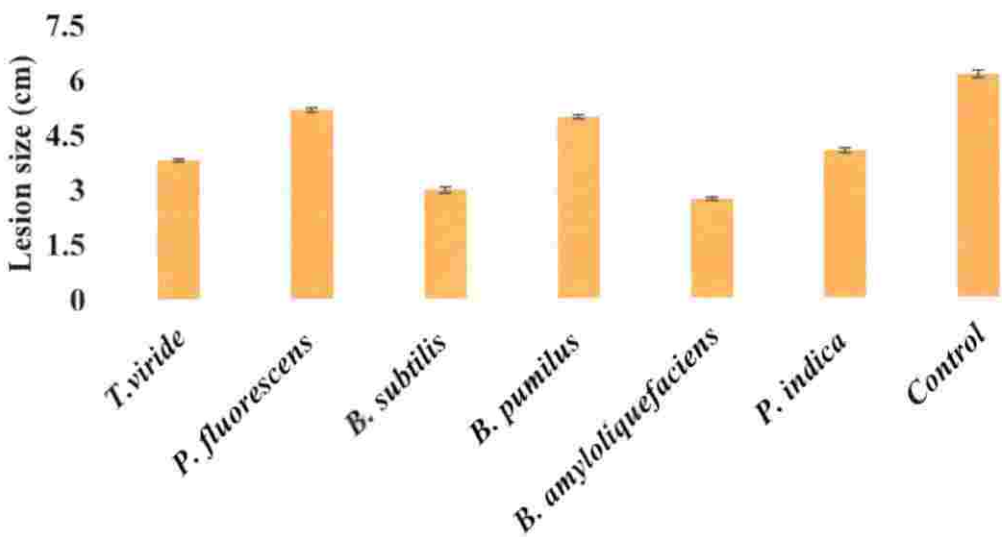

Figure 8. Lesion size produced by *C. capsici* isolates (10 DAI) on chilli fruits var. Vellayani Athulya after treatment with biocontrol agents; Error bars represent standard error of means of observed values.

Several workers have reported the *in vitro* antagonistic effect of biocontrol agents against fungal pathogens on fruit surfaces. *B. amyloliquefaciens* strain PPCB004 reduced the percentage disease incidence of anthracnose caused by *C. gloeosporioides* on harvested citrus fruits from 76.70 to 31.70 (reduction of 58.67 %) (Arrebola *et al.*, 2010a). *B. subtilis* isolates PMB-123 and PMB-183 reduced the lesion length of anthracnose in chilli caused by *C. capsici* in postharvest fruit bioassay by 61.69 and 55.41 per cent respectively. *B. pumilus* reduced the lesion size by 50.29 per cent (Ramanujam *et al.*, 2012). *B. amyloliquefaciens* strain LB01 with 94 per cent control efficiency was more effective in controlling *C. acutatum* on detached chilli fruits than *B. pumilus* strain LB15 with control efficiency of only 48.1 per cent (Han *et al.*, 2015).

T. viride successfully controlled stem-end rot of mango caused by *Botryodiplodia theobromae* on harvested mango (Kota *et al.*, 2006). *T. harzianum* when applied to harvested apple and subsequent challenge inoculation with *R. solnani* resulted in reduction of lesion size by 32.4 per cent (Batta, 2007). Citrus fruits dipped in a suspension of *P. fluorescens* and *T. atroviride* (1×10^9 cfu ml^{-1}) for two minutes and subsequent pathogen inoculation reduced citrus rot caused by *Penicillium digitatum* by 36.6 and 65.6 per cent respectively (Penebianco *et al.*, 2015). Competition for space and nutrients between the antagonist and the pathogen are the major mechanism by which the antagonists sprayed on fruit surface brings control. Along with that, direct parasitism, production of antibiotics and induced resistance are also noticed (Sharma *et al.*, 2009).

5.4.2. Peroxidase and Polyphenol oxidase enzyme activities

Detached chilli fruits treated with biocontrol agents and challenge inoculated with *C. capsici* exhibited higher peroxidase and polyphenol oxidase enzyme activities compared to those treated with only biocontrol agents, only pathogen and untreated control. Jayapala *et al.* (2019) also reported a similar increase in enzyme activity on pathogen challenge inoculation in bioprimed chilli

seedlings. They reported the induction of defence related enzymes such as phenylalanine ammonia lyase (PAL), PO, PPO, lipoxygenase, phenolics and chitinase and an additional increase in the synthesis was noted on further challenge inoculation with pathogen.

Peroxidase enzyme activity after treatment with biocontrol agents but before (Figure 9) and after (Figure 10) challenge inoculation with pathogen was found to be increasing upto 48 h. In the case of fruits treated with biocontrol agents and challenge inoculated with pathogen, the activity was increasing even after 48 h (Figure 10). But for fruits treated with biocontrol agents alone the activity decreased after 48 h (Figure 9). In the case of PPO, the activity increased upto 48 h of inoculation, reached maximum and decreased thereafter in all the cases (Figures 11 and 12). Untreated controls did not show much variation in the activities of the enzymes. The trend observed in the enzyme activity is in accordance with the findings of Anand *et al.* (2009) who observed that PO and PPO activities in chilli fruits inoculated with *C. capsici* were higher compared to uninoculated fruits. For inoculated fruits, the activities increased from the day of inoculation (initial stages of the disease) and reached the maximum on the 3rd day and thereafter a decrease in activity was noted coinciding with the disease progress and necrosis of the tissues. However the activities were higher than the initial level even on 5th day. Uninoculated fruits did not show significant difference in the enzyme activities during the experimental period.

Highest change in activity was observed for fruits treated with *B. amyloliquefaciens* followed by *B. subtilis* and *T. viride*. Treatment with *B. pumilus* and *P. indica* also showed changes in enzyme activities. The least activity was observed for fruits treated with *P. fluorescens*. There are many reports on the effect of biocontrol agents and pathogen challenge inoculation on PO and PPO enzyme activities in several crops. Gowtham *et al.* (2018) reported that chilli seedlings treated with *B. amyloliquefaciens* and challenge inoculated with *C. truncatum* exhibited one to two fold increase in PO and PPO activities compared with those inoculated with pathogen alone. Jayapala *et al.* (2019)

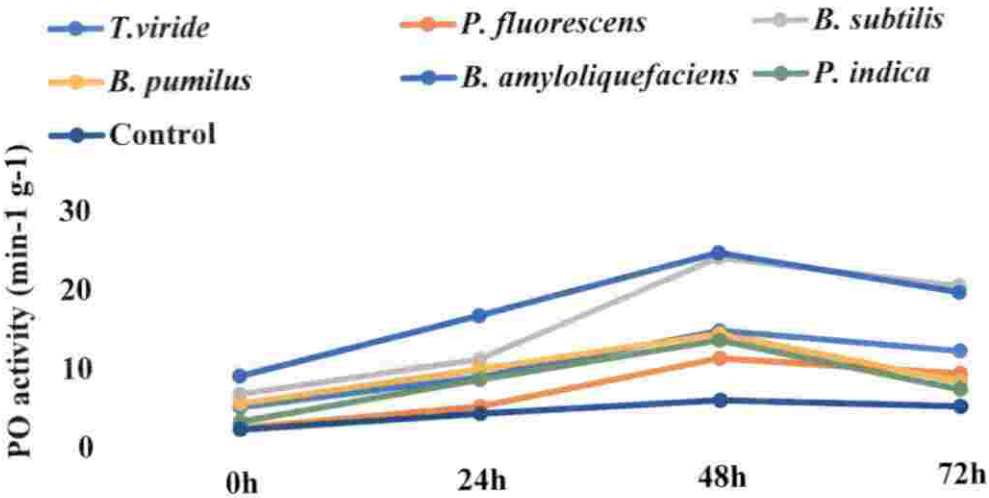

Figure 9. Peroxidase activities after treatment with biocontrol agents and before inoculation with *C. capsici* in detached chilli fruits var. Vellayani Athulya

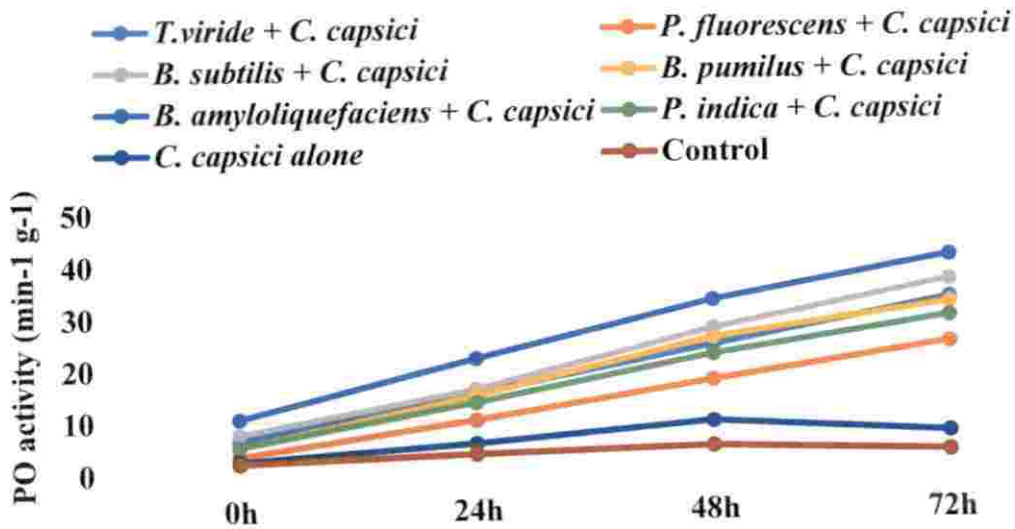

Figure 10. Peroxidase activities after treatment with biocontrol agents and inoculation with *C. capsici* in detached chilli fruits var. Vellayani Athulya

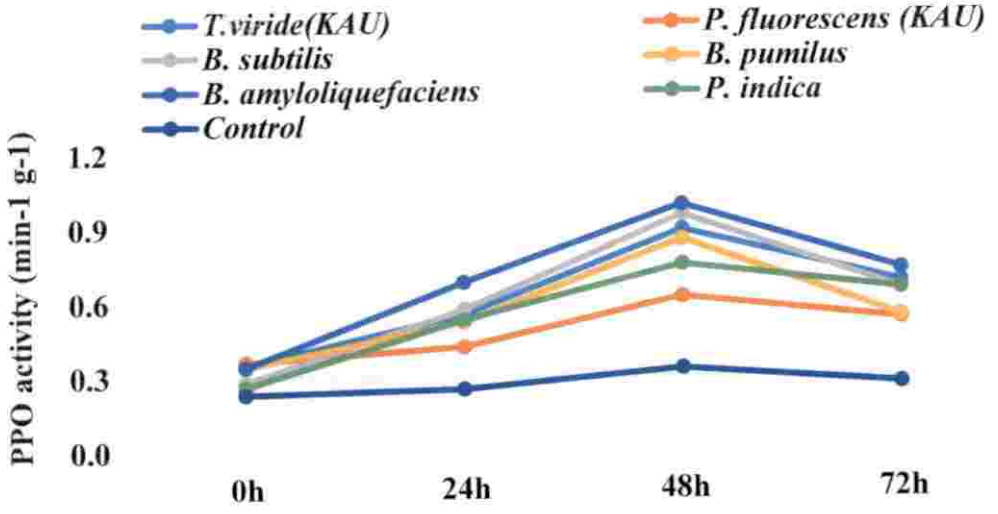

Figure 11. Polyphenol oxidase activities after treatment with biocontrol agents but before inoculation with *C. capsici* in detached chilli fruits var. Vellayani Athulya

