Characterization and management of fungal pathogens of cabbage (*Brassica oleracea* var. *capitata* L) and cauliflower (*Brassica oleracea* var. *botrytis* L)

by

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(2015-11-105)

THESIS
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DECLARATION

I, hereby declare that the thesis entitled "Characterization and management of fungal pathogens of cabbage (*Brassica oleracea* var. *capitata* L) and cauliflower (*Brassica oleracea* var. *botrytis* L)" is a bonafide record of research work done by me during the course of research and that this thesis has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled "Characterization and management of fungal pathogens of cabbage (*Brassica oleracea* var. *capitata* L) and cauliflower (*Brassica oleracea* var. *botrytis* L)" is a bonafide record of research work done independently by Ms. Nusrath Beegum C.H under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to her.

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Introduction

1. INTRODUCTION

Cabbage and cauliflower are the most economically important cole crops of the family Brassicaceae. Both cabbage and cauliflower have originated from a single parent known as wild cabbage (*Brassica oleraceae* var.*sylvestris*). Cabbage was introduced by Portuguese and the crop became more popular during British rule, while, cauliflower was introduced in 1882 by the British (Gopalakrishnan, 2007).

India is the second largest producer of cabbage next to China, accounting for 16.55 per cent of the world area (NHB, 2015). Among the cool season crops grown in India, it ranks second next to cauliflower in area and is top in production (NHB, 2015).It is grown in an area of 4.00 lakh hectare with an annual production of 8.9 million metric tonnes and productivity of 22 $MTha^{-1}$ (NHB, 2016-17).The major cabbage growing states are Uttar Pradesh, Orissa, Bihar, Assam, West Bengal, Maharashtra, Karnataka and Himachal Pradesh.

Cabbage is commonly used fresh as salad, as boiled vegetable, cooked in curries and processed as pickles. It is used against ailments like gout, diarrhoea, stomach coeliac troubles and even for diseases like scurvy disease. Also, cabbage juice was used as a remedy against poisonous mushrooms and also as gargle against hoarseness. In addition to several minerals such as calcium, phosphorus, potassium, sulphur and iron present in cabbage, it also contains high percentage of protein and vitamins like A, B and C, and its anti-cancer property is due to Indole-3-carbinol. Even though cauliflower is a poor source of nutrients compared to cabbage, its popularity in India is high compared to cabbage. It is grown in an area of 4.5 lakh hectare with an annual production of 8.5 million metric tonnes and productivity of 17 $MTha^{-1}$ (NHB, 2016-17). It is reported that a substantial amount of protein, carbohydrate, phosphorous and ascorbic acid is present in the curd of cauliflower.

In Kerala, cabbage and cauliflower are grown as seasonal crops during September to February months. Presently, availability of many tropical varieties

of cabbage and cauliflower suited to Kerala has led to an increase in the production of these crops to a commercial level in many districts. However, the cultivation of promising cultivars of cabbage and cauliflower are under great threat due to several biotic factors, among which disease causing pathogens are the most destructive ones. The warm humid tropical climatic conditions of Kerala attracts many fungal pathogens especially in the intensively cultivated tracts. Moreover detailed systematic studies for identification and characterization of fungal pathogens of cabbage and cauliflower were not undertaken so far. In this context, the present study is proposed to identify and characterize the fungal diseases of cabbage and cauliflower occurring in different selected districts of Kerala and to study the management of most severe and predominant disease under *in vitro* and *in vivo* conditions.

The study encompasses with the following objectives:

- Survey on the occurrence of fungal diseases of cabbage and cauliflower
- Isolation of pathogens
- Characterization of pathogens
- Symptomatology of diseases
- *In vitro* evaluation of fungicides and biocontrol agents
- *In vivo* evaluation of fungicides and biocontrol agents

Review of Literature

2. REVIEW OF LITERATURE

Important Brassica vegetables cultivated in Kerala include cabbage and cauliflower which are consumed all over the world. These vegetables possess both antioxidant and anti carcinogenic properties (Cohen *et al.*, 2000; Chu *et al.,* 2002). Both these crops are heavily challenged by various fungal pathogens, whereas bacterial and viral diseases have little effect on their yield (Abdel-Farida *et al.,* 2009). Systematic study in the identification and characterisation of fungal pathogens of cabbage and cauliflower was not done so far in Kerala. Therefore, the research on characterization and management of fungal diseases of cabbage and cauliflower is the first attempt to document the fine points of these diseases.

2.1 FUNGAL PATHOGENS

Various workers have reported different fungal pathogens found on cabbage and cauliflower *viz., Alternaria, Rhizoctonia* causing Leaf blights, , *Choanephora, Pythium,* causing head or curd rot, *Colletotrichum, Cercospora, Curvularia* causing leaf spots and *Fusarium* causing damping off.

Leaf blights in vegetables are causing serious crop losses in cole crops. *Alternaria* and *Rhizoctonia* are two important species of blight pathogens infecting cabbage and cauliflower. Genus *Alternaria* are extremely destructive plant pathogens on many vegetable families such as Cucurbitaceae, Brassicaceae, Solanaceae. Different kinds of symptoms are produced by *Alternaria* species on cabbage and cauliflower. Bassey and Gabrielson, (1983) reported that *Alternaria brassicicola* (Schw.) caused wilt disease to crucifer seed crop and resulted in reduced seed yield and germination, due to its seed born nature. In 2004, Robert *et al.*, recorded the infestation of *A. brassicicola* as black spot disease on cruciferous plants. Kubota *et al.* (2006) observed that *Alternaria brassicicola* infestation in seedlings of cabbage caused more than 50 percent of yield loss. In 2010, Sakhawat and Hossain found out that *Alternaria* blight is a serious problem in cauliflower seed crop and this caused 47.8 percent reduction in seed yield. Nowicki *et al.* (2012) reported that four important *Alternaria* species were causing

dark leaf spot of crucifers, which include *A. brassicae* (Berk.), *A. brassicicola* (Schw.), *A. raphanin* and *A. alternate*. Opinion of Mamgain *et al.* (2013) was that the necrotrophic nature of *Alternaria* species caused severe damage of the plant and harvested product, with an average yield loss in the range of 32-57 percent.

Rhizoctonia solani is a ubiquitous soil borne fungus which was described by Julius Gotthelf Kühn in 1858 (Ogosid 1996) that attacks a large number of plant species around the world with diverse symptoms. Abawi and Martin (1985) from New York, isolated *Rhizoctonia solani* from the necrotic lesions on the heads of cabbage. Keinath (1995) observed *Rhizoctonia* infection in cabbage grown under green-house conditions in South Carolina State of US. They enlisted pre-emergence and post-emergence damping off and wire stem diseases in cabbage by *Rhizoctonia solani*. Rehman *et al.* (2012) states that among the fungal diseases, damping off disease caused by *Rhizoctonia solani*. Kuehn is the major constraint in the production of cabbage seedlings. Grosch *et al.* (2003) observed bottom rot of lettuce in open fields of Germany and they isolated the causal agent as *Rhizoctonia solani.* Kuramae *et al.* (2003) reported infection of *Rhizoctonia solani* in lettuce, broccoli, spinach, melon and tomato from Brazil. It was in 2004, documentation of *Rhizoctonia solani* infection on cabbage was done by Yang *et al* for the first time from China. *Rhizoctonia solani* causing damping-off, wire stem, brown girdling root rot and head rot diseases in crucifers was reported by Rimmer *et al.*, (2007). According to Budge *et al.* (2009) wirestem caused by *Rhizoctonia* spp. remains was a sporadic problem of plants of *Brassica oleraceae* in the UK. They isolated *Rhizoctonia solani* from cabbage, cauliflower, broccoli, brussels sprouts and savoy cabbage. Zhang *et al.* (2009) reported an outbreak of cabbage head rot disease caused by *Rhizoctonia solani* AG-1 in Central China and recorded about 50 percent average yield reduction. Shim *et al.* (2013) studied occurrence of leaf rot and leaf ring spot disease, caused by *Rhizoctonia solani* in Chinese cabbage in seedling nursery and greenhouses in South Korea. Hua *et al.* (2014) recorded ninety seven isolates of Rhizoctonia causing diseases in crucifers from Vietnam. They isolated the pathogen from seven hosts including white

cabbage, Chinese cabbage, Chinese flowering cabbage, turnip cabbage, broccoli, mustard and park-choi.

Choanephora and *Pythium* are the two important soft rot causing fungal pathogens in vegetables. *Choanephora cucurbitarum* causes soft rot in economically important crop plants mainly on floral parts and fruits. Yu and Ko (1997) documented *C. cucurbitarum* causing rot disease on varieties of plants including okra, squash, pepper, pea, bean and cucumber. Kwon and Jee (2005) reported soft rot in eggplant caused by *Choanephora cucurbitarum* for the first time from South Korea. Kagiwada *et al.* (2010) noticed leaf and stem rot symptoms on ice plants (*Mesembryanthemum crystallinum*) grown in hydroponic green-house in Japan. They isolated *Choanephora cucurbitarum* from diseased plants. Saroj *et al.* (2012) reported wet rot disease in *Withania somnifera* (Aswagandha) caused by *Choanephora cucurbitarum* for the first time from India. On cauliflower, for the first time in India, leaf rot by *Choanephora cucurbitarum* was reported by Gogoi *et al.* (2016). *Choanephora cucurbitarum* can cause rot of floral parts on ornamental plants and it was reported in *Hibiscus syriacus* by Park *et al.* (2016). Pornsuriya *et al.,* 2017 documented wet rot on the leaves of *Brassic chinensis* by *Choanephora cucurbitarum* in Thailand and confirmed by morphological characters and molecular analysis.

Pythium sp. are ubiquitous soil borne pathogens causing damping off and root rot diseases on many plant species such as cabbage, chinese cabbage, broccoli, carrot, cucumber, melon, turf grass, cotton, wheat and others (Abdelzaher, 2001). The diseases caused by *P. aphanidermatum* varies with the host plant, it is the causal agent of pre and post emergence damping-off of various seedlings. It also causes seedling rots, root rot, cottony-leak, cottony blight, stalk rot etc. For the first time from United States of America, *Pythium* sp. causing head rot of cabbage was reported in 1925 itself by Dreschler. Mahmud (1950) documented damping off diseases on cabbage, cauliflower and kohlrabi seedlings due to *Pythium aphanidermatum* in Nagpur. Singh and Pavgi (1978) also isolated this pathogen from root rot disease of cabbage and cauliflower. Lira and See

(1982) isolated *P. aphanidermatum* from damping off disease of four Brassica species in Singapore. Tanina *et al.* (2004) reported rot of chingensai (*Brassica campestris L. chinensis* group) caused by *Pythium ultimum var. ultimum* and *Pythium aphanidermatum* in Japan. In a study by El-Mohamedy (2012), he remarked that *Pythium ultimum* is the main causal agent of root rot disease on green-house grown broccoli plants in Egypt. Damping-off and root rot caused by the *P. aphanidermatum* was considered among the most devastating diseases of greenhouse-grown crops by Parveen and Sharma in 2015. They also reviewed diseases caused by *Pythium* and its management.

Losses due to leaf spots are generally minor, but during favourable conditions they develop as blights and cause severe yield reduction in vegetables. *Cercospora, Colletotrichum* and *Curvularia* are some of the leaf spot causing pathogens reported by many mycologists. Not much studies have been carried out on *Cercospora* leaf spot on plants belonging to family Brassicacea however, Sinha and Singh (1995) reported the incidence of *Cercospora brassicola* leaf spot on Indian mustard for the first time from India. Mahmodi *et al.* (2013) isolated *Colletotrichum capsici* causing anthracnose in *Brssica chinensis* from Malasiya. He *et al.* (2016) reported *Colletotrichum truncatum* causing anthracnose of chinese flowering cabbage (*Brassica parachinensis* L. H. Bailey) for the first time in China. Sharma *et al.* (2011) reported leaf spot of *Amaranthus spinosus* caused by *Curvularia lunata* for the first time from India. They recorded up to 60 percent disease incidence. *Curvulria lunata* was reported as a blight pathogen in rice by Kamaluddeen *et al.,* in 2013 from Utter Pradesh. Aktar and Shamsi (2016) documented Curvularia blight of Marigold plants, *Tagetes erecta* and *T. patula* from Bangladesh. Chaudary *et al.* (2016) reported leaf spot of Eggplant caused by *C.lunata* from Pakistan.

The fungal pathogen *Fusarium* generally causes wilts and damping off in a number of vegetable crops. *Fusarium equiseti* causing wilt disease in cumin was reported by Reuveni (1982) from Israel. Dillard *et al* (2004) observed Fusarium infection on cabbage heads. Rimmer *et al.*, 2007 observed

yellows (*Fusarium* wilt) caused by *Fusarium* sp. in cabbage and cauliflower. First report of *Fusarium equiseti,* causing wilt of cumin, from India was in 2011 by Ramchandra *et al.* They estimated that there was 10 to 45 percent of crop loss in North Gujarat due to wilt.

2.2 PATHOGENICITY TEST OF FUNGAL ISOLATES

Rahimloo and Ghosta (2015) conducted pathogenicity test of different species of Alternaria on cabbage plants by detached leaf method where inoculation of agar blocks containing mycelia and spores were carried out on detached leaves. Zhang *et al.* (2009) carried out pathogenicity test of cabbage head rot by *Rhizoctonia solani.* On detached leaves, 6 mm diameter of mycelial plugs were placed and incubated at 28°C with 95 percent relative humidity.

Mahmodi *et al.* (2013) performed pathogenicity of *Colletotrichum capsici* on Bok choy (*Brassica chinensis* L.). On surface sterilized detached leaves 10µl conidial suspension was poured by wound/drop or non-wound/drop method. According to Chaudary *et al.* (2016), to confirm pathogenicity of *Curvularia lunata,* spore suspension ($4.5x10^5$ conidia/ml) spray to wounded leaves is an effective method. The test result showed typical necrotic spots on leaves after seven days of inoculation.

Johnson *et al.* (2014) demonstrated pathogenicity test of *Choanephora cucurbitarum* on different vegetables by mycelial bit inoculation on leaves. Gogoi *et al.* (2016) observed typical soft rot symptoms and signs of *Choanephora rot* on cauliflower after 5-7 days of inoculation by mycelial bit inoculation method.

Tanina *et al.* (2004) conducted studies on *Pythium ultimum* inciting rot of chinese cabbage. Wounding and placing of 5mm diameter agar plugs on midrib of plants showed symptoms after 48 hours of inoculation.

According to Ramchandra and Bhatt (2011), inoculation of sterilized soil with conidial suspension of *Fusarium equiseti* can be used for pathogenicity test

and planting seedling of cumin in inoculated soil resulted in wilting symptoms after 10-12 days of planting.

2.3. CULTURAL AND MORPHOLOGICAL CHARACTERS

The cultural and morphological studies are necessary for preliminary identification of different fungal pathogens. *Alternaria* is one of the most studied pathogen since it varies widely in cultural and morphological characters Rimmer *et al.* (2007) reported that conidia of *A.brassicicola* in cauliflower were with short beak , few longitudinal septa, up to 10 transverse septa, 50-130 µm length formed in long chains of 20 or more and golden or olive-brown or dark-brown colour. Sharma *et al.* (2013) studied morphology of the *Alternaria brassicae* from cauliflower and mustard. They observed the colour of the colonies, which were varied between light olive grey to olivaceous black on PDA media. Conidia were golden or brown, long obpyriform long beak and smooth surface. According to Kumar *et al.* (2014) *A. brasssicicola* from rapeseed and mustard had colonies of olive grey to greyish black with distinct zonations. Morphological studies revealed that conidiophores were septate, branched 44-55 µm x 11- 16 µm in dimension with 5-8 transverse septa. Sharma *et al.* (2014) studied the morphology of *A. brassicicola* and observed small conidia with short beak and 7-35 µm length. In study conducted by Rahimloo and Ghosta (2015) revealed that *A. brassicicola* of cabbage on PDA media after 7 days of inoculation the colony diameter reached 55mm at 23-25℃. Colony had olivaceous to dark brown colour with conidiophores appeared directly from surface of the media, 125 µm length with single conidiogenous site. Conidia were in long chains, older 30–45 × 10–12 µm in size, ellipsoid to ovoid in shape, formed without any longitudinal septa, transverse septa ranged from 5-7, thick and dark brown than the outer conidial wall. Younger conidia were smaller and 12–25 × 6–8 µm in size with ovoid shape.

According to Abawi and Martin (1985) on potato dextrose agar *Rhizoctonia* from cabbage initially produced white mycelium later turns brown-

grey with leathery texture. Rimmer *et al.* (2007) reviewed the morphology of *Rhizoctonia solani* from brassica vegetables and stated that mature hyphae 5-15µm thickness and branched at right angles, hyaline or brown. There was a septum formed with a slight constriction near to the point of branching. *Rhizoctonia solani* produced multinucleate irregular moniliform, barrel-shaped cells of up to 250 µm in length. Zhang *et al.* (2009) isolated *Rhizoctonia solani* from cabbage infected with head rot. The colony had white colour that turned brown after 5 days. Hyphae branched at right or acute angles near the septa with a thickness ranged from 4.9-8.0 µm. Cells had six to eight nuclei with characteristic dolipore septum. Isolates produced dark brown sclerotia after 10 days of incubation with a diameter in the range of 0.5-5 mm. Shim *et al.* (2013) observed light to dark brown colonies of *Rhizoctonia* from chinses cabbage hyphae with 5.01-11.12 µm diameter, dark brown sclerotia with 0.38-1.28mm diameter. The basic traditional methods of identification of different isolates of *Rhizoctonia* include colour of the colony, monilioid cells, sclerotia and other anastomosis behaviours (Prashanth *et al.*, 2016)

Gautam (2014) reviewed the morphological characters of *Colletotrichum gloeosporioides* pathogen attacking fruits and vegetable crops and stated that there is great variation in size and shape of the conidia is observed among different isolates of *C. gloeosporioides*. The morphological variations depend upon the host from which the pathogen is isolated and its area of origin. Chowdappa *et al.* (2012) studied the morphology of *C. gloeosporioides* on orchid and observed that the colony had white, grey, dark orange and pink-grey colour. The reverse side of the colonies appeared white, dark grey and orange with hyaline to brown coloured mycelium, sparse floccose, loose or compact growth. Conidia measured 7.57-15.50µm in length and 3.38-7.52µm in width. Conidia had cylindrical shape with both apices rounded or with one apex rounded and the other end pointed. Kulshrestha (2015) reviewed the morphology of *C. gloeosporioides* and found that the pathogen produced straight, cylindrical and oval conidia on well-

developed hyaline conidiphores. The size of the conidia differed from 11-16 x 4-6 µm and 13.8 x 4.8 µm.

Aktar and Shamsi (2016) observed *Curvularia lunata* on African marigold dark brown colony with zonations on the reverse side. Conidiophores were dark brown, septate, geniculate or unbranched. Under the microscope light to dark brown straight to pyriform conidia with 3-4 cells with large and curved central cells. Size of the conidia ranged from 18-26.5 x 8.5-14µm and were produced apically in a sympodial pattern.

Kwon and Jee (2005) isolated *Choanephora cucurbitrum* from eggplant on PDA media colonies appeared white to pale yellowish brown and they produced pediculate, elliptic fusiform or ovate monosporous sporangiola with striations and measured 12-20 x 6-14 µm. Sporangiospores , 14-22 x 7-10 µm sized and were produced at the head of each branch on the apex of long slender sporangiophore. They were attached with three or more appendages and had light brown to dark brown colour. Gogoi *et al.,* (2016) isolated the *C. cucurbitarum* from cauliflower and observed profuse and rapidly growing white colonies after 36 hours of incubation. Sporangiola were formed at the apex of sporangiophores. Monosporous sporangiola were elliptic, fusiform or ovoid and measured 8– 13× 11–22µm. Sporangia were sub-globose with 35-85µm diameter. Sporangiospores were brown coloured, with or without three or more thin appendages at the both ends, which were elliptic, fusiform or ovoid and measured 7–10.5 × 10-27µm. In a study by Pornsuriya *et al.* (2017) *C.cucurbitarum* produced white colonies later turned yellow or pale brown with abundant sporangiola. Monosporous sporangiola were ellipsoid to ovoid with brown to dark brown colour measuring 9-22µm in length and 8-15µm in width. The fungus produced erect, solitary, unbranched, non-septate sporangiophores with 5-13µm in length and 1-10mm in length, clavate vesicles at the tip.

Gherbawy *et al. (*2005) isolated P. aphanidermatum from soybean seedling and observed that colonies on cornmeal agar with cottony aerial mycelium, on

potato-carrot agar with some loose aerial mycelium without a special pattern. Main hyphae up to 10 mm wide. Zoosporangia consisted of terminal complexes of swollen hyphal branches of varying length and up to 22 mm wide. Zoospores formed at 15–308C. Encysted zoospores had12 mm diameter. Oogonia terminal, globose, smooth, 22–25mm diameter. Antheridia mostly intercalary, sometimes terminal, broadly sac-shaped, 11–15 mm long and 9–15 mm wide, one per oogonium and monoclinous or diclinous. Oospores aplerotic, 20–23 mm in diameter, walls 1–2 mm thick. Anees (2014) observed cultural and morphological characters of *P. aphanidermatum* isolated from cowpea. According to him the pathogen was a fast grower and colony had cottony aerial mycelium without any special pattern. The main hypha had 3.1-6.8 µm width. Sporangia consisted of terminal complexes of swollen hyphal branches of varying length. Oogonia terminl, globose, smooth, 18-21 µm diameter. Anthredia mostly intercalary, broadly sac shaped, 9.4-13.6 µm in length, 7.5-10.4 µm in width, one per oogonium, monoclinous or diclinous; oospores were aplerotic, 14.1-19.5 µm diameter with 1-2 µm wall thickness. Similar results were remarked by Parveen and Sharma (2015). The oospores are aplerotic. Rajalaksmi *et al.* (2016) observed white fluffy, dense mycelial growth of *P. aphanidermatum* on PDA within 24 hours. They produced coenocytic mycelium measuring 3 to 4 µm in diameter.

Ramchandra and Bhatt (2011) got the fungus *Fusarium equiseti* from wilted cumin plants and they cultured on PDA medium produced macro and micro conidia. Macroconidia had 28.0-30.5µm length and 3.5-5.25µm straight or slightly curved at the apex with 2-3 septa. Single celled microconidia were hyaline, non-septate with 9.5-12.5 x 3.5-5.25µm size. Lazreg *et al.* (2014) observed *Fusarium equiseti* from wilted aleppo pine plants with loosely floccose whitish aerial mycelium on PDA. Macroconidia were tapered having a pronounced curvature with elongated apical cell. Macroconidia had 5-6 septa and 31-45µm long. Chlamydospores observed were 7-13µm long and with globose shape formed intercalary and in solitary or in pairs and sometimes forming chains or clusters.

2.4 SYMPTOMATOLOGY OF FUNGAL DISEASES

Assessment of symptomatology is necessary for the systematic study of a pathogen. A detailed description of symptomatology of *Alternaria* leaf spot on crucifers was given by Singh (1987). The initial symptoms appear as small dark coloured spots which spread rapidly and form circular in shape with concentric circles size ranging from 1 cm diameter up to 23 inches. Under humid weather conditions these lesions may develop bluish growth in the centre. Mamgain *et al.* (2013) reviewed symptoms of *Alternaria* blight on mustard and reported presence of irregular, often circular brown to dark brown colour leaf spots with concentric lines which coalesce to form large patches causing the leaf blight. Kumar *et al.* (2014) observed initial symptoms of *Alternara* blight in rapeseed and mustard as the appearance of small black points which later enlarge and form prominent round spots with concentric rings. In some crucifers, lesions with constricting rings and zone of yellow halo are prominent. The leaf blight results in defoliation in many Brassica species. According to Tu *et al.* (2015) the first symptoms of the disease develop as minute yellow specks on the stems and oldest leaves of cabbage later enlarged to from circular, tan to dark brown spots. The spots had characteristic alternating light and dark concentric rings with a yellow halo around the lesion.

Wellman (1932) described leaf blight, bottom rot and head rot diseases incited by *Rhizoctonia solani* on cabbage crop. The blight disease first appeared on leaves near to the soil. On the midrib lesions appeared as sunken, black and sharply elliptical with long axes parallel to the sides of the midrib. On the leaf lamina round black spots developed and enlarged to form large blighted areas which had sparse web like surface mycelia over them. Finally general decay of the leaf base is observed and the tissues become black and easily torn. As a result distal parts turn yellow and the whole leaf droops or dry up and drop off. In the head rot, pathogen attacked bases of the first cover leaves of the head, lesions appeared as dark sunken spots with concentric zonation with a raised centre at the point of infection, and lesions expanded finally coalesced. Head rot advanced only

to the leaf tissues, whereas stem and core were not infected thus head become dark coloured, conspicuous, upright and covered with small brown sclerotia. Abawi and Martin (1985) observed initial symptoms of *Rhizoctonia* foliar blight on cabbage as small, irregularly shaped lesions that initially were light brown and elongated or circular lesions coalesced to form short streaks with dark brown to black colour. Yang *et al.* (2007) found that *Rhizoctonia solani* affected cabbages had a dark, sometimes wet decay at the bases of outer leaves and on emerging cabbage heads. A brown mycelium appeared on affected parts after damp weather with occasional small brown sclerotia on the cabbage head.

Gogoi *et al.* (2016) observed typical soft rot symptoms caused by *Choanephora cucurbitarum* with superficial fungal growth on cauliflower leaves. The disease appeared as water soaked areas on the margin of younger leaves which caused inward rolling and rapid rotting of diseased leaves. According to Pornsuriya *et al.* (2017) on Chinese cabbage the *Choanephora cucurbitarum* attack young and expanded leaves as water-soaked and rotted areas filled with dark mass of sporangiophores.

According to Drescler (1925) *Pythium* sp. cause head rot in cabbage as water soaked lesions on inner leaves which later discoloured and softened. Taina *et al.* (2004) observed initial symptoms of leaves and stems stem rot of chingensai (*Brassica campestris* L. *chinensis*) as water-soaked lesions at the base of the midrib. The diseased areas softened and turned dark brown which advanced to stem portion the plants leaving a fluffy white mass of fungal mycelium.

Smith (2012) observed *Cercospora* leaf spot on turnip, mustard, broccoli, colards and kale as pale green to grey or white lesions with a brown border. These lesions can be angular or circular in shape. Severe infection results in the defoliation of plants.

Mahmodi *et al.* (2013) reported symptoms of *Colletotrichum capsici* on *Brassica chinensis* as small water soaked spots on the leaf petioles of the young

plants. These spots enlarged and formed irregular to round sunken greyish brown spots with brownish border. On the well-developed lesions pale salmon colored conidial mass and acervuli were noticed. According to Goutham (2014) the initial symptoms of *Colletotrichum gloeosporioides* on vegetables were sunken spots with round to oval, water soaked appearance, later necrosis and death of infected tissues. Nelson (2008) observed symptoms of *C. gloeosporioides* as small, angular brown to black spots on leaves of mango. Later these spots enlarged to form extensive dead areas and in dry weather these lesions drop off leaving holes.

On rice the initial symptoms of *Curvularia lunata* blight appeared as elliptical brown spots which enlarged in size and formed large lesions on leaves. Later the colour of the spots changed to brownish black (Kamaluddeen *et al.* 2013). Akram *et al.* (2014) reported the blight on sorghum, *C. lunata* developed reddish brown circular spots on the leaves. Gradually the spots increased in size and formed large oblong lesions. Chaudary *et al.* (2016) recorded *Curvularia lunata* on eggplant which produced symptoms as dark brown circular spots, later coalesce and formed large oblong lesions.

According to Rimmer *et al.* (2007) the initial symptoms of seedling wilt of crucifers by Fusarium were yellow to yellow-grey discolouration of leaves with stunted growth of the seedlings. As the disease developed the yellow necrotic areas formed and fell off prematurely. The infected seedlings become brown, dry and brittle they often remain erect while retaining their uppermost leaves.

Ramachandra and Bhatt (2011) observed wilting in cumin. In open field condition the disease is characterized by the wilting of plants in patches. Due to the infection by *Fusarium equiseti* the whole plant wilted in cumin.

2.5. CHEMICAL CONTROL OF FUNGAL PATHOGENS

Singh and Rai (2003) stated that under *in vitro* conditions chlorothalonil (0.2 %) observed to be most effective fungicide reducing the mycelial growth of *Alternria alternata* causing blight in brinjal. Sidlauskiene *et al.* (2003) observed

88-93 percent disease reduction in *Alternaria* leaf spot on tomato, cucumber and cabbage after treatment with azoxistrobin. On *Alternaria alternata* causing blight of tomato Singh and Singh (2006) tested efficacy of seven fungicides viz., chlorothalonil, copper oxychloride, azoxystrobin, propineb, copper hydroxide, mancozeb at varying concentrations *viz.*, 2500, 2000, 1000, 500 and 250 ppm. Their observations revealed that the radial growth of the fungus significantly reduced by the use of all the concentrations for these seven fungicide. Anand *et al.* (2010) studied the efficacy of azoxystrobin against early blight of tomato caused by *Alternaria solani*. At 125 g a.i ha^{-1} azoxystrobin found to be effective with 92.82 per cent disease reduction. Meena *et al.* (2011) recorded 64.3-19.6% reduction in *Alternaria* leaf blight in oilseed brassica crop by mancozeb at (2052 kg ha-1). Viettu *et al.* (2015) studied the effect of different fungicides on leaf spot by *A.brassicicola* on cabbage. According to them the severity of the disease reduced in cabbage when treated with tebuconazole (4.62%), trifloxystrobin +tebuconazole (6.01%) and propiconazole (9.45%), followed by mancozeb (11.4%). Thakur (2015) observed 100 per cent growth inhibition of *A. brassicicola* on cabbage at ≥ 500ppm for zineb,captan, mancozeb and propineb. Singh *et al.* (2017) observed reduction in *Alternaria* leaf blight severity in mustard by mancozeb 75% WP at 0.25% and carbendazim 50WP at 0.2%.

Sundravadana (2007) observed complete inhibition of growth of *Rhizoctonia solani* causing sheath blight of rice by azoxystrobin at 1, 2, and 4 ppm. Field studies showed significant suppression of disease (> 60 %) and increased yield by foliar spraying of azoxystrobin at 125, 250, and 500 g/ha. Mishra *et al.* (2012) observed that for the management of Rhizoctonia leaf and inflorescence blight of cauliflower removal of lower infected leaves followed by spraying of mancozeb 75WP (0.2 %) was found to be best. According to Sriraj *et al.* (2014) even at the lowest concentration of 10ppm trifloxystrobin 25% + tebuconazole 50% and carbendazim completely inhibited the mycelial growth and sclerotia production of *Rhizoctonia* blight in turmeric.

Jignesh (2012) studied the effect of different chemicals on *P. aphanidermatum*. According to him Fenamidone (10%) + mancozeb (50%) (Sectin 60 WG) and metalaxyl (4%) + mancozeb (64%) (Ridomil MZ 68 WP) at 3000 ppm and 2000, ppm proved excellent in mycelial growth inhibition.

Pawar (2012) screened eight fungicides against *Curvularia lunata* causing blight in gladiolus and found complete inhibition of the pathogen by mancozeb at 0.2 % followed by tricyclazole (0.1%) and mancozeb + carbendazim (0.25%) with 83-84 % inhibition. According to his experiment carbendazim and chlorothalonil recorded less inhibition whereas Bordeaux mixture gave 73 per cent inhibition. Kithan and Daiho (2014) recorded inhibition of *Curvularia lunata var. aeria* from medicinal and aromatic plant *Etlingera linguiformis* by metalaxyl, mancozeb, carbendazim and hexaconazole at 0.1 per cent concentration. They suggested carbendazim and hexaconazole as alternative to metalaxyl and mancozeb.

Under *in vitro* against *Colletotrichum gloeosporioides* causing Blight in *Piper longum* maximum inhibition of 96.26 per cent was recorded in mancozeb + carbendazim (0.2%) followed by carbendazim (0.1%), mancozeb (0.25%) and copper oxychloride (0.3%). In field experiment, spraying of mancozeb + carbendazim (0.2%) was observed to be superior with 33.38 per cent disease control followed by carbendazim (0.1%), copper oxychloride (0.3%). Patel (2009) observed that propiconazole and carbendazim+mancozeb gave maximum disease control against fruit spot disease of pomegranate caused by *Colletotrichum gloeosporioides*. According to him the next best treatments were carbendazim and mancozeb with 67.22 and 62.54 per cent disease control. Parvathy and Girija (2016) observed complete inhibition of *Colletotrichum gloeosporioides* by tebuconazole (0.1 %) and combination fungicide mancozeb+ carbendazim (0.1 %) on black pepper.

Salam *et al.* (2016) studied the inhibitory effect of five fungicides against *Fusarium oxysporum* inciting Fusarium wilt disease of mango nursery. Among all the five fungicides trifloxystrobin 25% + tebuconazole 50% recorded significant

reduction of mycelial growth under *in vitro* conditions. Akhtar *et al.* (2017) observed 98 per cent reduction in growth of *Fusarium oxysporum* f.sp. *lycopersici* by trifloxystrobin 25% + tebuconazole 50% as compared to control on tomato.

George and Girija (2015) studied the effect of nine fungicides against *Choanephora cucurbitarum* in cowpea. Under *in vitro* condition at recommended concentration 100 per cent inhibition of growth was recorded by six chemicals, viz., mancozeb , copper oxy chloride, captan + hexaconazole, carboxin, carbendazim + mancozeb and propiconazole. According to them least effective fungicides were azoxystrobin and carbendazim with minimum inhibition of mycelial growth. They recorded 98.3 % inhibition of growth by copperhydroxide.

2.6 BIOLOGICAL CONTROL OF FUNGAL PATHOGENS

Sharma & Sharma (2006) observed 62.85 percent inhibition of mycelial growth of *Alternaria brassicae* by *Bacillus subtilis.* They also noticed the reduced percent of spore germinated from 100 percent in control to 82 percent. Under greenhouse condition the antagonist reduced percent leaf area spotted and disease severity in mustard plants. Thakur (2015) observed that *Trichoderma harzianum* proved inhibitory (74.2%) effect on *Alternaria brssicicola* followed by that of *T. viride* (72.4%) and *T. hamatum* (71.7%). Sabry, *et al.* (2015) tested the inhibitory action of different biocontrol agents on *Alternaria brassicicola* causing dark leaf spot of cabbage. Under greenhouse condition *Bacillus subtilis* showed highest reduction of disease severity of 90.44 per cent. *Trichoderma viride* and *T. harzianum* exhibited nearly similar disease reduction with 42.45 per cent and 42.35 per cent, respectively.