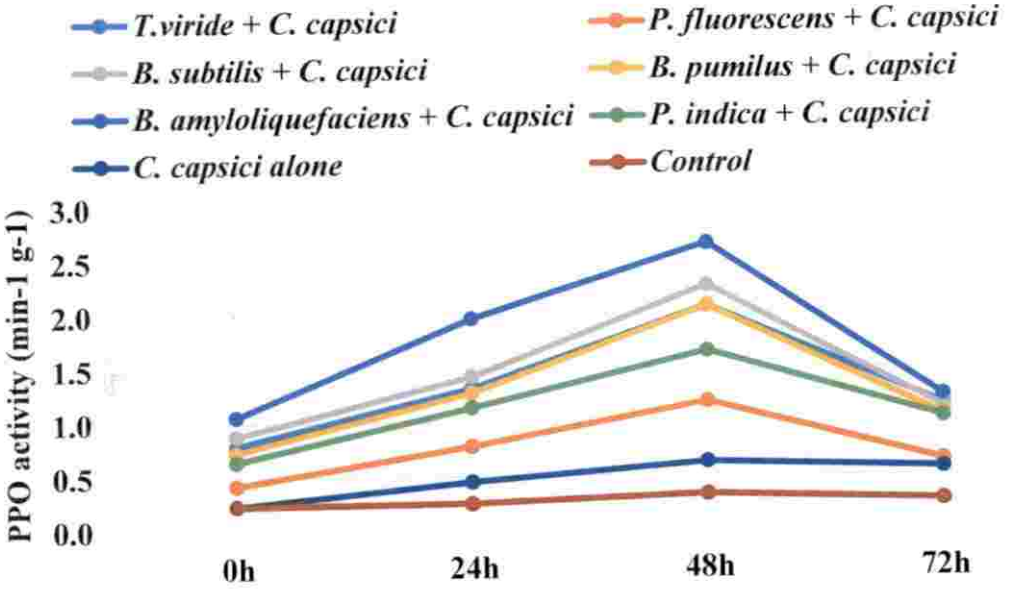

Figure 12. Polyphenol oxidase activities after treatment with biocontrol agents and inoculation with *C. capsici* in detached chilli fruits var. Vellayani Athulya

observed that seed biopriming with *Bacillus* sp. strain BSp.3/aM and subsequent challenge inoculation with *C. capsici* increased PO and PPO activities in 20 day old chilli seedlings. Treatment of black gram seeds with *T. viride* @ 4g / kg against dry root rot caused by *M. phaseolina* increased the PO and PPO activities. *P. indica* colonized tobacco plants after inoculation with *Phytophthora parasitica* var. *nicotiana* found 1.50 and 2.26 fold increase in PO and PPO activities for 2nd and 4th day of inoculation respectively (Bing *et al.*, 2015).

5.5. STANDARDISATION OF BIOPRIMING TECHNIQUES

5.5.1. Standardisation of Seed Soaking Time

Standardization of priming techniques revealed that soaking of seeds for 1 h in *T. viride* and 4 h in other biocontrol agents *viz.*, *P. fluorescens*, *B. subtilis*, *B. pumilus*, *B. amyloliquefaciens* and *P. indica* were found the best for biopriming. These treatments gave the highest values for all the biometric characters measured *viz.* germination percentage (Figure 13), shoot length (Figure 14), root length (Figure 15), seedling length (Figure 16), seedling dry weight (Figure 17), SVI - I (Figure 18) and SVI – II (Figure 19) compared to other durations of biopriming. Four hours of priming was also found the best for priming with carbendazim and hydropriming. All the priming treatments significantly increased the biometric characters measured compared to unprimed seeds. Soaking for 8 h and 16 h also improved the growth parameters beyond control but the effects were lesser than 4 h. This could be due to the seeds being subjected to imbibition injuries, thus 4 h of soaking was found to be better as it improved growth with lesser duration of priming.

Naik, (2015) also standardized the biopriming duration in garden pea. The seeds were soaked in *T. viride* (40 %) and *P. fluorescens* (40 %) and tap water (hydropriming) for 2, 4, 6, 8, 10 and 12 h separately. The maximum effect was noticed in priming for 4 h in all the treatments. Also soaking for more than 6 h reduced the quality of seedlings below the control. The lesser duration of priming (one hour) favoured for *T. viride* may be due to the fact that *T. viride* being an

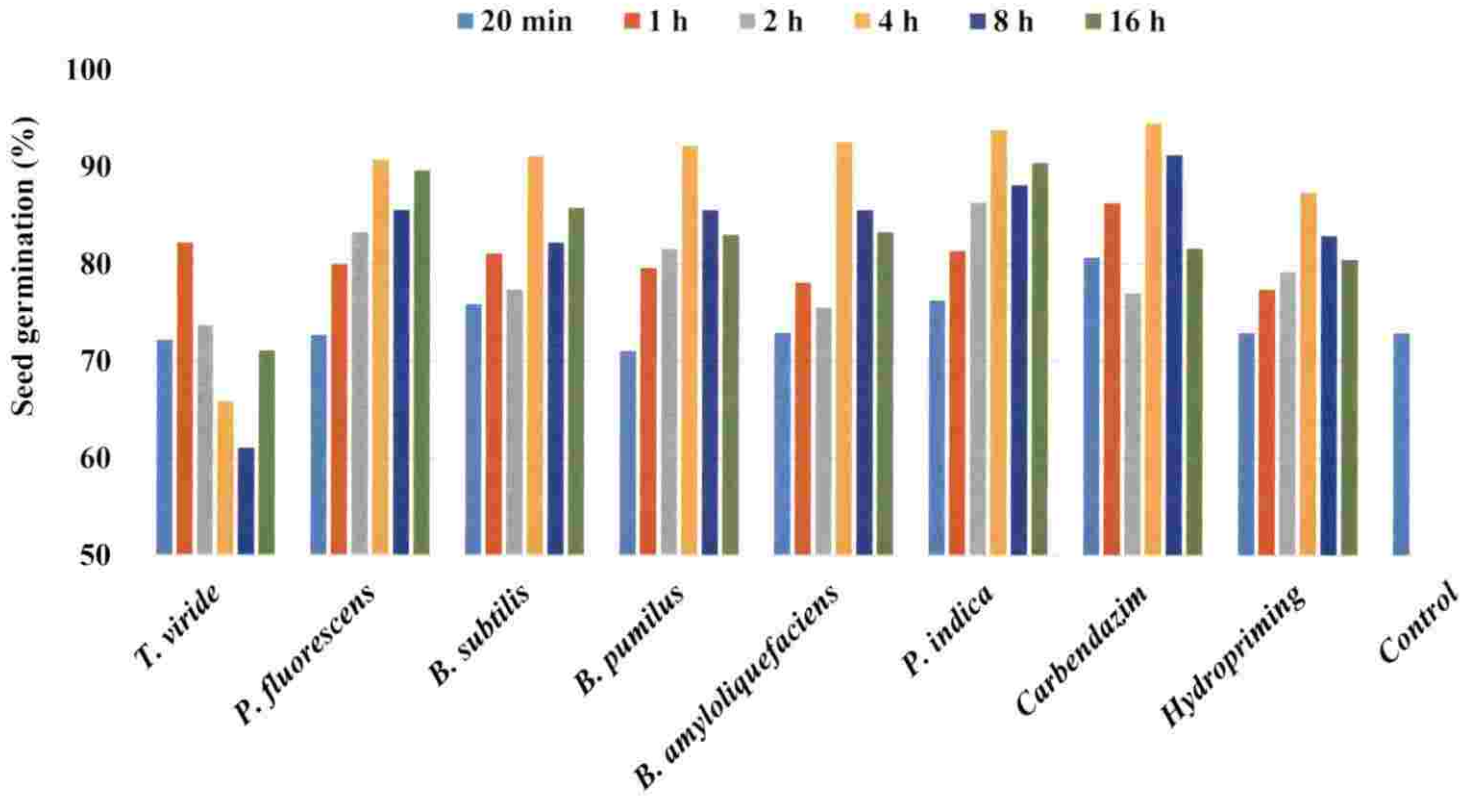

Figure 13. Effect of seed biopriming on germination percentage of chilli seedlings var. Vellayani Athulya

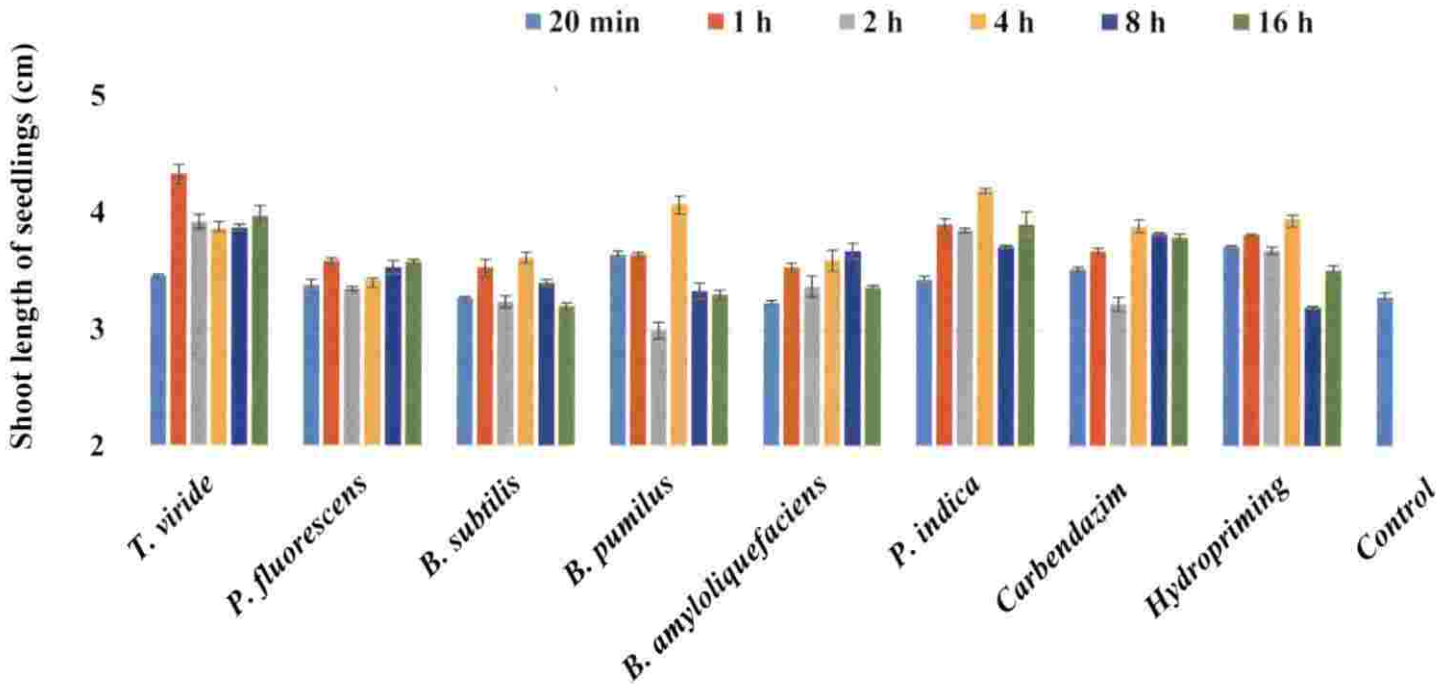

Figure 14. Effect of seed biopriming on shoot length of chilli seedlings var. Vellayani Athulya; Error bars represent standard error of means of observed values.

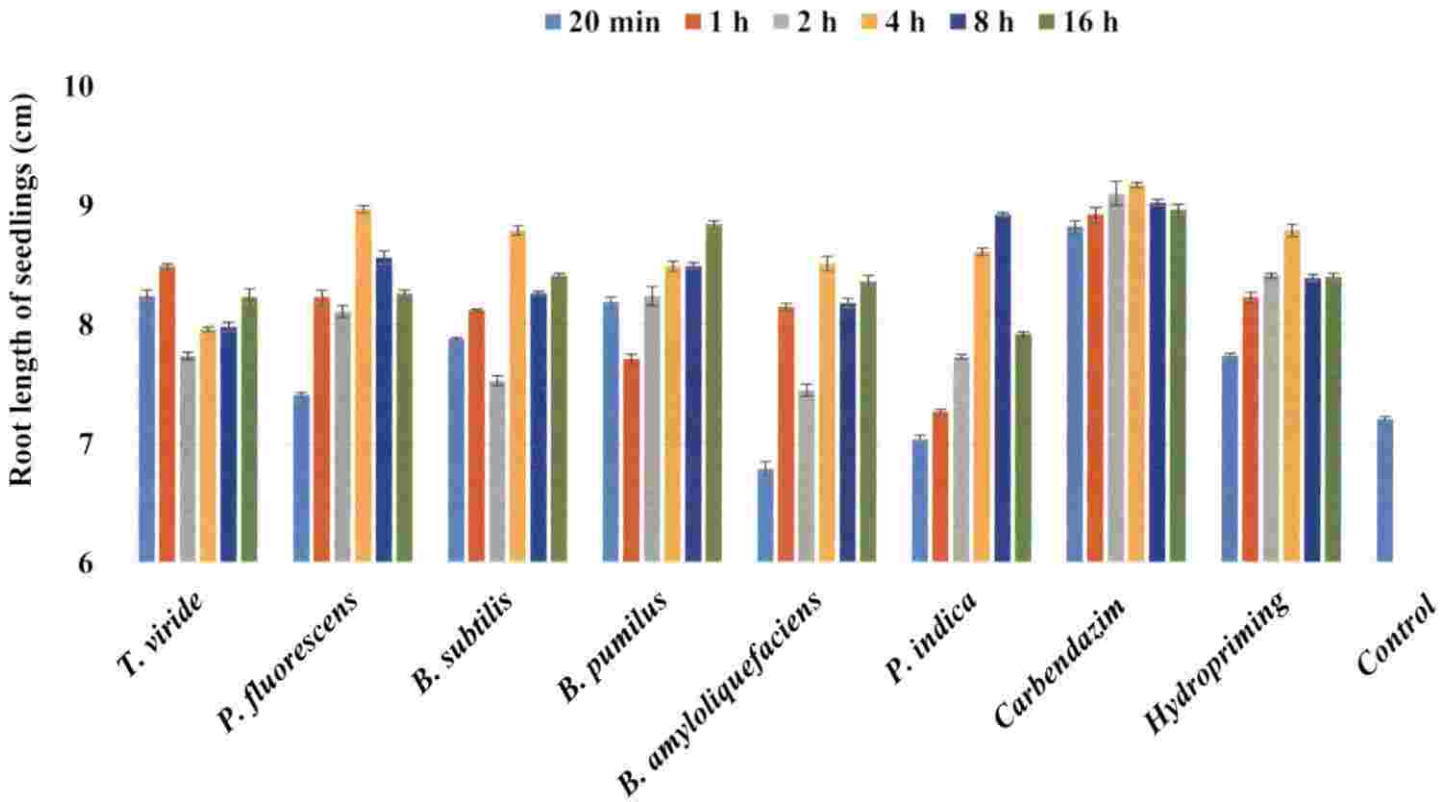

Figure 15. Effect of seed biopriming on root length of chilli seedlings var. Vellayani Athulya; Error bars represent standard error of means of observed values.

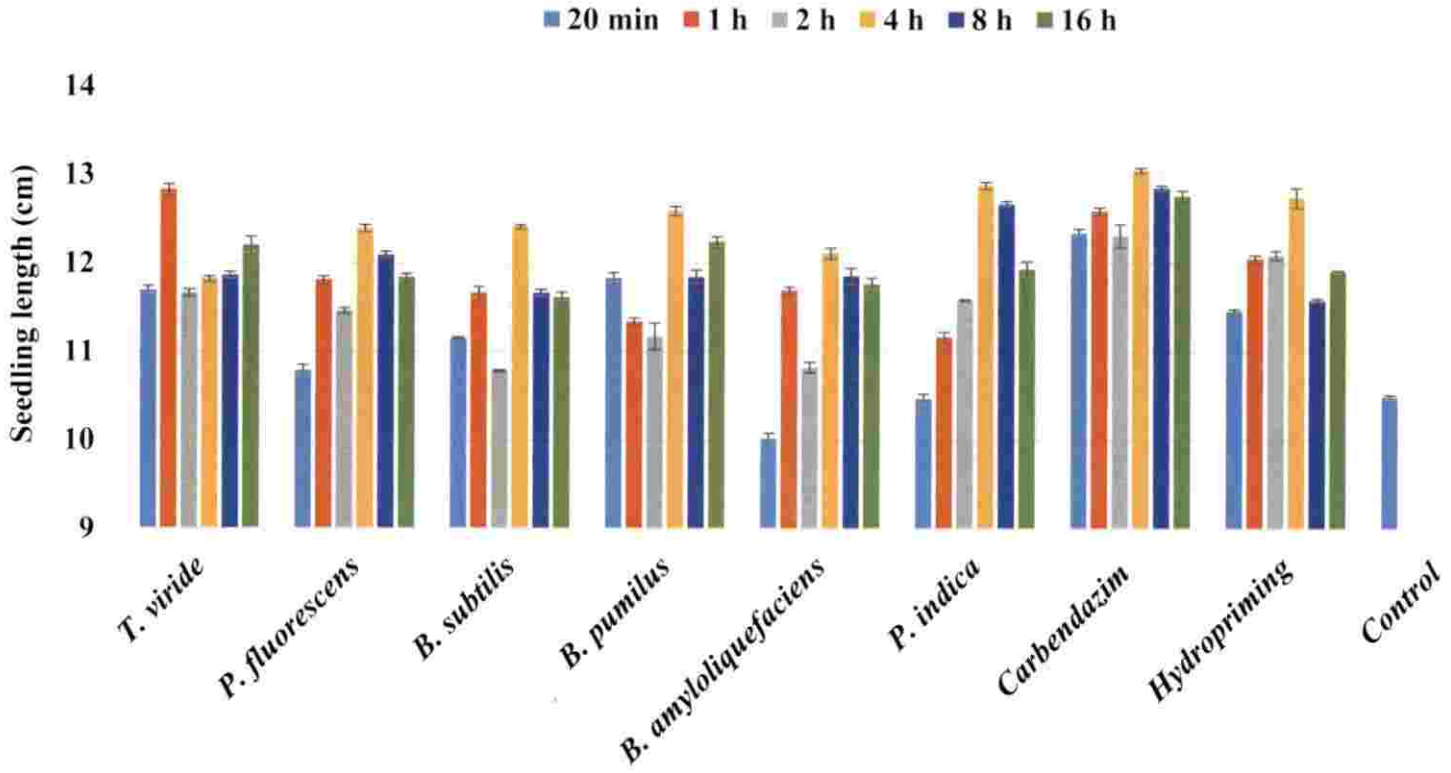

Fig 16. Effect of seed biopriming on seedling length of chilli var. Vellayani Athulya. Error bars represent standard error of means of observed values.

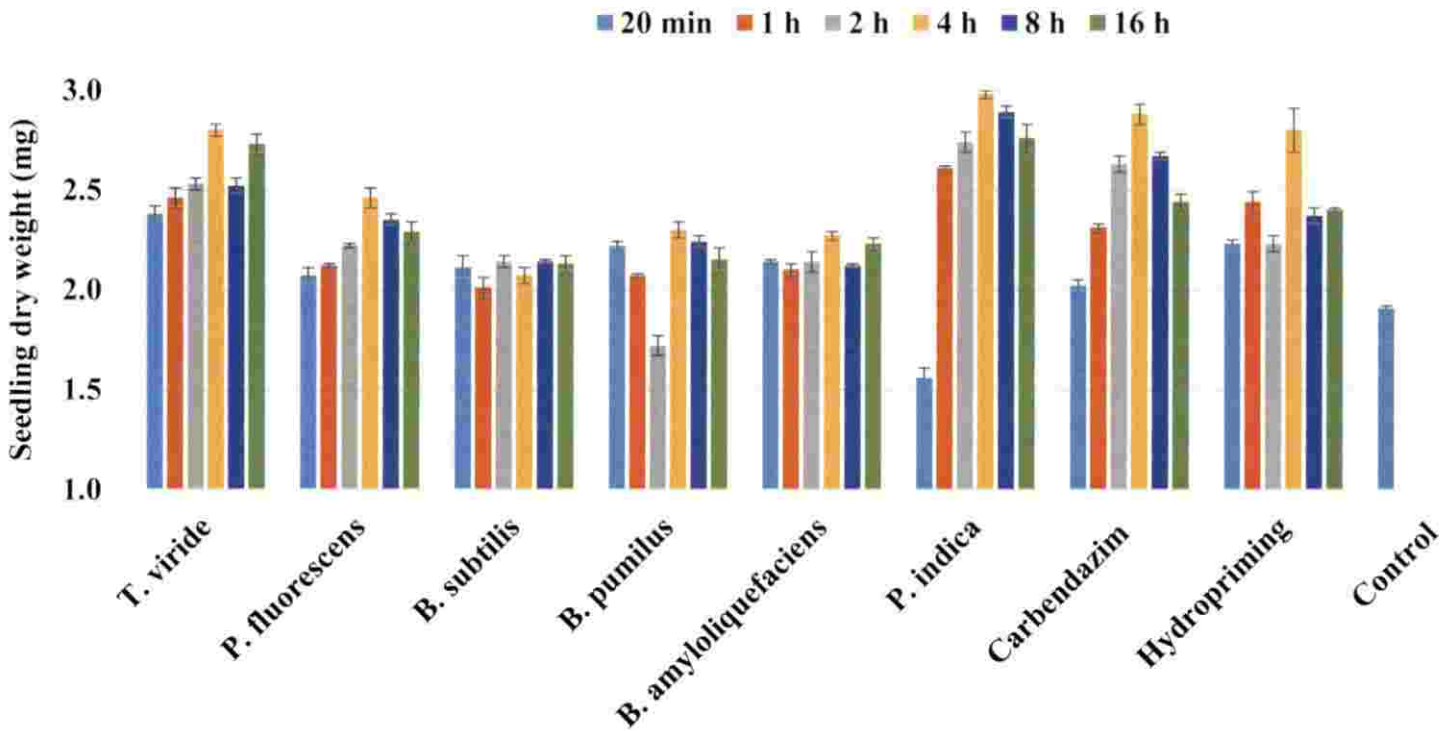

Figure 17. Effect of seed biopriming on dry weight of chilli seedlings var. Vellayani Athulya; Error bars represent standard error of means of observed values.