Rehman *et al.* (2012) studied the comparative effect of different biological control agents against *Rhizoctonia solani* causing damping off on cauliflower seedlings. They observed 85.5 and 83.0 per cent mycelial inhibition by *Trichoderma harzianum and T. viride* respectively. Under field conditions, biocontrol agents increased the seed germination, reduced damping-off incidence and improved plant growth vigour as compared to carbendazim and control. They

suggested seed treatment and seedbed treatment with Trichoderma sp., with combination farm yard manure for the management of the disease. Patil *et al.* (2009) recorded significant reduction of the mycelial growth of *Colletotrichum gloeosporioides* causing blight in *Piper longum* by *Trichoderma viride* (70.42 %). Which was followed by *Trichoderma harzianum* (66.90%). The least inhibition of the pathogen was observed in *Pseudomonas fluorescens* (20.72%). Kithan and Daiho (2014) conducted *in vitro* evaluation of biocontrol agents against *Curvularia lunata var.aeria* causing leaf blight of *E. linguiformis* by dual culture technique. Maximum inhibition of mycelial growth was found in *T. harzianum* 68.85 per cent inhibition, which was followed by *T. viride* (57.82%), and *P. fluorescens* (51.36%). Least effective bioagent was *Bacillus subtilis* with 30.32 per cent inhibition.

Siddiqui *et al.* (2008) studied the effect of *Trichoderma harzianum* on *Choanephora cucurbitarum*. There was 85.04 per cent disease reduction of okra wet rot treated with Trichoderma fortified rice straw extract and which was comparable to the conventional fungicide Dithane M-45. Abdelzaher *et al.* (2004) recommended soil treatment of *Pseudomonas fluorescens* against Pythium damping off on bush okra. In pot culture they observed reduced disease incidence and increased seedling emergence treated with *Pseudomonas fluorescens*. Under greenhouse condition the treatment gave 100 per cent seedling emergence. Jignesh (2012) recorded inhibitory effect of biocontrol agents against *Pythium aphanidermatum* causing damping off disease of chilli. By dual culture method maximum inhibition of 38.89 per cent was recorded by *Trichoderma harzianum*. Among four biocontrol agents *Bacillus subtilis* found to be the next best antagonist with 32.64 per cent inhibition. Under pot culture experiment significant reduction of the damping off disease was observed in *Bacillus substilis* (80.83%) and*Tricoderma harzianum* (72.62%). El-Mohamedy (2012) identified the effects of biocontrol agents isolated from rhizospheric soil of healthy broccoli plants. Biocontrol agents *Trichoderma harzianum, T. virde, Bacillus subtilis* and *Pseudomans fluorescens* completely inhibited the growth of *Pythium ultimum* on

PDA medium. Mixing the soil with suspension of biocontrol agents *ie.*, *Trichoderma harzianum, T. virde* ($5x10^6$ spores/ml), *Bacillus subtilis* and *Pseudomans fluorescens* ($8x10^7$ spore/ml) and dipping the roots of seedling in the same suspension gave the highest effect against Pythium root rot. In an experiment Jain *et al.* (2014) revealed the effectiveness of talc based formulation on damping off disease of chilli caused by *P. aphanidermatum*. Seed treatment of *Pseudomonas fluorescens* 0.5% W.P. formulation (TNAU Strain) @ 5, 10 and 20 g/kg of seeds resulted in efficient control of the damping off disease and also substantial improvement in the crop yield. Gholve *et al.* (2014) studied inhibiton of *Pythium ultimum* causing Brinjal damping off by different antagonists. Among the fungal and bacterial antagonists highest mean mycelial growth inhibition of 69.44 per cent was given by *Trichoderma viride. Trichoderma koningii* and *Trichoderma hamatum* were found to be good antagonists with 67.32 per cent and 63.99 per cent inhibition respectively. Bacterial biocontrol agents, *Bacillus subtilis* and *P. fluorescens* recorded inhibition of 59.71 and 56.27per cent respectively. Juma *et al.* (2015) studied the effect of *Trichoderma asperellum* and *B. subtilis* on Pythium damping off disease on Ethiopian kales (*Brassica carinata*). The incidence of post- emergence damping off reduced to range of 11 - 25.4 per cent on seeds coated with biocontrol agents whereas control recorded 64.8 per cent incidence. And they suggested that the use of *B. subtilis* and *T. asperellum* mixture can provide a potential control for damping off disease.

Sahar *et al.* (2013) observed moderate inhibition of *Fusarium oxysporum* f.sp. *melongenae* causing Fusarium wilt in eggplant by *Bacillus subtilis*. At the 6^{th} and 9^{th} day of inoculation *Bacillus subtilis* recorded 50 and 48 % inhibition. Seven days before transplanting the pathogen incorporated in soil and after 3 weeks of transplanting the antagonist applied directly into soil. Disease reduction of 71-72 % was recorded over the control. Ram *et al.* (2017) conducted *in vitro* and *in vivo* experiments for the management of root rot of fennel incited by *Fusarium oxysporum*. Under *in vitro T. harzianum* recorded 79.44 per cent inhibition of growth which was followed by *Trichoderma viride* (76.88%) and *Pseudomonas*

fluorescens (72.66%). Under *in vivo, Trichoderma harzianum* significantly reduced the disease with only 21per cent disease incidence, while *Trichoderma viride* and *Pseudomonas fluorescence* recorded 21.65 per cent and 25.27 per cent disease incidence respectively. Ashwini Srividya (2013) isolated a strain of *Bacillus subtilis* from the rhizosphere of Chilli which showed broad spectrum antagonism against many pathogens. In dual culture with it inhibited Alternaria spp. (55 %), *C. gloeosporioides* (57%), *Phytophthora capsici* (55 %), *Rhizoctonia solani* (42 %), *Fusarium solani* (42 %), *Fusarium oxysporum* (40 %) and *Verticillium* sp. (36 %). Under field condition against anthracnose of chilli (*C. gloeosporioides*), *B.subtilis* showed 65 per cent reduction in disease incidence by the treatment as compared to the seed treated with pathogen alone.

Materials & Methods

3. MATERIALS AND METHODS

The study entitled 'Characterization and management of fungal pathogens of cabbage (*Brassica oleracea* var. *capitate* L) and cauliflower (*Brassica oleraceae* var. *botrytis* L.)' was conducted in the Department of Plant Pathology, College of Agriculture, Padannakkad during the period of 2015-17. The detailed account of materials used and methods followed during the course of experiment are given below.

3.1 SURVEY ON THE OCCURRENCE OF FUNGAL DISEASES OF CABBAGE AND CAULIFLOWER

Purposive sampling surveys were carried out on the occurrence of fungal diseases in cabbage and cauliflower in Thrisur, Wayanad, Kasargod and Idukki districts of Kerala, grown during on-season in open field (August- November) and off-season in poly houses (March –September). Diseased plant samples were collected for the isolation of associated pathogens. The details regarding the places surveyed are given in Table.1

Table.1 Locations of sampling survey in various districts

District	Location
Thrissur	Vellanikkara
	Madakkathara
Wayanad	Ambalavayal
	Mananthavady
Kasaragod	Nileshwar
	Chullikkara
	Padannakkad
Idukki	Kanthalloor
	Marayoor

3.2. ASSESSMENT OF DISEASE INCIDENCE AND DISEASE SEVERITY

The disease incidence and disease severity was recorded from the surveyed areas based on different types of symptoms produced by different pathogens. Per cent disease incidence (PDI) was calculated using the formula given by Wheeler (1969).

$$\text{Per cent disease incidence (PDI)} = \frac{\text{Number of plants infected}}{\text{Total number of plants observed}} \times 100$$

The disease severity for all foliage diseases of cabbage and cauliflower was assessed by adopting a standard score chart of 0-5 scale as cited in Table.2.

Since the disease severity of two leaf blights were high, for recording the PDS two score cards were developed (Plate 1 and 2).

$$\text{Per cent disease severity (PDS)} = \frac{\text{Sum of all numerical ratings} \times 100}{\text{Total no.of leaves observed} \times \text{Maximum disease grade}}$$

The severity of diseases of head and curd were assessed by recording the per cent of area infected on head/curd using the following formula

$$\text{Per cent disease severity (PDS)} = \frac{\text{Head /curd area affected} \times 100}{\text{Total area of head/curd}}$$

Plate 1. Score card for leaf blight 1

Plate 2. Score card for leaf blight 2

Table.2 Score chart for severity of diseases on leaves

Grade	Description
0	No symptom
1	> 0 ≤ 10 per cent leaf area infected
2	> 10 ≤25 per cent leaf area infected
3	>25 ≤50 per cent leaf area infected
4	>50 ≤75 per cent leaf area infected
5	>75 per cent leaf area infected

3.3. ISOLATION OF PATHOGENS

Naturally infected plant parts *viz.,* leaf and head of cabbage and leaf and curd of cauliflower showing typical symptoms were collected as samples. For the isolation of fungal pathogens, these samples were washed under running tap water and cut into small bits consisting of both healthy and infected portions using a sterile blade and were disinfected with sodium hypochlorite (1%) for one minute. After three washings using sterilized distilled water, the samples were placed on solidified Potato Dextrose Agar (PDA) medium aseptically in sterile Petri dishes. After incubation at room temperature (28± 2°C), the fungal growth from second to sixth days of incubation was subsequently sub cultured to solidified PDA in sterile Petri dishes. Purification of isolates were done by single hyphal tip method and then periodic sub culturing and maintenance was done in PDA slants under refrigerated condition at 4°C for further studies.

3.4. PATHOGENICITY OF ISOLATES

The isolates from cabbage and cauliflower which were obtained from four districts of Kerala during the survey were selected for proving their pathogenicity. Artificial inoculation of cultures on healthy plants or plant parts was done to test pathogenicity by following Koch's postulates. The pathogenicity studies were carried out on both live plants as well as detached plant parts like leaf, head, curd or seedling according to the pathogens.

Pathogenicity test on live plants as well as on detached plant parts. Pathogenicity studies were carried out on live cabbage and cauliflower plants in pots. In order to prove the pathogenicity of the test fungus on live plant, the selected plant parts were surface sterilized by wiping with 70 per cent ethanol. Using a fine sterilized needle, pin pricks were made. Then the mycelium of the test fungus was placed on the injured area and covered with moist cotton. The whole plants were covered with polythene bag to maintain high humidity and incubated at room temperature till the symptoms appeared. The healthy plant parts with injury but without inoculation served as control.

For pathogenicity studies on detached plant parts, fresh, healthy non infected plant parts of cabbage and cauliflower collected were brought to the laboratory and washed under running tap water followed by surface sterilization using 70 percent ethanol. Artificial inoculation of pathogen was carried out for which injury was given on concerned plant parts using sterile needle followed by placing the fungal mycelium at the site of injury. The site of inoculation was then covered with moist cotton. After inoculation, the plant parts were kept under high humidity and were incubated at room temperature till the symptoms appeared on inoculated portions. The healthy plant parts with injury but without inoculation with the fungal mycelium served as control (Rocha *et al.*, 1998).

For proving the pathogenicity of fungal pathogens which could not be isolated in culture, an infected leaf was detached which containing a single colony

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and inoculated onto a fresh healthy leaf. Humidity was maintained for this leaf sample for symptom development. (Warkentin *et al.*, 1995).

3.5. SYMPTOMATOLOGY OF DISEASES

During the survey, symptom development of fungal diseases of cabbage and cauliflower in different plant parts under natural conditions were studied. The symptoms developed during artificial conditions were also recorded.

For this study, the fungal pathogens causing diseases in cabbage and cauliflower were artificially inoculated as per standard procedure as mentioned in 3.4.

3.6. VIRULENCE OF PATHOGENS

The virulence of isolates of various fungal pathogens obtained in multiples from cabbage and cauliflower were tested by observing the variation in symptom development by the artificial inoculation of pathogens on respective host plants. Based on this virulence test, most virulent isolate from each of the pathogen was selected for further studies. In the case of single isolates, they were directly taken for identification and advance studies. The isolates were numbered by giving code numbers. Inoculation was done by Mycelial Bit Inoculation Method. High relative humidity was maintained using polythene sheets over the plants. Observations were taken on the lesion development on 3rd day and 10th day after inoculation. Days of first symptom development were also taken. Based on the lesion size and period of symptom development, most virulent isolates were selected for further studies. These selected cultures were purified by frequent transferring of hyphal tips to PDA slants. The isolates were maintained in PDA slants and sub cultured at two month intervals and stored under refrigeration for further studies.

3.7. CHARACTERISATION AND IDENTIFICATION OF PATHOGENS

Characterisation of fungal pathogens isolated were identified based on their and morphological and cultural characters

3.7.1 Morphological characters

Morphological characters were studied based on various fungal structures *viz.*, type of mycelium, branching pattern, type of spores, their shape, size and presence of sexual structures if any. Photomicrographs and measurements of fungal structures were taken assisted with the help of ZEN imaging software. For further confirmation for the identity of each pathogen by ITS sequencing, the isolates were sent to Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram.

3.7.2 Cultural characters

The cultural characters were studied by recording the visual observations on the growth of the respective pathogens. The culture discs of the pathogen on PDA with a diameter of 5mm was placed at the centre of PDA plates and incubated at 28±2°C. Variations in colony characteristics, pigmentation, and growth pattern of each isolate were studied.

3.7.3 Identification of fungal pathogens

Selected fungal pathogens based on their morphological and cultural characters were temporarily identified up to their genus level. Identification species level identification was carried out at Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram by ITS sequencing.

3.8. MOLECULAR CHARACTERISATION OF MAJOR PATHOGENS OF CABBAGE AND CAULIFLOWER

Selected seven pathogens were subjected to molecular characterisation at Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram by ITS sequencing to identify at species level. Sequence analysis and nucleotide homology of each pathogen were analysed through the BLASTn programme of NCBI (http:// ncbi.nlm.nhm.gov/blast).

3.8.1 DNA isolation using NucleoSpin® Plant II Kit (Macherey-Nagel)

About 100 mg of the tissue/mycelium is homogenized using liquid nitrogen and the powdered tissue is transferred to a microcentrifuge tube. Four hundred microlitres of buffer PL1 is added and vortexed for 1 minute. Ten microlitres of RNase A solution is added and inverted to mix. The homogenate is incubated at 65°C for 10 minutes. The lysate is transferred to a Nucleospin filter and centrifuged at 11000 x g for 2 minutes. The flow through liquid is collected and the filter is discarded. Four hundred and fifty microlitres of buffer PC is added and mixed well. The solution is transferred to a Nucleospin Plant II column, centrifuged for 1 minute and the flow through liquid is discarded. Four hundred microlitre buffer PW1 is added to the column, centrifuged at 11000 x g for 1 minute and flow though liquid is discarded. Then 700 µl PW2 is added, centrifuged at 11000 x g and flow through liquid is discarded. Finally 200 µl of PW2 is added and centrifuged at 11000 x g for 2 minutes to dry the silica membrane. The column is transferred to a new 1.7 ml tube and 50 µl of buffer PE is added and incubated at 65°C for 5 minutes. The column is then centrifuged at 11000 x g for 1 minute to elute the DNA. The eluted DNA was stored at 4°C.

3.8.2 Agarose Gel Electrophoresis for DNA Quality check

The quality of the DNA isolated was checked using agarose gel electrophoresis. 1µl of 6X gel-loading buffer (0.25% bromophenol blue, 30% sucrose in TE buffer pH-8.0) was added to 5µl of DNA. The samples were loaded to 0.8% agarose gel prepared in 0.5X TBE (Tris-Borate-EDTA) buffer containing 0.5 µg/ml ethidium bromide. Electrophoresis was performed with 0.5X TBE as electrophoresis buffer at 75 V until bromophenol dye front has migrated to the bottom of the gel. The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad).

3.8.3 PCR Analysis

PCR amplification reactions were carried out in a 20 µl reaction volume which contained 1X Phire PCR buffer (contains 1.5 mM $MgCl_2$), 0.2mM each

dNTPs (dATP, dGTP, dCTP and dTTP), 1 µl DNA, 0.2 µl Phire Hotstart II DNA polymerase enzyme, 0.1 mg/ml BSA and 3% DMSO, 0.5M Betaine, 5pM of forward and reverse primers.

Primers used

Target	Primer Name	Direction	Sequence (5' → 3')
ITS	ITS-1F	Forward	TCCGTAGGTGAACCTGCGG
	ITS-4R	Reverse	TCCTCCGCTTATTGATATGC

The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems).

PCR amplification profile

ITS & LSU

98 °C - 30 sec

98 °C - 5 sec
60 °C - 10 sec
72 °C - 15 sec
} 40 cycles

72 °C - 60 sec
4 °C - ∞

3.8.4 Agarose Gel electrophoresis of PCR products

The PCR products were checked in 1.2% agarose gels prepared in 0.5X TBE buffer containing 0.5 µg/ml ethidium bromide. 1 µl of 6X loading dye was mixed with 5 µl of PCR products and was loaded and electrophoresis was performed at 75V power supply with 0.5X TBE as electrophoresis buffer for about 1-2 hours, until the bromophenol blue front had migrated to almost the bottom of the gel. The molecular standard used was 2-log DNA ladder (NEB).

The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad).

3.8.5 ExoSAP-IT Treatment

ExoSAP-IT (GE Healthcare) consists of two hydrolytic enzymes, Exonuclease I and Shrimp Alkaline Phosphatase (SAP), in a specially formulated buffer for the removal of unwanted primers and dNTPs from a PCR product mixture with no interference in downstream applications. Five micro litres of PCR product is mixed with 2 µl of ExoSAP-IT and incubated at 37°C for 15 minutes followed by enzyme inactivation at 80°C for 15 minutes.

3.8.6 Sequencing using BigDye Terminator v3.1

Sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA) following manufactures protocol.

The PCR mix consisted of the following components:

PCR Product (ExoSAP treated)	-	10-20 ng
Primer	-	3.2 pM
Sequencing Mix	-	0.28 µl
5x Reaction buffer	-	1.86 µl
Sterile distilled water	-	make up to 10µl

The sequencing PCR temperature profile consisted of a 1st cycle at 96°C for 2 minutes followed by 30 cycles at 96°C for 30 sec, 50°C for 40 sec and 60°C for 4 minutes for all the primers.

3.8.7 Post Sequencing PCR Clean up

1. Make master mix I of 10µl milli Q and 2 µl 125mM EDTA per reaction
2. Add 12µl of master mix I to each reaction containing 10µl of reaction contents and are properly mixed.

3. Make master mix II of 2 µl of 3M sodium acetate pH 4.6 and 50 µl of ethanol per reaction.
4. Add 52 µl of master mix II to each reaction.
5. Contents are mixed by inverting.
6. Incubate at room temperature for 30 minutes
7. Spin at 14,000 rpm for 30 minutes
8. Decant the supernatant and add 100 µl of 70% ethanol
9. Spin at 14,000 rpm for 20 minutes.
10. Decant the supernatant and repeat 70% ethanol wash
11. Decant the supernatant and air dry the pellet.

The cleaned up air dried product was sequenced in ABI 3500 DNA Analyzer (Applied Biosystems).

3.8.8 Sequence Analysis

The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 (Drummond et al., 2010).

3.9. EFFICACY OF FUNGICIDES AND BIOCONTROL AGENTS AGAINST PATHOGENS UNDER *IN VITRO* CONDITIONS

The present investigation was carried out to evaluate fungicidal effect of following fungicides as depicted in Table 3 against fungal pathogens of cabbage and cauliflower at three different concentrations. The three concentrations selected were that of the recommended dosage as per Package of Practices by Kerala Agricultural University and each of one lower and higher concentration from the recommended dosage. The experimental design was Completely Randomized Design with eleven treatments and three replications. Poison food

technique (Nene and Thapliyal, 1993) was used for the *in vitro* evaluation of fungicides against the pathogens.

3.9.1 *In vitro* evaluation of fungicides by Poison Food technique

The fungus was grown on PDA medium for eight days prior to the experiment. The fungicides each at three different concentrations were added to 100ml molten PDA medium to obtain the required concentration (Table 3). About 20ml of poisoned medium was poured in each sterilized Petri dishes and thoroughly mixed. Five mm diameter of the mycelial disc was taken from periphery of eight days old colony and placed in the centre of Petri plates and incubated at 28 $\pm 1^0$C for15 days. Three replications were maintained for each treatment. The diameter of the colony was measured when maximum growth was attained in control plates. The per cent inhibition was calculated using the following formula given by Vincent (1927).

$$I = \frac{C - T}{C} X100$$

Where, I: Percent inhibition C: Mycelial growth in control (mm) T: Mycelial growth in treatment (mm)

The fungicides which showed more than 70 per cent inhibition were selected for further studies.

3.9.2 *In vitro* evaluation of biocontrol agents

The reference cultures of KAU *viz, Trichoderma viride, Pseudomonas fluorescens* and *Bacillus subtilis* were tested against isolated pathogens by following the dual culture technique (Dickinson and Skidmore, 1976).

Table.3 Fungicides tested against pathogens *(in vitro)*

Sl.no	Fungicides	Trade Name	Manufacturer	Concentrations (%)		
				Low	Recommended	High
1	Bordeaux mixture	-	-	0.5	1.0	1.5
2	Chlorothalonil 75% WP	Kavach	Syngenta India Ltd, Pune	0.05	0.1	0.15
3	Copper hydroxide 77% WP	Kocide	DuPont India Pvt.Ltd,Mumbai	0.1	0.2	0.3
4	Copper oxychloride 50% WP	Blitox	Indofil Chemicals Company, Mumbai	0.1	0.2	0.3
5	Mancozeb 75% WP	Mega-M 45	K.P.R Fertilisers Ltd, Andhra Pradesh	0.2	0.3	0.4
6	Carbendazim 50% WP	Megastin	K.P.R Fertilisers Ltd, Andhra Pradesh	0.05	0.1	0.15
7	Tebuconazole 5% EC	Folicur	Bayer Crop Science Ltd. Thane	0.05	0.1	0.15
8	Trifloxystrobin 25% + Tebuconazole 50%	Nativo	Bayer Crop Science Ltd, Gujarat	0.02	0.03	0.04
9	Azoxystrobin 23% SC	Amistar	Syngenta India Ltd, Gujarat	0.05	0.1	0.15
10	Propineb 75% WP	Antracol	Bayer Crop Science Ltd, Mumbai	0.2	0.3	0.4

3.9.2.1 *In vitro* evaluation of the fungal antagonist

The fungal antagonist *Trichoderma viride* was tested for the antagonistic effect against the pathogens by dual culture technique (Dickinson and Skidmore, 1976). Agar discs of 5mm diameter were cut from the edge of the vigorously growing seven days old colonies of pathogen and the antagonistic fungi were placed 4cm apart and 2.5cm from the periphery of Petri dish on PDA in a Petri dish of 90 mm diameter and incubated at room temperature (28± 1 °C) for five days. Three replications were maintained for each treatment. The nature of parasitism of *Trichoderma viride* on the pathogen was studied at regular intervals. Petri dishes inoculated with 5mm agar discs of respective pathogens served as control. Growth measurements were taken at regular intervals after 24 h of inoculation of the antagonist up to five days till the control plates reached full growth.

The percentage inhibition of mycelial growth was calculated using the formula,

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

Where,

I: Inhibition of mycelial growth

C: Growth of the pathogen in control plates (mm)

T: Growth of the pathogen in dual culture (mm) (Vincent, 1927)

Assessment of nature of parasitism against the pathogens was done by categorisation in four groups using the method of Purkayastha and Bhattacharya (1982).

- Homogenous - Free intermingling hyphae
- Overgrowth - Pathogen overgrown by test organism

- Cessation of growth - Cessation of growth at the line of contact
- Aversion - Development of clear zone of inhibition

3.9.2.2 *In vitro* evaluation of the bacterial antagonists

Isolates of bacterial antagonists, *Pseudomonas fluorescens* and *Bacillus subtilis* were tested for antagonistic property against the isolated fungal pathogens by dual plate method (Dickinson and Skidmore, 1976), by simultaneous antagonism.The bacterial antagonists were streaked on both the ends of Petri dish in the PDA medium three cm away from the edge of the plate prior to pathogen inoculation. Then 5mm sized culture disc of pathogen was cut out from the edge of pure culture was placed on the centre of the Petri dish. Plates were incubated at room temperature (28± 1 °C) for five days. Three replications were maintained for each isolate. Petri dishes inoculated with pathogen alone served as control. Observations on growth of the fungus were recorded at regular intervals till full growth of the pathogen was attained in control plates. Per cent inhibition of mycelial growth of the pathogen was calculated as mentioned in 3.8.2.1.

3.10. EVALUATION OF FUNGICIDES AND BIOCONTROL AGENTS ON MAJOR FUNGAL DISEASES OF CABBAGE AND CAULIFLOWER UNDER *in vivo* CONDITIONS

A pot culture experiment was laid out for the management of three most severe and predominant fungal pathogens of cabbage and cauliflower using the promising treatments obtained under *in vitro* studies. Selection of fungal pathogens in this experiment was done based on the observations in the severity of disease during the survey period at various locations. The treatments were decided based on the *in vitro* efficacy of fungicides and biocontrol agents as carried out in 3.8 (Table 4 and 5). The experiment was carried out during December 2016 at College of Agriculture, Padannakkad. Observations on disease incidence and per cent disease reduction were recorded. Effect of different

treatments on the growth parameters of cabbage and cauliflower during the management of these three major pathogens were also evaluated and biometric observations were taken.

The details of the experiment are as follows:

Design	: CRD
Number of treatments	: 13
Replications	: 3

Varieties selected: Cabbage-F1 Hybrid NS 43(Namdhari),
Cauliflower-F1 Hybrid NS 60 N (Namdhari)

3.10.1. Preparation of potting mixture and planting

Seedlings were raised in grow bags of size 35×20×20 cm. The bags were filled with sterilized potting mixture which consisted of soil, sand and cow dung at 1:1:1. The grow bags were kept in net house and irrigated regularly. All the cultural operations were carried out as per the Package of Practice of Recommendations (KAU, 2016).

3.10.2. Inoculation of pathogens and application of fungicides and biocontrol agents

For *Alternaria* and *Rhizoctonia* leaf blights, plants sixty days after transplanting, were inoculated with spore suspension of pathogen obtained from the culture grown on PDA broth at 1x 10^6 spores ml^{-1} concentration using a hand sprayer. For Pythium curd rot plants of 75 days after transplanting were inoculated. The plants were kept in moist chamber for 48 h. Periodical observations were made for the development of symptoms on the plant parts. First observation of disease severity was taken 7 days after inoculation. Then first spray of fungicides were given. The details of different treatments given are shown in Table 4.

Table.4 Details of *in vivo* experiment of *Rhizoctonia* and *Alternaria* blight

Sl. No.	Treatments		Conc. (%)
	Chemical name	Trade name	
1.	Control	-	-
2.	Chlorothalonil 75WP	Kavach	0.1
3.	Propineb 75WP	Antracol	0.3
4.	Trifloxystrobin 25%+ Tebuconazole 50 %	Nativo	0.03
5.	Mancozeb 75WP	Mega M-45	0.3
6.	Tebuconazole 5EC	Folicure	0.1
7.	COC 50 WP	Blitox	0.2
8.	Copperhydroxide 77WP	Kocide	0.2
9.	Carbendazim 50WP	Megastin	0.1
10	Bordeaux mixture	-	1
11	*Pseudomonas fluorescens*	-	2
12	*Trichoderma viride*	-	2
13	*Bacillus subtilis*	-	$1x10^6$cfu/ml

Table.5 Details of *in vivo* experiment of *Pythium* curd rot

Sl. No.	Treatments		Conc. (%)
	Chemical name	Trade name	
1.	Trifloxystrobin 25 % + Tebuconazole 50%	Nativo	0.03
2.	Bordeaux mixture	-	1
3.	COC 50WP	Blitox	0.2
4.	Copper hydroxide 77WP	Kocide	0.2
5.	Mancozeb 75 WP	Mega M-45	0.3
6.	*Bacillus subtilis*	-	$1x10^6$cfu/ml
7.	*Trichoderma viride*	-	2
8.	*Pseudomonas fluorescens*	-	2
9.	Control	-	-

Second observations on PDS were taken ten days after first spray. Second spray was given ten days after the first spray and third observations were taken after ten days of second spray. The biocontrol agents *Trichoderma viride* and *Pseudomonas fluorescens* were applied prophylactically @ 20g per litre of water (talc based formulation) 10 days after transplanting as soil drench. Two foliar sprays were also given @20g per litre, first one immediately after symptom development and second 30 days after first spray. The culture of *Bacillus subtilis* purchased from KAU was grown in broth of nutrient medium and applied at concentration of $1x10^6$CFU/ml in the same method as mentioned above.

3.11. STATISTICAL ANALYSIS

Data was subjected to analysis of variance (ANOVA). Data sets were analyzed using OPISTAT software. Levels of significance, means and standard error were obtained for various data sets. Multiple comparisons between the treatments means, where the F test was significant was done with Duncan's Multiple Range Test (DMRT). The data wherever needed was subjected to appropriate transformation as suggested by Gomez and Gomez (1984).

Results

4. RESULTS

The present investigations on the 'Characteriztion and management of fungal pathogens of cabbage (*Brassica oleracea* var.*capitata* L.) and cauliflower (*Brassica oleracea* var. *botrytis* L.)' was carried out to identify various fungal pathogens infecting cabbage and cauliflower and to study the symptomatology, characterization and management of diseases. The results of the investigation carried out during 2015-17 are presented below:

4.1 SURVEY AND COLLECTION OF DISEASED SAMPLES

A purposive sampling survey was conducted in four districts *viz.,* Kasargod, Thrissur, Wayanad and Idukki for the collection of diseased samples of cabbage and cauliflower and the disease incidence and severity was recorded during the cropping season of 2015-17 (Plate 3). The diseased specimens were collected from these locations and the pathogens were isolated. The diseases observed in each location of each district and also under poly house and open filed conditions are presented in Table 6.

4.2. ASSESSMENT OF DISEASE INCIDENCE AND DISEASE SEVERITY

The incidence and intensity of fungal diseases of cabbage and cauliflower in nine different locations of four districts were noted and the diseased plant samples such as leaves, heads and curds were collected. Based on the occurrence of fungal diseases in both crops, in open field during on- season and poly house during off season, 25 samples were selected (16 in cabbage and 9 in cauliflower)

The data revealed that the incidence of different fungal diseases of cabbage were noticed with in a range of 5.4 to 69.3 per cent and disease severity with a range of 8.1 to 68.3. In the case of cauliflower PDI ranged from 3.1 to 52.2 and PDS ranged from 4.9 to 44.2 per cent. The practice of cultivating cabbage and cauliflower in poly houses were not observed during the survey except in Mananthavady. The disease incidence was maximum in Leaf spot-1 of cabbage in Ambalavayal (69.3 %) and disease severity was maximum for Leaf bligt-2 of

Table .6 Diseases of cabbage and cauliflower observed in different districts during the survey period

District	Locations	Period of survey		
		On season (open field)		Off season (poly house)
		September - February		March – September
		Cabbage	Cauliflower	
Thrissur	Vellanikkara	Leaf blight -1	-	No disease occurrence
	Madakkathara	Leaf blight -1	Leaf blight -1	No crop
Wayanad	Ambalavayal	Leaf blight -1 Leaf blight -2	Leaf blight-1	No crop
	Mananthavady	Leaf blight -1 Leaf spot -1	Leaf blight -1	No disease occurrence
Kasaragod	Nileshwar	Leaf blight -1 Leaf blight -2	Leaf blight -1	No crop
	Chullikkara	Leaf blight-2 Head rot -1 Leaf spot-2	Curd rot -1 Curd rot-2	No crop
	Padannakkad	Leaf blight-1 Head rot -1 Leaf spot-3	Leaf blight-1 Leaf blight -2 Damping off	No crop
Idukki	Kanthalloor	Leaf blight-1	-	No crop
	Marayoor	Leaf blight -1	-	No crop

Marayoor, Idukki

Kanthalloor, Idukki

Padannakkad, Kasargod

Nileshwar, Kasargod

Chullikkara, Kasargod

Mananthavady, Wayanad

Plate 3. Survey conducted in different locations

cabbage in Chullikkara (68.3 %). For cauliflower, both disease incidence and severity were maximum for curd rot-2 (52.2 and 44.2).

In two locations of Thrissur district (Table 7) leaf blight -1 (CA-1, CA-2 and CFA-1 –suspected as *Alternaria* sp.) was the single fungal disease recorded from two locations of Thrissur district from both cabbage and cauliflower. Leaf blight-1 (CA-2) from cabbage had high PDI and PDS of 8.1 and 8.3 respectively in Madakkathara compared to Vellanikkara.

Survey was conducted in two locations of Wayanad district where diseases like Leaf blight-1, Leaf blight-2 (suspected as *Rhizoctonia* sp.) and Leaf spot-1(suspected as *Cercospora* sp.) were observed. Leaf blight-1 (CA-3, CA-4 and CFA-2) was present in both cabbage and cauliflower in Ambalavayal area and the highest PDI and PDS was recorded with Leaf blight-1 (CA-3) in cabbage with 69.3 and 64.8 per cent respectively. In Mananthavady area, Leaf spot-1 (CCE) was observed only in cabbage with a PDI and PDS of 11.4 and 12.4 per cent PDI and PDS respectively (Table 7).

Survey results of two locations in Idukki district showed that from Kanthalloor area, Leaf blight-1(CA-5, CFA-3) was observed in both cabbage and cauliflower. From Marayoor, Leaf blight-1(CA-6) was observed only in cabbage. High PDI and PDS was recorded from Kanthalloor in cabbage with 18.2 and 28.3 per cent disease respectively (Table 7).

During the survey maximum fungal diseases were obtained from three locations of Kasargod district. The fungal diseases recorded from cabbage were, Leaf blight-1 (CA-6), Leaf blight-2 (CR-2 & CR-3) Head rot-1 (CCH-1 & CCH-2- suspected as *Choanephora* sp.), Leaf spot-2(CCO- suspected as *Colletotrichum* sp.) and Leaf spot-3(CCU- suspected as *Curvularia* sp.) (Table 8). Whereas the diseases such as Leaf blight-1 (CA-5, CFA-4, CFA-5) was alone observed in both cabbage and cauliflower. From cauliflower, Curd rot-1 (CFCH), Curd rot-2 (CFP-suspected as *Pythium* sp.) and damping off (CFF- suspected as *Fusarium* sp.) were recorded. High PDI and PDS was recorded in Leaf blight-2 (CR-3) with 65.5

Table.7 Per cent disease incidence and severity of fungal diseases of cabbage and cauliflower in Thrissur, Wayanad and Idukki district

Sl. No	District	Locations	Disease	Cabbage		Cauliflower	
				PDI	PDS	PDI	PDS
1	Thrissur	Vellanikkara	Leaf blight-1(CA-1)	5.4	4.6	-	-
		Madakkathara	Leaf blight-1(CA-2)	8.1	8.3	3.1	4.9
2	Wayanad	Ambalavayal	Leaf blight -2(CR-1)	41.2	36.4	-	-
			Leaf blight -1(CA-3)	69.3	64.8	13.5	12.2
		Manathavady	Leaf blight -1(CA-4)	13.3	16.9	10.3	12.4
			Leaf spot -1(CCE)	11.4	12.4	-	-
3	Idukki	Kanthalloor	Leaf blight -1 (CA-7)	18.1	28.3	5.1	4.9
		Marayoor	Leaf blight -1 (CA-8)	8.5	8.1	-	-

Table.8 Per cent disease incidence and severity of fungal diseases of cabbage and cauliflower in Kasaragod district

Sl. No.	Locations	Disease	Cabbage		Cauliflower	
			PDI	PDS	PDI	PDS
1.	Nileswar	Leaf blight -2(CR-2)	12.4		-	-
		Leaf blight -1(CA-5)	63.5	64.7	23.3	32.2
2.	Chullikkara	Leaf blight-2(CR-3)	65.5	68.3	-	-
		Head rot -1(CCH-1)	37.6	40.9	-	-
		Leaf spot-2(CCO)	51.8	64.8	-	-
		Curd rot -1(CFCH)	-	-	13.3	12.4
		Curd rot-2(CFP)	-	-	60.2	58.2
3.	Padannakkad	Leaf blight-1(CA-6)	14.7	24.7	-	-
		Head rot -1(CCH-2)	24.6	28.2	-	-
		Damping off(CF)			36.2	28.4
		Leaf blight-2(CFR-1)			27.3	20.2
		Leaf spot-3(CCU)	23.4	24.6		

and 68.3 in Chullikkara and Leaf blight-1 (CA-5) 63.5 and 64.7 from cabbage in Nileswar. Leaf spot-2(CCO) recorded a PDI and PDS of 51.8 and 64.8 percent respectively in Chullikkara. For leaf spot -3 (CCU), 23.4 and 24.6 percent PDI and PDS was recorded.

Out of the four districts, Leafblight-1 and 2 in cabbage were having higher severity in locations like Ambalavayal of Wayanad and Nileswar and Chullikkara of Kasargod district. Considering the diseases of cauliflower, disease severity of curd rot was found to be maximum in Chullikkara.

The diseased specimens were brought to the laboratory and based on the observations on the symptomatology and microscopic studies, preliminary identification was done. The eight diseases were categorized and according to the suspected pathogens, code numbers were given (Table 9)

4.3. ISOLATION OF PATHOGENS

Different fungal pathogens were isolated from naturally infected samples of cabbage and cauliflower collected from various locations. The pathogens were isolated from leaves, curd and head, purified by single hyphal tip method and maintained on PDA by periodic sub culturing

4.4. PATHOGENICITY OF ISOLATES

The pathogenicity of different isolates from cabbage and cauliflower were proved both on live plant as well as detached plant parts like leaf, head, curd or seedling according to the pathogen by artificial inoculation. The method followed for inoculation of pathogenicity tests on leaf, head, curd and seedling diseases was Mycelial Bit Inoculation Method. Details of the symptoms observed after inoculation of each pathogen are described below.

4.4.1 .Leaf blight -1

The pathogenicity test of leaf blight -1 showed that the pathogen could infect the leaves of cabbage and cauliflower and could produce typical symptoms

Table.9 Details of isolates obtained during the survey

Sl. No.	Diseases	Suspected pathogens	No. of isolates obtained and code	
			Cabbage	Cauliflower
1	Leaf blight -1	*Alternaria* sp.	8- (CA-1,CA-2,CA-3,CA-4,CA-5,CA-6,CA-7,CA-8)	5- (CFA-1,CFA-2,CFA-3,CFA-4,CFA-5)
2	Leaf blight -2	*Rhizoctonia* sp.	3- (CR-1,CR -2,CR-3)	1- (CFR-1)
3	Leaf spot-1	*Cercospora* sp.	(could not be isolated)	-
4	Leaf spot -2	*Colletotrichum* sp.	1- (CCO)	-
5	Leaf spot-3	*Curvularia* sp.	1- (CCu)	
6	Head rot - 1/Curd rot-1	*Choanephora* sp.	2-(CCH-1,CCH-2)	1- (CFCH)
7	Curd rot-2	*Pythium* sp.		1- (CFP)
8	Damping off -1	*Fusarium* sp.		1- (CFF)
TOTAL ISOLATES			15	9

of the disease after two days of inoculation. Initial symptom started as small dark to brown spots from pin point to 6.2 cm diameter which later enlarged in concentric circles causing blightening of the entire leaf lamina. Symptoms of infection were similar in cabbage and cauliflower. Cross inoculation of isolates of cabbage and cauliflower could also produce same symptoms in both the crops.

4.4.2 .Leaf blight- 2

Symptoms of infection were similar in cabbage and cauliflower except that yellowish halo in cauliflower leaves was having more width than cabbage leaves. Small water soaked dark green lesions were developed initially on the lamina which later produced blightening of the whole area. Initial symptoms were produced within two to three days of inoculation and after seven days it extended up to 6.2 cm. Cross inoculation of isolates of cabbage and cauliflower could also produce same symptoms in both the crops.

4.4.3 .Leaf spot 1

All the leaf spots were found to be attacked on cabbage only. Leaf spot -1 was observed only in Mananthavady of Wayanad. The pathogen causing leaf spot-1 was inoculated which produced small circular tan spots on second day of inoculation later turning to papery white on second day of inoculation.

4.4.4. Leaf spot 2

Leaf spot-2 was observed in cabbage from the field of Chullikkara in Kasargod district. Symptoms appeared on the third day of inoculation as small round spots with dry straw coloured centre on leaf lamina. Later these spots started to elongate and became sunken.

4.4.5. Leaf spot 3

Leaf spot -3 also was observed in cabbage from the fields of Padannakkad of Kasargod district.After second day of inoculation, symptom started as grey coloured round spots which later enlarged to form leaf blight.

4.4.6. Head rot / Curd rot -1

Head rot was infected on cabbage head for which the pathogenicity test was done by MBIM. Symptoms initially appeared as water soaked lesions on head which was developed three days after inoculation and later rotted completely.

Curd rot -1 in cauliflower developed similar symptoms as head rot in cabbage. For proving its pathogenicity MBIM was used and the symptoms appeared three days after inoculation. Initial symptoms were greyish brown spots on curd which later appeared as soft wet decay.

4.4.7. Curd rot -2

Curd rot-2 was also observed in cauliflower. Pathogenicity of this disease was confirmed by observing the symptom produced in curd three days after inoculation. Initially brown discolouration of the curd noticed later the curd rotted completely.

4.4.8. Damping off

Post emergence damping off was noticed in the seedlings of cauliflower only, for which pathogenicity was proved by inoculating the mycelial disc of the pathogen on the collar region of the seedling. Symptom started as water soaked spots which later converted to darkened shrunken area.

4.5. SYMPTOMATOLOGY OF DISEASES

Symptomatology of fungal diseases of cabbage and cauliflower were studied by observing the symptoms produced and their development under natural conditions as well as artificial conditions.

4.5.1 Symptomatology in nature

Survey was conducted in nine locations in four districts and different symptoms of fungal diseases of cabbage and cauliflower and their variations were

noted. The different types of symptoms observed were two types of leaf blights, three types of leaf spots, two types of curd rots, one head rot and one damping off.

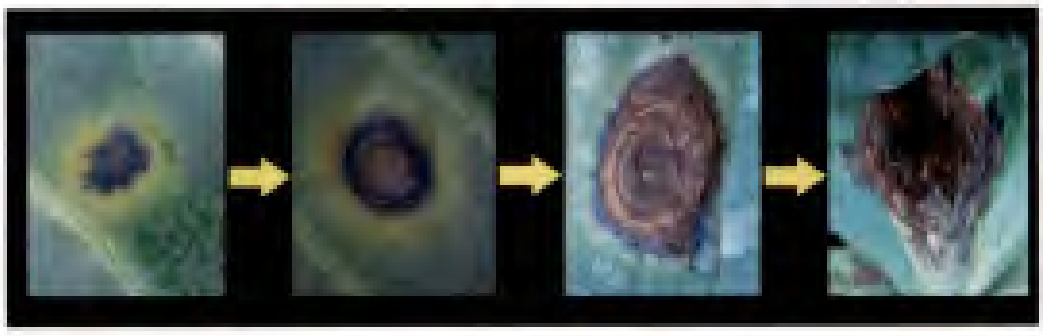
4.5.1.1. Leaf blight-1

The leaf blight initially appeared as small dark brown to black coloured spots with yellow halo on the leaves of both cabbage and cauliflower which spread rapidly to form circular lesions and got enlarged. On maturity concentric zonations with target like appearance were developed and fading of yellow halo occurred. As spots matured, the tissue became dry, brittle and often fell out, resulting in a 'shot hole' appearance of the leaf. Usually leaf spots were concentrated on margins and spots coalesce to form blighted marginal patches. Severe attack caused brittleness and deformity of leaves. If the pathogen attacks on head, blighted appearance was observed at first causing dry rot of head. Infection on head slowly progressed inwards into the deeper layers. Often, black velvety spore masses appeared in concentric manner on the blighted portions.

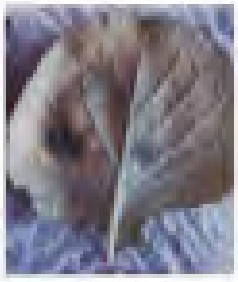
In the case of cauliflower, similar symptoms were noticed on the leaves. The pathogen attack on curd produced black dry rot with offensive smell (Plate 4).

4.5.1.2. Leaf blight-2

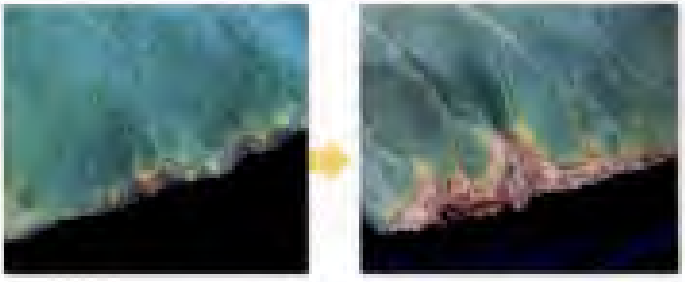
Initial symptom of foliar blight-2 of cabbage was appearance of small, irregular bluish green lesions and later changing to light brown in colour. Many lesions coalesced to form large patches causing blighting and drying in later stage. Generally older leaves were first affected. In severe cases, the diseased portion became papery and got withered away leading to shot hole symptoms. At this stage, yellowing around the blighted portion was also observed. Sometimes infection appeared as wet decay of base of outer leaves. Infection produced in the unopened leaves caused head rot in cabbage. On the heads bluish green lesions developed, which enlarged in size. Wet rot was seen which extended to deeper layers of head causing complete rotting. Under lower humidity, leaves dried up and defoliation occurred. In higher humidity infected leaves turned black in colour due to severe rotting. Creamy white to light brown mycelia could be observed on



a. Development of *Alternaria* leaf blight on cabbage leaf

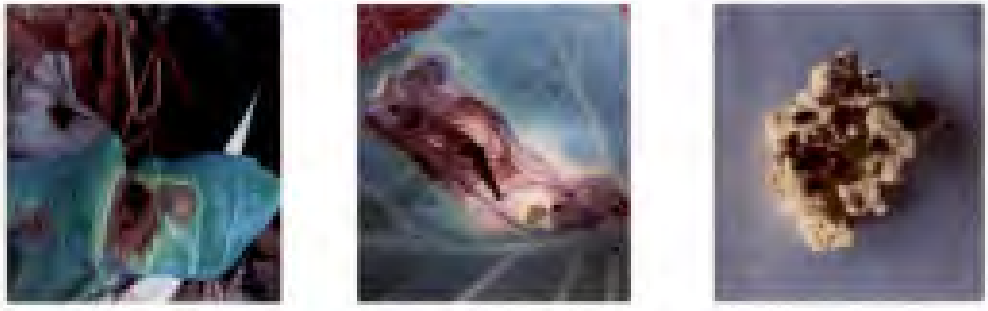


b. Pathogenicity test



c. Development of symptoms on leaf margin

d. Development of symptoms on head of the cabbage



e. Deformation f. Withering g. Curd rot

Plate 4. Symptomatology of *Alternaria* leaf blight

the affected foliage. Often such mycelia aggregated to form hard globular irregular sclerotial bodies which were initially white turning to brown with a size of 1-4 mm in diameter. In rare cases, petioles of leaves also showed sunken lesions. Similar symptoms were noticed on leaves of cauliflower but curd was not infected (Plate 5 and 6).

This leaf blight was differed from leaf blight-1 in that; yellow halo produced was more prominent in the former one.

4.5.1.3. Leaf spot-1

Initially symptom appeared as brown spots later enlarged to have greyish colour with white centre delimited by major veins.The spots on older leaves were usually circular, about 2mm with a papery texture. Enlarged lesions tend to be less regular in shape, larger and darker with well defined margins. (Plate 7).

4.5.1.4. Leaf spot-2

In Leaf spot-2 initial symptom was noted as brown spots with round shape in irregular manner which later turned to lesions with dark brown border on leaves and further coalesced to form large blighted areas. Spots were mostly concentrated on the margin of leaves (Plate 7).

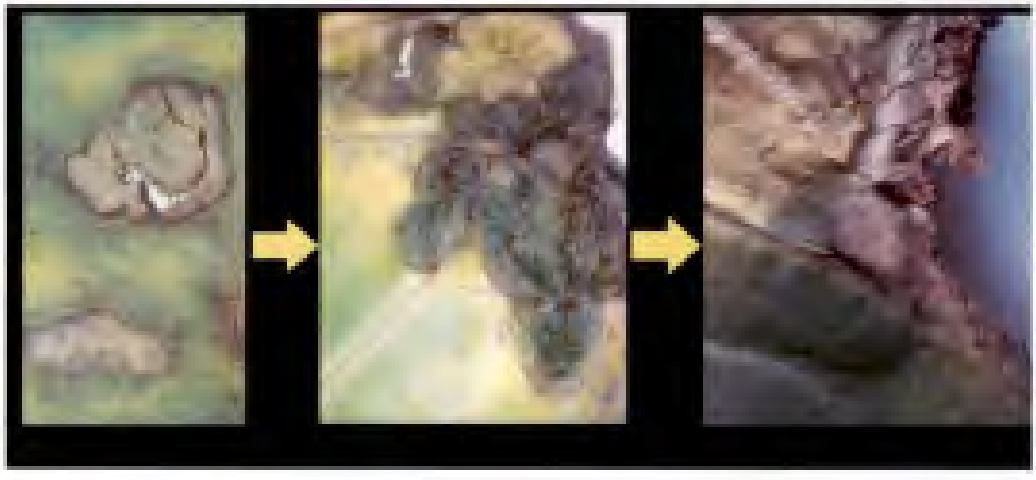
4.5.1.5. Leaf spot-3

Leaf spot -3 started in the margin of leaves with light brown colour which slowly coalesced to cause drying of leaves. During head emergence, spots developed on younger leaves which later produced blighting symptoms. Severe attack caused inward curling of leaves (Plate 7).

4.5.1.6. Head rot /Curd rot

The symptoms started on lower leaves of cabbage as water soaked, depressed lesions with papery white, grey centre, having pale green border. Lesions were in irregular shape and distribution but with specific margin. As the infection progressed, spots coalesced to cause blighting of leaves. Later stage inward curling of the leaf was observed. Black sporulation of the fungus could be

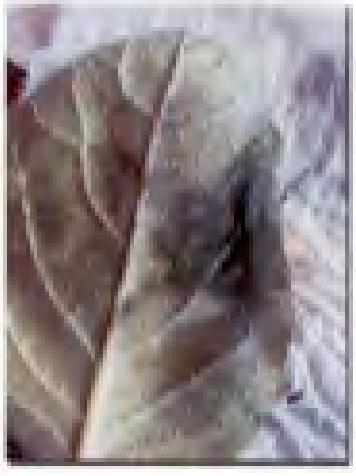
a. Symptom expression of *Rhizoctonia* leaf of cabbage and cauliflower



b. Development stages of *Rhizoctonia* leaf blight

c. Withering

d. Infection on petiole



e. Pathogenicity test

Plate 5. Symptomatology of *Rhizoctonia* leaf blight

a. *Rhizoctonia* head rot

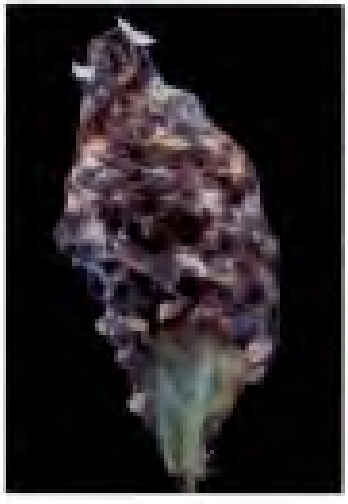
b. Mycelium and sclerotium on infected head

c. Development stages of *Rhizoctonia* head rot

Plate 6. Symptomatology of *Rhizoctonia* head rot

a. Symptoms of *Cercospora* leaf spot

b. Symptoms of *Colletotrichum* leaf spot



c. Symptoms of *Curvularia* leaf spot

d. Pathogenicity test of *Colletotrichum* leaf spot

e. Pathogenicity test of Curvularia leaf spot

Plate 7. Symptomatology and pathogenicity of *Cercospora*, *Colletotrichum* and *Curvularia* leaf spot

observed throughout the infected parts. Infection on head produced similar symptoms with soft rot of the head causing severe yield loss.

In cauliflower, infection on leaves caused water soaked lesions usually near the margin causing folding of leaves. On curd pathogen caused watery soft rot forming white puffy growth. This wet rot produced offensive smell also.

Both in cabbage and cauliflower, it was found that disease started from the lower leaves touching the soil (Plate 8).

4.5.1.7. Curd rot-2

Curd rot -2 was observed only on cauliflower. Infection started on upper surface of curd as small black discolouration. Pathogen produced watery soft rot white puffy cottony mycelium (Plate 9). Disease covered throughout the florets and stalk causing complete collapse of the plant. Rotted portions produced offensive smell. In rare cases infection started from the interior of the curd which could not be detected early.

4.5.1.8. Damping off

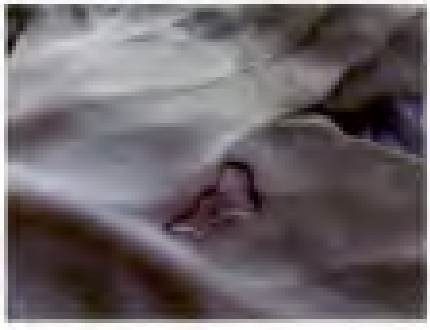
Pathogen produced post emergence damping off only in cauliflower. Initial symptom appeared as water soaked spots in the collar region of seedling which later converted to darken shrunk area due to which wet rot was noticed with constriction. Later the stem could not support the seedling and this caused wilting and death of seedling (Plate 10).

4.5.2 Symptomatology under artificial conditions

Symptom development in different plant parts like leaves, head and curd and collar region of seedling of cabbage and cauliflower were recorded under artificial conditions. The symptoms noticed were similar to that observed in field under natural conditions. Details of symptom development on different plant parts after inoculation of pathogens are shown in Table 10.

Table.10 Variation on the symptom development for fungal pathogens of cabbage and cauliflower

Sl. No.	Symptom	Lesion size (cm^2)		Days of first symptom development
		Days after inoculation		
		2	10	
1.	Leaf blight – 1 (cabbage)	1.2	7.8	2
2.	Leaf blight – 2 (cabbage)	1.2	7.0	2
3.	Leaf spot – 1 (cabbage)	0.5	2.1	3
4.	Leaf spot – 2 (cabbage)	0.5	2.3	2
5.	Leaf spot - 3 (cabbage)	0.3	2.1	3
6.	Head rot (cabbage)	1.1	7.5	2
7	Curd rot -1 (cauliflower)	0.5	5.9	2
8.	Curd rot - 2 (cauliflower)	1.2	6.9	2
9.	Damping off (cauliflower)	1.2	3.5	2



a. Pathogenicity test

b. Symptom expression of leaf rot

c. Symptom expression of curd rot on cauliflower

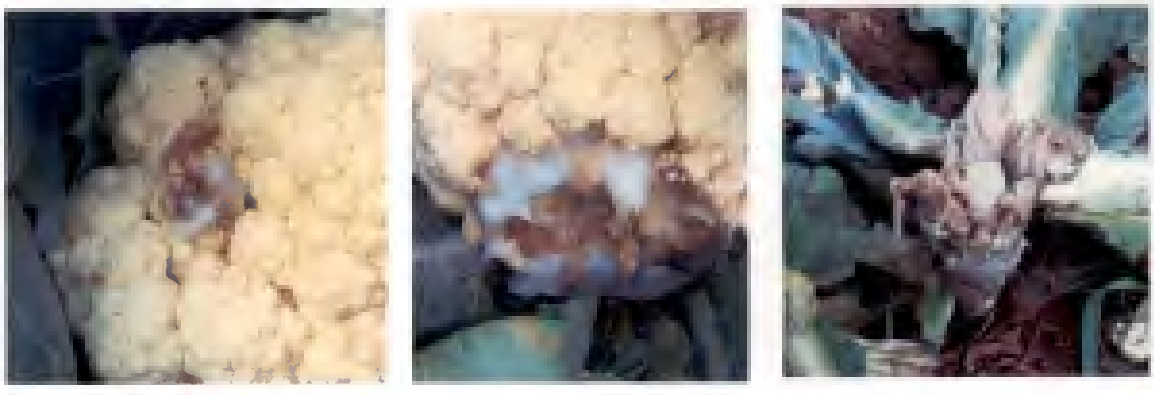
d. Symptom expression of head rot on cabbage

e. Symptom expression of leaf rot on cabbage

Plate 8. Symptomatology of *Choanephora* rot

a. Pathogenicity test

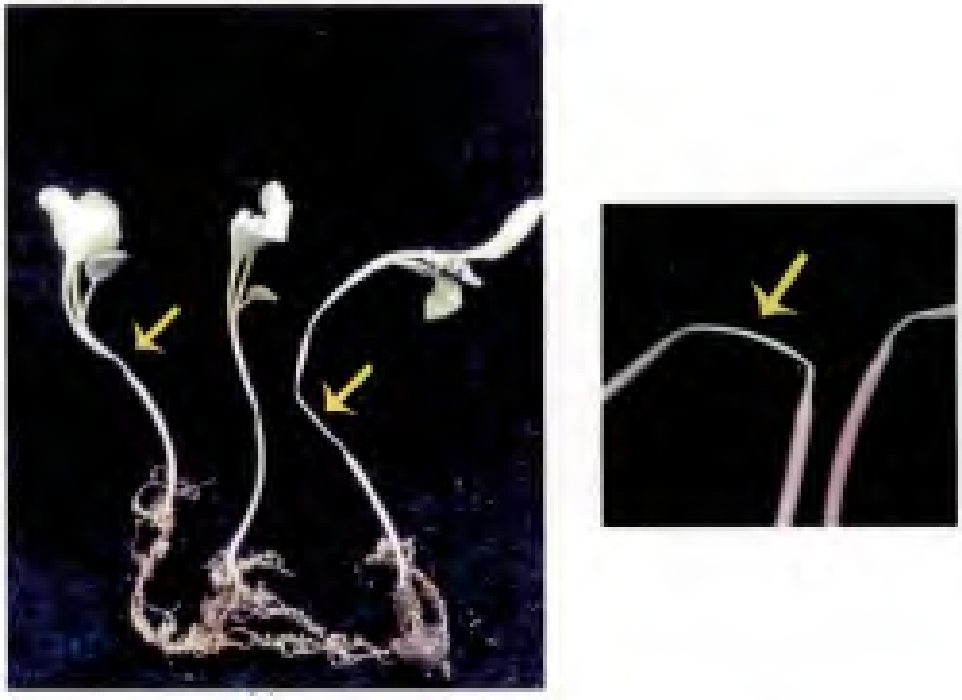
b. Symptom expression of *Pythium* curd rot



c. Development stages of *Pythium* curd rot

Plate 9. Symptomatology of *Pythium* curd rot

a. Pathogenicity test



b. Symptom expression of damping off

Plate 10. Symptomatology of *Fusarium* damping off

Leaf blight -1 produced small brown spot on second day with an area of 1.2 cm^2 which expanded up to 7.8cm^2 on 10^{th} day leaf blight-2 also had similar performance where symptom developed on second day as 1.2cm^2 and enlarged up to 7 cm2 on 10^{th} day. But colour of the spot was bluish green with water soaked appearance. The inoculated leaf of leaf spot-1 and leaf spot-3showed first symptom of disease development after 3^{rd} day of inoculation with a size of 0.5 cm^2 and 0.3 cm^2 respectively. They increased their size upto 2.1 cm^2 on 10^{th} day of inoculation. But leaf spot -2 started symptom production on 2^{nd} day with a size of 0.5cm^2 and extended upto 2.3cm^2 on 10^{th} day. The curd/head rot-1 and curd rot-2 produced symptom on 2^{nd} day in both crops. Rotted area expanded on the head and curd with an area upto 7.5cm^2 which defoliated within one to two weeks on live plants. In the case of damping off disease in cauliflower, the inoculated seedling showed initial symptom development on second day of inoculation.

4.6. VIRULENCE OF THE PATHOGENS

The virulence of the isolates of various fungal pathogens obtained in multiples from cabbage and cauliflower were tested by observing the differential response of artificial inoculation of pathogens on respective host plants. Since there were more than one isolate of leaf blight-1, leaf blight-2 and curd rot-1 pathogens obtained during survey, these three pathogens were selected for virulence studies. Based on the results of virulence test most virulent isolate from leaf blight-1, leaf blight-2 and curd rot-1were selected for further studies. Rest of the pathogens which were obtained as single isolate were also selected for continuation of the research. Variation in the symptom development in each of the above mentioned pathogens are shown in Table 11, 12 and 13.

4.7. CHARACTERISATION AND IDENTIFICATION OF PATHOGEN

The fungal pathogens of cabbage and cauliflower isolated from different diseased samples from nine locations in four districts were screened for virulence test based on the lesion area at seven days after inoculation (cm^2) and days of first

Table.11 Virulence test of different isolates of leaf blight-1 pathogen on cabbage and cauliflower plants

Crop	Isolate number	Isolate code	Lesion area (cm^2)		Days of first symptom development
			2 days after inoculation	10 days after inoculation	
Cabbage	1	CA-1	0.9	5.1	3
	2	CA-2	0.7	4.6	3
	3	CA-3	1.2	7.8	2
	4	CA-4	0.5	5.5	2
	5	CA-5	0.9	5.2	2
	6	CA-6	0.8	4.1	2
	7	CA-7	0.7	5.8	3
	8	CA-8	0.5	5.3	3
Cauliflower	1	CFA-1	0.5	6.0	3
	2	CFA-2	0.5	6.1	3
	3	CFA-3	0.9	4.9	3
	4	CFA-4	0.5	5.5	3
	5	CFA-5	0.2	5.3	4

Table.12 Virulence test of different isolates of leaf blight-2 pathogen on cabbage and cauliflower plants

Crop	Isolate code	Lesion area (cm^2)		Days of first symptom development
		2days after inoculation	10days after inoculation	
Cabbage	CR-1	0.7	5.1	3
	CR -2	0.9	4.1	2
	CR-3	1.2	7.0	2
Cauliflower	CFR -1	0.9	4.8	3

Table.13 Virulence test of different isolates of head/curd rot-1 pathogen on cabbage and cauliflower plants

Crop	Isolate code	Lesion area at (cm^2)		Days of first symptom development
		2 days after inoculation	10 days after inoculation	
Cabbage	CH-1	0.9	7.5	2
	CH-2	0.5	6.4	2
Cauliflower	CFH -1	0.5	5.9	3

symptom development. Out of the 15 isolates of different fungi in cabbage and nine isolates from cauliflower, eight isolates were subjected to cultural and morphological studies for characterisation and thereby identification of the isolates. Two leaf blights and curd rot- 1 were detected both in cabbage and cauliflower. However, all the three leaf spots were found only in cabbage. It was observed that curd rot-2 and damping off were present only in cauliflower. A detailed description regarding each isolate is presented below.

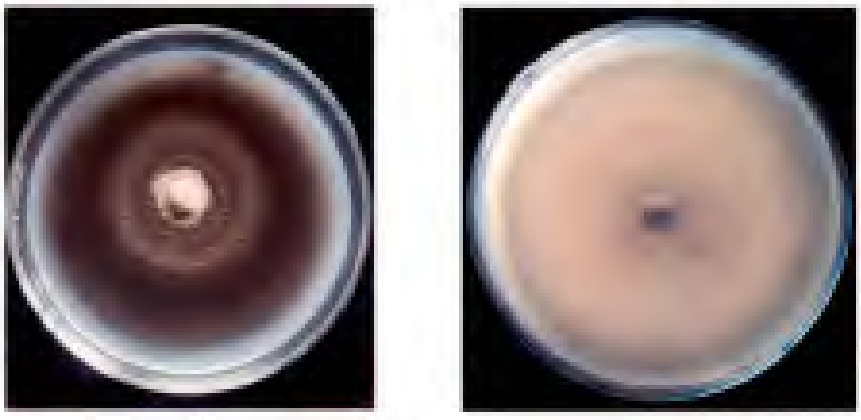
4.7.1 Cultural and morphological characters

4.7.1.1 Leaf blight-1

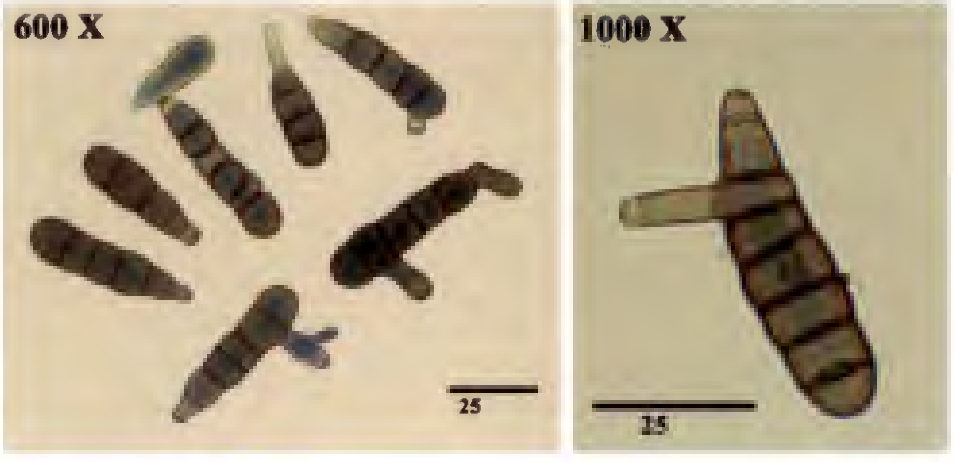
The pathogen was observed in cabbage in all the locations except Chullikkara location.

On studying the cultural characters on PDA medium the pathogen was found to be a slow grower, since it had taken almost thirteen days of incubation to complete growth in a 90 mm diameter Petri plate. The colony had a deep olive green colour with concentric zonations at regular intervals.

Morphological studies revealed that hyphae were septate, conidiophores brown, septate, branched and 2.68 - 6.31 µm wide. Mature conidia were golden to brown, cylindrical to oblong, muriform and produced in chains of three to four spores. Younger conidia were oval, without beak and usually without transverse septa or with 1-2 transverse septa. Size of young conidia ranged from 8.96-17.77 µm x 5.93-10.09 µm. Size of the mature conidia ranged from 21.78 – 50.47µm x 6.33 – 18.32 µm with 2-6 transverse septa. Beak was absent in some of the conidia if present a short beak of length ranging from 3.47 to 15.62 µm was observed. Usually conidia were formed with or without any longitudinal septa and when longitudinal septa were present, it ranged from 1-2 in number. Based on these cultural and morphological characters, the fungal pathogen was identified as *Alternaria* sp (Plate 11).



a. Culture plates of *Alternaria* sp.



b. Conidia of *Alternaria* sp.

c. Conidiophore

d. Conidia in chains

e. Germination

Plate 11. Colony morphology and conidial characters of *Alternaria* sp.

4.7.1.2 Leaf blight-2

The pathogen was observed in cabbage from locations like Ambalavayal, Nileswar, Chullikkara and Padannakkad.

The colony initially appeared as creamy white in colour later turning into light brown with fluffy aerial mycelium. The underside of the plate appeared dark brown in the centre and light brown in the periphery. The pathogen produced light brown to coffee brown, globular to irregular shaped, 1-4 mm diameter sclerotial bodies mostly on the centre medium after seven days of inoculation. It had taken three days for full growth of 90 mm in a Petri dish.

The mycelia of the pathogen showed right angled branching and a characteristic septum was observed at the point of origin of the right angle. Hyphae showed a characteristic constriction at the branching point. The hyphal cells were barrel shaped and light brown in colour. The thickness of hyphae ranged from 5.46-8.24µm (Plate 12). The pathogen was identified as *Rhizoctonia* sp. Based on above mentioned characters.

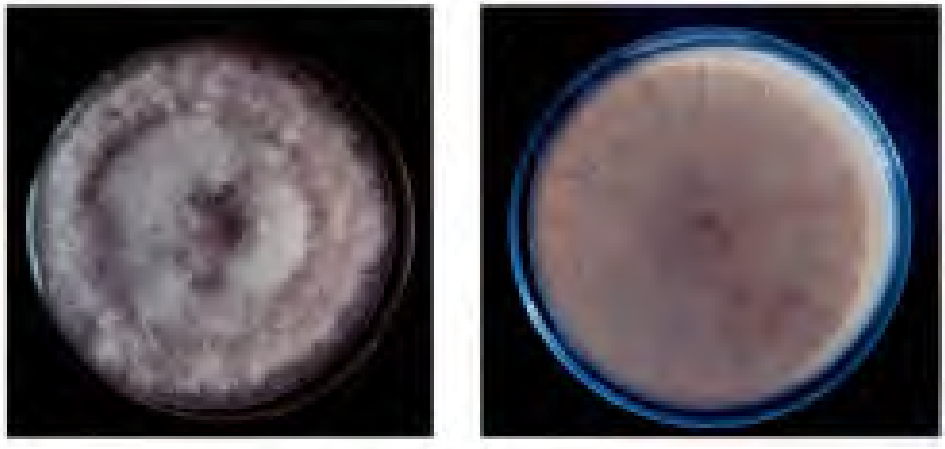
4.7.1.3 Leaf spot-1

The fungal pathogen causing leaf sot-1 from cabbage could not be recovered as culture isolate.

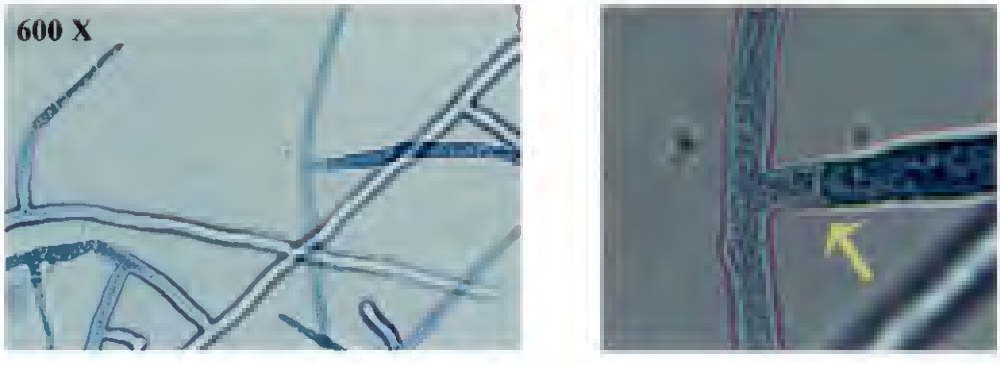
Microscopic studies were conducted for identification of the pathogen. Long filiform shaped spores with one broad end and narrow tip was observed. Conidiophores were brown in colour with 3.63-6.53µm width. Size of the conidia ranged from 57.52-160.40µm in length and 2.23-4.49µm width (Plate 13). The pathogen based on its morphological characters was tentatively identified as *Cercospora* sp.