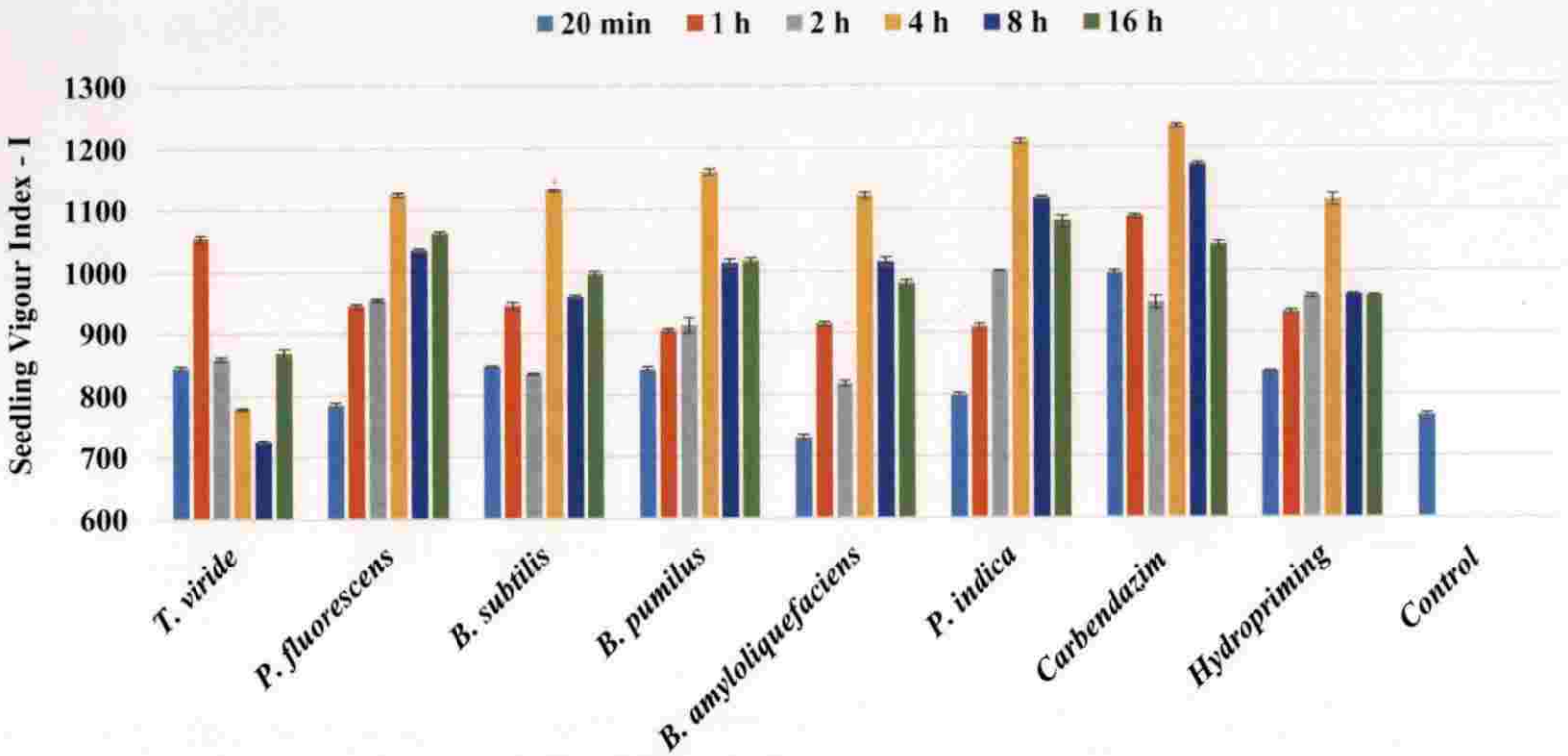

Figure 18. Effect of seed biopriming on seedling vigour index – I (SVI - I) of chilli var. Vellayani Athulya. Error bars represent standard error of means of observed values.

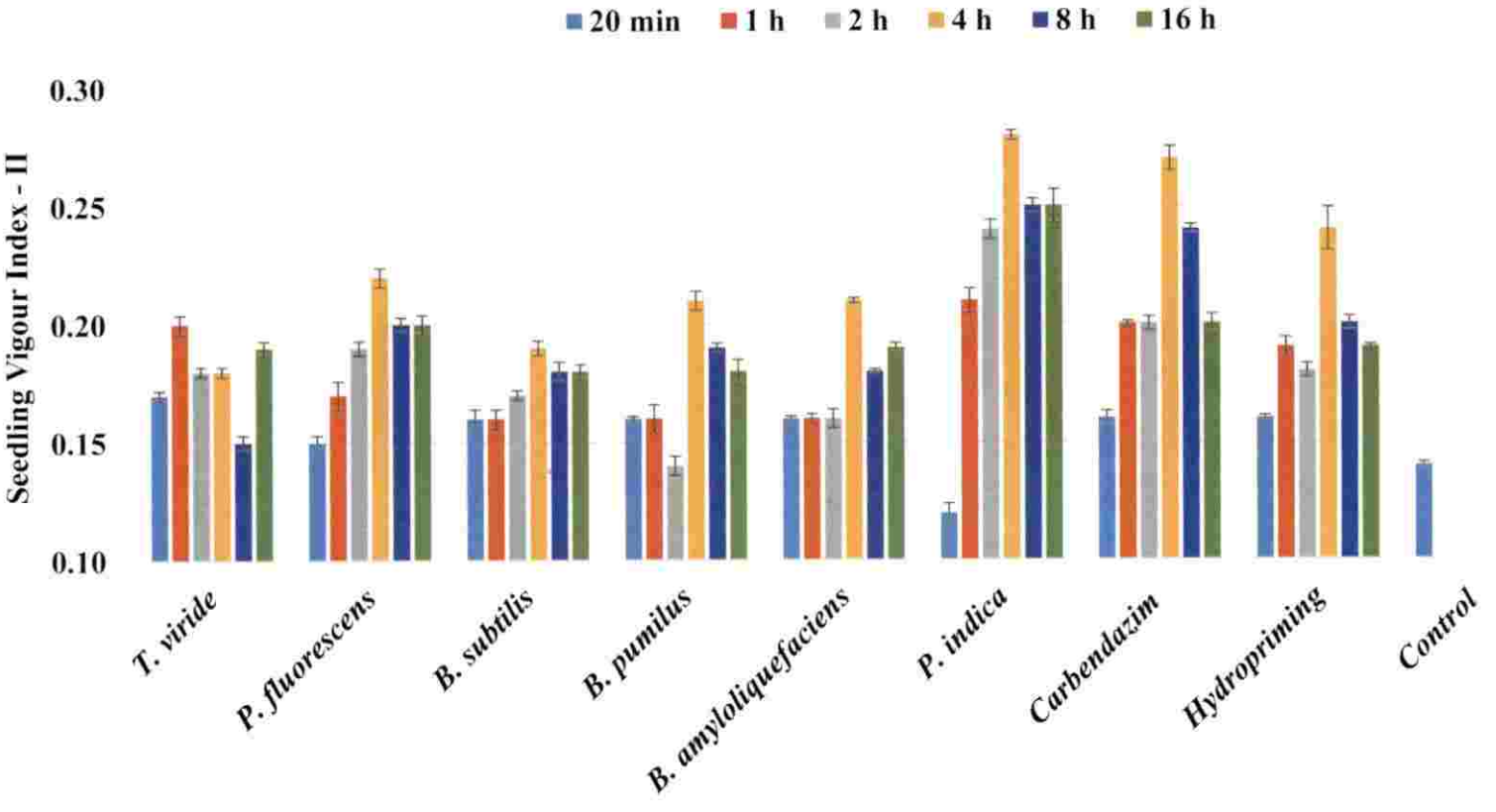

Figure 19. Effect of seed biopriming on seedling vigour index - II (SVI - II) of chilli var. Vellayani Athulya; Error bars represent standard error of means of observed values.

aerobic fungi, it could not survive in the biopriming suspension for longer duration and hence the effect decreased, while other biocontrol agents tested *viz.*, *P. fluorescens*, *Bacillus* spp. and *P. indica* can survive anaerobic conditions. *Bacillus* spp. produces endospores which can survive for longer periods in adverse conditions.

Several workers have reported the enhanced effects of seed biopriming in chilli. Biopriming chilli seeds with *T. viride*, *P. fluorescens* and *B. subtilis* (Bharathi *et al.*, 2004; Dhanalakshmi, 2013; Machenahalli *et al.*, 2014; Ilyas *et al.*, 2015; Chauhan and Patel, 2017a; Rai and Behera, 2019), *B. amyloliquefaciens* (Sathya *et al.*, 2016; Gowtham *et al.*, 2018), *B. pumilus* (Amaresan *et al.*, 2012), enhanced the germination percentage, root length, shoot length, drymatter production and vigour index over non-primed seeds.

5.6. EFFECT OF SEED BIOPRIMING AND SPRAYING AT FRUIT SET ON CHILLI UNDER POT CULTURE STUDIES

5.6.1. *In vivo* Effect of Seed Biopriming on Chilli

Biopriming significantly improved all the growth parameters measured. Priming with carbendazim also improved the growth parameters. Among the biopriming treatments, plant height was the highest for biopriming with *B. amyloliquefaciens* (11.91 % increase over control) followed by *T. viride* (6.09 %) and *B. subtilis* (2.65 %) 120 DAS (Figure 20). The maximum number of tertiary branches per plant (Figure 21), fruits per plant (Figure 22), 100 seed weight (Figure 23) and seed yield per plant (Figure 24) was observed for biopriming with *T. viride* (44.30, 52.36, 19.35, and 65.34 % increase respectively over control) followed by *B. subtilis* (24.05, 38.86, 17.74 and 44.91 % increase respectively) and *B. amyloliquefaciens* (12.68, 38.37, 14.52 and 33.10 % increase respectively).

Biopriming significantly reduced the severity of fruit rot (Figure 25) and anthracnose in leaves (Figure 26). Biopriming with *B. subtilis* gave the maximum control of fruit rot (29.58 % over control) and leaf anthracnose (41.11 %)

followed by *T. viride* (24.26 % for fruit rot and 37.22 % for leaf anthracnose respectively) and *B. amyloliquefaciens* (24.26 % and 33.89 % respectively) over inoculated control and were better than priming with carbendazim (11.83 % and 23.89 % over control). Several workers have reported about the growth enhancing and disease controlling effects of seed biopriming.

Seed biopriming with *B. subtilis* strain IN937b in pearl millet enhanced the yield by 33 per cent (Raj *et al.*, 2003). *B. subtlils* isolate AB17 increased the plant height of chilli by 39 per cent, root length by 40.44 per cent, shoot fresh weight by 42 per cent and root biomass by 47 per cent over control (Lamsal *et al.*, 2012). Meena *et al.* (2012) studied the effect of seed biopriming with *T. harzianum* ($2X10^9$ cfu/ml for 24 h) in sorghum against anthracnose caused by *C. graminicola* under field conditions and observed a reduction in disease severity, increase in plant height and yield by 28.1, 28.2 and 6.59 per cent respectively. Chauhan and Patel (2017b) observed that PDI of anthracnose or fruit rot caused by *C. capsici* in chilli 30 DAS was reduced by seed biopriming with *T. viride* (PDI - 6.2) and *B. subtilis* (PDI - 7.2) compared to hydropriming (PDI - 13.4) and control (PDI - 18.4). Seed biopriming with *B. amyloliquefaciens* in chilli resulted in significant protection of 71 per cent against anthracnose caused by *C. truncatum* and also the maximum plant height (18.32 cm), shoot fresh weight (3.52g), dry weight (1.53g) and number of leaves per plant (15.25), 30 DAS (Gowtham *et al.*, 2018). Biopriming chilli seeds with *Bacillus* sp. strain BSp.3/AM for 6 h reduced the incidence of anthracnose by 20 per cent under greenhouse conditions (Jayapala *et al.*, 2019).