4.7.1.4 Leaf spot-2

On PDA medium, the pathogen causing leaf spot-2 from cabbage produced greyish brown growth with concentric zonations. Underside of the plate



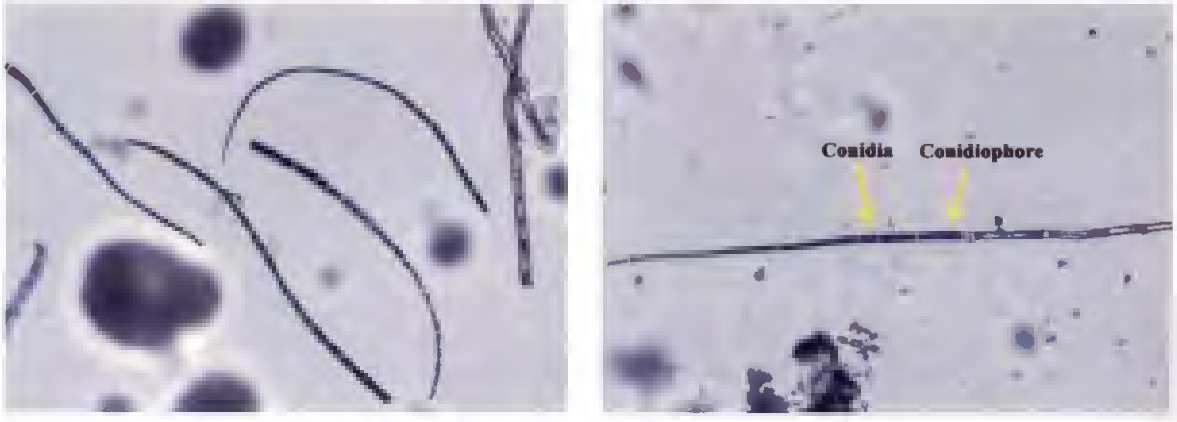
a. Culture plates of *Rhizoctonia* sp.



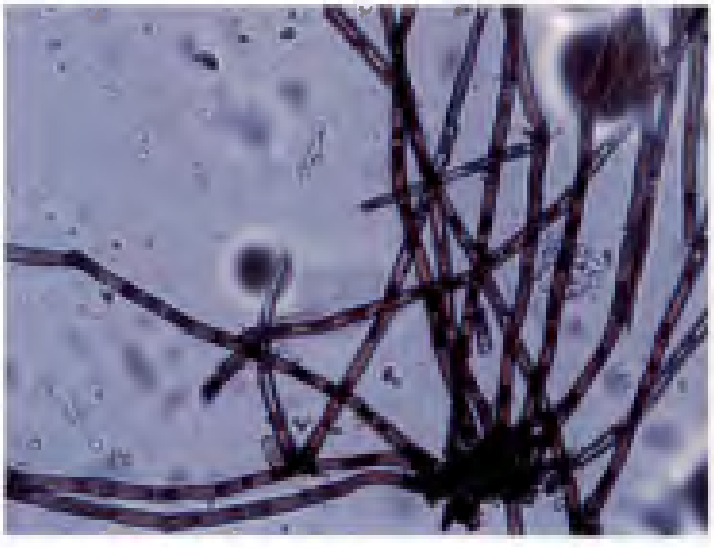
b. Branching characters of hypha

c. Hyphal anastomosis

Plate 12. Colony and hyphal morphology of *Rhizoctonia* sp.



a. Conidia and conidial attachment of *Cercospora* sp.



b. Conidiophore

Plate 13. Conidial morphology of *Cercospora* sp.

was dark grey to black colour and produced pink coloured spore mass on the media. It had taken ten days for full growth of 90 mm in a Petri dish.

Hyphae were hyaline with 1.20-3.1µm width. Conidia were aseptate, hyaline, cylindrical and oval with both apices rounded on well-developed hyaline conidiphores. Size of the conidia ranged from 7.76-23.24µm in length and 4.28-6.17µm in breadth (Plate 14). The pathogen based on its cultural and morphological characters was tentatively identified as *Colletotrichum* sp.

4.7.1.4 Leaf spot-3

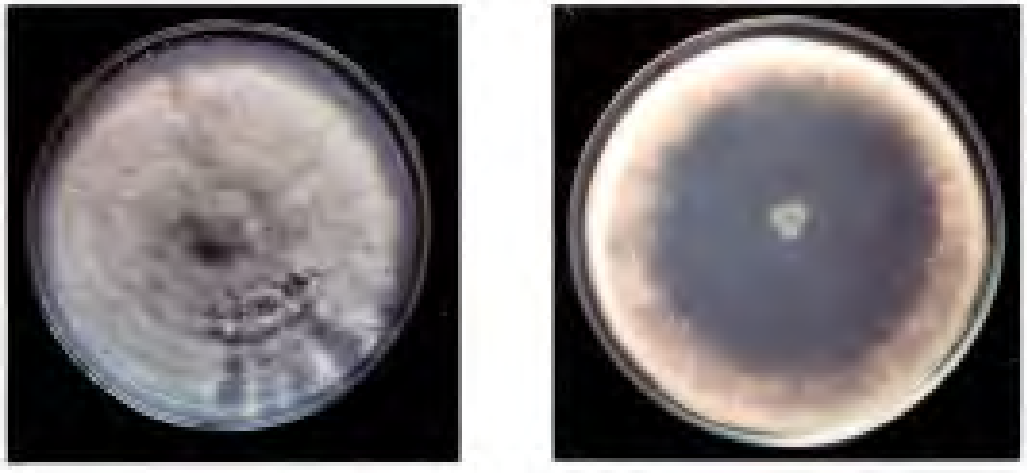
On PDA media, colony of the pathogen of leaf spot-3 from cabbage appeared as brown to dark brown growth, black on back side with irregular borders and greyish white cottony growth appeared with concentric zonations. It had taken eight days for full growth of 90 mm in a Petri dish.

Hyphae measured 2.5-5.9µm width. Conidiophores were dark brown and septate. Under the microscope light to dark brown straight to pyriform conidia were observed. Conidia fusiform, conidia with a prominent curve .The cells at the centre bigger compared to those on tips. Size of the conidia ranged from 18.9-30.12µm x 7.84-12.43µm (Plate 15). Based on these characters, the pathogen was tentatively identified as *Curvularia* sp.

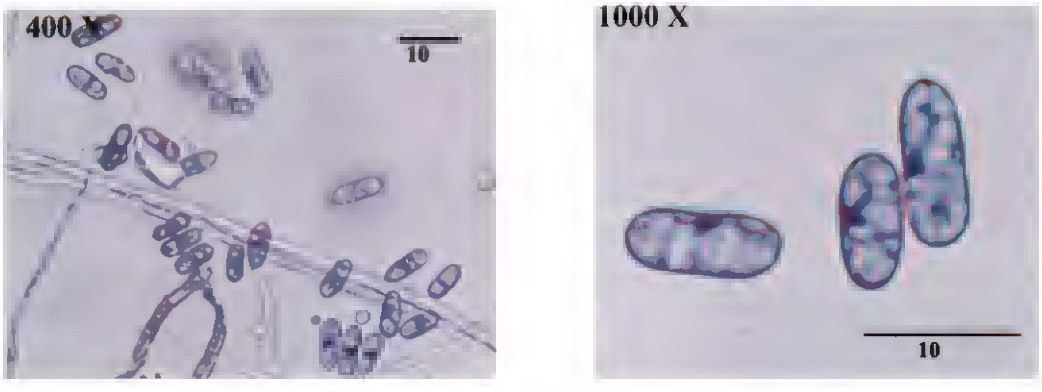
4.7.1.5 Head rot / Curd rot-1

The pathogen was found to be fast grower covering a radial growth of 50 mm after 24 hours of inoculation with cottony white aerial mycelium. Sporulation was observed on the periphery of the petri plate and creamy white colour on under surface.

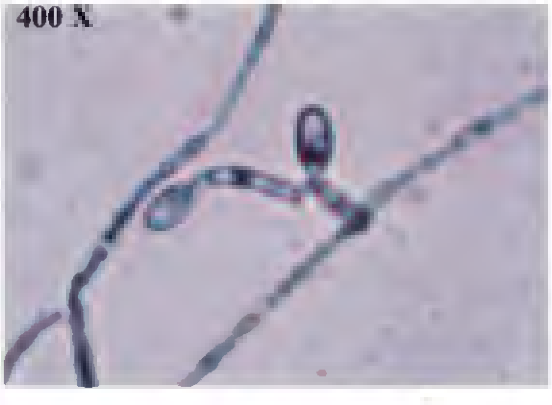
Sporangiophores bearing apical sporangiola were observed. Sporangiola were formed at the apex of 14.3-16.41 µm wide sporangiophores. Monosporous sporangiola were ovoid with brown to dark brown colour with striations on the surface. They measured 12.22-14.85µm x 6.66-9.61 µm in size. Sporangia were globose to sub-globose with 32.80-74.91µm diameter. Sporangiospores were



a. Culture plates of *Colletotrichum* sp.

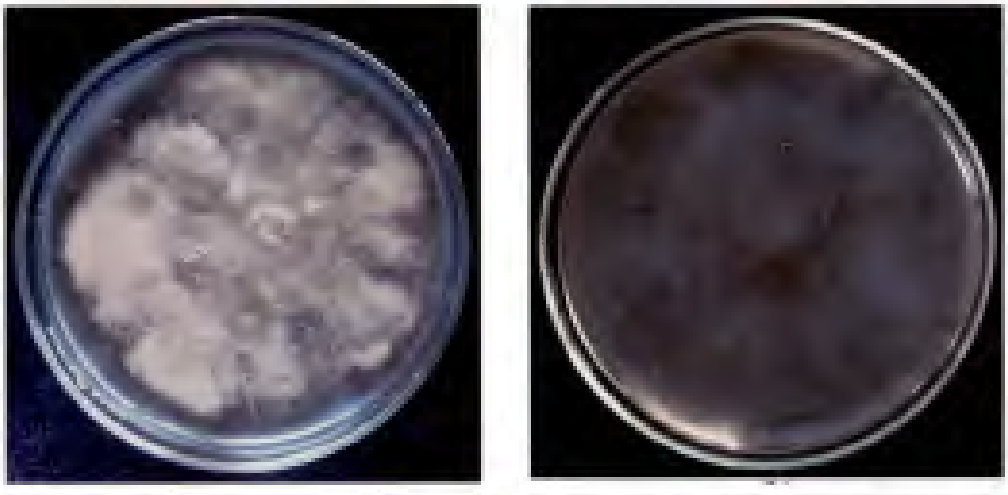


b. Conidia of *Colletotrichum* sp.



c. Development of Conidia

Plate 14. Colony and conidial morphology of *Colletotrichum* sp.



a. Culture of *Curvularia* sp

b. Conidia of *Curvularia* sp

Plate 15. Colony and conidial morphology of *Curvularia* sp.

brown coloured, elliptic to ovoid and measured 12.68-18.38µm x 6.48-7.99µm (Plate 16). Based on these cultural and morphological characters the pathogen was tentatively identified as *Choanephora* sp.

4.7.1.6 Curd rot -2

The pathogen was found to be a fast growing fungus and measured 9 cm diameter after 48 hours of inoculation. Colony had cottony white fluffy mycelium without any special pattern (Plate 17).

It produced hyaline hypha with 0.83-3.71µm width. Sporangia consisting of terminal complexes of swollen hyphal branches of varying length and upto 7.6µm wide. Oogonia terminal, globose, smooth, 16.0-21.3µm diameter. Most of the anthredia were intercalary, broadly sac shaped, 7.4-11.61µm long and 6.27-8.96µm wide, one per oogonium, monoclinous or dinoclinous. Oospores were plerotic 12.65-3.18µm diameter with wall thickness of 1.57-3.18µm. Based on the characters the pathogen was tentatively identified as *Pythium* sp.

4.7.1.7. Damping off

Initially the colony had dense white mycelium with irregular borders which later turned to brownish orange colour. On the underside it appeared as light orange colour. It had taken seven days for full growth of 90 mm in a Petri dish.

Hyphae were hyaline with 2.21-4.14µm thickness. Both micro and macro conidia were observed. Where macroconidia were elongated with pronounced curved foot cell which measured 14.09-28.01 µm x 2.50-4.51 µm in sizewith 2-5 septa. Microconidia elongated, oval shaped with one to two cells and measured 4.17-4.51 µm in length and 1.99-3.32 µm width (Plate 18). Based on these cultural and morphological characters the pathogen was tentatively identified as *Fusarium* sp.

a. Culture plates of *Choanephora* sp.

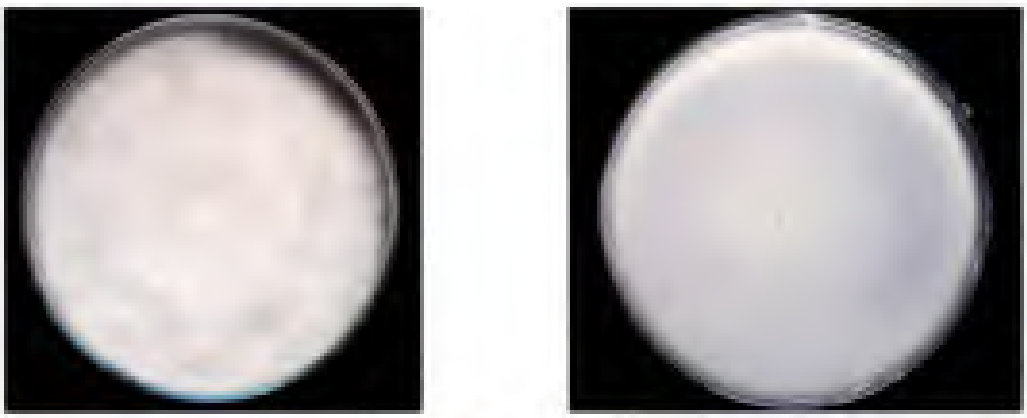
b. Morphology of sporangia

c. Vesicles of sporangiophore

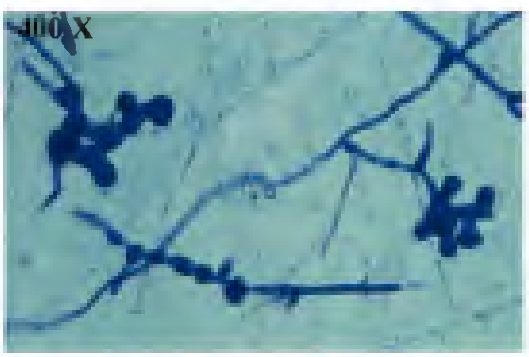
d. Sporangiospore

e. Monosporous sporangiola

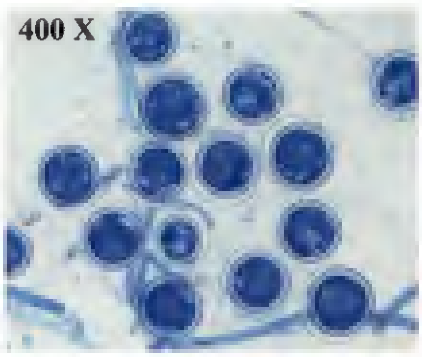
Plate 16. Colony and spore morphology of *Choanephora* sp.



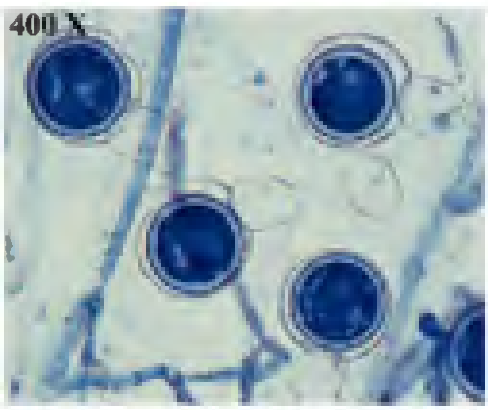
a. Culture plates of *Pythium* sp.



b. Lobbed sporangium



c. Oospores



d. Anthredium and oogonium

Plate 17. Colony and sporangial morphology of *Pythium* sp.

a. Culture plate of *Fusarium* sp.

b. Macroconidia of *Fusarium* sp.

c. Microconidia of *Fusarium* sp.

Plate 18. Colony and conidial morphology of *Fusarium* sp.

4.8. MOLECULAR CHARACTERISATION OF PATHOGENS ISOLATED FROM CABBAGE AND CAULIFLOWER.

Preliminary identification of the pathogen up to genus level was done based on cultural and morphological characters. Further species level identification was done by molecular analysis. The molecular characterization of the pathogens was carried out at Rajiv Gandhi Centre for Biotechnology (RGCB), Thriuvananthapuram. Sequence analysis and nucleotide homology of each pathogen were analysed through the online BLASTn programme of NCBI. Details of the result of sequence comparison of these isolates are presented in Tables 14-19. The gel electrophotresis of genomic DNA and PCR product are given in Plate 19.

The ITS sequence of pathogens are as follows

1. *Alternaria* sp. (F1 & F2)

AGGGATCATTACACAATATGAAAGCGGGCTGGACTCACCTCAGCAGC
ATCTGCTGTTGGGGCCAGCCTTGCTGAATTATTCACCCGTGTCTTTTGC
GTACTTCTTGTTTCCTTGGTGGGCTCGCCCACCACAAGGACAAACCAT
AAACCTTTTGTAATTGCAATCAGCGTCAGTAACAACATAATAATTACA
ACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGC
GAAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAAT
CTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTC
GAGCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCT
CCAGTTTGCTGGAGACTCGCCTTAAAGTCATTGGCAGCCGGCCTACTG
GTTTCGGAGCGCAGCACAAGTCGCGCTCTCTTCCAGCCAAGGTCAGCA
TCCATAAAGCCTTTTTTCAACTTTTGACCTCGGATCAGGTAGGGATACC
CGCTGAACTTAA

2. *Rhizoctonia* sp. (F8)

AATTCCATCACCCATTTGCTGTCTACTTAATTTACACACACTCTACTTA
ATTTAAACTGAATGTAATTGATGTAACGCATCTAATACTAAGTTTCAA

94

a. Gel electrophoresis of genomic DNA

b. Gel electrophoresis of PCR product

Plate 19 DNA amplification profile of selected pathogens

CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCG
ATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAAC
GCACCTTGCGCTCCTTGGTATTCCTTGGAGCATGCCTGTTTGAGTATCA
TGAAATCTTCAAAGTAAACCTTTTGTTAATTCAATTGGTCTTTTTTACTT
TGGTTTTGGAGGATCTTATTGCAGCTTCACACCTGCTCCTCTTTGTGCA
TTAGCTGGATCTCAGTGTTATGCTTGGTTCCACTCGGCGTGATAAGTTA
TCTATCGCTGAGGACACCCGTAAAAAAGGTGGCCAAGGTAAATGCAG
ATGAACCGCTTCTAATAGTCCATTGACTTGGACAATATTT

3. *Colletotrichum* sp. (F5)

AGGGATCATTACTGAGTTTACGCTCTATAACCCTTTGTGAACATACCTA
TAACTGTTGCTTCGGCGGGTAGGGTCTCCGCGACCCTCCCGGCCTCCC
GCCTCCGGGCGGGTCGGCGCCCGCCGGAGGATAACCAAACTCTGATTT
AACGACGTTTCTTCTGAGTGGTACAAGCAAATAATCAAAACTTTTAAC
AACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGA
TAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACG
CACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCA
TTTCAACCCTCAAGCTCTGCTTGGTGTTGGGGCCCTACAGCTGATGTAG
GCCCTCAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTA
ACTTTACGTCTCGCACTGGGATCCGGAGGGACTCTTGCCGTAAAACCC
CCCAATTTTCCAAAGG

4. *Curvularia* sp. (F3)

AGGGATCATTACACAAATGAAAATATGAAGGCCCTTCAAACCGGCTG
GATTATTTTTCTTCACCCTTGTCTTTTGCGCACTTGTTGTTTCCTGGGCG
GGTTCGCTCGCCACCAGGACCACACCATAAACCTTTTTGTTAATGCAA
TCAGCGTCAGTAAAAAGTAATAATTATTTTACAACTTTCAACAACGGA
TCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATACGTA
GTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATT
GCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCGTCATTTGTAC
CCTCAAGCTTTGCTTGGTGTTGGGCGTTGTTTGTCTTTGGCCTTGCCCA

96

AAGACTCGCCTTAAAACAATTGGCAGCCGGCCTACTGGTTTCGCAGCG
CAGCACATTTTTGCGCTTGCAATCAGCAAAAGAGGTTGGCCATCCATC
AAGACTACATTTATACGTTTGACCTCGGATCAGGTAGGGATACCCGCT
GAACTTAA

5. *Choanephora* sp. (F4)

AAGGATCATTAAATAAATAAGAAGTATTTCGGGCTTGTCCTGATCTAC
TATCTATTTTACTGTGAACTGTTTTATTTCATGGCGTTTGAGGGATGTT
CTTGTGCTATATGGGTAGGCATGAGGAATGTTAACCGAGCTATGGTCA
AGCTTAGGCTTGGTACCCTGTTTATATACTTTCAATTGATCAGATTATA
AAATGTAACATAGGTAGTAATATCTATAAAACAACTTTTAACAATGGA
TCTCTTGGCTTTTGCATCGATGAAGAACGTAGCAAATTGCGATAACTA
GTGTGAATTGCAAATTCAGTGAATCATCGAGTCTTTGAACGCATCTTG
CGCTCATTGGTATTCCAGTGAGCACGCCTGTTTCAGTATCAAAAACAA
CCCTCATTCAAAATTTTTTTTTGAATGGTCATGAAGGAAGCTAGCAATG
GCGACCTTTTAAATTGAGTAAGGCCTGAATCTGTTCATCTAGCCTGAA
CTTTTTTTTAATATAAAGGAAAGCTCTTGCGACTTGGACTTTGTTGGGG
CCTCCCAAATAAAACTCTTTCATCTTGATCTGAAATCAGGTGGGACTA
CCCGCTGAACTTAA

6. *Fusarium* sp. (F6)

CCTGTGAACATACCTATACGTTGCCTCGGCGGATCAGCCCGCGCCCCG
TAAAACGGGACGGCCCGCCCGAGGACCCCTAAACTCTGTTTTTAGTGG
AACTTCTGAGTAAAACAAACAAATAAATCAAAACTTTCAACAACGGAT
CTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATAAGTAA
TGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTG
CGCCCGCCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAAC
CCTCAAGCTCAGCTTGGTGTTGGGACTCGCGGTAACCCGCGTTCCCCA
AATCGATTGGCGGTCACGTCGAGCTTCCATAGCGTAGTAATCATACAC
CTCGTTACTGGTAATCGTCGCGGCCACGCCGTTAAACCCCAACTTCTG
AATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAA

97

4.8.1 Sequence comparison of *Alternaria* sp.

Comparison of nucleotide sequence of *Alternaria* culture revealed that the fungus showed 100 per cent identity with *Alternaria brassicicola* iolate R5 (Accesssion JN10901.1), *A. brassicicola* isolate T4 (Accession JF439455.1), *A. brassicicola* isolate T3 (Accession JF439454.1), *A. brassicicola* isolate T2 (Accession JF439453.1) and *A. brassicicola* isolate D7 (Accession JF439452.1). Hence sequence analysis of the *Alternaria* culture showed homology with *A. brassicicola* having 100 percent identity and 100 percent query coverage as the same was identified earlier through cultural and morphological characterization

4.8.2. Sequence comparison of *Rhizoctonia* sp.

Comparison of nucleotide sequence of *Rhizoctonia* culture revealed that it showed 100 per cent identity with *Rhizoctonia solani* strain HPSnG (Accession KF959672.1) and *R. solani* strain AG 1-IA (Accession JX089962.1). With *R. solani* AG-1 IA isolate CSU8 (Accession KX674527.1), *R. solani* AG-1 IA isolate CSU4 (Accession KX674526.1), *R.solani* AG-1 IA isolate CSU1 (Accession KX674525.1) it showed 99 percent identity. Hence sequence analysis of the *Rhizoctonia* culture showed homology with *R. solani* having 100 per cent identity and cent per cent query coverage as the same was identified earlier through cultural and morphological characterization.

4.8.3. Sequence comparison of *Colletotrichum* sp.

Sequence homology of *Colletotrichum* culture showed 100 per cent identity with *Colletotrichum gloeosporioides* (Accession LC169644.1, LC169638.1 and LC169630.1), *C. gloeosporioides* isolate A15 (Accession KY962995.1) and *C. gloeosporioides* isolate A14 (Accession KY962994.1). Hence, the isolate which was earlier identified as *Colletotrichum gloeosporioides* by cultural and morphological characters was further confirmed with the result of molecular characterization.

Table.14 Sequence homology observed for *Alternaria* sp. in BLASTn analysis as per BLAST results

Sl. No.	Description	Max. score	Query coverage (%)	E value	Identity (%)	Accession
1.	*Alternaria brassicicola* isolate R5	1003	100%	0.0	100%	JN108901.1
2.	*Alternaria brassicicola* isolate T4	1003	100%	0.0	100%	JF439455.1
3.	*Alternaria brassicicola* isolate T3	1003	100%	0.0	100%	JF439454.1
4.	*Alternaria brassicicola* isolate T2	1003	100%	0.0	100%	JF439453.1
5.	*Alternaria brassicicola* isolate D7	1003	100%	0.0	100%	JF439452.1

Table.15 Sequence homology observed for *Rhizoctonia* sp. in BLASTn analysis as per BLAST results

Sl. No.	Description	Max. score	Query coverage (%)	E value	Identity (%)	Accession
1.	*Rhizoctonia solani* strain HPSnG	880	99%	0.0	100%	KF959672.1
2.	*Rhizoctonia solani* strain AG 1-IA	880	99%	0.0	100%	JX089962.1
3.	*Rhizoctonia solani* AG-1 IA isolate CSU8	874	99%	0.0	99%	KX674527.1
4.	*Rhizoctonia solani* AG-1 IA isolate CSU4	874	99%	0.0	99%	KX674526.1
5.	*Rhizoctonia solani* AG-1 IA isolate CSU1	874	99%	0.0	99%	KX674525.1

4.8.4 Sequence comparison of *Curvularia* sp.

Nucleotide of *Curvularia* culture recorded 100 per cent identity with *Curvularia lunata* strain 25C (Accession KU715134.1), *C. verruculosa* strain WS3L (Accession KT923467.1), *C. verruculosa* WS2L (Accession KT923466.1), *C. verruculosa* strain WS1L (Accession KT923465.1) and *C. verruculosa* (Accession HF934909.1). Hence, the pathogen showed 100 per cent identity and query coverage with *Curvularia lunata* and therefore the cultured pathogen is identified to be the same.

4.8.5. Sequence comparison of *Choanephora* sp.

Sequence homology search for *Choanephora* culture revealed 100 per cent identity with *Choanephora infundibulifera* isolate B.C-12 (Acccession KX980520.1), *C. cucurbitarum* isolte Colo 16 (Accession KU877802.1), *C. infundibulifera* f. *cucurbitarum* voucher KUS-F27540 (Accession KM200034.1), *C. infundibulifera* f. *cucurbitarum* isolate KA47637 (Accession KJ461159.1) and *C. cucurbitarum* clone C7 (Accession KY080447.1). Hence sequence analysis of the *Choanephora* culture showed homology with *C. cucurbitarum* having 100 percent identity and 100 per cent query coverage as the same was identified earlier through cultural and morphological characterization.

4.8.6. Sequence comparison of *Fusarium* sp.

Nucleotide analysis of Fusarium culture gave 100 per cent identity with *Fusarium equiseti* strain NAR08 (AccessionMF039884.1), *Fusarium* sp. (Accession KU886151.1, LC184219.1 and KX953459.1) and *F. equiseti* isolate PAK54 (Accession KY523100.1). Hence it showed 100 per cent identity and query coverage with *Fusarium equiseti*, the pathogen confirmed to be the same.

Table.15 Sequence homology observed for *Colletotrichum* sp. in BLASTn analysis as per BLAST results

Sl. No.	Description	Max. score	Query coverage (%)	E value	Identity (%)	Accession
1.	*Colletotrichum gloeosporioides*	920	100%	0.0	100%	LC169644.1
2.	*Colletotrichum gloeosporioides*	920	100%	0.0	100%	LC169638.1
3.	*Colletotrichum gloeosporioides*	920	100%	0.0	100%	LC169630.1
4.	*Colletotrichum gloeosporioides* isolate A15	920	100%	0.0	100%	KY962995.1
5.	*Colletotrichum gloeosporioides* isolate A14	920	100%	0.0	100%	KY962994.1

Table.16 Sequence homology observed for *Curvularia* sp. in BLASTn analysis as per BLAST results

Sl. No.	Description	Max. score	Query coverage (%)	E value	Identity (%)	Accession
1.	*Curvularia lunata* strain 25C	996	100%	0.0	100%	KU715134.1
2.	*Curvularia verruculosa* strain WS3L	996	100%	0.0	100%	KT923467.1
3.	*Curvularia verruculosa* strain WS2L	996	100%	0.0	100%	KT923466.1
4.	*Curvularia verruculosa* strain WS1L	996	100%	0.0	100%	KT923465.1
5.	*Curvularia verruculosa*	996	100%	0.0	100%	HF934909.1

Table.18 Sequence homology observed for *Choanephora* sp. in BLASTn analysis as per BLAST results

Sl. No.	Description	Max. score	Query coverage (%)	E value	Identity (%)	Accession
1.	*Choanephora infundibulifera* isolate B.C-12	1098	100%	0.0	100%	KX980520.1
2.	*Choanephora cucurbitarum* isolate *Colo16*	1098	100%	0.0	100%	KU877802.1
3.	*Choanephora infundibulifera* f. *cucurbitarum* voucher KUS-F27540	1098	100%	0.0	100%	KM200034.1
4.	*Choanephora infundibulifera* f. *cucurbitarum* isolate KA47637	1098	100%	0.0	100%	KJ461159.1
5.	*Choanephora cucurbitarum* clone C7	1092	100%	0.0	99%	KY080447.1

Table.19 Sequence homology observed for *Fusarium* sp.in BLASTn analysis as per BLAST results

Sl. No.	Description	Max. score	Query coverage (%)	E value	Identity (%)	Accession
1.	*Fusarium equiseti* strain NAR08	876	100%	0.0	100%	MF039884.1
2.	*Fusarium* sp	876	100%	0.0	100%	KU886151.1
3.	*Fusarium equiseti* isolate PAK54	876	100%	0.0	100%	KY523100.1
4.	*Fusarium* sp	876	100%	0.0	100%	LC184219.1
5.	*Fusarium* sp	876	100%	0.0	100%	KX953459.1

4.9 EFFICACYOF FUNGICIDES AND BIOCONTROL AGENTS AGAINST PATHOGENS UNDER *in vitro* CONDITIONS

In vitro studies were conducted for the evaluation of fungicides and bio control agents against the selected isolates of different pathogens obtained from cabbage and cauliflower. Fungicides were tested by Poison food technique and those with bioagents were evaluated by dual culture technique respectively as described in 3.8.1 and 3.8.2.

4.9.1 *In vitro* evaluation of fungicides

Ten fungicides at three concentrations were selected for *in vitro* evaluation against the seven selected pathogens. *viz., Alternaria brassicicola., Rhizoctonia* solani, *Colletotrichum gloeosporioides, Curvularia lunata, Choanephora cucurbitarum., Pythium aphanidermatum* and *Fusarium equiseti.*

4.9.1.1 Alternaria brassicicola

In vitro evaluation studies of fungicides showed that among ten fungicides tested, best three fungicides against *Alternaria* were found to be trifloxystrobin 25% + tebuconazole 50% (Nativo), tebuconazole 5EC (Folicur) and Bordeaux mixture. Since they showed 100 per cent inhibition in all the three concentrations tested (Table 20, Plate 20A and 20B). Next effective fungicide noticed was copper oxychloride 50WP (Blitox) which gave 78.51, 83.70 and 88.89 per cent inhibition for all the three concentrations. Copper hydroxide 77WP (Kocide) and propineb 75WP (Antracol) showed almost similar type of performance with 74 and 75 per cent inhibition at the recommended dosage. Similarly mancozeb 75WP,

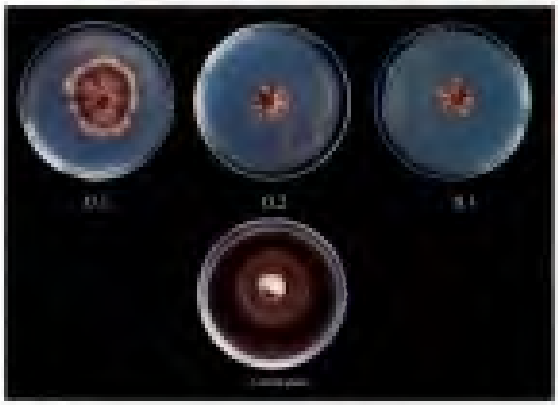
Table.20 *In vitro* evaluation of chemical fungicides on the inhibition of mycelial growth of *Alternaria brassicicola*

Treatments	Chemical Fungicides (conc. %)	Trade name	Inhibition of mycelial growth of *Alternaria brassicicola* (%)*		
			C-1	C-2	C-3
T1	Mancozeb 75WP (0.2, 0.3,0.4)	Mega M-45	57.04 (49.03)	58.15 (49.63)	61.48 (51.62)
T2	Copper hydroxide 77WP (0.1,0.2,0.3)	Kocide	58.15 (49.72)	74.07 (59.40)	80.37 (63.70)
T3	Propineb 75WP (0.2,0.3,0.4)	Anthracol	69.26 (56.32)	75.56 (60.35)	81.85 (69.01)
T4	Trifloxystrobin 25%+ Tebuconazole 50 % (0.02, 0.03,0.04)	Nativo	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T5	Copper oxychloride 50WP (0.1, 0.2,0.3)	Blitox	78.52 (62.37)	83.70 (66.17)	88.89 (70.50)
T6	Chlorothalonil 75WP (0.05, 0.1, 0.15)	Kavach	57.78 (49.46)	64.81 (53.60)	67.04 (54.94)
T7	Carbendazim 50WP (0.05, 0.1, 0.15)	Megastin	17.78 (24.71)	20.00 (26.32)	42.59 (40.71)
T8	Azoxystrobin 23SC (0.05, 0.1, 0.15)	Amistar	63.70 (52.94)	65.56 (54.04)	70.37 (57.10)
T9	Tebuconazole 5EC (0.05, 0.1, 0.15)	Folicur	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T10	Bordeaux mixture (0.5, 1.0, 1.5)		100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
CD			3.876	10.591	1.606
SE			1.305	3.565	0.541

*Mean of three values

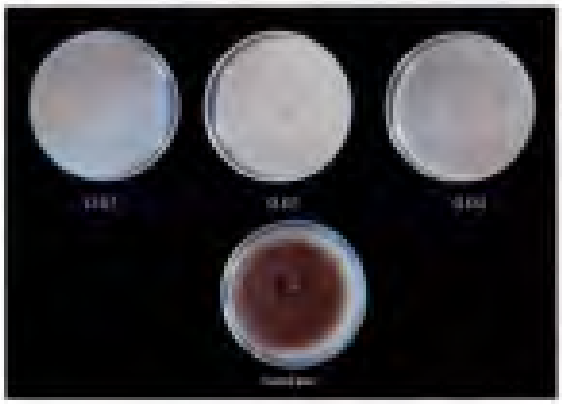
**Values in parenthesis are angular transformed values C -concentration

a. Mancozeb 75WP



b. Copper hydroxide 77WP

c. Propineb 75WP



d. Trifloxystrobin 25% + Tebuconazole 50%

e. Copper oxychloride 50WP

Plate 20 A. Effect of different levels of fungicides on radial growth of *Alternaria brassicicola*

a. Chlorothalonil 75WP

b. Carbendazim 50 WP

c. Azoxystrobin 23SC

d. Tebuconazole 5EC

e. Bordeaux mixture

Plate 20 B. Effect of different levels of fungicides on radial growth of *Alternaria brassicicola*

cholorothalonil 75WP, and azoxystrobin 23SC showed no significant difference (61, 67 and 70 per cent inhibition respectively). Carbendazim 50WP (Megastin) was the least effective chemical in all the three concentrations tested against *Alternaria brassicicola*.

4.9.1.2 Rhizoctonia solani

The data revealed that the fungicides likes tebuconzole 5EC (Folicur), carbendazim 50WP, copperoxychloride 50WP (Blitox), trifloxystrobin 25% + tebuconazole 50% (Nativo) and propineb 70WP (Antracol) were significantly superior among all the ten fungicides because at all the three concentrations they showed 100 per cent inhibition of the pathogen (Table 21, Plate 21A and 21B). At the recommended concentration copper oxychloride 50WP (Blitox) was on par with superior fungicides. At the higher concentration Bordeaux mixture and copper hydroxide 77 WP (Kocide) showed complete inhibition of the pathogen. Azoxystrobin 23SC (Amistar) found to be least effective fungicide with lowest inhibition of the mycelial growth at all the three concentrations

4.9.1.3 Colletotrichum gloeosporioides

Among the fungicides tested, lower concentration of trifloxystrobin 25% + tebuconazole 50% (Nativo), carbendazim 50WP (Bavistin), tebuconazole 5EC (Folicure) showed significantly higher inhibition (Table 22, Plate 22A and 22B). At the recommended concentration and higher concentration, mancozeb 75WP (Mega M-45), trifloxystrobin 25% + tebuconazole 50% (Nativo), carbendazim 50WP (Megastin), tebuconazole 5EC (Folicure) and Bordeaux mixture were found superior to other fungicides with maximum inhibition of the mycelial growth. The next best fungicide noticed was copper oxychloride 50WP (Blitox) which gave 60.00, 78.15 and 93.70 per cent inhibition for all the three concentrations tested. Azoxystrobin 23SC (Amistar) showed significantly lower inhibition of the pathogen at all the three concentrations.

Table.21 *In vitro* evaluation of chemical fungicides on the inhibition of mycelial growth of *Rhizoctonia solani*

Treatments	Chemical Fungicides (conc. %)	Trade name	Inhibition of mycelial growth of *Rhizoctonia solani* (%)*		
			C-1	C-2	C-3
T1	Mancozeb 75WP (0.2, 0.3,0.4)	Mega M-45	86.67 (68.60)	87.78 (69.54)	94.81 (76.93)
T2	Copper hydroxide 77WP (0.1,0.2,0.3)	Kocide	55.19 (47.96)	96.30 (83.50)	100.00 (90.00)
T3	Propineb 75WP (0.2,0.3,0.4)	Anthracol	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T4	Trifloxystrobin 25%+ Tebuconazole 50 % (0.02, 0.03,0.04)	Nativo	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T5	Copper oxychloride 50WP (0.1, 0.2,0.3)	Blitox	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T6	Chlorothalonil 75WP (0.05, 0.1, 0.15)	Kavach	91.48 (73.10)	93.33 (75.01)	93.33 (75.00)
T7	Carbendazim 50WP (0.05, 0.1, 0.15)	Megastin	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T8	Azoxystrobin 23SC (0.05, 0.1, 0.15)	Amistar	12.22 (20.44)	39.24 (38.72)	42.59 (40.67)
T9	Tebuconazole 5EC (0.05, 0.1, 0.15)	Folicur	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T10	Bordeaux mixture (0.5, 1.0, 1.5)		77.40 (61.60)	100.00 (90.00)	100.00 (90.00)
CD			1.803	6.84	2.967
SE			0.607	2.302	0.999

*Mean of three values

**Values in parenthesis are angular transformed values C -concentration

Table.22 *In vitro* evaluation of chemical fungicides on the inhibition of mycelial growth of *Colletorichum gloeosporioides*

Treatments	Chemical Fungicides (conc. %)	Trade name	Inhibition of mycelial growth of *Colletorichum gloeosporioides* (%)*		
			C-1	C-2	C-3
T1	Mancozeb 75WP (0.2, 0.3,0.4)	Mega M-45	98.52 (83.08)	98.89 (86.48)	99.63 (87.97)
T2	Copper hydroxide 77WP (0.1,0.2,0.3)	Kocide	45.93 (42.63)	68.52 (55.99)	72.22 (58.43)
T3	Propineb 75WP (0.2,0.3,0.4)	Anthracol	51.85 (46.04)	60.37 (51.00)	63.70 (52.96)
T4	Trifloxystrobin 25%+ Tebuconazole 50 % (0.02, 0.03,0.04)	Nativo	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T5	Copper oxychloride 50WP (0.1, 0.2,0.3)	Blitox	60.00 (50.76)	78.15 (62.11)	93.70 (76.08)
T6	Chlorothalonil 75WP (0.05, 0.1, 0.15)	Kavach	47.78 (43.70)	53.33 (46.89)	59.26 (50.32)
T7	Carbendazim 50WP (0.05, 0.1, 0.15)	Megastin	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T8	Azoxystrobin 23SC (0.05, 0.1, 0.15)	Amistar	20.37 (26.42)	19.63 (26.25)	37.41 (37.51)
T9	Tebuconazole 5EC (0.05, 0.1, 0.15)	Folicur	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T10	Bordeaux mixture (0.5, 1.0, 1.5)		82.59 (65.32)	100.00 (90.00)	100.00 (90.00)
CD			4.843	4.974	7.073
SE			1.630	1.674	2.381

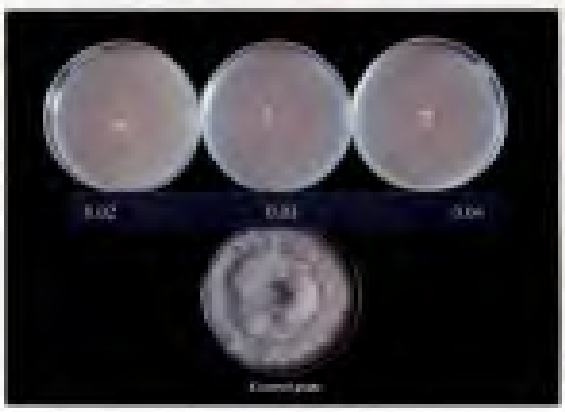
*Mean of three values

**Values in parenthesis are angular transformed values C –concentration

a. Mancozeb 75WP

b. Copper hydroxide 77WP

c. Propineb 75WP



d. Trifloxystrobin 25% + Tebuconazole 50%

e. Copper oxychloride 50WP

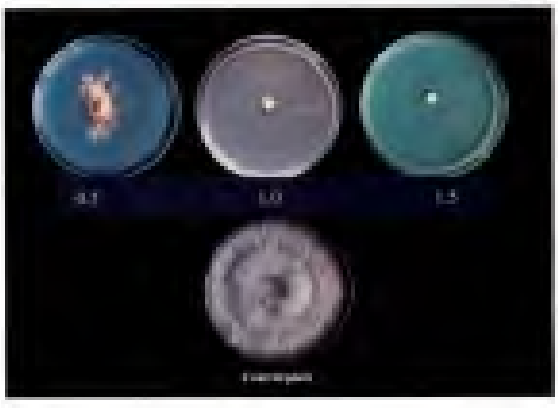
Plate 21 A. Effect of different fungicides on radial growth of *Rhizoctonia solani*

a. Chlorothalonil 75WP

b. Carbendazim 50 WP

c. Azoxystrobin 23SC

d. Tebuconazole 5EC



e. Bordeaux mixture

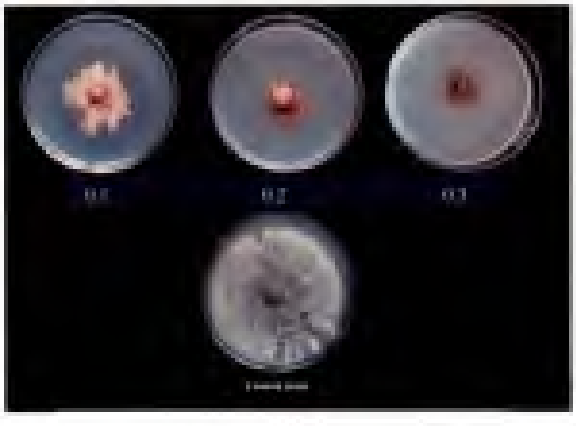
Plate 21 B. Effect of different fungicides on radial growth of *Rhizoctonia solani*

a. Mancozeb 75WP

b. Copper hydroxide 77WP

c. Propineb 75WP

d. Trifloxystrobin 25% + Tebuconazole 50%



e. Copper oxychloride 50WP

Plate 22 A. Effect of different levels of fungicides on radial growth of *Colletotrichum gloeosporioides*

a. Chlorothalonil 75WP

b. Carbendazim 50WP

c. Azoxystrobin 23SC

d. Tebuconazole 5EC

e. Bordeaux mixture

Plate 22 B. Effect of different levels of fungicides on radial growth of *Colletotrichum gloeosporioides*

4.9.1.4 Curvularia lunata

The data revealed that propineb 70WP (Antracol), trifloxystrobin 25% + tebuconazole 50% (Nativo) and tebuconazole 5EC (Folicur) were the most superior fungicide at all the three concentrations and they showed 100 per cent inhibition of the pathogen (Table 23, Plate 23A and 23B). At the lower concentration copper oxychloride 50WP (Blitox) was the next best fungicide noticed with 84.81 per cent inhibition. Copper oxychloride 50WP (Blitox) and Bordeaux mixture at the recommended and higher concentrations were on par with the superior fungicides. At the recommended concentration and higher concentration mancozeb 75WP (Mega M-45) and copper hydroxide 77WP (Kocide) recorded more than 80 per cent inhibition of the pathogen. At all the concentrations, least inhibition of growth was given by carbendazim 50WP (Megastin).

4.9.1.5 Choanephora cucurbitarum

Evaluation of fungicides with *Choanephora* revealed that at the lower concentration of copper oxychloride 50WP (Blitox), tebuconazole 5EC (Folicur) and Bordeaux mixture were significantly superior to other fungicides (Table 24 Plate 24A and 24B). At the higher concentrations along with the superior fungicides, copper hydroxide 77WP (Kocide) and trifloxystrobin25% + tebuconazole 50% (Nativo) gave 100 per cent inhibition of the mycelial growth. Propineb 75WP (Antracol) recorded 98.89 per cent inhibition of mycelial growth at the field concentration and which was on par with the superior fungicides whereas at the higher concentration it recorded 100 per cent inhibition of the pathogen. From the experiment, azoxystrobin 23SC (Amistar) was found to be the least effective fungicide against *Choanephora cucurbitarum*

4.9.1.6 Pythium aphanidermatum

Evaluation of fungicides against *Pythium aphanidermatum* showed that among the six fungicides viz, mancozeb 70WP (Mega M-45), copper oxychloride 50WP (Blitox), copper hydroxide 77WP (Kocide) and Bordeaux mixture were

Table.23 *In vitro* evaluation of chemical fungicides on the inhibition of mycelial growth of *Curvularia lunata*

Treatments	Chemical Fungicides (conc. %)	Trade name	Inhibition of mycelial growth of *Curvularia lunata* (%)*		
			C-1	C-2	C-3
T1	Mancozeb 75WP (0.2, 0.3,0.4)	Mega M-45	77.78 (61.85)	80.00 (63.43)	82.96 (65.67)
T2	Copper hydroxide 77WP (0.1,0.2,0.3)	Kocide	66.30 (54.49)	80.74 (63.98)	85.93 (67.94)
T3	Propineb 75WP (0.2,0.3,0.4)	Anthracol	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T4	Trifloxystrobin 25%+ Tebuconazole 50 % (0.02, 0.03,0.04)	Nativo	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T5	Copper oxychloride 50WP (0.1, 0.2,0.3)	Blitox	84.81 (84.82)	100.00 (90.00)	100.00 (90.00)
T6	Chlorothalonil 75WP (0.05, 0.1, 0.15)	Kavach	47.78 (43.71)	53.33 (46.89)	59.26 (50.32)
T7	Carbendazim 50WP (0.05, 0.1, 0.15)	Megastin	16.3 (23.65)	37.78 (37.81)	37.04 (37.43)
T8	Azoxystrobin 23SC (0.05, 0.1, 0.15)	Amistar	62.59 (52.28)	69.26 (56.31)	65.19 (53.83)
T9	Tebuconazole 5EC (0.05, 0.1, 0.15)	Folicur	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T10	Bordeaux mixture (0.5, 1.0, 1.5)		75.93 (60.62)	100.00 (90.00)	100.00 (90.00)
CD			2.667	3.521	2.813
SE			0.898	1.185	0.947

*Mean of three values

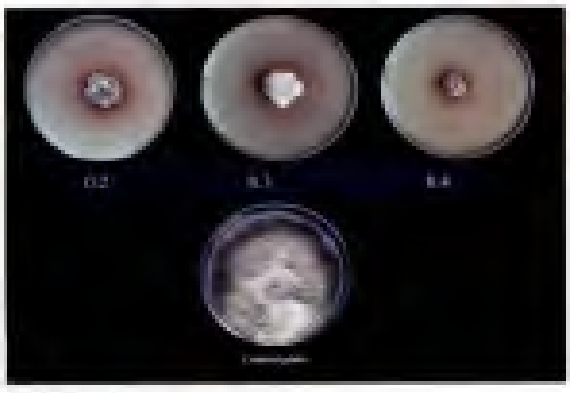
**Values in parenthesis are angular transformed values C -concentration

Table.24 *In vitro* evaluation of chemical fungicides on the inhibition of mycelial growth of *Choanephora cucurbitarum*

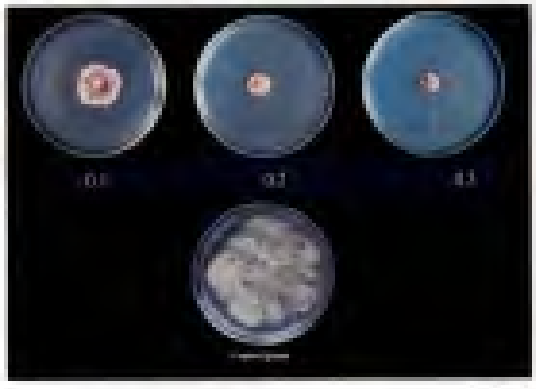
Treatments	Chemical Fungicides (conc. %)	Trade name	Inhibition of mycelial growth of *Choanephora cucurbitarum* (%)*		
			C-1	C-2	C-3
T1	Mancozeb 75WP (0.2, 0.3,0.4)	Mega M-45	49.24 (44.56)	58.52 (55.00)	69.26 (56.39)
T2	Copper hydroxide 77WP (0.1,0.2,0.3)	Kocide	44.44 (41.78)	100.00 (90.00)	100.00 (90.00)
T3	Propineb 75WP (0.2,0.3,0.4)	Anthracol	98.52 (84.26)	98.89 (85.10)	100.00 (90.00)
T4	Trifloxystrobin 25%+ Tebuconazole 50 % (0.02, 0.03,0.04)	Nativo	96.30 (80.89)	100.00 (90.00)	100.00 (90.00)
T5	Copper oxychloride 50WP (0.1, 0.2,0.3)	Blitox	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T6	Chlorothalonil 75WP (0.05, 0.1, 0.15)	Kavach	31.11 (33.88)	52.22 (46.27)	58.15 (49.69)
T7	Carbendazim 50WP (0.05, 0.1, 0.15)	Megastin	18.15 (18.15)	53.70 (47.10)	68.89 (56.08)
T8	Azoxystrobin 23SC (0.05, 0.1, 0.15)	Amistar	0.00 (0.00)	31.48 (34.09)	52.59 (46.47)
T9	Tebuconazole 5EC (0.05, 0.1, 0.15)	Folicur	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T10	Bordeaux mixture (0.5, 1..0, 1.5)		100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
CD			5.703	17.684	1.546
SE			1.920	5.953	0.521

*Mean of three values

**Values in parenthesis are angular transformed values C -concentrations



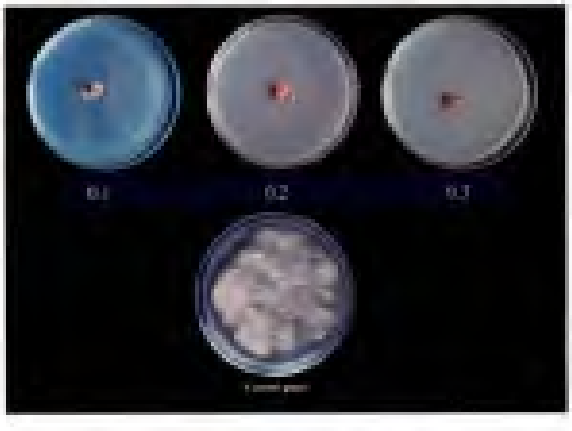
a. Mancozeb 75WP



b. Copper hydroxide 77WP

c. Propineb 75WP

d. Trifloxystrobin 25% + Tebuconazole 50%



e. Copper oxychloride 50WP

Plate 23 A. Effect of different levels of fungicides on radial growth of *Curvularia lunata*

a. Chlorothalonil 75WP

b. Carbendazim 50 WP

c. Azoxystrobin 23SC

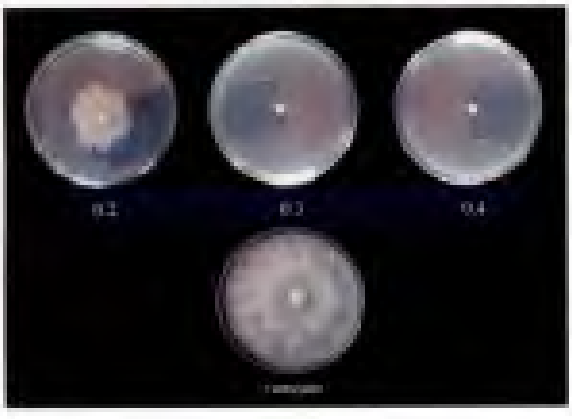
d. Tebuconazole 5EC

e. Bordeaux mixture

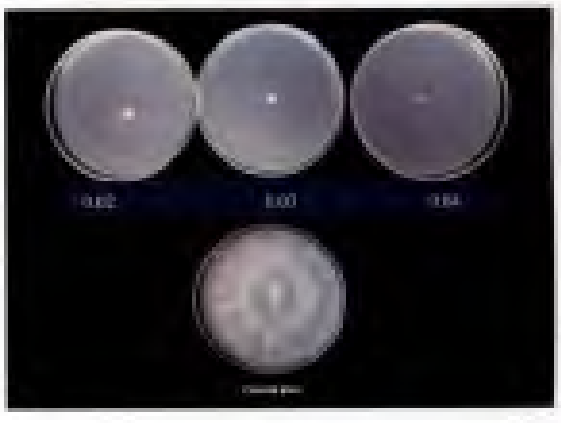
Plate 23 B. Effect of different levels of fungicides on radial growth of *Curvularia lunata*

a. Mancozeb 75WP

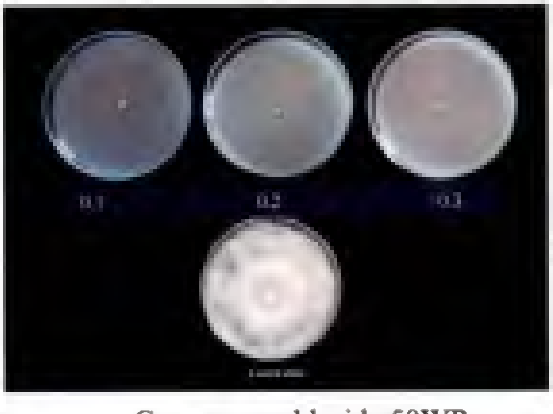
b. Copper hydroxide 77WP



c. Propineb 75WP



d. Trifloxystrobin 25% + Tebuconazole 50%

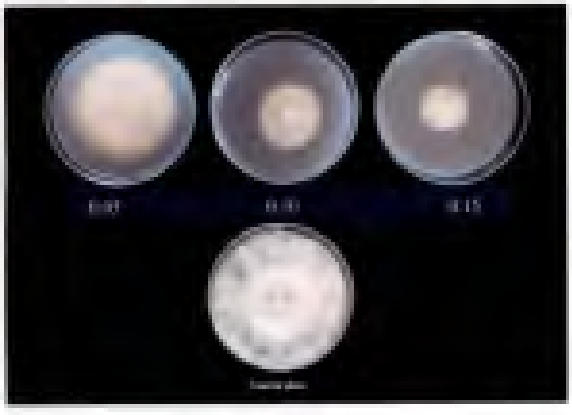


e. Copper oxychloride 50WP

Plate 24 A. Effect of different fungicides on radial growth of *Chonephora curcubitarum*

a. Chlorothalonil 75WP

b. Carbendazim 50 WP



c. Azoxystrobin 23SC

d. Tebuconazole 5EC

e. Bordeaux mixture

Plate 24 B. Effect of different fungicides on radial growth of *Chonephora curcubitarum*

superior and showed significantly higher inhibition of the pathogen at the field and higher concentration (Table 25 Plate 25). Even at the lower concentration mancozeb 70WP (Mega M-45) and copper oxychloride 50WP (Blitox) were significantly differed from other fungicides and recorded 100 per cent inhibition of the pathogen. Azoxystrobin 23SC (Amistar) was the least effective fungicide under *in vitro* conditions.

4.9.1.7. Fusarium equiseti

From the *in vitro* studies at the lower concentration propineb 70WP (Antracol), trifloxystrobin 25%+ tebuconazole 50% (Nativo), copper oxychloride 50WP (Blitox), carbendazim 50WP (Megastin) and tebuconazole 5EC (Folicur) were found to be superior fungicides with significantly higher inhibition of growth (Table 26 Plate 26A and 26B). Along with these fungicides, at the field and higher concentration Bordeaux mixture recorded cent per cent inhibition of the pathogen. Chlorothalonil 75WP (Kavach) at all the three concentrations showed the least inhibition against *Fusarium equiseti*.

4.9.2 *In vitro* evaluation of biocontrol agents

In vitro evaluation of fungal antagonist *Trichoderma viride* and bacterial antagonists *Pseudomonas fluorescens* and *Bacillus subtilis* were tested against seven fungal pathogens isolated from cabbage and cauliflower were done and is presented in Tables 27, 28 and 29 (Plate 27, 28 and 29). The method followed was dual culture technique (Dickinson and Skidmore, 1976).

4.9.2.1 Alternaria brassicicola

When *Trichoderma viride* were paired with, *Alternaria brassicicola* there was 66.6 per cent inhibition. Among the bacterial antgonists *Bacillus subtilis* recorded 48 per cent inhibition, whereas *Pseudomonas fluorescens* recorded only 21 per cent inhibition of the pathogen.

Table.25 *In vitro* evaluation of chemical fungicides on the inhibition of mycelial growth of *Pythium aphanidermatum*

Treatments	Chemical Fungicides (conc. %)	Trade name	Inhibition of mycelial growth of *Pythium aphanidermatum* (%)*		
			C-1	C-2	C-3
T1	Mancozeb 75WP (0.2, 0.3,0.4)	Mega M-45	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T2	Copper hydroxide 77WP (0.1,0.2,0.3)	Kocide	64.07 (53.24)	100.00 (90.00)	100.00 (90.00)
T3	Trifloxystrobin 25% + Tebuconazole 50% (0.02, 0.03,0.04)	Nativo	77.78 (61.97)	86.67 (68.63)	82.59 (65.32)
T4	Copper oxychloride 50WP (0.1, 0.2,0.3)	Blitox	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T5	Azoxystrobin 23SC (0.05, 0.1, 0.15)	Amistar	0.00 (0.00)	0.00 (0.00)	33.70 (35.47)
T6	Bordeaux Mixture (0.5, 1.0, 1.5)		78.15 (62.10)	100.00 (90.00)	100.00 (90.00)
CD			4.649	1.787	0.761
SE			1.492	0.574	0.244

*Mean of three values

**Values in parenthesis are angular transformed values C –concentration

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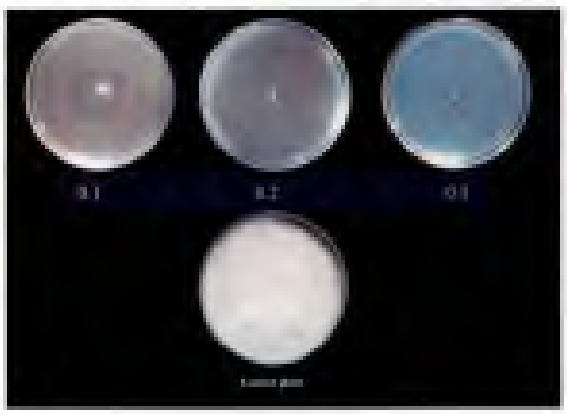
Table.26 *In vitro* evaluation of chemical fungicides on the inhibition of mycelial growth of *Fusarium equiseti*

Treatments	Chemical Fungicides (conc. %)	Trade name	Inhibition of mycelial growth of *Fusarium equiseti* (%)*		
			C-1	C-2	C-3
T1	Mancozeb 75WP (0.2, 0.3,0.4)	Mega M-45	70.00 (56.77)	76.30 (60.84)	99.63 (56.77)
T2	Copper hydroxide 77WP (0.1,0.2,0.3)	Kocide	51.85 (46.04)	78.15 (62.10)	80.37 (46.04)
T3	Propineb 75WP (0.2,0.3,0.4)	Anthracol	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T4	Trifloxystrobin 25%+ Tebuconazole 50 % (0.02, 0.03,0.04)	Nativo	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T5	Copper oxychloride 50WP (0.1, 0.2,0.3)	Blitox	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T6	Chlorothalonil 75WP (0.05, 0.1, 0.15)	Kavach	38.52 (38.31)	44.82 (42.00)	45.19 (42.00)
T7	Carbendazim 50WP (0.05, 0.1, 0.15)	Megastin	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T8	Azoxystrobin 23SC (0.05, 0.1, 0.15)	Amistar	67.40 (55.20)	67.78 (55.20)	68.52 (55.86)
T9	Tebuconazole 5EC (0.05, 0.1, 0.15)	Folicur	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T10	Bordeaux mixture (0.5, 1.0, 1.5)		76.67 (61.10)	100.00 (90.00)	100.00 (90.00)
CD			1.957	2.042	2.949
SE			0.659	0.687	0.993

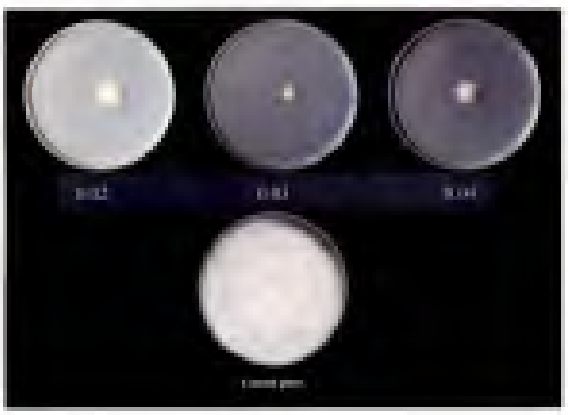
*Mean of three values

**Values in parenthesis are angular transformed values C -concentration

a. Mancozeb 75WP

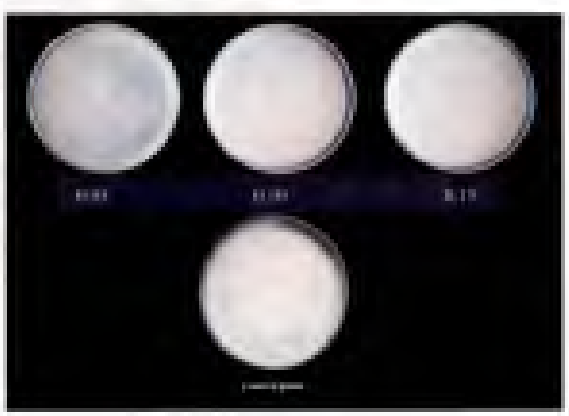


b. Copper hydroxide 77WP

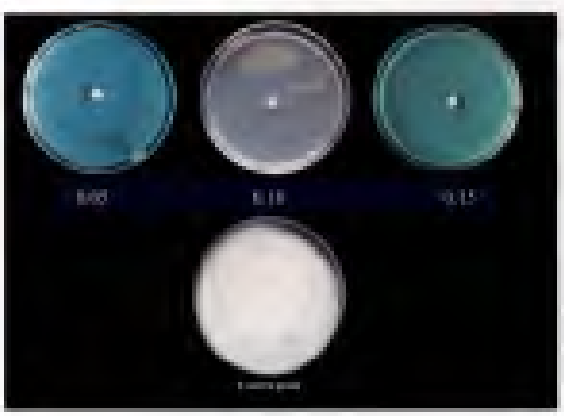


c. Trifloxystrobin 25% + Tebuconazole 50%

d. Copper oxychloride 50WP



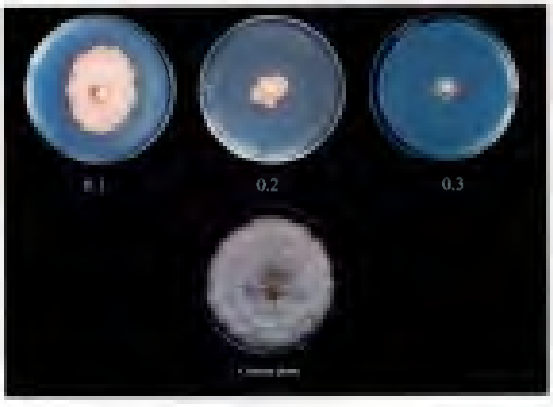
e. Azoxystrobin 23SC



f. Bordeaux mixture

Plate 25. Effect of different fungicides on radial growth of *Pythium aphanidermatum*

a. Mancozeb 75WP



b. Copper hydroxide 77WP

c. Propineb 75WP

d. Trifloxystrobin 25% + Tebuconazole 50%

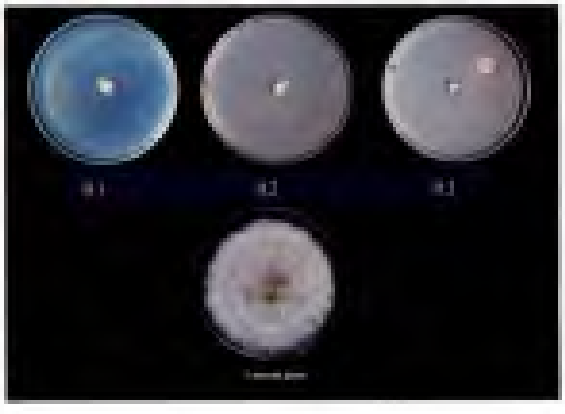


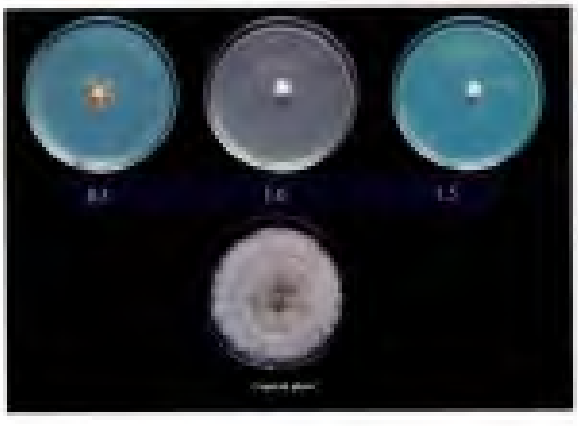
Plate 26 A Effect of different levels of fungicides on radial growth of *Fusarium equiseti*

a. Chlorothalonil 75WP

b. Carbendazim 50 WP

c. Azoxystrobin 23SC

d. Tebuconazole 5EC



e. Bordeaux mixture

Plate 26 B Effect of different levels of fungicides on radial growth of *Fusarium equiseti*

4.9.2.2 Rhizoctonia solani

In the case of *Rhizoctonia solani* bacterial antagonist, *Bacillus subtilis* showed higher inhibition of 48 per cent. Whereas *Pseudomonas fluorescens* and fungal antagonist *Trichoderma viride* showed 33 and 21 per cent inhibition of the pathogen.

4.9.2.3. Colletotrichum gloeosporioides

The response of *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* to *Colletotrichum gloeosporioides* showed almost similar per cent inhibition *ie*., 52, 48 and 44 per cent respectively.

4.9.2.4 Curvularia lunata

In vitro evaluation of biocontrol agents revealed that the fungal antagonist *Trichoderma viride* restricted the fungal growth by only 50 per cent. The bacterial antagonists were also recorded similar inhibition with 46 per cent by *Pseudomonas fluorescens* and 19 per cent by *Bacillus subtilis.*

4.9.2.5 Choanephora cucurbitarum

Both *Trichoderma viride* and *Bacillus subtilis* recorded more than 45 per cent inhibition of mycelial growth. While the bacterial antagonist *Pseudomonas fluorescens* inhibited only 30 per cent growth of pathogen.

4.9.2.6 Pythium aphanidermatum

In vitro evaluation of biocontrol agents revealed that among the three biocontrol agents, *Trichoderma viride* gave better inhibition of *Pythium aphanidermatum* and restricted its growth to 75 per cent. Both *Pseudomonas fluorescens* and *Bacillus subtilis* recorded more than 45 per cent inhibition of mycelial growth.