Biocontrol agents can improve plant growth by several mechanisms. Endophytic bacteria such as *Bacillus* spp. can increase the plant growth by the production of plant growth regulators like auxins (eg. Indole Acetic Acid (IAA)) or cytokinins or by the degradation of the ethylene precursor ACC by ACC deaminase, phosphorus solubilization and siderophore production. IAA improves the root growth and development and thus enhances nutrient uptake (Amaresan *et al.*, 2012). For the successful seed or root bacterization, high level of plant habitat

competence should be exhibited by the treatment bacteria (Amaresan *et al.*, 2014). As stated by Ananthi *et al.* (2017), an increase in growth parameters with biopriming can be attributed to suppression of deleterious microorganisms and pathogens, production of plant growth regulators like gibberellins, cytokinins and indole acetic acid, increased availability of minerals and ions and extensive rooting which helps in water and nutrient uptake. The disease suppression effect of biopriming has been attributed to the induction of systemic resistance in plants by the biopriming agent which colonizes the roots of plants (Bharathi *et al.*, 2004; Anand and Bhaskaran, 2009). *Bacillus* species colonize the root surface and increase the plant growth (Turner and Backman, 1991; Podile and Prakash, 1996; Takayanagi *et al.*, 1991).

Priming with carbendazim increased the growth parameters in plants as it stimulates plant growth (Debergh *et al.*, 1993). Carbendazim can act as a growth regulator in tissue culture, had cytokinin like activity in soybean biotest and it stimulated shoot development in micropropagated asparagus. The disease suppressive effect of carbendazim was lower than biopriming. This can be due to the fact that chemicals used for seed treatment mostly act as contact / systemic fungicides and cannot protect the plants from foliar pathogens in later stages of the plant growth (Prasad *et al.*, 2016).

5.6.2. *In vivo* Effect of Seed Biopriming and Spraying during Fruit Set on Chilli

There was no significant difference between bioprimng, and biopriming and spraying treatments on growth and yield parameters *viz.* plant height (Figure 20), number of branches per plant (Figure 21), number of fruits per plant (Figure 22), 100 seed weight (Figure 24) and seed yield per plant (Figure 25). The increase in these parameters was brought about by the effect of biopriming alone. This could be due to the fact that the spraying treatment was given after fruit set only. But there was increase in the fruit yield per plant (g) in biopriming and spraying treatments compared to biopriming alone (Figure 23). This could be due to the

fact that biopriming and spraying gave direct protection to fruits than biopriming alone and more fruits yielded to harvest maturity without the attack of pathogen.

Biopriming of seeds, and spraying at fruit set exhibited significant reduction in the severity of fruit rot (Figure 26) and anthracnose in leaves (Figure 27) than biopriming alone. *B. amyloliquefaciens* gave maximum control of fruit rot (46.48 % reduction over control) and it was higher than the carbendazim treatment (44.10 %). This was followed by treatment with *B. subtilis* (40.54 %) and *T. viride* (38.17 %). The reduction in anthracnose of leaf was maximum for treatment with *B. subtilis* (55.56 %) followed by *B. amyloliquefaciens* (55 %) and *T. viride* (52.78 %). Carbendazim gave 67.78 per cent reduction over inoculated control.

Many workers have reported the reduction in disease incidence and severity by biopriming and spraying treatments. Anand and Bhaskaran (2009) observed that spraying of *T. viride* against fruit rot of chilli caused by *C. capsici* reduced the disease incidence by 47.5 per cent (PDI - 37.52) and severity by 44.22 per cent (PDI - 40.76) over inoculated control. Two sprays with biocontrol agents was found to be more effective than one spray. Seed biopriming (@ 2×10^9 cfu / ml for 24 h) followed by two spraying treatments with *T. harzianum* in sorghum reduced anthracnose caused by *C. graminicola* by 43.9 per cent under field conditions (Meena *et al.*, 2012). Christopher *et al.* (2014) studied the effect of seed treatment @ 10g / kg followed by prophylactic spraying @ 0.2 per cent of *B. subtilis* at 25 and 75 days after transplanting on chilli under greenhouse conditions. Incidence of fruit rot was decreased by 28.97 per cent. *B. subtilis* treatment was found better than the chemical check mancozeb.

Amaresan *et al.* (2014) studied the effect of seed treatment and foliar application of talc based formulations of *B. subtilis* strain BECL11 and *B. amyloliquefaciens* strain BECR2 on the yield and incidence of anthracnose in chilli and compared it with seed treatment of thiram (2.5g/kg seed) and foliar spray of fytolan (COC) (2.5g/L) as the chemical check. The PDI of anthracnose on leaves was 31.33 for strain BECL11 and 28.00 for strain BECR2 while the PDI

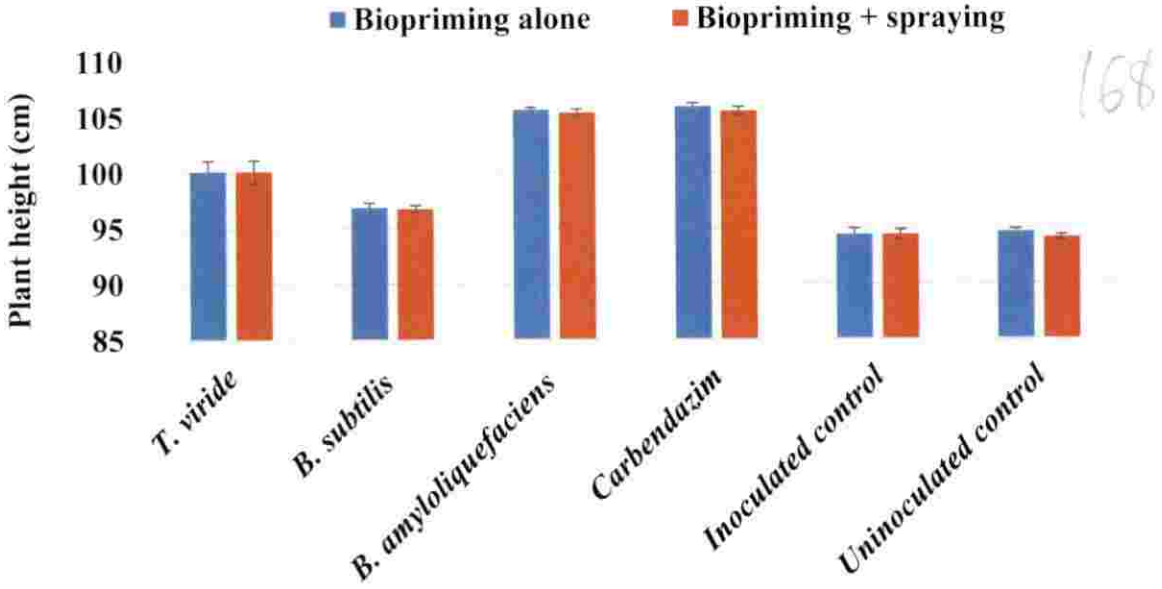

Figure 20. Comparison of seed biopriming; and seed biopriming and spraying on height of chilli plants of variety Vellayani Athulya; Error bars represent standard error of means of observed values.

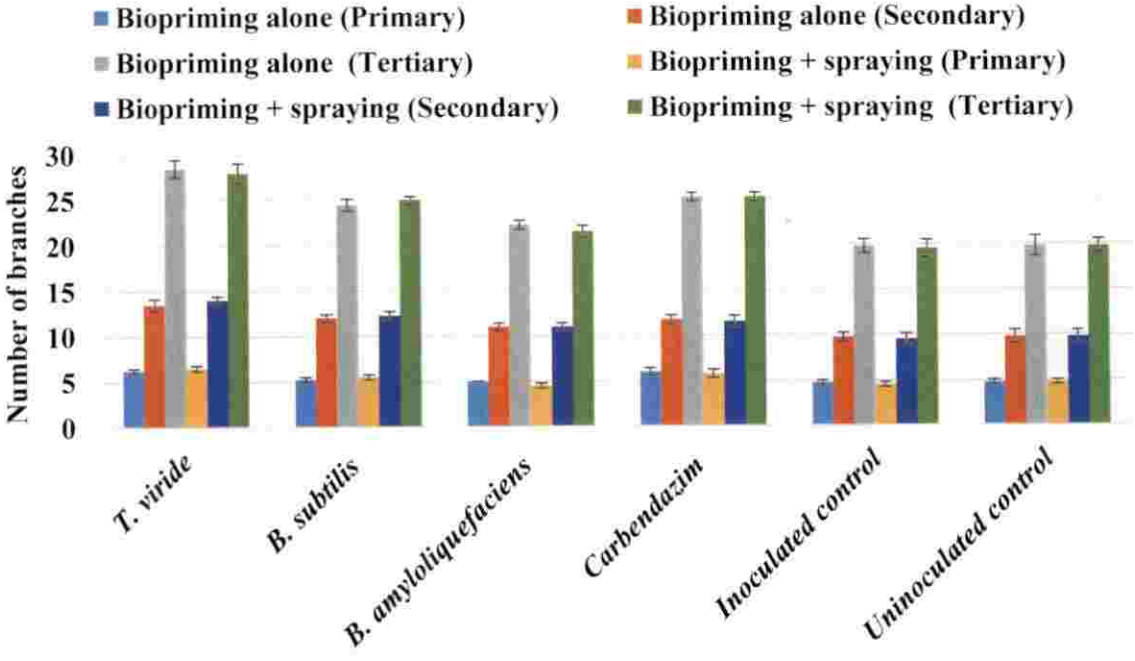

Figure 21. Comparison of seed biopriming and seed biopriming and spraying on number of branches per plant of chilli variety Vellayani Athulya;Error bars represent standard error of means of observed values.

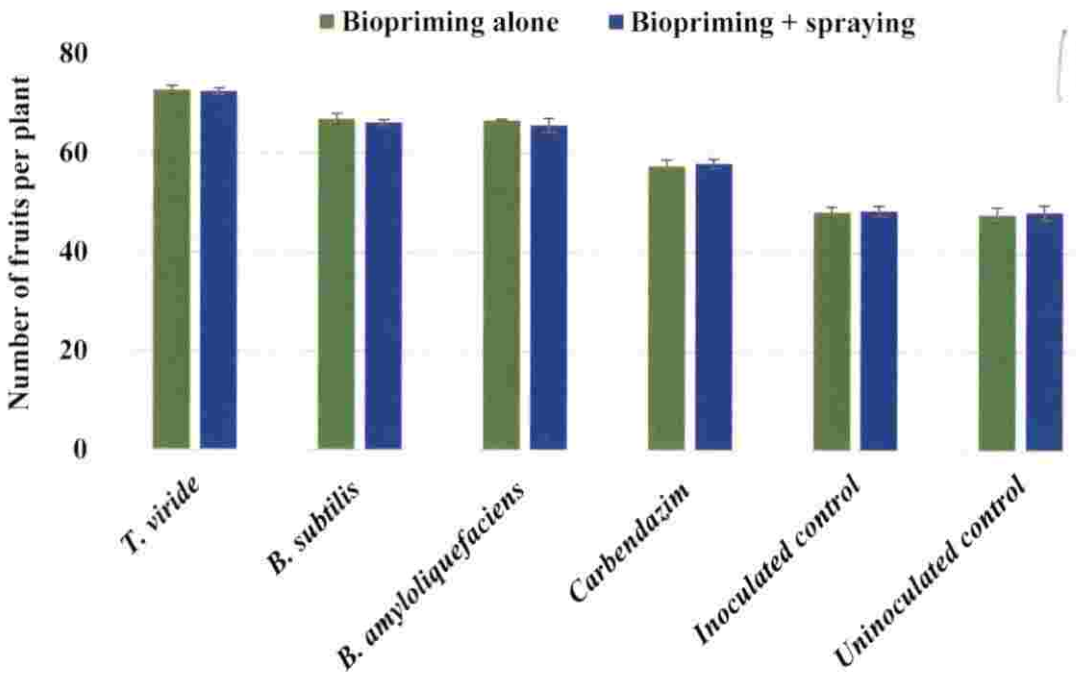

Figure 22. Comparison of seed biopriming; and seed biopriming and spraying on number of fruits per plant of chilli variety Vellayani Athulya; Error bars represent standard error of means of observed values

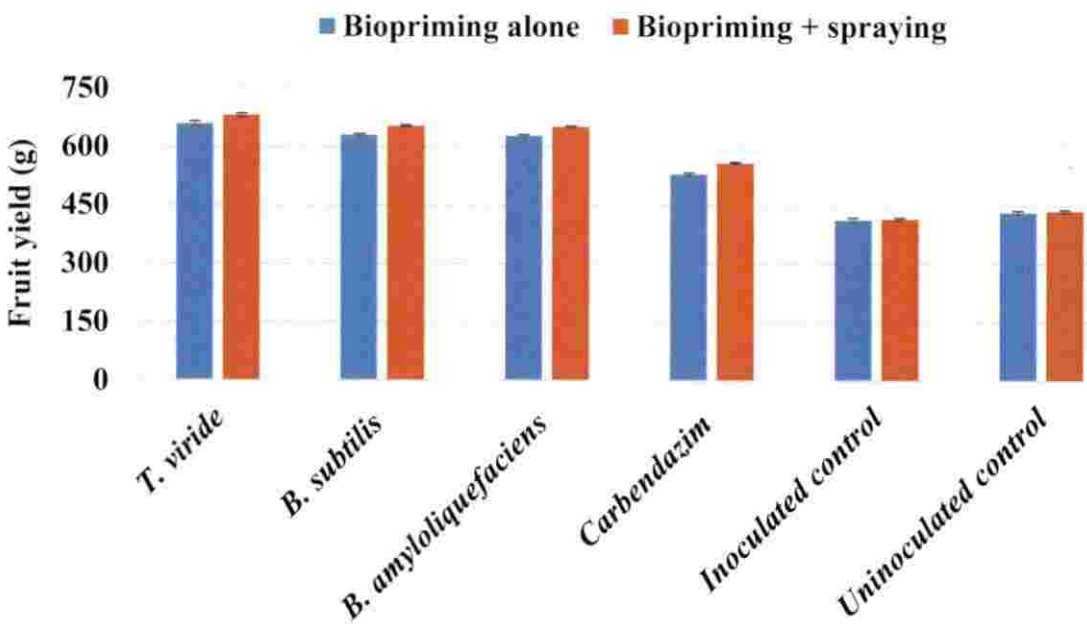

Figure 23. Comparison of seed biopriming and seed biopriming and spraying on fruit yield per plant of chilli variety Vellayani Athulya; Error bars represent standard error of means of observed values.

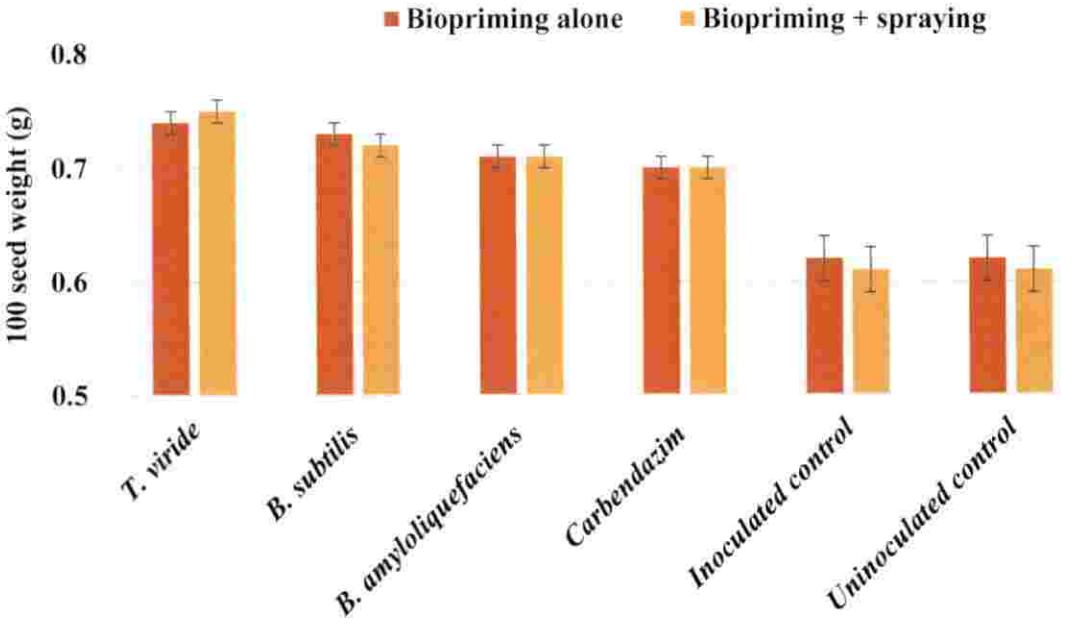

Figure 24. Comparison of seed biopriming; and seed biopriming and spraying on hundred seed weight of chilli variety Vellayani Athulya; Error bars represent standard error of means of observed values.

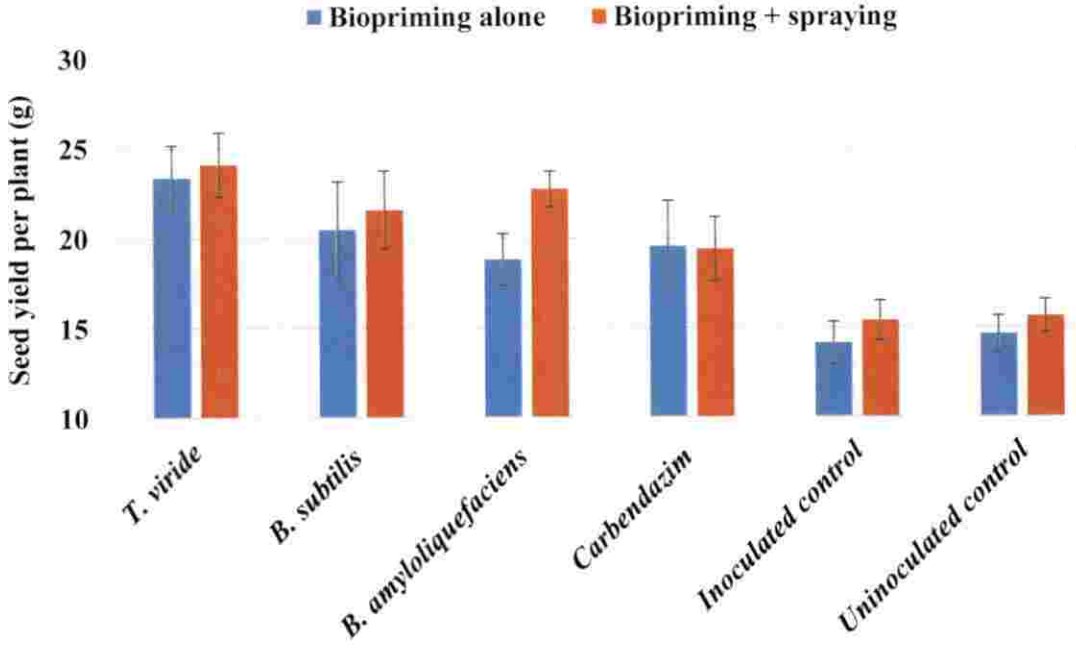

Figure 25. Comparison of seed biopriming; and seed biopriming and spraying on seed yield per plant of chilli variety Vellayani Athulya; Error bars represent standard error of means of observed values.

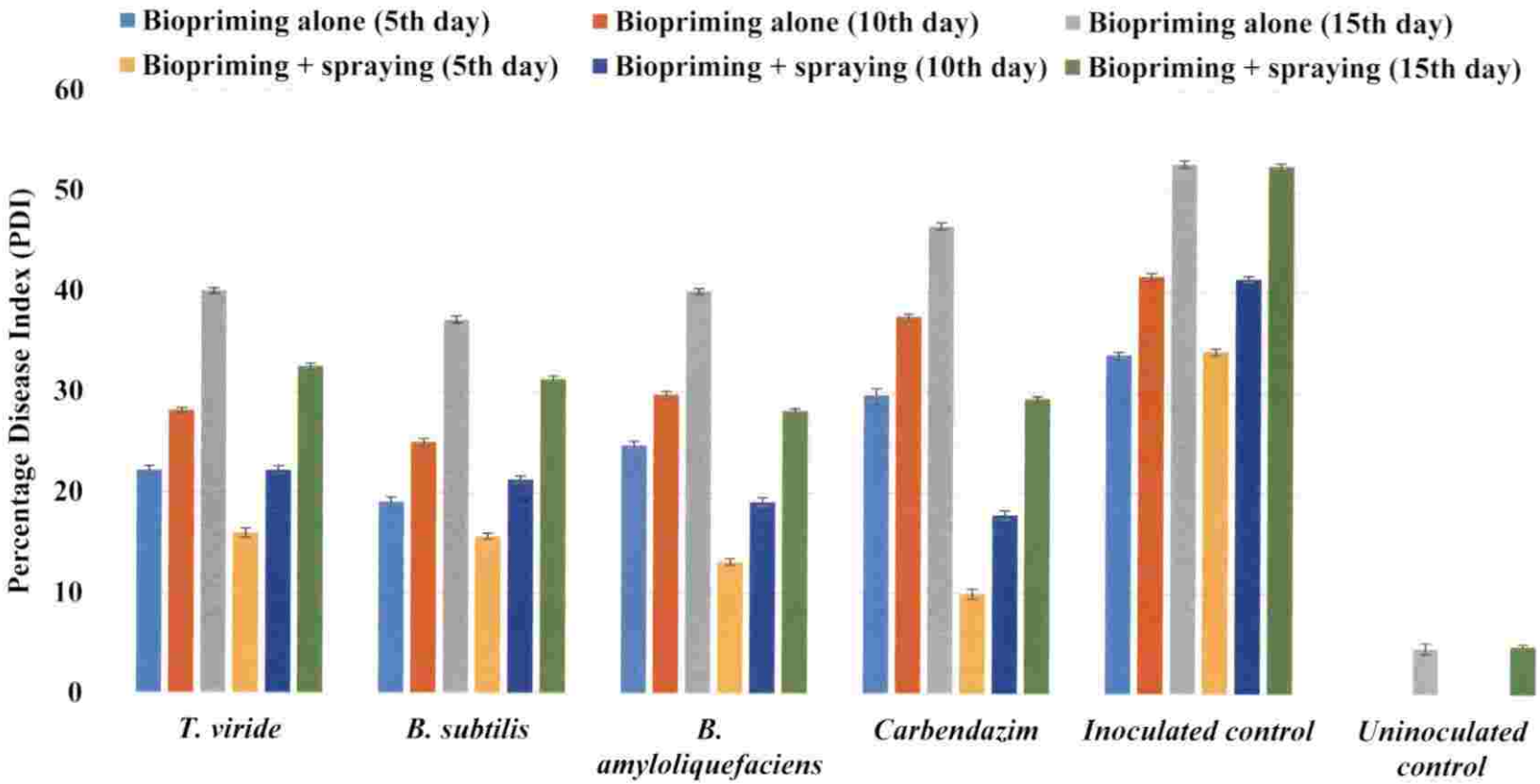

Figure 26. Comparison of seed biopriming and seed biopriming and spraying on severity of fruit rot in chilli variety Vellayani Athulya; Error bars represent standard error of means of observed values.