Table.27 Inhibition of selected pathogens by *Trichoderma viride* on PDA under dual culture

Sl. No.	Pathogen	Per cent inhibition of pathogen	Antagonistic reaction
1.	*Alternaria brassicicola*	66.66	O
2.	*Rhizoctonia solani*	21.12	O
3.	*Colletotrichum gleosporiodes*	51.94	C
4.	*Curvularia lunata*	50.18	O
5.	*Choanephora cucurbitarum*	46.21	O
6.	*Pythium aphanidermatum*	75.00	O
7.	*Fusarium equiseti*	48.33	O

O - Overgrowth
C - Cessation of growth

Table.28 Inhibition of selected pathogens by *Pseudomonas fluorescens* on PDA under dual culture

Sl. No.	Pathogen	Per cent inhibition of pathogen
1.	*Alternaria brassicicola*	48.30
2.	*Rhizoctonia solani*	33.89
3.	*Colletotrichum gleosporioides*	48.06
4.	*Curvularia lunata*	46.00
5.	*Choanephora cucurbitarum*	30.83
6.	*Pythium aphanidermatum*	50.28
7.	*Fusarium equiseti*	3.06

Table.29 Inhibition of selected pathogens by *Bacillus subtilis* on PDA under dual culture

Sl. No.	Pathogen	Per cent inhibition of pathogen
1.	*Alternaria brassicicola*	45.50
2.	*Rhizoctonia solani*	48.94
3.	*Colletotrichum gleosporiodes*	43.89
4.	*Curvularia lunata*	19.00
5.	*Choanephora cucurbitarum*	47.50
6.	*Pythium aphanidermatum*	45.83
7.	*Fusarium equiseti*	22.50

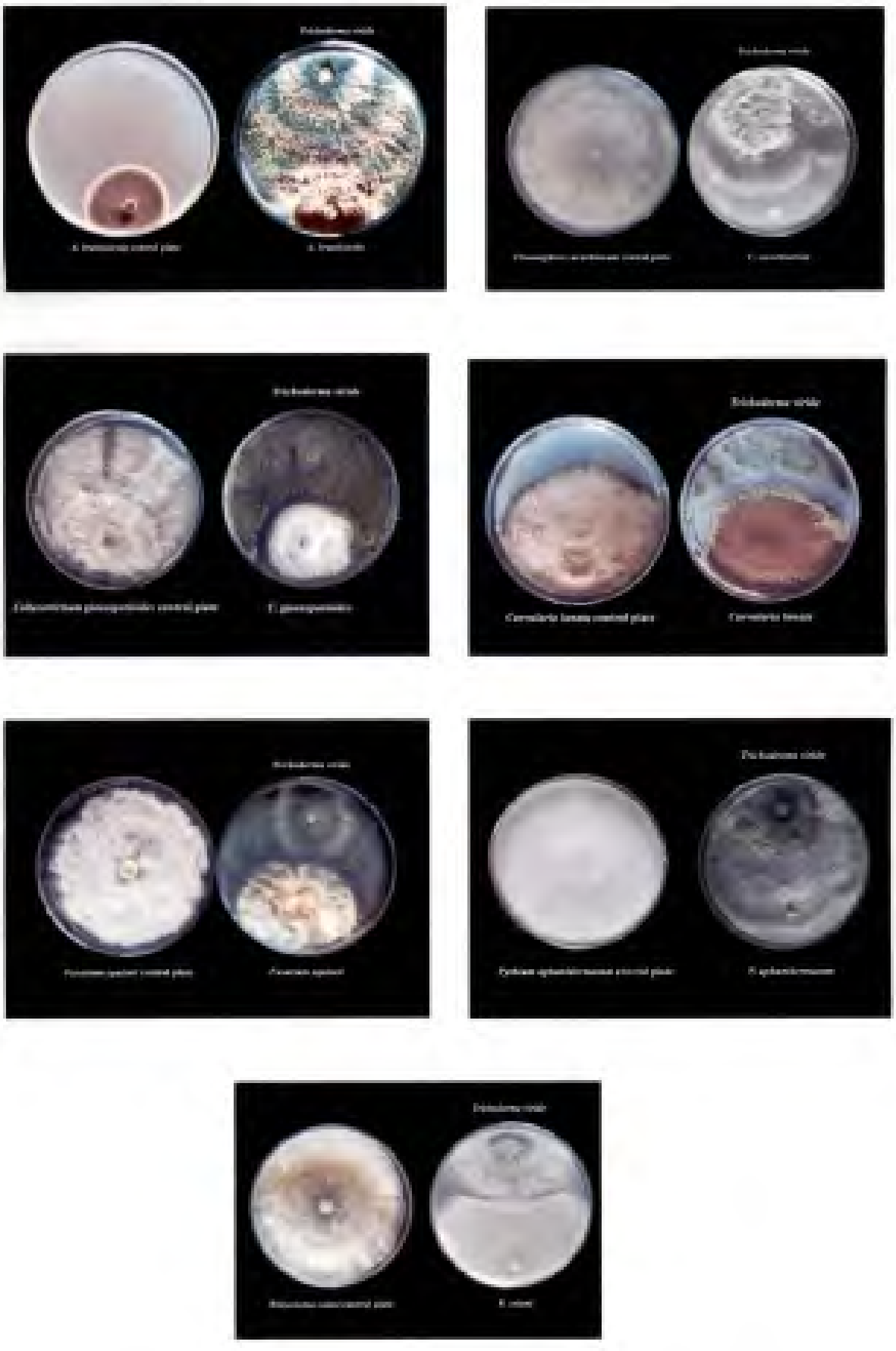


Plate 27 *In vitro* antagonism of *Trichoderma viride* against pathogens

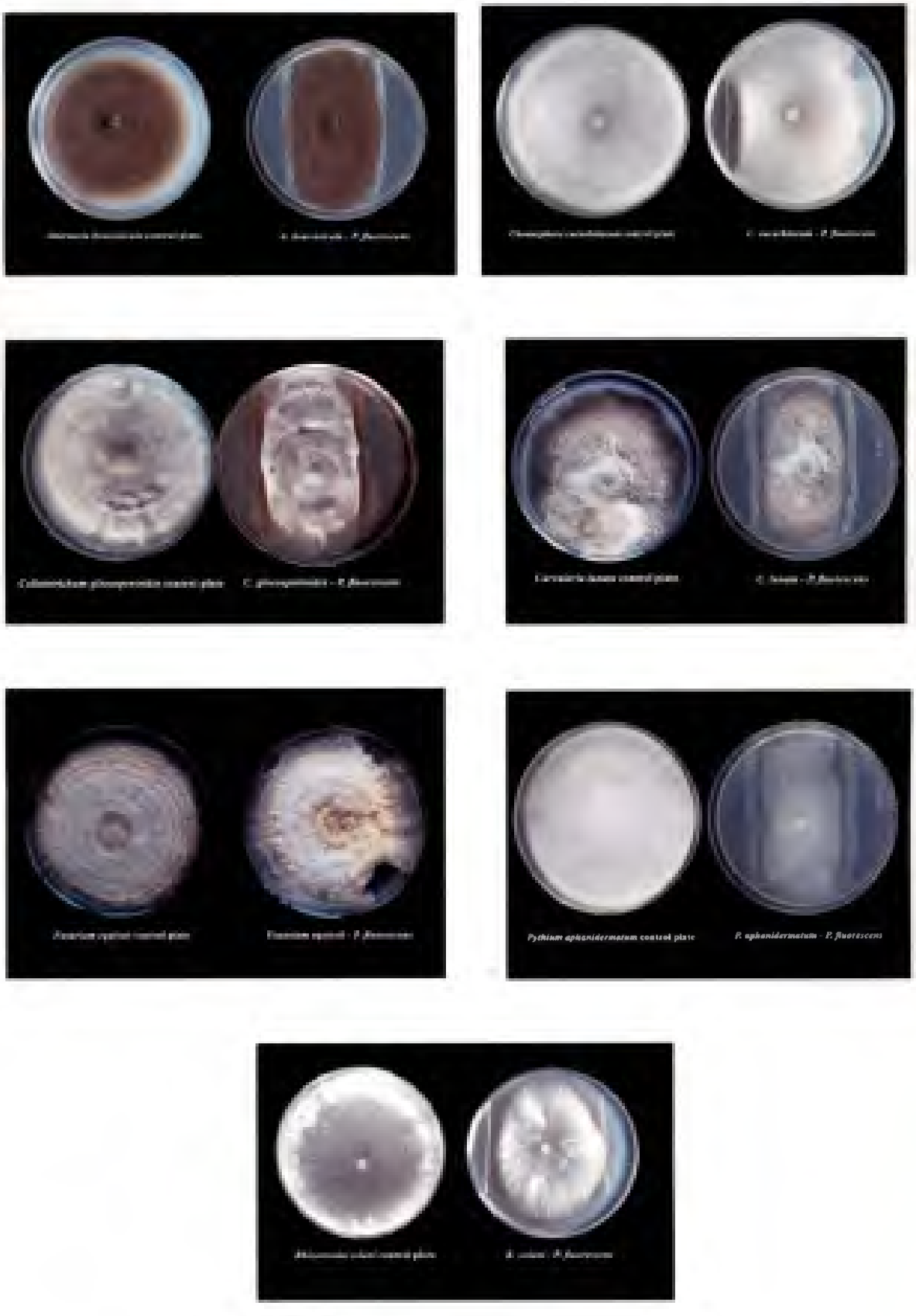


Plate 28 *In vitro* antagonism of *P. flourescens* against pathogens

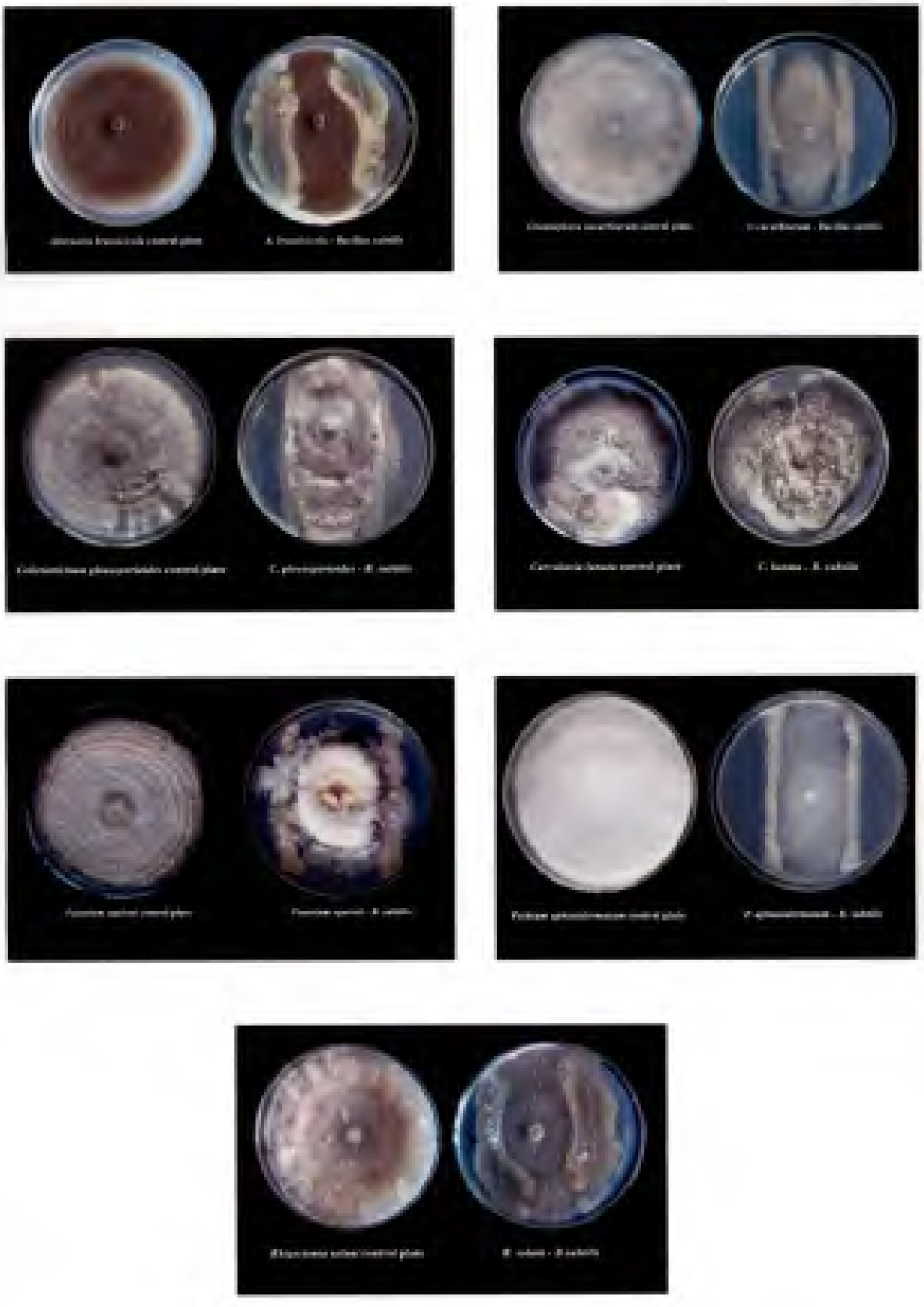


Plate 29 *In vitro* antagonism of *Bacillus subtilis*

4.9.2.7 Fusarium equiseti

Under *in vitro* studies it was observed that both the bacterial antagonists *Pseudomonas fluorescens* and *Bacillus subtilis* were less effective against *Fusarium equiseti*. But in case of *Trichoderma viride* 48.33 per cent inhibition was noticed against the pathogen *Fusarium equseti*.

4.10. EVALUATION OF FUNGICIDES AND BIOCONTROL AGENTS ON MAJOR FUNGAL DISEASES OF CABBAGE AND CAULIFLOWER UNDER *in vivo* CONDITIONS

Based on the purposive survey in nine locations of four districts, estimation of per cent disease incidence (PDI) and per cent disease severity (PDS) were carried out and three major pathogens *viz., Alternaria brassicicola, Rhizoctonia solani* and *Pythium aphanidermatum* were selected as the major disease causing pathogens. Among these three pathogens, *Alternaria brassicicola* and *Rhizoctonia solani* were common pathogens of both cabbage and cauliflower. Both the isolates were cross inoculated to confirm their pathogenicity. *Pythium aphanidermatum* had not taken infection on cabbage. Hence the efficacy of selected fungicides and biocontrol agents on the management of these three major fungal diseases of cabbage and cauliflower was done under *in vivo* conditions. Treatments were selected based on the efficacy shown in the *in vitro* evaluation and was applied as described in material and methods. Fungicides which showed inhibition above 60 per cent were selected for *in vivo* studies. Observations on disease incidence and per cent disease reduction were recorded. Effect of different treatments on the growth parameters of cabbage and cauliflower during the management of these three major pathogens were also taken (Plate 30). The number of leaves on 30th day and 60th day after plating as well as yield data were recorded for comparison. Details of the results are furnished below.

Plate 30 *In vivo* studies under green net house

4.10.1.1 *In vivo* evaluation of fungicides and biocontrol agents for management of *Alternaria brassicicola*

The pathogen was inoculated on cabbage plants of 60 days after transplanting and the disease symptoms appeared three days after inoculation and the first observation was made on the seventh day. There was no significance difference in disease severity and it ranged from 20.2-31.46 per cent (Table 30). From the data it is clear that ten days after the first spray of fungicides, T_1 (trifloxystrobin 25%+tebuconazole 50%) (0.03%) found to be the most effective with 38.13 per cent disease severity 48.05 per cent disease reduction over control. T_1 (trifloxystrobin25%+ tebuconazole 50 %) was on par with other four fungicides *viz.*,T_2 (Bordeaux mixture -1%), T_5 (mancozeb 75WP-0.3%), T_8 (carbendazim 50WP-0.1%), T_9 (tebuconazole 5EC-0.1%) with 42.86, 36.36, 31.17 and 46.75 per cent of reduction over control. The biocontrol agents T_{10} (*Bacillus subtilis*), T_{11} (*Trichoderma viride*) and T_{12} (*Pseudomonas fluorescens*) were equally effective with on par results of 40.26, 41.56, 32.47 per cent of reduction over control.

The remaining fungicides like copper hydroxide 77WP-0.2%, (12.9 per cent disease reduction over control) copper oxichloride 50WP -0.2% (7.79%), propineb 75WP -0.3% (27.27%) and chlorothalonil 75WP-0.1% (29.87%) were recorded least effective fungicide against *Alternaria brassicicola*.

After the second spray, further advancement of disease was observed among all the treatments with highest in the control (T_{13}). It was proved that the most effective fungicide against *Alternaria brassicicola* was T_1 (trifloxystrobin 25%+ tebuconazole 50%)-0.03% with least PDS of 49.57 with highest per cent disease reduction over control (50.48). This effect was on par with T_2 (Bordeaux mixture-1%), T_5 (mancozeb 75WP-0.3%), T_8 (carbendazim-0.1%), T_9 (tebuconazole 5EC-0.1%), and three of the antagonists, T_{10} (*Bacillus subtilis*), T_{11} (*Trichoderma viride*) and T_{12} (*Pseudomonas fluorescens*). These three biocontrol agents showed 39.07, 42.86 and 33.33 per cent of disease reduction over control.

Table.30 *In vivo* evaluation of fungicides and biocontrol agents for management of *Alternaria brassicicola* Leaf blight

Treatment no.	Treatments (Foliar spray)	Conc. (%)	7 days after inoculation	10 days after first spray		10 days after second spray	
			PDS	PDS	Per cent disease reduction over control	PDS	Per cent disease reduction over control
T_1	Trifloxystrobin 25%+ Tebuconazole 50 %	0.03	23.83	38.13	48.05	49.57	50.48
T_2	Bordeaux mixture	1	25.74	41.94	42.86	52.43	47.62
T_3	Copperhydroxide 77WP	0.2	22.88	63.87	12.99	94.38	5.71
T_4	COC 50 WP	0.2	24.79	67.69	7.79	90.57	9.52
T_5	Mancozeb 75WP	0.3	24.79	46.71	36.36	64.82	35.24
T_6	Propineb 75WP	0.3	25.74	53.39	27.27	70.55	29.52
T_7	Chlorothalonil 75WP	0.1	22.88	51.48	29.87	75.31	24.76
T_8	Carbendazim 50WP	0.1	23.83	50.52	31.17	66.73	33.33
T_9	Tebuconazole 5EC	0.1	24.79	39.09	46.75	50.52	49.52
T_{10}	*Bacillus subtilis*	$1x10^6$cfu/ ml	25.74	43.85	40.26	60.99	39.07
T_{11}	*Trichoderma viride*	2	24.79	42.90	41.56	57.20	42.86
T_{12}	*Pseudomonas fluorescens*	2	21.93	49.57	32.47	66.73	33.33
T_{13}	Control	-	25.74	73.40	0.00	100.1	0
	CD			12.81		20.62	

Disease severity was maximum of more than 90 per cent for treatments, T_3 (copper hydroxide 77WP-0.2%) andT_4 (Copper oxichloride-0.2% 50WP) and were found less effective against the *Alternaria brassicicola*.

4.10.1.2 Effect of different treatments on growth parameters of Cabbage during the management studies of *Alternaria* leaf blight

During *in vivo* studies on management of *Alternaria* leaf blight of cabbage, comparison of biometric observations were taken to get the effect of different fungicides and biocontrol agents. As per the results shown in the Table.31, the number of leaves observed on 30DAP had no significant effect among the treatments. But during 60DAP, all the fungicides and bio control agents were significantly superior to control except copper oxychloride and copper hydroxide which are having 9 and 11 leaves per plant. Regarding the yield of cabbage, highest yield was for T_9 (tebuconazole 5EC-0.1%) yield of 702.5 g/plant followed by trifloxystrobin 25%+ tebuconazole 50% (00.03%) (698.2g/plant) and T_2 Bordeaux mixture-1% (682.5 g/plant). All the treatments showed significant difference compared to control except, T_3 (copper hydroxide 77WP-0.2%) T_4 (copper oxichloride 50WP-0.2%), T_6 (propineb 75WP-0.3%) which showed poor performance in yield.

4.10.2 *In vivo* evaluation of fungicides and biocontrol agents for management of *Rhizoctonia solani*

Inoculation of *Rhizoctonia solani* was done on seedlings of 60 days after transplanting. On the second day symptom started and after seven days of inoculation, observation on disease severity for each treatment was taken (Table 32). Per cent disease severity ranged from 28.6 to 37.18 per cent after seven days of inoculation. First spray of fungicides were given as foliar treatment.

Based on disease severity of *Rhizoctonia* leaf blight, ten days after first spray, treatment T_4 (trifloxystrobin 25%+ tebuconazole 50%) -0.03%found to be

Table 31 Effect of different treatments on growth parameters of cabbage during the management studies of *Alternaria* leaf blight

Treatment No.	Treatments	Conc. (%)	Biometric observations		
			Number of leaves/plant		Yield (g/plant)
			30 days after planting	60 days after planting	
T_1	Trifloxystrobin 25%+ Tebuconazole 50 %	0.03	6.6	16.0	698.2
T_2	Bordeaux Mixture	1	6.3	16.6	682.5
T_3	Copper hydroxide 77WP	0.2	5.3	11.0	470.8
T_4	COC 50 WP	0.2	5.3	9.3	430.6
T_5	Mancozeb- 75WP	0.3	6.3	16.6	572.6
T_6	Propineb 75WP	0.3	6.6	14.6	490.7
T_7	Chlorothalonil 75WP	0.1	6.0	14.0	522.2
T_8	Carbendazim 50WP	0.1	6.3	15.6	540.8
T_9	Tebuconazole 5EC	0.1	6.6	16.3	702.5
T_{10}	*Bacillus1subtilis*	$1x10^6$cfu/ml	6.6	16.0	596.4
T_{11}	*Trichoderma viride*	2	6.6	15.3	656.6
T_{12}	*Pseudomonas fluorescens*	2	6.6	15.6	583.7
T_{13}	Control	-	6.0	10.3	488.9
	CD		1.7	2.5	20.3

Table. 32 *In vivo* evaluation of fungicides and biocontrol agents for management of *Rhizoctonia solani* Leaf blight

Treatment no.	Treatments (Foliar spray)	Conc. (%)	7 days after inoculation	10 days after first spray		10 days after second spray	
			PDS	PDS	Per cent disease reduction over control	PDS	Per cent disease reduction over control
T_1	Control	-	35.27	93.42	-	99.14	-
T_2	Chlorothalonil 75WP	0.1	31.46	49.57	46.93	67.68	31.73
T_3	Propineb 75WP	0.3	32.41	50.52	45.91	64.82	34.61
T_4	Trifloxystrobin 25%+ Tebuconazole 50 %	0.03	28.6	36.22	61.22	54.34	45.19
T_5	Mancozeb- 75WP	0.3	37.18	72.45	22.44	87.70	11.53
T_6	Tebuconazole 5EC	0.1	34.32	48.62	47.95	61.96	37.49
T_7	COC 50 WP	0.2	28.6	68.64	26.53	94.38	4.80
T_8	Copperhydroxide 77WP	0.2	30.50	57.2	38.77	77.22	22.11
T_9	Carbendazim 50WP	0.1	32.41	50.52	45.91	63.45	36.00
T_{10}	Bordeaux mixture	1	33.36	67.68	27.55	90.53	8.68
T_{11}	*Pseudomonas fluorescens*	2	36.22	60.06	35.71	76.26	23.07
T_{12}	*Trichoderma viride*	2	33.36	56.24	39.79	78.17	21.15
T_{13}	*Bacillus subtilis*	$1x10^6$cfu/ ml	31.46	52.43	43.87	70.54	28.84
	CD			12.414		14.845	

superior and recorded least severity of 36.22 per cent with maximum disease reduction (61.22%) over control. Treatments, T_6 (tebuconazole 5EC), T_2 (chlorothalonil 75WP), T_3 (propineb 75WP), T_9 (carbendazim 50WP) were having on par effect among chemicals. It was also observed that three of the biocontrol agents T_{11} (*Pseudomonas fluorescens*), T_{12} (*Trichoderma viride*) and T_{13} (*Bacillus subtilis*) were equally effective and are on par with the above four fungicides. Whereas treatments T_5 (mancozeb 75WP), T_7 (copper oxichloride 50WP) and T_{10} (Bordeaux mixture) recorded least disease severity and were less effective because disease reduction over control were only 22, 26 and 27 per cent.

After the second spray, it was confirmed that T_4 (trifloxystrobin 25%+ tebuconazole 50%)-0.03% was the best chemical for control of *Rhizoctonia* blight with 45 per cent disease severity reduction over control. The treatments T_3 (propineb 75WP), T_6 (tebuconazole 5EC), T_9 (carbendazim 50WP), and T_2 (chlorothalonil 75WP) were on par with each other with above fungicide. Treatments, T_5 (mancozeb 75WP), T_7 (copper oxichloride 50WP) and T_{10} (Bordeaux mixture) were least effective chemicals with a lower percent of severity reduction over control. The biocontrol agents *Trichoderma viride* (T_{12}), (T_{11}) *Pseudomonas fluorescens* and *Bacillus subtilis* (T_{13}) recorded disease reduction over control with a range of 21.15 to 28.46 per cent which were on par with the chemical copper hydroxide (T_8).

4.10.2.3 Effect of different treatments on growth parameters of Cabbage during the management studies of *Rhizoctonia* blight

Biometric observations were taken to get the effect of different fungicides and biocontrol agents in the *in vivo* studies on management of *Rhizoctonia* leaf blight of cabbage. *Rhizoctonia* had taken the infection earlier than *Alternaria* during challenge inoculation. Therefore the results of Table.33 showed a significant difference among the treatments in the number of leaves at 30 DAP compared to control except mancozeb, copper oxychloride and Bordeaux mixture.

Table 33 Effect of different treatments on growth parameters of cabbage during the management of *Rhizoctonia* leaf blight

Treatment No.	Treatments	Conc. (%)	Biometric observations		
			Number of leaves		Yield (g/plant)
			30 days after planting	60 days after planting	
T_1	Control	-	4.3	10.0	562.5
T_2	Chlorothalonil 75WP	0.1	6.3	15.3	673.9
T_3	Propineb 75WP	0.3	6.0	16.3	584.5
T_4	Trifloxystrobin 25%+ Tebuconazole 50 %	0.03	7.0	16.0	700.5
T_5	Mancozeb- 75WP	0.3	5.3	11.6	570.3
T_6	Tebuconazole 5EC	0.1	6.0	13.6	687.6
T_7	COC 50 WP	0.2	5.3	11.6	531.5
T_8	Copper hydroxide 77WP	0.2	6.3	15.3	565.5
T_9	Carbendazim	0.1	6.3	16.3	683.3
T_{10}	BM	1	5.6	11.6	587.7
T_{11}	*Pseudomonas fluorescens*	2	7.3	15.3	610.1
T_{12}	*Trichoderma viride*	2	7.0	16.3	652.8
T_{13}	*Bacillus subtilis*	$1x10^6$cfu /ml	6.3	15.3	596.6
	CD		1.50	2.1	20.4

The number of leaves on 60 DAP also had a similar significant effect among the treatments. When result data was analyzed, the highest yield of 700.5 g/plant, was for T_4 (trifloxystrobin 25%+ tebuconazole 50%) followed by T_6 (tebuconazole 5EC) (687.6) T_9 (carbendazim 50WP) (683.3 g/plant). All the treatments showed significant difference in the yield data compared to control, except T_5 mancozeb75WP, T_8 (copper hydroxide 77WP) T_7(copper oxychloride 50WP) Three of the biocontrol agents showed significantly higher yield compared to control, and T_{12} (*Trichoderma viride*) had given a higher value of head weight(652.8 g/plant).

4.10.3.1 *In vivo* evaluation of fungicides and biocontrol agents for management of *Pythium aphanidermatum*

Pythium aphanidermatum was inoculated on curds of cauliflower plants seventy five days after transplanting. On the second day symptom started and after seven days of inoculation, observation on disease severity for each treatment were taken (Table 34). The fungicides which are not effective against *Pythium* were not taken for the study, *viz.,* chlorothalonil 75WP, propineb 75WP, tebuconazole 5EC, carbendazim 50WP and azoxystribin 23SC. From the data it is clear that there was no significant difference of PDS after challenge inoculation with the pathogen and the PDS ranged from 10.67 to 20 per cent.

Treatment, T_1 (trifloxystrobin 25 % + tebuconazole 50%)-0.03% observed to be the best fungicide tested with 71.42 per cent disease reduction over control after first spray. Second effective fungicide was mancozeb 75WP (T_5) with 60 per cent reduction over control. Effect of biocontrol agents for the management of *Pythium aphanidermatum* were on par result with the above two fungicides. Treatment T_7 (*Trichoderma viride*), T_6 (*Bacillus subtilis*), T_8 (*Pseudomonas fluorescens*) were having 51.49, 58.28, and 51.42 per cent disease reduction respectively. Other treatments, T_3 (copper hydroxide 77WP) and r T_4 copper oxichloride 50WP recorded 42.85 and 28.57 per cent disease reduction. Among

Table.34 *In vivo* evaluation of fungicides and biocontrol agents for management of *Pythium* curd rot

Treatment no.	Treatments (Foliar spray)	Conc. (%)	7 days after inoculation	10 days after first spray		10 days after second spray	
			PDS	PDS	Per cent disease reduction over control	PDS	Per cent disease reduction over control
T_1	Trifloxystrobin 25 % + Tebuconazole 50%	0.03	15.00	16.67 (23.84)	71.42	20.00 (26.55)	78.57
T_2	Bordeaux mixture	1	11.00	46.67 (42.98)	20.00	73.33 (60.76)	21.42
T_3	COC 50WP	0.2	20.00	41.67 (40.15)	28.57	63.33 (52.84)	32.14
T_4	Copper hydroxide 77WP	0.2	17.67	33.33 (34.98)	42.85	60.00 (51.83)	35.71
T_5	Mancozeb 75 WP	0.3	10.67	23.33 (28.65)	60.00	43.33 (41.05)	68.57
T_6	*Bacillus subtilis*	$1x10^6$cfu/ml	13.00	16.67 (23.97)	51.49	23.33 (28.65)	57.00
T_7	*Trichoderma viride*	2	13.33	15.00 (22.00)	58.28	23.33 (28.65)	62.00
T_8	*Pseudomonas fluorescens*	2	15.00	28.33 (31.76)	51.42	50.00 (44.69)	46.42
T_9	Control	-	18.33	58.33 (49.81)	0.00	93.33 (75.21)	0.00
	CD			11.01		19.76	

the fungicides T_2 (Bordeaux mixture) was least effective with only 20 per cent disease reduction.

After the second spray treatment T_1 (trifloxystrobin 25 % + tebuconazole 50%) was leading in the case effectiveness with 78.57 per cent disease reduction. T_5 (mancozeb 75WP) was the next best fungicide with 68.57 per cent disease reduction. The antagonists T_7 (*Trichoderma viride*), T_6 (*Bacillus subtilis*) and *Pseudomonas fluorescens* were again proved to be better performers with 62.00, 57.00 and 46.42 per cent disease reduction respectively. T_2 (Bordeaux mixture) was least effective with less than 50 percent reduction in the disease.

4.10.3.2 Effect of different treatments on growth parameters of Cabbage during the management studies of *Pythium* curd rot

During the management studies of Pythium curd rot, effects of treatments on growth parameters of cauliflower were taken. Pythium was not found to be attacking on leaves. Therefore effects of different treatments on number leaves were not directly affecting the disease severity. The results of Table.35 showed that there is no significant difference among the treatments in the number of leaves at 30 DAPS as well as 60DAP, compared to control. The yield data showed significant differences among treatments. Highest yield of curd weight 524.7 g/plant, was for T_5 (mancozeb 75WP)-0.3% followed by T_1 (trifloxystrobin 25%+ tebuconazole 50%) -0.03% (505.5 g/plant) and T_7 (*Trichoderma viride*) (503.7 g/plant). T_2 Bordeaux Mixture and T_6 *Bacillus subtilis* were also on par with the above treatments. All the remaining treatments like T_3, copper hydroxide 77WP, T_4 copper oxychloride 50WP and T_8 *Pseudomonas fluorescens* showed non-significant difference in the yield data compared to control.

Table 35 Effect of different treatments on growth parameters of Cauliflower during the management of *Pythium* curd rot

Treatment No.	Treatments	Conc. (%)	Biometric observations		
			Number of leaves		Yield (g/plant)
			30 days after planting	60 days after planting	
T_1	Trifloxystrobin 25 % + Tebuconazole 50%	0.03	6.6	18.3	505.5
T_2	Bordeaux mixture	1	6.0	18.3	482.8
T_3	Copper hydroxide 77WP	0.2	5.3	17.6	468.5
T_4	COC 50WP	0.2	5.3	17.6	461.7
T_5	Mancozeb 75 WP	0.3	6.0	14.6	524.7
T_6	*Bacillus subtilis*	$1x10^6$cfu /ml	6.0	16.3	473.3
T_7	*Trichoderma viride*	2	6.6	16.0	503.7
T_8	*Pseudomonas fluorescens*	2	6.0	16.3	441.4
T_9	Control	-	5.2	16.0	450.2
	CD		1.5	2.6	19.6

Discussion

5. DISCUSSION

Cabbage and cauliflower are the most widely cultivated cole crops in India. As per the Annual report (2015) of Indian Institute of Vegetable Research, India stand in the second position in the global ranking with reference to area, production and productivity in cabbage and cauliflower. Previously cabbage and cauliflower were the sole crops of high ranges of Kerala. But by the development of tropical hybrids, cultivation of these temperate vegetables can be possible anywhere in Kerala.

One of the main constraints in the production of these crops is the occurrence of diseases, mainly of fungal origin. Detailed systematic studies on the identification and characterization of these fungal pathogens were not undertaken in Kerala. It is in the light of this reasons, the present study on the Characterization and management of fungal diseases of cabbage and cauliflower was undertaken. The studies were carried out as per the technical programme during the period 2015-2017 at the Department of Plant Pathology, College of Horticulture, Vellanikkara, Thrissur, and College of Agriculture, Padannakkad, Kasargod, Kerala and the results are discussed in this chapter.