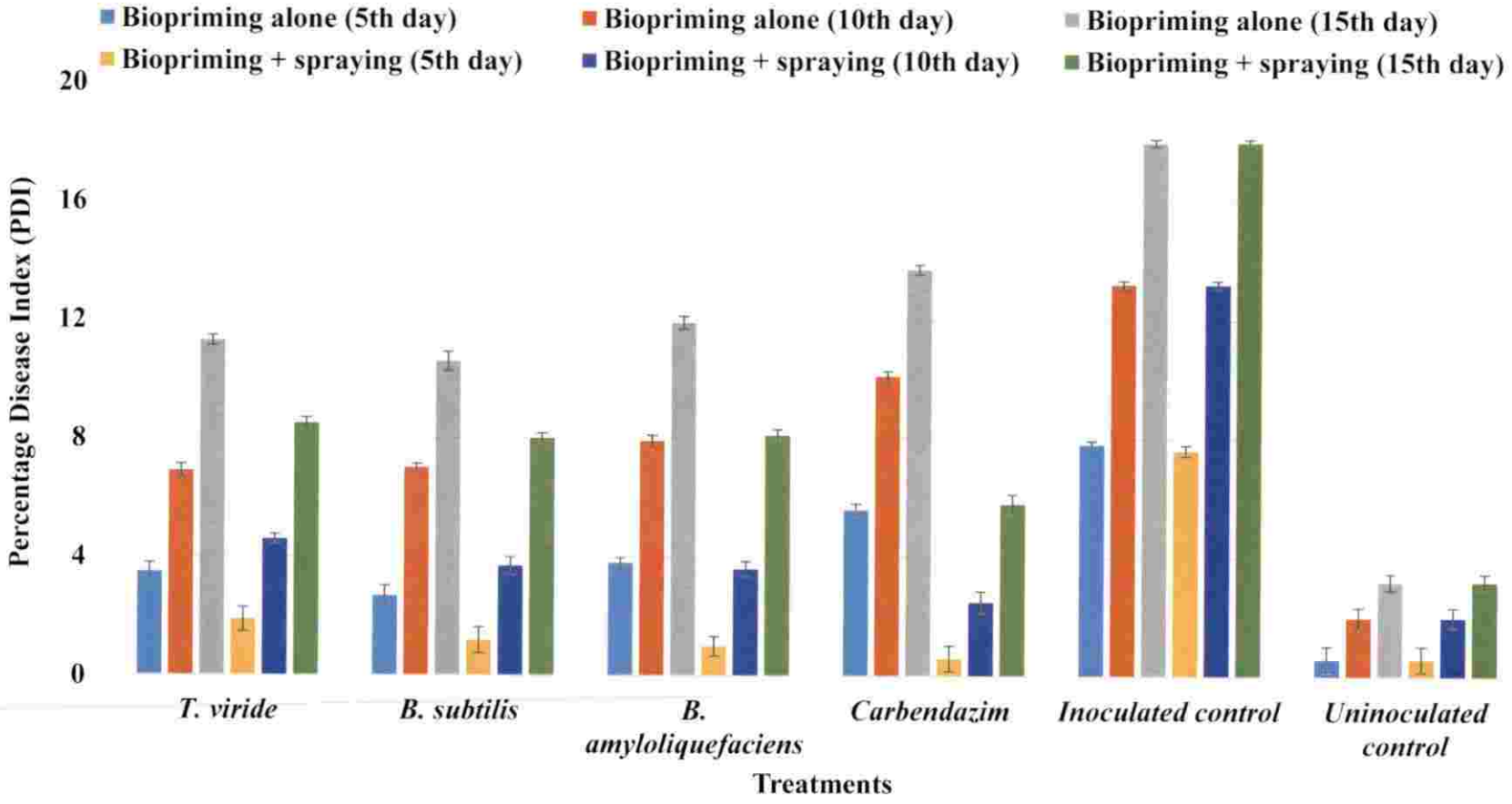

Figure 27. Comparison of seed biopriming; and seed biopriming and spraying on severity of anthracnose in chilli variety Vellayani Athulya; Error bars represent standard error of means of observed values.

for chemical check was 14.67 and control was 28.67. Considerable reduction (66.67 %) in anthracnose of fruits treated with strain BECL11 (PDI - 8.00) compared to control (PDI - 24.00) was observed and was lower than the chemical check (PDI - 12.00). The strain BECR2 reduced the anthracnose on fruits by 44.46 per cent (PDI – 13.33). The yield was also increased by the treatments BECL11 (7.72 t / ha) and BECR2 (7.05 t / ha) compared to control (5.15 t / ha). Spray application resulted additional benefits over biopriming because it allows the antagonists to pre-colonise on the surface before the arrival of the pathogen (Ippolito and Nigro, 2000).

Bacillus spp. were found to be more effective in reducing the disease. This was because *Bacillus* spp. forms biofilms on the surfaces of fruits. Biofilms are multicellular matrixes of bacteria surrounded by extracellular polysaccharides called as glycocalyx. This glycocalyx acts as a physical barrier and is strongly anionic, thereby protecting the microcolony from external agents (Arrebola *et al.*, 2010b). Biofilm mode is important for the bacteria to act as biocontrol agents (Bais *et al.*, 2004). Biopriming of seeds and spray application of *B. amyloliquefaciens* during fruit set was found to be the best for managing the fruit rot caused by *C. capsici* in chilli. Yamamoto *et al.* (2014) also observed that spray application of *B. amyloliquefaciens* strain S13-3 ($1.5x10^8$ cfu / ml) reduced the severity of anthracnose of strawberry caused by *C. gloeosporioides* and reported that the lipopeptide antibiotics produced by this strain identified such as iturin A, fengycin, mixirin, pumilacidin and surfactin were responsible for the induction of defence related components like pathogenesis-related proteins, chitinase and β-1, 3-glucanase in strawberry leaves. It was observed from the study that biopriming followed by spraying with *B. amyloliquefaciens* VLY 24 gave effective protection to chilli from fruit rot and anthracnose disease also significantly improved the plant growth and yield.

Thus the present study revealed that seed biopriming combined with spraying of biocontrol agents during fruit set is an effective technique to improve the growth parameters of plants, yield and bring significant decrease in disease

incidence and severity. This was found comparable with the application of chemical fungicides and can be used as an eco-friendly alternative to raise safe-to-eat crop.

Summary

6. SUMMARY

Chilli is an important cash crop grown worldwide. India is one of the leading producer and exporter of chilli. It is an inevitable spice in the daily cuisine in all parts of the world. Chilli contains high amount of vitamins, minerals, fibres and protein. It is also known to boost the immune system and lower the cholesterol levels. Anthracnose/ fruit rot caused by *C. capsici* is one of the important diseases in chilli. It affects the economic part i.e., the fruit and causes yield loss up to 50 per cent in chilli. Chemical fungicides are widely used to curb the disease but are perilous to humans and the environment. They also possess the threat of the pathogen developing resistance against the fungicides. Biological control is a promising and eco-friendly alternative. The method of application of the biocontrol agents to the plants is an important aspect to be considered. Seed biopriming is an emerging technique of application of biocontrol agents to plants. It is a biological and physiological method which primes the seeds with biocontrol agent thus protecting the seeds from pathogen. It also improves the vigour of the seedlings. Thus the present study was conducted to study the effect of seed biopriming as well as spraying with biocontrol agents at fruit set for the control of anthracnose/ fruit rot of chilli caused by *C. capsici*.

Twenty sets of samples of chilli seeds were collected from five agro-ecological zones of Kerala. The infection percentage was calculated; and the externally and internally seedborne microflora were isolated. The infection percentage ranged from 6.62 to 43.33. Six species of *Aspergillus*, three species of *Penicillium*, and other fungi such as *Pestalotia*, *Curvularia*, *Alternaria*, *Mucor*, *Fusarium* and *C. capsici* were found associated with the seeds. *Aspergillus* spp. and *Penicillium* spp. were the major fungi associated. Among the fungi isolated four species of *Aspergillus*, one species of *Penicillium*, *Alternaria* spp., *Fusarium* spp. and *C. capsici* were found to be both externally and internally seed borne, *Pestalotia* spp. and *Curvularia* spp., were found to be externally seed borne only and *Mucor* spp. was internally seed borne. Eighteen different isolates of bacteria

were also isolated from the seeds. Three of them were both externally and internally seed borne and others were externally seed borne only.

A survey was conducted during June – November 2018 in five locations of Kerala *viz.* Vellayani-Thiruvanathapuram, Kumarakom-Kottayam, Vellanikkara-Thrissur, Ambalavayal-Wayanad and Padannakkad-Kasaragod. The incidence and severity of anthracnose / fruit rot of chilli was recorded at each location. It was found that fruit rot / anthracnose was a severe disease in chilli and the pathogen *C. capsici* was associated majorly with the disease. The incidence of fruit rot ranged from 20 - 75 per cent and the severity ranged from 43.50 – 67.25 per cent. The symptoms observed during the survey included leaf spot, leaf blight and brown necrotic lesions on the stem. Fruit rot symptom began as sunken water soaked lesions which later enlarged to straw or dark brown coloured elliptical lesions. These lesions later covered the entire fruit surface causing rot. Black coloured acervuli were seen on the rotted area as dots arranged in concentric rings. Gradually fruits got deformed, dried and turned black. The infection spread to seeds and such seeds initially had white mycelial growth and at advanced stage turned dark brown to black with acervuli on the surface. Severely infected plants completely dried up.

Five isolates of *C. capsici* were obtained from the survey. The morphological and cultural characters were studied. All the isolates exhibited concentric zonations with acervuli. The cultures were either white, off white or grey on the upper side and white, cream, yellow or yellow to dark brown on the reverse side. The margins were either regular or irregular. The radial mycelial growth at 7th day of growth in PDA medium ranged from 7.95 to 8.80 cm. Mycelium was hyaline, septate and branched with width ranging from 2.19 to 3.32 µm. The conidia were sickle shaped with an oil globule at the centre with length ranging from 19.55 to 24.85 µm and the width ranging from 2.72 to 3.42 µm. Acervuli were black with diameter ranging from 121.23 to 147.03 µm. The number of setae in acervuli ranged from 13 - 56. Appressoria were oval shaped

and brown coloured. Appressoria length varied from 11.71 to 15.66 µm and the width varied from 6.26 to 8.56 µm.

Pathogenicity of the isolates were proved. Virulence test was done by artificial inoculation on tender, mature and ripe fruits of chilli variety Vellayani Athulya. Typical fruit rot symptoms were observed. Symptoms started as brown water soaked lesions which later enlarged to entire fruit surface with black concentric zonations having acervuli. The size of the lesions produced by the most and least virulent isolates were 3.81 and 0.61cm on tender fruits, 4.02 and 0.43 cm on mature fruits at 7th day of inoculation and 2.80 and 0.48 cm at 10th day of inoculation for ripe fruits respectively. The most virulent isolate obtained was used for further *in vitro* and *in vivo* studies.

Six biocontrol agents *viz. T. viride* (KAU isolate), *P. fluorescens* PN 026 (KAU isolate), *B. subtilis* VLY 62, *B. pumilus* VLY 17, *B. amyloliquefaciens* VLY 24 and *P. indica* were screened *in vitro* against *C. capsici* by dual culture technique and fruit assays. Dual culture assay revealed that the maximum percentage inhibition of mycelial growth of *C. capsici* was by *B. amyloliquefaciens* (62.96) followed by *B. subtilis* (56.30) and *T. viride* (51.85). The mycelial growth inhibition shown by *P. indica* was 46.67 per cent and *B. pumilus* was 45.19 per cent. *P. fluorescens* did not inhibit of mycelial growth of *C. capsici in vitro*.

In vitro fruit assays revealed that the lesion size of anthracnose in chilli fruits treated with biocontrol agents were significantly lower than untreated fruits. Fruits treated with *B. amyloliquefaciens* exhibited the lowest lesion size with highest disease reduction (55.64 %) over control. This was followed by *B. subtilis* (51.39 % reduction) and *T. viride* (37.64 % reduction). Reduction in lesion size shown by *P. indica* and *B. pumilus* were 34.04 and 18.65 per cent respectively. The least effective was *P. fluorescens* with only 15.22 per cent reduction over control. The activities of the defence enzymes PO and PPO in the fruits before and after treatment were measured. All the treated fruits exhibited higher enzyme

activities than the untreated control. Fruits treated with biocontrol agents and subsequently challenge inoculated with *C. capsici* exhibited higher PO and PPO enzyme activities than those treated with biocontrol agents or pathogen alone. PO activity after treatment with biocontrol agents but before challenge inoculation was found to be increasing upto 48 h and then decreasing. PO activity after challenge inoculation was increasing even after 48 h. PPO activity in biocontrol agent treated fruits increased upto 48 h and then decreased, both before and after challenge inoculation. Untreated control did not show much variation in enzyme activity throughout the experiment. Highest change in activity was observed in case of treatment with *B. amyloliquefaciens* followed by *B. subtilis* and *T. viride*. The least activity was observed on treatment with *P. fluorescens*.

An experiment was undertaken to standardize the duration of biopriming in chilli. Seeds were bioprimed with all the six biocontrol agents *viz.*, *T. viride*, *P. fluorescens*, *B. subtilis*, *B. pumilus*, *B. amyloliquefaciens* and *P. indica* along with carbendazim and hydropriming. The different durations tested were 20 min, 1 h, 2 h, 4 h, 8 h and 16 h. It was revealed that soaking of seeds for 1 h in *T. viride* and 4 h in other biocontrol agents *viz.*, *P. fluorescens*, *B. subtilis*, *B. pumilus*, *B. amyloliquefaciens* and *P. indica* were found to be the best duration of biopriming. These treatments gave the highest values for all the parameters measured *viz.*, germination percentage, shoot length, root length, seedling length, seedling dry weight, SVI - I and SVI - II compared to other durations of biopriming. Four hours of priming was also found best for carbendazim and hydropriming. All the priming treatments significantly increased the biometric parameters measured compared to unprimed seeds. The effective soaking durations were followed for the respective treatments in further *in vivo* studies.

Two pot culture experiments were conducted to test the effectiveness of seed biopriming, and seed biopriming along with spraying during fruit set for the control of fruit rot / anthracnose, growth and yield in chilli. The best three treatments from the dual culture assay *viz., B. amyloliquefaciens* strain VLY 24,

B. subtilis strain VLY 62 and *T. viride* KAU T_6 and best durations of soaking *viz.*, 1 h for *T. viride* and 5 h for *Bacillus* spp. were chosen for the *in vivo* studies.

Biopriming significantly improved all the growth parameters and reduced the severity of fruit rot / anthracnose. Among the treatments, plant height was maximum for biopriming with *B. amyloliquefaciens* (11.91 % increase over control) followed by *T. viride* (6.09 %) and *B. subtilis* (2.65 %) at 120 DAS. The maximum number of tertiary branches per plant, fruits per plant, 100 seed weight and seed yield per plant were recorded for *T. viride* with increase percentage of 44.30, 52.36, 19.35, and 65.34 respectively over control followed by *B. subtilis* and *B. amyloliquefaciens*. Biopriming with *B. subtilis* gave the maximum control of fruit rot (29.58 % reduction over control) and leaf anthracnose (41.11 %) followed by *T. viride* and *B. amyloliquefaciens* and was better than priming with carbendazim.

There was no significant difference between biopriming alone and biopriming combined with spraying during fruit set on the growth and yield parameters of chilli like plant height, number of branches per plant, number of fruits per plant, 100 seed weight and seed yield per plant. Biopriming along with spraying exhibited significant reduction in the severity of fruit rot and anthracnose in leaves than biopriming alone. Biopriming and spraying with *B. amyloliquefaciens* resulted maximum control of fruit rot (reduction of 46.48 % over inoculated control) and was on par with carbendazim (44.10 %). This was followed by *B. subtilis* (40.54 %) and *T. viride* (38.17 %). The reduction in anthracnose of leaves was maximum for treatment with *B. subtilis* (55.56 %) followed by *B. amyloliquefaciens* (55 %) and *T. viride* (52.78 %). Carbendazim gave a reduction of 67.78 per cent over inoculated control.

Thus the present study revealed that seed biopriming and spraying with *B. amyloliquefaciens* VLY 24 at fruit set was found to be most effective for the management of fruit rot / anthracnose of chilli. It also improved the growth and yield of the crop. Thus it can be concluded that seed biopriming along with

spraying of biocontrol agents at fruit set could be used as an eco-friendly measure to produce safe-to-eat crop.

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Appendices

APPENDIX - I

Compostion of media used

1. Potato Dextrose Agar (PDA)

Potatoes (sliced)	- 200.00 g
Agar-agar	- 20.00 g
Dextrose	- 20.00 g
Distilled water	- 1000 mL

2. Nutrient Agar

Beef extract	- 3 g
Peptone	- 5 g
Sodium chloride	- 1 g
Agar-agar	- 20 g
Distilled water	- 1000 mL

3. King's B medium

Peptone	- 20 g
Dipotassium hydrogen phosphate	- 1.5 g
Magnesium sulphate heptahydrate	- 1.5 g
Agar-agar	- 20 g
Final pH	- 7.2 ± 2
Distilled water	- 1000 mL

APPENDIX - II

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Composition of stain used

4. Lactophenol cotton blue

Anhydrous lactophenol - 67.0 ml

Distilled water - 20.0 ml

Cotton blue - 0.1 g

Anhydrous lactophenol prepared by dissolving 20 g phenol in 16 ml lactic acid in 3 ml glycerol.

APPENDIX - III

Buffers for enzyme analysis

1. 0.1 M Sodium phosphate buffer (pH 6.5)

Stock solutions

A: 0.2 M solutions of monobasic sodium phosphate (27.8 g in 1 litre)

B: 0.2 M solutions of dibasic sodium phosphate (53.65 g in 1 litre)

68.5 ml of A mixed with 31.5 ml of B diluted to a total of 200 ml.

Abstract

SEED BIOPRIMING AND SPRAYING AT FRUIT SET FOR THE MANAGEMENT OF CHILLI ANTHRACNOSE CAUSED BY *Colletotrichum capsici* (Sydow) Butler and Bisby

by

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ABSTRACT OF THE THESIS

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ABSTRACT

The study entitled 'Seed biopriming and spraying at fruit set for the management of chilli anthracnose caused by *Colletotrichum capsici* (Sydow.) Butler and Bisby' was conducted at College of Agriculture, Vellayani and Coconut Research Station (CRS), Balaramapuram during 2017- 2019 with the objective to study the seed borne nature of chilli anthracnose/fruit rot caused by *Colletotrichum capsici* and the effect of seed biopriming and spraying during fruit set for its management.

Samples of chilli seeds were collected from five agro-ecological zones of Kerala *viz.* Northern, High range, Central, Special problem and Southern zones. The lowest percentage of infection was found in seed samples collected from Vellayani (6.62) and the highest in Parassala (43.33). Both externally and internally seed borne fungi and bacteria were isolated from the infected seeds.

Fruit rot samples were collected from five locations of Kerala *viz.* Vellayani, Kumarakom, Thrissur, Wayanad and Kasargod. Five isolates (C1 to C5) of *C. capsici* were obtained. Vellyani isolate (C1) was the most virulent isolate which recorded a lesion size of 3.81 cm in detached fruit assay at seven days after inoculation (DAI). The isolate C1 was used for further studies.

Six biocontrol agents *viz. Trichoderma viride* (KAU isolate), *Pseudomonas fluorescens* PN 026 (KAU isolate), *Bacillus subtilis* VLY 62 *Bacillus pumilus* VLY 17, *Bacillus amyloliquefaciens* VLY 24 and *Piriformospora indica* were screened *in vitro* against *C. capsici* using dual culture method. *B. amyloliquefaciens* recorded the highest percentage suppression of mycelial growth of *C. capsici* (62.96) followed by *B. subtilis* (56.30) and *T. viride* (51.85). *P. fluorescens* did not show the mycelial growth suppression of *C. capsici*.

In vitro assays on chilli fruits revealed that *B. amyloliquefaciens* was most effective in controlling fruit rot with a lesion size of 2.71 cm 10 DAI followed by

B. subtilis (lesion size - 2.97 cm) and *T. viride* (lesion size – 3.81 cm). Peroxidase and polyphenol oxidase activities were also higher in *B. amyloliquefaceins* treated chilli fruits before and after treatment.

Standardization of priming techniques revealed that soaking of seeds for 1 h was found effective for *T. viride* and 4 h for other biocontrol agents. The effective soaking durations were followed for the respective treatments in further *in vivo* studies.

Based on the *in vitro* assays, the best three treatments *viz., B. amyloliquefaciens*, *B. subtilis* and *T. viride* were taken for *in vivo* studies. *In vivo* evaluation of seed biopriming on the control of fruit rot revealed that the lowest disease severity was recorded in *B. subtilis* (PDI – 37.19) followed by *B. amyloliquefaciens* and *T. viride* (PDI in both - 40.00). The severity of anthracnose in leaves was lowest in *B. subtilis* (PDI – 10.60) which was on par with *T. viride* (PDI – 11.30) and *B. amyloliquefaciens* (PDI – 11.90). The number of fruits per plant, 100 seed weight and seed yield per plant were higher in *T. viride*.

In vivo studies on the effect of seed biopriming and spraying during fruit set recorded the lowest disease severity for *B. amyloliquefaciens* (PDI – 28.13 at 15 DAI) which was on par with carbendazim 0.1 per cent (PDI - 29.38) and was followed by *B. subtilis* (PDI - 31.25) and *T. viride* (PDI - 32.5). The severity of anthracnose in leaves was lowest in *B. subtilis* (PDI - 8.00) which was on par with *B. amyloliquefaciens* (PDI - 8.10) and *T. viride* (PDI - 8.50). The number of fruits per plant, 100 seed weight and seed yield per plant were highest in *T. viride* treated plants.

Thus, the present study indicated that seed biopriming and spraying with *B. amyloliquefaciens* VLY 24 at fruit set was found to be the most effective for the management of fruit rot / anthracnose of chilli which could be used as an eco-friendly measure to produce safe-to-eat crop.