5.1 SURVEY AND COLLECTION OF DISEASED SAMPLES AND ISOLATION

A purposive sampling survey was conducted in nine locations of four districts *viz.,* Kasargod, Thrissur, Wayanad and Idukki for the collection of diseased samples of cabbage and cauliflower and recorded the disease incidence and severity during crop season of 2015-17. The diseased specimens were collected from these locations and the pathogens were isolated. The survey revealed that, the maximum disease incidence was in Leaf spot-1 of cabbage in Ambalavayal of Wayanad distrcict (69.3 per cent) and maximum disease severity was in Leaf bligt-2 of cabbage in Chullikkara of Kasargod district. (68.3 per cent). Out of the eight types of diseases in cabbage Leaf blight-1 and Leaf blight -2 were the most severe diseases. But in the case of cauliflower, curd rot was identified as most destructive one because both disease incidence and severity were maximum

for curd rot-2 in Chullikkara area. In both the places, intense cultivation and poor management practices might be the reason for severe occurrence of the fungal diseases. Due to the recent climatic changes in Kerala, many fungal pathogens which were reported as minor, are now emerging as new threats for the vegetable cultivation. Based on the symptoms found in the diseased samples and microscopic studies the different isolates were grouped into eight categories *viz.*, Leaf blight -1 and 2, leaf spots -1, 2 and 3, head/curd rot- 1, curd rot-2, damping off and were given names according to the name of suspected pathogens. Leaf blights were the major pathogens during the survey and they were identified as *Alternaria* sp. and *Rhizoctonia* sp. Elven isolates of *Altenaria* were isolated from both cabbage and cauliflower from selected districts of Kerala. These results are in accordance with the studies of Gopalakrishnan (2007) and Ajitkumar *et al.* (2014). Several researchers reported dark leaf spot of crucifers caused by *Alternaria* sp. from India (Shrama *et al.* 2004, Sharma *et al.* 2014, Thakur 2015).

Leaf blight-2 suspected as *Rhizoctonia* sp. was isolated from blight symptoms on cabbage and cauliflower. Many researchers recorded Rhizoctonia blight from cabbage around the world (Zhang *et al.* 2009, Hua *et al.* 2014, Yang *et al.* 2004, Abawi and Martin, 1985).

Three types of leaf spots were observed from surveyed localities, which were suspected as *Cercospora, Colletotrichum* and *Curvularia.* Sinha and Singh (1995) reported the incidence of *Cercospora brassicola* on mustard. Mahmodi *et al.* (2013), He *et al.* (2016) isolated *Colletotrichum truncatum* from Chinese cabbage and Chinese flowering cabbage. Different species of this leaf spot pathogen *Curvularia brassicae* was observed as early as 1979 by Mohan and Mukerji.

One pathogen caused leaf / head /curd rot which was suspected as *Choanephora* sp. was noticed on both cabbage and cauliflower. A number of workers isolated *Choanephora* sp. from Brassica family. Pornsuriya *et al.* (2017) reported *Choanephora cucurbitarum* infection on Brassica vegetables. From India Gogoi *et al.* (2016) reported leaf rot by *Choanephora cucurbitarum* on cauliflower.

Curd rot-2 of cauliflower, which was suspected as *Pythium* sp.was observed during the survey. Curd rot complex by many fungal and bacterial pathogens was reported by Chakrabarty (1993). The fungal pathogens reported by them were *A.brassicicola, B.cinerea, F.equiseti, P.aphnidermatum, R.solani, S.sclerotiorum, P. tropica* and the bacteria *Erwinia crotovora.* But the curd rot pathogen obtained from cauliflower at Chullikkara of Kasargod was *Pythium* and it was observed as a single pathogen causing curd rot in cauliflower. *Pythium* infecting in curd and causing rot is a first report in India.

Damping off of cauliflower seedling was a noted disease during the study, in which, *Fusarium* was the suspected pathogen. Rimmer *et al.* (2007), Srivastava *et al.* (2011) reported fusarium yellows on Brassica vegetables caused by many *Fusarium* species .Humanuan *et al.,* 2012 reported the foot and root rot disease of cauliflower caused by *Fusarium* sp.

5.2. PATHOGENICITY OF ISOLATES

Artificial inoculation of suspected pathogens *viz., Alternaria, Rhizoctonia, Colletotricum, Curvularia, Choanephora, Pythium, Fusarium* on whole plant and detached leaves were done and pathogenicity was proved for each isolate. The leaf spot -1 pathogen, which was suspected as *Cercospora* sp.could not be isolated in the medium. The methods employed for testing pathogenicity varied with the nature of isolate. The symptoms produced by both the leaf blight pathogens during pathogenicity tests were similar to those in the survey fields. Rahimloo and Ghosta (2015) experimentally proved pathogenicity of *Alternaria brassicicola* by mycelial bit inoculation on detached leaves of cabbage crop. Sharma *et al.*, 2013, could prove the pathogenicity of *Alternaria brassicae* on cauliflower leaves and they found that isolates from Kerala were less pathogenic with a lesion size less than 0.5cm, than Tamil Nadu, Utter Pradesh and Delhi. This result showed that after three years from 2013 to 2017 the pathogen *Alternaria* could establish in fields of Kerala and came out as a major pathogen.

The results of the present study are in line with the findings of Zhang *et al.* (2009) where they could prove pathogenicity of *Rhizoctonia solani* in

Cabbage. *Rhizoctonia solani* is having wide host range and this fungus causes diseases on economically important crops. During the survey it was observed that most of the vegetables grown near cabbage and cauliflower were attacked by this pathogen. Wide host range of *Rhizoctonia* was reported earlier in 1996 by Tu *et al*. They have reported that *Rhizoctonia solani* attacked a number of crops under the families *viz*., Papilionaceae, Solanaceae, Cucurbitaceae, Chenopodiaceae, Cruciferae and Umbelliferae. In the upcoming years, *Rhizoctonia* will become a serious threat to crops by emerging as a universal pathogen.

Leaf spot -1 was observed with a lesser severity and was suspected as *Cercospora*. Smith (2012) observed *Cercospora* attack on turnip, mustard, broccoli, colards and kale. The leaf spot -2 pathogen was suspected as *Colletotrichum*. Different species of *Colletotricum* was observed by many workers *viz*., *Colletotrichum capsici* on *Brassica chinensis* (Mahmodi*et al*., 2013), *Colletotrichum truncatum* on Chinese flowering cabbage (He *et al*., 2016). The leaf spot pathogen -3 was suspected as *Curvularia*. In 1979, Mohan and Mukerj reported different species of this leaf spot pathogen *Curvularia brassicae*. Chaudary *et al*. (2016) proved pathogenicity of *Curvularia lunata* by spraying spore suspension on healthy Brinjal plants.

Gogoi *et al*. (2016) observed typical soft rot symptoms and signs of Choanephora rot on cauliflower by mycelial bit inoculation method. Moreover Pornsuriya *et al*. (2017) confirmed the pathogenicity of *Choanephora cucurbitarum* in Chinese cabbage by spraying spore suspension on healthy plants.

Likewise, the Koch postulates as pathogenicity test for fungal pathogen Pythium is in congruence with the results of Tanina *et al*. (2004) where they observed isolates of *Pythium ultimum* pathogenic on Chinese cabbage by wounding and placing of 5mm diameter agar plugs on midrib of the plants. Ramchandra and Bhatt (2011) could isolate and prove the pathogenic nature of *Fusarium equiseti* from wilted cumin plants. Hence the pathogenicity test carried out in the present investigation proved that all the isolates were disease causing

agents in cabbage and cauliflower reproducing distinct symptoms observed as in naturally infected plants.

5.3 SYMPTOMATOLOGY OF THE PATHOGENS

Symptomatology of each pathogen was studied under field conditions. As observed in the present study, Singh (1987) observed initial small dark spots of Alternaria infection on crucifers. Later the lesion size increased and characterized by concentric circle and yellow halo around them. Many authors reported similar symptoms by Alternaria on Brassica vegetables (Kumar *et al.* 2014, Viet *et al.* 2015).

Symptoms of the *Rhizoctonia solani* infection on cabbage and cauliflower was observed as brown lesions on leaves which resulted in blighting and decay of leaves and head. Such kind of disease symptoms were also reported by Wellman (1932), Abawi and Martin (1985), Yang *et al.* (2007).

Among the leaf spot diseases symptoms of *Cercospora* appeared as irregular tan spots with yellow halos and brown margins. Smith (2012) observed similar symptoms of *Cercospora* disease on turnip, mustard and broccoli. Symptoms of *Colletotrichum* infection was characterized by small circular gray to straw coloured spots with definite margin.Goutham (2014) also reported similar symptoms of *Colletotrichum gloeosporioides* on vegetable crops.Mahmodi*et al.* (2013) observed similar symptoms of infection by *Colletotrichum capsici* on *Brassica chinensis*. Likewise, He *et al.* (2016) reported the same by *Colletotrichum truncatum* on Chinese flowering cabbage.

Leaf spot by *Curvularia* appeared as dark greyish brown spots on the margin of the leaves. Occurrence of this pathogen in vegetables is very rare in Kerala. Similar symptoms were reported by Chaudary *et al.* (2016) on brinjal leaves as dark brown circular spots later coalesced and formed large oblong lesions.

Typical soft rot symptoms were noticed on both cabbage and cauliflower leaves. The pathogen *Choanephora cucurbitarum* was isolated from rotted leaves of cabbage and cauliflower, which at later stages develop as head/curd rot. Similar symptoms of rotting and curling of cauliflower leaves were observed by Gogoi *et al.* (2016). In another study by Pornsuriya *et al.* (2017) reported soft rot symptoms by the pathogen on Chinese cabbage which is also having analogous results with the present study.

The pathogen associated with curd rot in cabbage was suspected as *Pythium aphanidermatum*. The pathogen caused water soaked lesions on the base of the curd. Affected areas get softened and turned brown. A fluffy white mycelium was visible on the surface of rotted areas. The occurrence of *Pythium aphanidermatum* on cauliflower has not been reported so far, however similar symptoms were reported from other Crucifers (Drescler 1925, Taina *et al.* 2004).There are several reports of *Pythium aphanidermatum* attacking on cabbage and cauliflower but all these reports were about damping off symptoms. Emergence of *Pythium aphanidermatum* as a major pathogen at a different site of attack can be due to the changes in the adaptability of this fungus. This alteration may be caused by different factors *viz.*, variations in the genetic levels of the pathogen or the climatic changes in Kerala.

Damping off of seedling was a specifically observed symptom during this study. The pathogen was suspected as *Fusarium* and wilting of the seedlings with yellowing of the lower leaves was noticed as initial symptoms later rotting of collar area was seen. Similar observation was made by Ramachandra and Bhatt (2011) on cumin plants.

Among the eight fungal pathogens isolated, *Alternaria*, *Rhizoctonia* and *Choanephora* when cross inoculated in cabbage and cauliflower, they were found to attack both the crops. Ismail *et al.* (2012) reported that the fungal pathogens causing dark stem lesions and damping off symptoms in seedlings of cauliflower are *Fusarium*, *Alternaria* and *Curvularia*. These reports showed the polyphagous

nature of these pathogens for better survival due to the changes in the micro and macroclimate.

5.4. CULTURAL AND MORPHOLOGICAL CHARACTERISTICS OF PATHOGENS

Cultural and morphological characters are important tools in identification and classification of fungi. The fungal pathogens were temporarily identified up to the genus level based on the studies of symptomatology, cultural and morphological characters. The molecular characterization of seven selected pathogens were carried out at Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram by ITS sequencing to identify at species level. Leaf blight -1and Leaf blight-2 were identified as *Alternaria brassicicola* and *Rhizoctonia solani,* Leaf spot -2 and 3 as *Colletotrichum gleosporioides* and *Curvularia lunata,* head/curd rot-1 as *Choanephora cucurbitarum*, curd rot -2 as *Pythium aphanidermatum* and damping off as *Fusarium equiseti.*

The pathogen *Alternaria* produced deep olive green colour colony with concentric zonations at regular intervals and golden to brown coloured conidia in chains in both on cabbage and cauliflower. Characters of condiophores, conidia were well studied and they are comparable with the reports of Sharma *et al.* (2013). They observed that the isolates of *Alternaria brassicae* on cauliflower from Rajastan, Kerala and Tamilnadu were closey related. All the characters were in accordance with those reported by Kumar *et al.* (2014), Sharma *et al.* (2014), Rimmer *et al.* (2007), Rahimloo and Ghosta (2015) on cauliflower, mustard and cabbage.

Cultural and morphological characters of the leaf blight causing pathogen in cabbage and cauliflower, *Rhizoctonia* were also undertaken and these results are in accordance with the description given by Rimmer *et al.* (2007), Zhang *et al.* (2009) and Shim *et al.* (2013) on cabbage and Chinese cabbage.

All the leaf spot pathogens were isolated from cabbage only. The leaf spot -1 pathogen *Cercospora* was identified based on conidial characters and it could

not be recovered in culture. Therefore based on the characters of conidiophores, conidial size and their filiform shape the pathogen was identified as *Cercospora* sp. Similar observations were made by Meeboon *et al.,* 2007 from the brassica vegetables.

The leaf spot pathogen -2 was identified as *Colletotrichum* with the help of colony colour as whitish grey colonies with concentric zonations and conidial characters as, cylindrical to oval with both apices rounded. Chowdappa *et al.* (2012) and Sharma and Kulshrestha (2015) also described these characters, while studying the fungus on orchid and vegetables.

The morphological and cultural characters of causal agent of leaf spot-3, *Curvularia* was also studied in detail. Characterisation of *Curvularia brassicae* was done in 1979 itself by Mohan and Mukerji. They reported the characters of conidia as dark brown, 20-27.5 x7.5-12.5 µm size, three septate, which was similar to the noted characters in this study. The results were supported by Aktar and Shamsi (2016) during their study on *Tagetes* spp.

Study on head /curd rot pathogen *Choanephora* got from cabbage and cauliflower produced creamy white colony with cottony aerial mycelium and sporulation was observed on the periphery of the petri plate. Characters of sporangiophores, monosporous sporangiola and sporangiospores were as described by Kwon and Jee (2005), Gogoi *et al.* (2016) and Pornsuriya*et al.* (2017) during their studies in brinjal, cauliflower and Chinese cabbage respectively.

Tanina *et al* (2004), studied the characters of oogonia, antheridia, oospore of the fungus *Pythium aphanidermatum*. These characters are analogous to the findings obtained in the present study. Similar observations were also made by Gherbawy *et al.* (2005) on beans. Al-Sheik *et al.* (2012). Parveen and Sharma (2015) also reviewed morphology of *Pythium aphanidermatum*, from crucifers and found that the pathogen produced aplerotic oospores, which was exactly same observation as in the present study. Rajalaksmi *et al.* (2016) observed white

fluffy, dense mycelial growth of *Pythium aphanidermatum* from ginger on PDA within 24 hours, with coenocytic mycelium measuring 3 to 4 µm in diameter, which was also a related observation in the present study.

The damping off pathogen *Fusarium* produced dense white mycelium with irregular borders and produced both micro and macro conidia in the culture. These results are according to the findings of Ramchandra and Bhatt (2011) and Lazreg *et al.* (2014).

5.5. EFFICACY OF FUNGICIDES AND BIOCONTROL AGENTS AGAINST PATHOGENS UNDER *in vitro* CONDITIONS

The seven pathogens obtained in culture were used for *in vitro* evaluation of fungicides and biocontrol agents. Efficacy of ten selected fungicides and three biocontrol agents were tested against these pathogens.

5.5.1. *In vitro* evaluation of fungicides

In vitro evaluation of fungicides on the inhibition of the pathogen, provides preliminary information and serves as a guide for field testing. *In vitro* evaluation of fungicides against *Alternaria brassicicola* showed that among ten fungicides tested best three fungicides were trifloxystrobin 25% + tebuconazole 50% (Nativo), tebuconazole 5EC (Folicur) and Bordeaux mixture. Because they showed 100 percent inhibition in all the three concentrations.Singh and Rai (2003) stated that under *in vitro* conditions chlorothalonil (0.15 %) observed to be most effective fungicide reducing the mycelial growth of *Alternria alternata* causing blight in brinjal. Sidlauskiene *et al* (2003) observed 88-93 per cent disease reduction in Alternaria leaf spot on tomato, cucumber and cabbage after treatment with azoxistrobin (0.15%). In the current study, chlorothalonil and azoxystrobin showed 67 and 70 per cent inhibition over control. These results are in accordance with the present studies eventhough they were not the best effective fungicides. Similar studies were undertaken by other workers also. which had shown comparable results.On *Alternaria alternate* causing blight of tomato Singh and Singh (2006) observed the efficacy of seven fungicides viz., chlorothalonil,

copper oxychloride, azoxystrobin, Propineb, copper hydroxide, mancozeb at varying concentrations *viz*, 2500, 2000, 1000, 500 and 250 ppm. From this study these three fungicides found to be most effective followed by Mancozeb (11.4%). From this studies of TU *et al.* (2015) the severity of the disease reduced in cabbage when treated with tebuconazole (4.62%), trifloxystrobin +tebuconazole (6.01%) and propiconazole (9.45%).

In vitro studies with Rhizoctonia revealed that the fungicides ,tebuconzole 5EC (Folicure), carbendazim, copper oxychloride 50 WP (Blitox), trifloxystrobin 25% + tebuconazole 50% (Nativo) and propineb 70 WP (Antracol) at all the three concentrations showed 100 per cent inhibition of the pathogen. Similar results were obtained for Sriraj *et al.* (2014) even at the lowest concentration of 10ppm Nativo and Bavistin recorded effectiveness against *Rhizoctonia.*

Among the ten fungicides tested, five chemicals showed complete inhibition of mycelial growth of *Colletotrichum gloeosporioides. viz.,* trifloxystrobin 25% + tebuconazole 50% (Nativo), copper oxychloride 50 WP (Blitox), carbendazim, tebuconazole 5EC (Folicure), and Bordeaux mixture recorded 100 per cent inhibition at all the three concentrations. Prashanth *et al.* in 2009, showed inhibition of 68.34 per cent by carbendazim (0.1%), 67.51 per cent by mancozeb (0.25%) and 64.88 per cent by copper oxychloride (0.3%) against *Colletotrichum* sp. From pomegranate.

In vitro studies for *Curvularia lunata* revealed that fungicides such as tebuconazole 5 EC (Folicure), trifloxystrobin 25% + tebuconazole 50% (Nativo) and propineb 70 WP (Antracol) at all the three concentrations showed 100 per cent inhibition. Similar observation was obtained for Pawar (2012) as the complete inhibition of the pathogen by mancozeb (0.2 %) tricyclazole (0.1%) and mancozeb + carbendazim (0.25%) inhibition.

Under *in vitro* condition at the recommended dose 100 per cent inhibition of growth was recorded by six chemicals, viz., mancozeb , copper oxy chloride, captan + hexaconazole, carboxin, carbendazim + mancozeb and propiconazole for

Choanephora cicurbitarum. Cent per cent inhibition of the fungal growth was achieved by chemicals like copper oxychloride 50 WP (Blitox), tebuconazole 5EC (Folicure) and Bordeaux mixture. The results of *in vitro* evaluation of fungicides against *Pythium aphanidermatum* revealed that at field conditions, six chemicals *viz.* mancozeb 70WP (Mega M-45), copper hydroxide 77 WP(Kocide), propineb 70 WP (Antracol), copper oxychloride 50 WP (Blitox), tebuconazole 5EC (Folicure) and Bordeaux mixture gave cent per cent inhibition to the growth of the pathogen.

In vitro evaluation of five fungicides such as propineb 70 WP (Antracol), trifloxystrobin 25% + tebuconazole 50% (Nativo), copper oxychloride 50 WP (Blitox), carbendazim and tebuconazole 5 EC (Folicure) recorded cent per cent inhibition of the *Fusarium equiseti.* Akhtar *et al.* (2017) observed 98 per cent reduction in growth of *Fusarium oxysporum* f.sp. *lycopersici* by Nativo as compared to control on Tomato.

Among the fungicides Bordeaux mixture, trifloxystrobin 25% + tebuconazole 50% (Nativo) was the most effective fungicides for all the seven genus of fungi tested, followed by tebuconazole 5EC (Folicure) in all three concentrations under *in vitro* conditions.This shows that even after years of fungicide research, Bordeaux mixture is stands foremost in its effectiveness. Inhibition of various biosynthesis processes in the fungus will be the reason for effectiveness of both the new generation fungicides, trifloxystrobin 25% + tebuconazole 50% (Nativo) and tebuconazole 5EC (Folicure).

5.5.2. *In vitro* evaluation of bio control agents

The use of fungicides to control diseases is not advisable especially in vegetables. Chemical methods are not ecofriendly and therefore biological control of diseases had gained a momentum. Fungal and bacterial antagonists can be effectively used for management of vegetable diseases.

In vitro evaluation of fungal antagonist *Trichoderma viride* and bacterial antagonists *Pseudomonas fluorescens* and *Bacillus subtilis* were tested against seven fungal pathogens isolated from cabbage and cauliflower.

When *Trichoderma viride* was paired under dual technique with *Alternaria brassicicola* there was 66.6% inhibition. But for bacterial antagonists the *Pseudomonas fluorescens* and *Bacillus subtilis* per cent of inhibition was only 48 % and 45 % respectively. Sharma and Sharma (2006) observed 62.85 % inhibition of mycelial growth of *Alternaria brassicae* by *Bacillus subtilis.* Thakur (2015) found out that among the different antagonists evaluated, *Trichoderma harzianum* was most effective (74.2% inhibition) on *Alternria brssicicola* followed by that of *T. viride* (72.4%) and *T. hamatum* (71.7%). Sabry, *et al* (2015) tested the inhibitory action of different biocontrol agents on *Alternria brssicicola* causing dark leaf spot of cabbage and also got similar results.

In the case of *Rhizoctonia solani* bacterial antagonists, *Pseudomonas fluorescens* and *Bacillus subtilis* were more superior with 34% and 48% inhibition than fungal antagonist *Trichoderma viride* which showed only 21 % inhibition. Rehman *et al* (2012) studied the comparative effect of different biological control agents against *Rhizoctonia solani* damping off on cauliflower seedlings. They observed 85.5-83.0 per cent mycelial inhibition by *Trichoderma harzianum and T. viride.*

The response of *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* to *Colletotrichum gloeosporioides* showed almost similar per cent inhibition *ie.*52, 48 and 44 per cent respectively. Patil *et al* (2009) recorded significant reduction of the mycelial growth of *Colletotrichum gloeosporioides* causing blight in *Piper longum* by *Trichoderma viride* (70.42 %), which was followed by *Trichoderma harzianum* (66.90%). The least inhibition of the pathogen was observed in *Pseudomonas fluorescens* (20.72%).

In vitro evaluation of biocontrol agents against *Curvularia lunata* revealed that the fungal antagonist *Trichoderma viride* restricted the fungal growth by 50

per cent. The bacterial antagonists were also recorded similar inhibition with 46 per cent by *Pseudomonas fluorescens* and 19 per cent by *Bacillus subtilis.* Kithan and Daiho (2014) observed 57.82 per cent inhibition of *Curvularia lunata* var. *aeria* by *Trichoderma viride*. He also recorded inhibition of the pathogen by *Trichoderma harzianum* (68.85 %), *Pseudomonas fluorescens* (51.36 %) and *Bacillus subtilis* (30.32%)

Both the fungal and bacterial anatagonists were effective against *Choanephora cucurbitarum. Trichoderma viride* was with 46 % inhibition of mycelial growth of by overgrowth mechanism. *Pseudomonas fluorescens* inhibited 30 per cent growth of pathogen whereas the other bacterial biocontrol agent *Bacillus subtilis* restricted fungal growth by 47.5 percent. Siddiqui *et al* (2008) studied the effect of *Trichoderma harzianum* on *Choanephora cucurbitarum*. There was 85.04 per cent disease reduction of okra wet rot treated with Trichoderma fortified rice straw extract and which was comparable to the conventional fungicide Dithane M-45.

In vitro evaluation of biocontrol agents revealed that among the three biocontrol agents *Trichoderma viride* gave better inhibition of *Pythium aphanidermatum* and restricted its growth to 75 per cent. Both *Pseudomonas fluorescens* and *Bacillus subtilis* recorded 50 and 45 per cent inhibition of mycelial growth. Biocontrol agents *Trichoderma harzianum T. virde, Bacillus subtilis* and *Pseudomonas fluorescens* completely inhibited the growth of *Pythium ultimum* on PDA medium. (El-Mohamedy, 2012)

Under *in vitro* studies it was observed that both the bacterial antagonists *Pseudomonas fluorescens* and *Bacillus subtilis* were less effective against *Fusarium equiseti.* But in case of *Trichoderma viride* 48.33 per cent inhibition was noticed against the pathogen *Fusarium equseti*. Ram *et al* (2017) conducted *in vitro* and *in vivo* experiments for the management of root rot of fennel incited by *Fusarium oxysporum*. Under *in vitro* conditions *T. harzianum* recorded 79.44%

inhibition of growth which was followed by *Trichoderma viride* (76.88%) and *Pseudomonas fluorescens* (72.66%).

The antagonistic reaction by *Trichoderma viride* was over growth on the test pathogen in all the six pathogens, except for *Colletotricum*, where it was cessation of growth at the line of contact. Coiling and lysis of pathogenic hyphae might be the main mechanisms of action by the *Trichoderma* sp. which was reported by Singh and Singh (2000). Effectiveness of Trichoderma attributes to its action as a competitor, mycoparasite, and also suppresser of the pathogen by producing volatile and nonvolatile antibiotics (Humauan *et al.*, 2012). The suppression of fluorescent pseudomonad on the fungal pathogens might be by way of competition for space and nutrients, production of antibiotics, volatile and antimicrobial substance and compounds such as iron chelating siderophores and HCN as per the observations of Rosales *et al.*, 1995. Inhibition of growth of fungal pathogens might be due to the antifungal compounds produced by *Bacillus subtilis* as reported by Abdelzaher (2003)

5.6. EVALUATION OF FUNGICIDES AND BIOCONTROL AGENTS ON MAJOR FUNGAL DISEASES OF CABBAGE AND CAULIFLOWER UNDER *in vivo* CONDITIONS

All the observations obtained in the *in vitro* evaluation may not be in line with the field conditions. Physiology of crop and micro and macro climate will influence the efficacy. Therefore evaluation of selected fungicides and biocontrol agents were done under *in vivo* conditions also.

During the *in vivo* studies on the management of *Alternaria* leaf blight of cabbage, it was proved that the most effective fungicide against *Alternaria brassicicola* was trifloxystrobin 25%+ tebuconazole 50 % (Nativo) (0.03%) with least PDS of 49.57 and with highest per cent disease reduction over control (50.48). This effect was on par with (Bordeaux mixture) (1%), (mancozeb - 75WP) (0.3%), (carbendazim) (0.1%), (tebuconazole 5EC) (0.1%), and three of the antagonists, (*Bacillus subtilis*), (*Trichoderma viride*) and (*Pseudomonas*

fluorescens). These three biocontrol agents showed 39.07, 42.86 and 33.33 percent of disease reduction over control (Fig 1). The results are in accordance with findings of different workers. Yadav *et al.* (2014) could get successful control of *Alternaria* blight in cabbage by seed treatment of the antagonists *Trichoderma viride, Peudomonas fluorescens*. This study also reported the superior effect of carbendazim and mancozeb in controlling *Alternaria* blight under *in vivo* conditions. Thakur (2015) reported that under field conditions soil application of *Trichoderma harzianum* @2.5 kg/50 kg of FYM could limit the *Alternaria* leaf spot by enhancing the yield.

While taking the biometric observations, regarding the number of leaves, all the fungicides and bio control agents were significantly superior to control except copper oxichloride and copper hydroxide. Regarding the yield of cabbage, highest yield was for tebuconazole (0.1%) (702.5 gram/plant) followed by trifloxystrobin 25%+ tebuconazole 50 % (0.03%) (698.2 g/plant) and Bordeaux mixture (1%) (682.5g/plant). Treatments, (copper hydroxide) (0.2%), (copper oxychloride) (0.2%), (propineb) (0.3%) showed poor performance in yield. Number of leaves and yield were having a direct correlation with reduction in disease severity and yield. Among the bio control agents by application of *Trichoderma viride* comparable yield of 657g/plant was obtained (Fig 2).

In vivo studies on the management of Rhizoctonia leaf blight of cabbage, it was observed that trifloxystrobin 25%+ tebuconazole 50 % (Nativo)(0.03%) was the best fungicide for control of Rhizoctonia blight with 45 per cent disease severity reduction over control. The treatments propineb 75WP (0.3), tebuconazole 5EC (0.1%), carbendazim (0.1%), and chlorothalonil 75WP (0.1%) were on par with each other with above fungicide. The biocontrol agents *Trichoderma viride, Pseudomonas fluorescens* and *Bacillus subtilis* recorded disease reduction over control with a range of 21.15 to 28.46 per cent which were on par with the chemical copper hydroxide (0.2%) (Fig 3). Effect of bio control agents *Trichoderma virens, Pseudomonas fluorescens,* and *Bacillus subtilis*

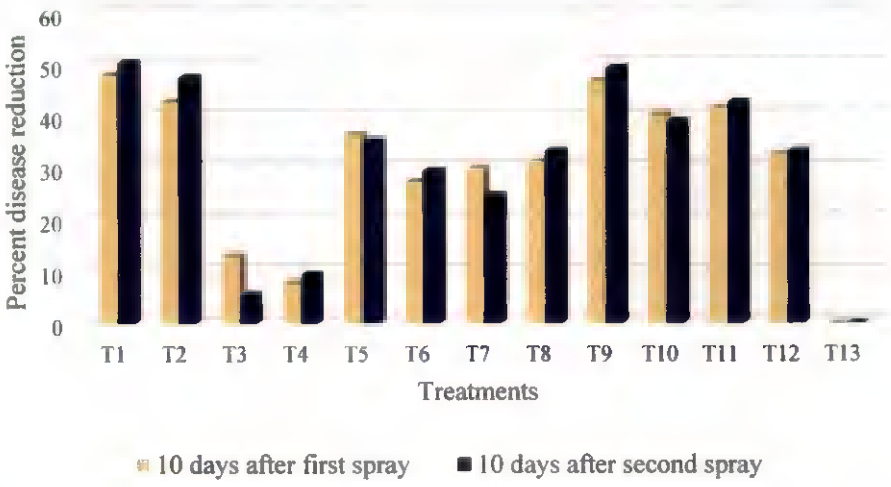


Fig 1. *In vivo* evaluation of fungicides and biocontrol agents for management of *Alternaria brassicicola* Leaf blight

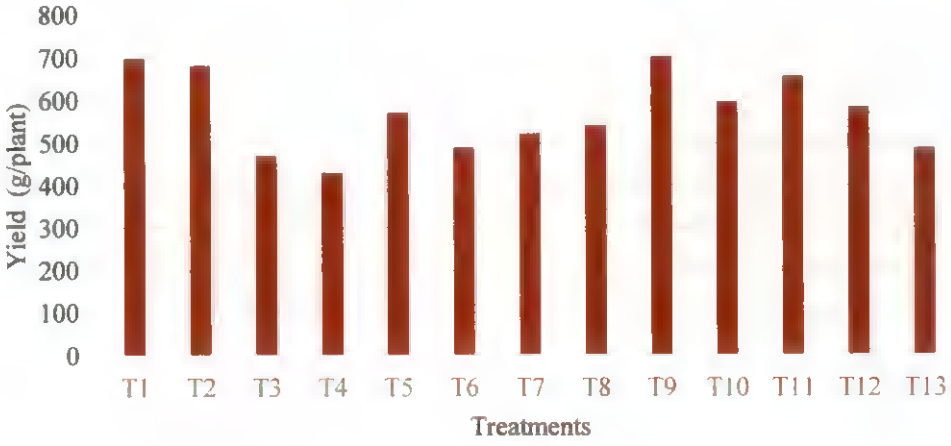


Fig 2. Effect of different treatments on growth parameters of cabbage during the management studies of *Alternaria* leaf blight

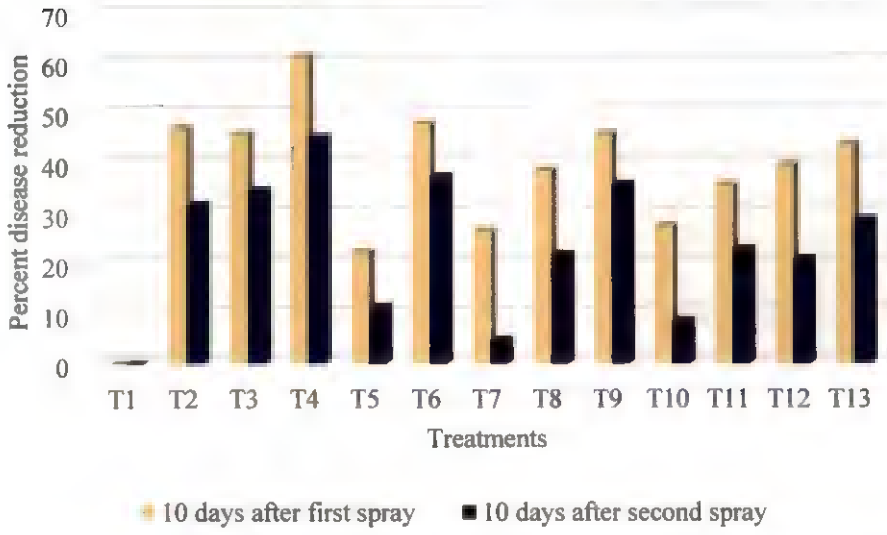


Fig 3. *In vivo* evaluation of fungicides and biocontrol agents for management of *Rhizoctonia solani* Leaf blight

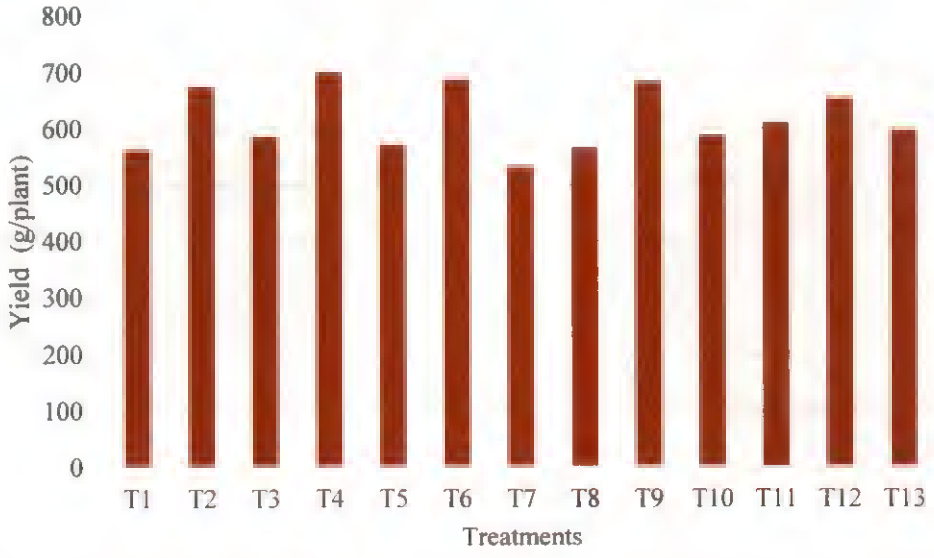


Fig 4. Effect of different treatments on growth parameters of cabbage during the management of *Rhizoctonia* leaf blight

against *Rhizoctonia solani* causing root rot and damping off of tomato seedlings in green houses was studied by Nihad *et al.* (2015) and obtained similar results.

The results of biometric observations showed highest yield of 700.5 gram/plant, was for trifloxystrobin 25%+ tebuconazole 50 % (0.03%) followed by tebuconazole(0.1%) (687.6 g/plant) carbendazim (0.1%) (683.3 g/plant). All the treatments showed significant difference in the yield compared to control, except mancozeb (0.3%) copper hydroxide (0.2%) copper oxychloride (0.2%).Three of the biocontrol agents showed significantly higher yield compared to control, and *Trichoderma viride* had given a higher value of head weight(652.8 g/plant) (Fig 4).

During the management studies of *Pythium* curd rot it was found that treatment, trifloxystrobin 25 % + tebuconazole 50% (0.03%) was leading in the case effectiveness with 78.57 per cent disease reduction. Mancozeb 75WP (0.3%) was the next best fungicide with 68.57 per cent disease reduction. The antagonists *Trichoderma viride*, *Bacillus subtilis* were again proved to be better performers with 62, 57 and 46.42 percent disease reduction respectively. Bordeaux mixture (1%) was least effective with less than 50 per cent reduction in the disease (Fig 5).

Regarding the biometric observations, there is no significant difference among the treatments in the number of leaves compared to control. The highest yield of curd weight 524.7 g/plant, was for mancozeb(0.3%) followed by trifloxystrobin 25%+ tebuconazole 50 % (0.03%)(505.5g/plant) and *Trichoderma viride*) (503.7g/plant). Bordeaux mixture (1%) and *Bacillus subtilis* were also on par with the above treatments (Fig 6). Similar results were obtained for different workers. Soil application of *Bacillus subtilis* could effectively control *Pythium ultimum* causing root rot of cauliflower was reported by Abdelzaher (2003).Lumsden and Loke (1983) observed that damping off caused by *Pythium ultimum* and *Rhizoctonia solani* from cabbage was effectively controlled by *Gliocladium virens*. Hasan *et al.*, 2014 reported that fungal and bacterial

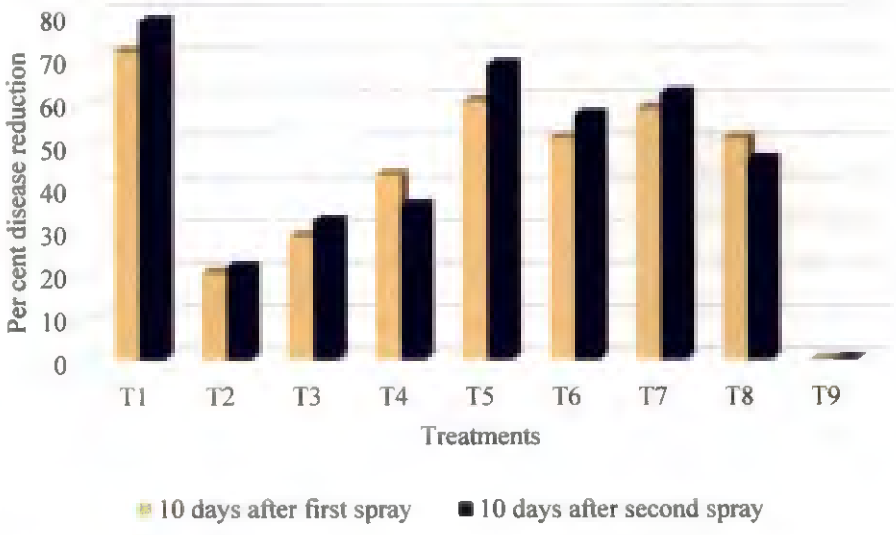


Fig 5. *In vivo* evaluation of fungicides and biocontrol agents for management of *Pythium* curd rot

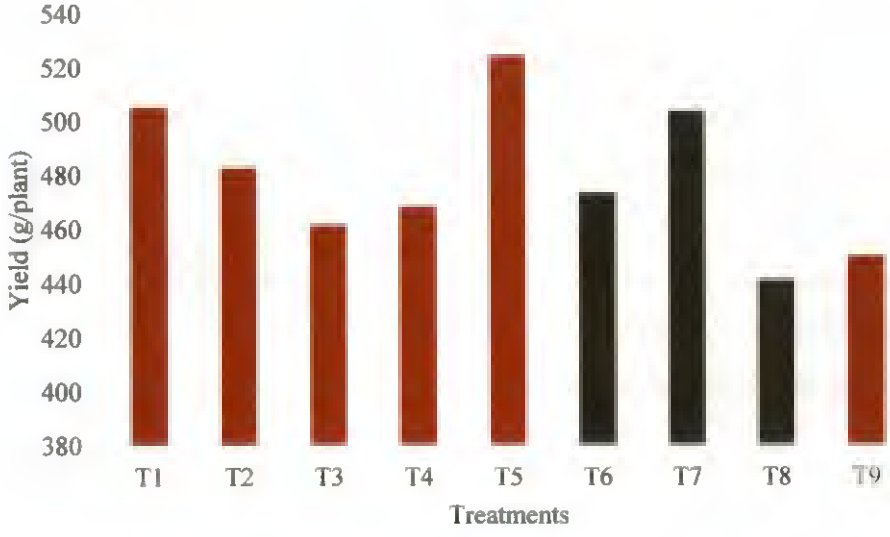


Fig 6. Effect of different treatments on growth parameters of Cauliflower during the management of *Pythium* curd rot

antagonists *viz., Trichoderma virens, Pseudomonas fluorescens,* and *Bacillus subtilis* could effectively control *Pythium, Rhizoctonia* and *Fusarium* of crucifers.

The yield data showed significant differences among treatments. The highest yield of curd weight 524.7 g/plant, was for mancozeb (0.3%) followed by trifloxystrobin 25% + tebuconazole 50 % (0.03%) (505.5 g/plant) and *Trichoderma viride* (503.7g/plant). Bordeaux mixture (1%) and *Bacillus subtilis* were also on par with the above treatments.

Results of *in vivo* experiment can be summarized as follows. In the upcoming years, leaf blights and curd rot will be major threats to cabbage and cauliflower cultivation especially in Kerala. Therefore an integrated disease management strategy must be followed in near future. For the management of *Alternaria* and *Rhizoctonia* leaf blights of cabbage, trifloxystrobin 25%+ tebuconazole 50 % at 0.03 per cent can be recommended as effective fungicide with a better yield. It is a systemic broad-spectrum fungicide with protective and curative action which offers not only a disease control but also improves quality and yield of crop. From the point of view of an organic farming or bio intensive management approach, Bordeaux mixture (1%) can be suggested as an effective fungicide. Application of *Trichoderma viride* starting from the time of planting as drenching and later two foliar spray can successfully manage both these leaf blights. For the management of Pythium curd rot, mancozeb (0.3%) and trifloxystrobin 25% + tebuconazole 50 % (0.03) were the best fungicides. For organic cultivation Bordeaux mixture (1%), biocontrol agents, *Trichoderma viride* and *Bacillus subtilis* are the better choices for disease management of Pythium curd rot.

Use of eco-friendly techniques is one of the emerging strategies for managing plant diseases with the aim of minimum usage of pesticides, production of non-polluted produce and eventually to safeguard human health and environment. The present study convince that field application of bio-control agents and fungicides can be effectively utilized and a biointensive management

strategy can be worked out for the control of fungal diseases of cabbage and cauliflower. Future line work should be concentrated on multi location trials, varietal evaluation and the residue analysis of the effective fungicides.

Summary

6. SUMMARY

Cabbage and cauliflower are the most economically important cole crops of the family Brassicaceae. The warm humid tropical climatic conditions of Kerala attracts many fungal pathogens especially in the intensively cultivated tracts. Moreover detailed systematic studies for identification and characterization of fungal pathogens of cabbage and cauliflower were not undertaken so far in Kerala. In this context, the present study is proposed to identify and characterize the fungal diseases of cabbage and cauliflower occurring in different selected districts of Kerala and to study the management of most severe and predominant disease under *in vitro* and *in vivo* conditions.

1. A purposive sampling survey in four districts *viz.,* Thrissur, Wayanad, Idukki and Kasargod during three seasons under open field and polyhouse conditions revealed the incidence of leaf blights, leaf spots, curd rot, head rot and damping off diseases.
    - Based on the distinct symptoms, diseases were categorized into leaf blight 1, leaf blight 2, leaf spot 1 and leaf spot 2, leaf spot 3, head rot 1, curd rot 1, curd rot 2 and damping off diseases.
    - In Thrissur, the disease leaf blight 1 was observed on both cabbage and cauliflower. Maximum PDI and PDS of 8.1 and 8.3 was recorded on cabbage from Madakkathara location.
    - Survey in Idukki district revealed that only one disease, leaf blight 1 was the most severe disease prevailed in the district with maximum PDI and PDS of 18.1 and 28.3.
    - In Wayanad district, diseases like leaf blight 1 and leaf blight 2 were observed where leaf blight 1 recorded maximum PDI and PDS of 69.3 and 64.8 on cabbage.
    - Survey from the three locations of Kasargod revealed that among leaf blight 1, leaf blight 2, leaf spot 1, leaf spot 2, leaf spot 3, head rot/curd rot 1, curd rot 2 and damping off , leaf blight 2 recorded highest PDI and PDS of 65.5 and 68.3 on cabbage followed by curd rot 2 on cauliflower with PDI and PDS of 60.2 and 58.2

2. Isolation of pathogens from diseased samples collected during the survey yielded eight isolates and pathogenicity was established by Mycelial Bit Inoculation Method (MBIM) for foliage, curd/ head and seedling diseases.
3. Symptomatology studies were carried out both under natural and artificial conditions.
    - Leaf blight 1 under natural conditions, exhibited large dark coloured blighted areas with concentric zonations and yellow halo on foliage and head of the cabbage.
    - Leaf blight 2 showed large bluish green lesions on lower leaves without yellow halo. Advanced stages webbing of the leaves and the dark brown sclerotial bodies were noticed.
    - Leaf spot 1 appeared as brown spot later enlarged and had greyish colour. The spots were characterized by white centre and delimited by veins.
    - Leaf spot 2 developed as small brown, round to irregular spots which later turned to dark brown in colour. Numerous spots coalesced and appeared as large blighted patches.
    - Leaf spot 3 produced small light brown spots. Numerous spots coalesced and formed larger spots. In severe cases inward curling of leaves observed.
    - Head rot/ curd rot 1 disease developed as water soaked depressed lesions with pale green border and papery white centre. Inward curling of leaves and pinhead sporangia were also noticed.
    - Curd rot 2 showed brown discolouration and soft rot of the curd. Fluffy white mycelial growth of the pathogen was also noticed on rotted areas.
    - Damping off developed as water soaked spot at the collar region and later converted to shrunken areas. Later stages yellowing and wilting of the seedlings observed.
4. Cultural and morphological characterization of pathogens were undertaken for genus level identification. Species level confirmation was done by molecular

characterization which was carried out at Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram

5. Leaf blight 1 pathogen was identified as *Alternaria brassicicola*, leaf blight 2 as *Rhizoctonia solani*, leaf spot 1 as *Cercospora* sp., leaf spot 2 as *Colletotrichum gloeosporioides*, leaf spot 3 as *Curvularia lunata,* head/curd rot 1 as *Choanephora cucurbitarum*, curd rot 2 as *Pythium aphanidermatum*, and damping off pathogen as *Fusarium equiseti*.
6. *In vitro* evaluation of fungicides and bioagents against fungal pathogens revealed that:
    - Against leaf blight and head rot pathogens *ie., Alternaria brassicicola, Rhizoctonia solani* and *Choanephora cucurbitarum* fungicides trifloxystrobin 25% + tebuconazole 50% , tebuconazole 5EC and Bordeaux mixture showed 100 per cent inhibition at all the three different concentrations.
    - Against leaf spot pathogens *Colletotrichum gloeosporioides* and *Curvularia lunata* chemicals mancozeb 75 WP, trifloxystrobin 25% + tebuconazole 50%, carbendazim 50WP, tebuconazole 5EC, propineb 70 WP and Bordeaux mixture recorded complete inhibition of mycelial growth in all the three concentrations.
    - The growth of curd rot 2 pathogen *Pythium aphanidermatum* was completely inhibited by mancozeb 70WP, copper oxychloride 50 WP and copper hydroxide77 WP in all the three concentrations.
    - The mycelial growth of damping off pathogen *Fusarium equiseti* was completely inhibited by propineb 70 WP, trifloxystrobin 25% + tebuconazole 50 %, copper oxychloride 50 WP, carbendazim 50WP and tebuconazole 5 EC.
    - Evaluation of biocontrol agents revealed that, the fungal antagonist *Trichoderma viride* and *Pseudomonas fluorescens* recorded highest inhibition against curd rot 2 pathogen *Pythium aphanidermatum*. Whereas *Bacillus subtilis* showed highest inhibition against *Rhizoctonia solani.*

7. *In vivo* evaluation of management of *Alternaria* leaf blight, *Rhizoctonia* leaf blight and *Pythium* curd rot were carried out using selected fungicides and bioagents which showed promising result under *in vitro* evaluation.
    - Against *Alternaria* leaf blight, trifloxystrobin 25% + tebuconazole 50 % (0.03%) recorded highest disease reduction of 50.48 per cent against *Alternaria* leaf blight. Whereas highest yield of 702.5g/plant was given by plants sprayed with tebuconazole 5EC (0.1%) but trifloxystrobin 25% + tebuconazole 50 % at 0.03% showed on par results.
    - Among biocontrol agents against *Alternaria* leaf blight, *Bacillus subtilis*, *Trichoderma viride* and *Pseudomonas fluorescens* were equally effective with on par results of 40.26, 41.56 and 32.47 per cent of disease reduction and over control and with higher yield.
    - Against *Rhizoctonia* leaf blight, highest disease reduction of 45.19 per cent and yield of 700.5 g/plant were recorded for trifloxystrobin 25% + tebuconazole 50 % at 0.03%.
    - The biocontrol agents *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* recorded disease reduction over control with a range of 21.15 to 28.46 per cent, but *Trichoderma viride* had given a higher value of head weight (652.8 g/plant).
    - Maximum disease reduction of *Pythium* curd rot (78.57%) was noticed in plants sprayed with trifloxystrobin 25% + tebuconazole 50% at 0.03%. The highest yield was obtained from plants sprayed with mancozeb 75WP at 0.3%.
    - For Pythium curd rot, the antagonists, *Trichoderma viride*, *Bacillus subtilis* and *Pseudomonas fluorescens* were again proved to be better performers with 62, 57 and 46.42 per cent disease reduction respectively. *Trichoderma viride* showed best result in yield of 503.7 g/plant compared to other two bioagents.

References

7. REFERENCES

Abawi, G.S. and Martin, S.B. 1985. *Rhizoctonia* foliar blight of cabbage in New York State. *Plant disease (USA)*.69:158-161 Vol-2.

Abdel-Farida, I.B., Jahangira,M., Van den Hondelc C.A.M.J.J.,. Kima, H.K., Choi, Y.H. and. Verpoorte, R. 2009. Fungal infection-induced metabolites in *Brassica rapa. Plant Sci.*, **176**: 608-615.

Abdelzaher, H.M. 2004. Occurrence of damping-off of wheat caused by *Pythium diclinum* Tokunaga in El-Minia, Egypt and its possible control by *Gliocladium roseum* and Trichoderma harzianum. *Arch. Phytopathol. and Plant Prot*, *37*(2), pp.147-159.

Abdelzaher, H.M.A. 2003. Biological control of root rot of cauliflower caused by *Pythium ultimum* var. *ultimum* using selected antagonistic rhizospheric strains of *Bacillus subtilis. NewZealand Journal of crop and Horticulture Science.*31 209-220

Ajay Kumar, G. 2014. *Colletotrichum gloeosporioides*: Biology, pathogenicity and management in India. *J Plant Physiol Pathol 2, 2,* p.2.doi.org/10.4172/2329-955X.1000125

Ajithkumar, B., Karthika, V. P., and Rao, V.U .M. 2014. *Crop weather relationships in Cauliflower (Brassica oleracea* var *botrytis L.) in the central zone of Kerala.* AICRP on Agrometeorology, Depatment of Agricultural Meteorology, College of Horticulture, Kerala Agricultural University.85p.

Akhtar, T., Shakeel, Q., Sarwar, G., Muhammad, S., Iftikhar, Y., Ullah, M.I., Mubeen, M. and Hannan, A. 2017. Evaluation of fungicides and biopesticides for the control of *Fusarium* wilt of tomato. *Pak. J. Bot*, *49*(2), pp.769-774.

Aktar, M. and Shamsi, S. 2016. Report on blight of *Tagetes* spp. caused by *Curvularia lunata* (Wakker) Boedijn. *Bangladesh J. Bot*, *45*(1), pp.167-173.

Akwaji, P.I., Johnson, U.E., Effiong, U.S., Aniedi-Abasi, M., Ntui, O.E. and Johnson, U.I. 2014. Determination of pathogenicity of *Choanephora cucurbitarum* (Berkeley and ravenel) Thaxt, amongst commonly cultivated vegetables in calabar, cross river state, Nigeria. *Int. J. Phytopathol*, *3*(2), pp.55-61.

Al-Sheikh, H. and Abdelzaher, H.M. 2012. Occurrence, Identification and Pathogenicity of *Pythium aphanidermatum, P. diclinum, P. dissotocum and Pythium*" Group P" Isolated from Dawmat Al-Jandal Lake, Saudi Arabia. *Res. J. Environ. Sci*, *6*(6), p.196.

Anand, T., Chandrasekaran, A., Kuttalam, S. and Samiyappan, R. 2010. Evaluation of azoxystrobin (Amistar 25 SC) against early leaf blight and leaf spot diseases of tomato. *J. Agric. Technol*, *6*(3), pp.469-485.

Anees, M. M.2014. Integrated Management of *Pythium* stem rot of vegetable cowpea (*Vigna unguiculata* sub.sp. *sesquipedalis* (L.) Verdcourt).M.Sc. (Ag) thesis, Kerala Agricultural University, Thrissur, 100p.

Annual Report-2015-16, Indian Institute of Vegetable Research, Varanasi www.iivr.org.in

Bassey, E.O. and Gabrielson, R.L. 1983. The effects of humidity, seed infection level, temperature and nutrient stress on cabbage seedling disease caused by *Alternaria brassicicola. Seed sci. and technol*, *11*(2), pp.403-410.

Bécot, S., Pajot, E., Le Corre, D., Monot, C. and Silué, D. 2000. Phytogard® (K_2HPO_3) induces localized resistance in cauliflower to downy mildew of crucifers. *Crop Prot*, *19*(6), pp.417-425.

BigDye Terminator v3.1 Cycle sequencing Kit – User Manual, Applied Biosystems

Budge, G.E., Shaw, M.W., Lambourne, C., Jennings, P., Clayburn, R., Boonham, N. and McPherson, M. 2009. Characterization and origin of infection of *Rhizoctonia solani* associated with *Brassica oleracea* crops in the UK. *Plant pathol*, *58*(6), pp.1059-1070.

Chakrabarty, P.K. 1993. Chemical management of curd-rot complex of cauliflower (Brassica oleracea convar botrytis var. botrytis). *Ind. J.Agric. Sci, 63*(1), pp.50-55.

Chaudary, T., Shahid, A.A., Asif, M., Asghar, F., Majeed, R.A., Ali, M. and Anwar, W. 2016. First report of *Curvularia lunata* causing leaf spot disease of *Solanum melongena* (Eggplant) in Lahore, Pakistan. *Plant Disease, 100*(11), pp.2326-2326.

Chowdappa, P. 2012. Integrated Disease Management in Vegetable Crops. Indian Institute of Horticulture Research, Bangalore, ICAR. Available at: http://nhm.nic.in Archieve/ICAR-11.pdf. Retrieved on January 15, 2016.

Chowdappa, P., Chabanahalli, S.C., Bharghav, R., Sandhya, H. and Rajendra, P.P. 2012. Morphological and molecular characterization of *Colletotrichum gloeosporioides* (Penz) Sacc. isolates causing anthracnose of orchids in India. *Biotechnol. Bioinf. Bioeng, 2*, pp.567-572.

Chu Y.F., Sun J., Wu X., Liu R.H. (2002): Antioxidant and antiproliferative activities of common vegetables. *Journal of Agricultural and Food Chemistry*, 50: 6910–6916.

Cohen, J., Kristal, R and. Stanford, J. 2000. Fruit and vegetable intakes and prostate cancer. *J. National Cancer Institute*, **9**: 61-68.

Consortium of Barcode of Life (CBOL) http://www.barcodeoflife.org

Dillard, H.R., Cobb, A.C. and Lamboy, J.S. 1998. Transmission of *Alternaria brassicicola* to cabbage by flea beetles (*Phyllotreta cruciferae*). *Plant disease, 82*(2), pp.153-157.

Dinh, V.T., Somasekhara, Y.M. and Govindaraju, C. 2015. Evaluation of new molecules of fungicides against leaf spot (*Alternaria brassicicola* (Schw.) Wiltshire) of cabbage (*Brassica oleracea* var. *capitata* L.). *Int. J. Agric. Sci. and Res. (IJASR), 5*(3), pp.349-354.

Drechsler, C. 1925. Pythium infection of cabbage heads. *Phytopathol, 15*(8), pp.482-485.

Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Heled J, Kearse M, Moir R, Stones-Havas S, Sturrock S, Thierer T and Wilson A (2010) Geneious v5.1, Available from http://www.geneious.com

El-Mohamedy, R.S.R. 2012. Biological control of Pythium root rot of broccoli plants under greenhouse conditions. *J. Agri. Technol*, *8*, pp.1017-1028.

ExoSAP-IT – User Guide, GE Healthcare

Ganeshan, G and Kumar, A.M.2015.*Pseudomonas fluorescens*- A potential bacterial antagonist to control plant diseases *Journal of plant interactions.* 1(3) 123-134

Gautam, A.K., 2005. The genera Colletotrichum: an incitant of numerous new plant diseases in India. *Phytopathology*, *58*(1), p.125.

George, M. and Girija, V.K. 2015. Pod rot of cowpea and its management using fungicides. *Internl J. Applied Pure Sci. Ag*, *106*, p.111.

Gherbawy, Y.A., Abdelzaher, H.M., Meens, J. and El-Hariry, H. 2005. Morphological and molecular identification of some closely related Pythium species in Egypt. *Arch. Phytopathol. and Plant Prot*, *38*(3), pp.193-208.

Gogoi, R., Kulanthaivel, S., Rai, S.N. and Ahuja, D.B. 2016. Leaf rot disease of cauliflower caused by *Choanephora cucurbitarum* in India. *Australasian Plant Disease Notes*, *11*(1), p.27.DOI 10.1007/s13314-016-0214-5.

Gomez, K.A. and Gomez, A.A. 1984. *Statistical procedures for agricultural research.* John Wiley & Sons.

Gopalakrishnan, T.R 2007. *Vegetable crops* (No.4). New India Publishing. New Delhi.p.208.

Grosch, R., Schneider, J.H.M. and Kofoet, A. 2004. Characterisation of *Rhizoctonia solani* anastomosis groups causing bottom rot in field-grown lettuce in Germany. *Eur. J. Plant Pathol*, *110*(1), pp.53-62.

He, Y., Chen, Q., Shu, C., Yang, M. and Zhou, E. 2016. *Colletotrichum truncatum*, a new cause of anthracnose on Chinese flowering cabbage (*Brassica parachinensis*) in China. *Tropical Plant Pathol 41*(3), pp.183-192.DOI 10.1007/s40858-016-0086-4

Hossain, M.S. and Hossain, M.M. 2010. Effect of *Alternaria* blight on the seed yield of cauliflower (*Brassica oleracea* L.). *Bangladesh J. Agric. Res, 35*(3), pp.381-385.

Hua, G.K.H., Bertier, L., Soltaninejad, S. and Höfte, M. 2014. Cropping systems and cultural practices determine the *Rhizoctonia* anastomosis groups associated with *Brassica* spp. in Vietnam. *PloS one, 9*(11), p.e111750.

Humanuan, M.R., Khalequzzaman,K.M., Akhter, B.,Alam, M.J.Islam, M.R. 2012. Role of biocontrol agent for the management of foot and root rot disease of cauliflower.*Bull.Inst.Trop.Agr.Kyushu.Univ.*35 41-45

Jambhulkar, P.P., Lakshman, D.K., Roberts, D.P. and Sharma, P. 2016. The biology, identification and management of *Rhizoctonia* pathogens. *Ind. Phytopathol, 69*(4s), pp.93-106.

Kagiwada, S., Kayano, Y., Hoshi, H., Kawanishi, T., Oshima, K., Hamamoto, H., Horie, H. and Namba, S. 2010. First report of *Choanephora* rot of ice plant (Mesembryanthemum crystallinum) caused by *Choanephora cucurbitarum* in Japan. *J. Gen. Plant Pathol, 76*(5), pp.345-347.

Kamaluddeen, S. S., & Abhilasha, A. L. 2013. A new blight disease of rice caused by Curvularia lunata from Uttar Pradesh. *Int. J. Agric. Sci. and Res, 3*, 13-16.

Kanaiyalal, P.J. 2010. Management of damping-off *[Pythium aphanidermatum (Edson) Fitz]* in chilli and residues of fungicides (Doctoral dissertation, Anand Agricultural University).

Kapoor, K. S. 1999. Fungal and Bacterial diseases of Crucifers. In: *Diseases of Horticulture Crops, Vegetables, Ornamentals and Mushrooms*. Verma, L. R. and Sharma, R.C (eds.). Indian Publishing Co., New Delhi, pp.184-221

KAU (Kerala Agricultural University) 2016. Package of Practices Recommendations: Crop (15th Ed.). Kerala Agricultural University, Thrissur, 360p.

Keinath, A.P. 1995. Relationships between inoculum density of *Rhizoctonia solani*, wirestem incidence and severity, and growth of cabbage. *Phytopathol*, *85*(12), pp.1487-1492.

Khedher, S.B., Kilani-Feki, O., Dammak, M., Jabnoun-Khiareddine, H., Daami-Remadi, M. and Tounsi, S. 2015. Efficacy of Bacillus subtilis V26 as a biological control agent against *Rhizoctonia solani* on potato. *Comptes rendus biologies*, *338*(12), pp.784-792.

Kithan, C. and Daiho, L. 2014. *In vitro* evaluation of botanicals, bio-agents and fungicides against leaf blight of *Etlingera linguiformis* caused by *Curvularia lunata* var. *aeria*. *Journal of Plant Pathology & Microbiology*, *5*(3), p.1.

Kubota, M., Abiko, K., Yanagisawa, Y. and Nishi, K. 2006. Frequency of *Alternaria brassicicola* in commercial cabbage seeds in Japan. *J. Gen. Plant Pathol*, *72*(4), pp.197-204. DOI 10.1007/s10327-006-0272-1

Kumar, D., Maurya, N., Bharati, Y.K., Kumar, A., Kumar, K., Srivastava, K., Chand, G., Kushwaha, C., Singh, S.K., Mishra, R.K. and Kumar, A. 2014. *Alternaria* blight of oilseed Brassicas: a comprehensive review. *Afr. J. Microbiol. Res*, *8*(30), pp.2816-2829.

Kuramae, E.E., Buzeto, A.L., Ciampi, M.B. and Souza, N.L. 2003. Identification of Rhizoctonia solani AG 1-IB in lettuce, AG 4 HG-I in tomato and melon, and AG 4 HG-III in broccoli and spinach, in Brazil. *Eur. J. Plant Pathol*, *109*(4), pp.391-395.

Kwon, J.H. and Jee, H.J., 2005. Soft rot of eggplant (Solanum melongena) caused by *Choanephora cucurbitarum* in Korea. *Mycobiology*, *33*(3), pp.163-165.

Lazreg, F., Belabid, L., Sanchez, J., Gallego, E., Garrido-Cardenas, J.A. and Elhaitoum, A. 2014. First report of Fusarium equiseti causing damping-off disease on aleppo

pine in Algeria. *Plant Disease*, *98*(9), pp.1268-1268.doi.org/10.1094/PDIS-02-13-0194-PDN

Lim, G. and See, G.K. 1982. Damping-off ofBrassica seedlings. *Mycopathologia*, *79*(3), pp.133-135.

Mahmodi, F., Kadir, J.B., Wong, M.Y., Nasehi, A., Soleimani, N. and Puteh, A. 2013. First report of anthracnose caused by *Colletotrichum capsici* on Bok choy (*Brassica chinensis*) in Malaysia. *Plant Disease*, *97*(5), pp.687-687.doi.org/10.1094/PDIS-09-12-0843-PDN

Mahmud, K.A. 1950. Damping-off of Cabbage, Cauliflower and Knolkohl caused by *Pythium aphanidermatum* (Eds.) Fitz. *Curr. Sci*, *19*(2).

Mamgain, A., Roychowdhury, R. and Tah, J. 2013. *Alternaria* pathogenicity and its strategic controls. *Res J Biol*, *1*, pp.1-9.

Meeboon, J., Hidayat, I. and To-anun, C. 2007. An annotated list of cercosporoid fungi in Northern Thailand. *Journal of Agricultural Technology*, *3*, pp.51-63.

Meena, P.D., Chattopadhyay, C., Kumar, A., Awasthi, R.P., Singh, R., Kaur, S., Thomas, L., Goyal, P. and Chand, P. 2011. Comparative study on the effect of chemicals on *Alternaria* blight in Indian mustard-A multi-location study in India. *J. Environ. Biology*, *32*(3), p.375.

Mishra, P.K., Saha, S., Singh, R.P., Singh, A. and Rai, A.B. 2012. Integrated approach for the management of blight of cauliflower. *Int. J. Agric. Environ. and Biotechnol*, *5*(4), pp.373-376.

Mohan, M and Mukerji, K.G.1979. Seed borne fungi. Three new records and a new species of Curvularia. Proc. *Indian, natn. Sci. Acad. B.* 45(2) 147-149

Nelson, S.C. 2008. Mango anthracnose (*Colletotrichum gloeosporiodes*). *Plant Disease*

Nene, Y.L. and Thapliyal, P.N. 1993. *Fungicides in plant disease control* (No. Ed. 3). International Science Publisher.

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NHB [National Horticulture Board].2015.NHB home page. Available: http:// www. nhb.gov.in

NHB [National Horticulture Board].2016.NHB home page. Available: http:// www. nhb.gov.in

Nihad, H.M and AL-Elzerjawi.2015.Assessment of effect of some biocontrol agents and organi Cul-ITM for controlling root rot and damping off disease of Tomato causing *Rhizoctonia solani. International Journal of scientific and engineering research. 6(1) 1250-1255*

Nowicki, M., Nowakowska, M., Niezgoda, A. and Kozik, E. 2012. *Alternaria* black spot of crucifers: symptoms, importance of disease, and perspectives of resistance breeding. *Veg. Crops Res. Bull, 76*, pp.5-19.

Pankaj, S., Singh, N. and Verma, O.P. 2011. First report of *Curvularia lunata* associated with leaf spot of *Amaranthus spinosus*. *Asian J. Plant Pathol*, *5*(2), pp.100-101.DOI.10.3923/ajppaj.2011.100.101

Park, J.H., Cho, S.E., Suyama, M., Degawa, Y. and Shin, H.D. 2016. Identification and characterization of *Choanephora* spp. causing *Choanephora* flower rot on *Hibiscus syriacus*. *Eur. J. Plant Pathol*, *146*(4), pp.949-961.

Parvathy, R. and Girija, V.K. 2016. *In vitro* Evaluation of fungicides and organic preparations against *Colletotrichum gloeosporioides* causing anthracnose of black pepper (*Piper Nigrum* L.*). Int. J. Appl and Pure Sci. and Agric (IJAP.SA),* 2(6).e-ISSN: 2394-5532, p-ISSN: 2394-823X

Parveen, T. and Sharma, K. 2014. Pythium diseases, control and management strategies: a review. *Int. J. Plant Anim. Env. Sci*, *5*(1).

Pawar, D.M. 2012. In vitro evaluation of fungicides and organics against *Curvularia lunata* and *Curvularia pallescens* causing leaf blight in gladiolus. *Int. J. Plant Prot*, *5*(2), pp.442-443.

Pornsuriya, C., Chairin, T., Thaochan, N. and Sunpapao, A. 2017. *Choanephora* rot caused by *Choanephora cucurbitarum* on *Brassica chinensis* in Thailand. *Australasian Plant Disease Notes*, *12*(1), p.13.

Prashanth, A., Sataraddi, A. R., Naik, M. K., Patil, M. B., and Patil, R.S. 2008. Evaluation of fungicides, bioagents and botanicals against pomegranate anthracnose. *Indian Journal of Plant Protection. 36* (2):283-7.

Purkayastha, R.P. and Bhattacharyya, B. 1982. Antagonism of micro-organisms from jute phyllosphere towards *Colletotrichum corchori. Trans. Br. Mycological Soc*, *78*(3), pp.509-513.

Rahimloo, T. and Ghosta, Y. 2015. The occurrence of *Alternaria* species on cabbage in Iran. *Žemdirbystė (Agriculture)*, *102*(3), pp.343-350.

Rajalakshmi J, Durgadevi D, Harish S and Raguchander T. 2016. Morphological and molecular characterization of *Pythium aphanidermatum* the incitant of rhizome rot in turmeric. *Int. J. Environ, Ecol, Family and Urban Stud (IJEEFUS)* ISSN(P): 2250-0065; ISSN(E): 2321-0109 Vol. 6, Issue 4, Aug 2016, 1-8

Ramchandra, S.S. and Bhatt, P.N. 2011. Morphological and molecular identification of Fusarium isolated from cumin wilt. *Internat. J. Plant Protec*, *4*(2), pp.359-362.

Ramchandra, S.S. and Bhatt, P.N. 2011. Morphological and molecular identification of Fusarium isolated from cumin wilt. *Internat. J. Plant Protec*, *4*(2), pp.359-362.

Rehman, S. U., Lewrence, R., Ebnezer, J., Kumar, E. J. and Badri, Z. A. 2012. Comparative efficiency of *Trichoderma vide*, *T. harzianum* and carbendazim against damping off disease of cauliflower caused by *Rhizoctonia solani* Kuhn. *J. Biopest.* **5**(1):23-27

Reuveni, R. 1982. *Fusarium equiseti* - a new cause of cumin spice plant wilt in Israel. *Plant disease*. 66(6), pp.498-499

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Rimmer, S. R., Shattuck, V.I. and Buchwaldt, L. 2007. *Compendium of brassica diseases*. American Phytopathological Society (APS Press), pp.15-58.

Rocha, J. R. S., Oliveira, N. T. and Menezes, M. 1998. Comparison of inoculation methods efficiency for evaluation of *Colletotrichum gloeospoiroides*isolates pathogenicity on passion fruits (*Passiflora edulis*). *Braz. Arch. Biol. Technol.* 41(1), pp. 145-153.

Rosales, A. M., Thomashow, L.S., Cook, R. J. and Mew, T.W.1995. Isolation and identification of antifungal metabolites produced by rice associated antagonistic *Pseudomonas* sp.*Phytopathology*.85:1028-1032

Saroj, A., Kumar, A., Qamar, N., Alam, M., Singh, H.N. and Khaliq, A. 2012. First report of wet rot of Withania somnifera caused by *Choanephora* cucurbitarum in India. *Plant Disease*, *96*(2), pp.293-293.

Saseetharan, M., Huda, N. and Zakaria, L. 2014. Occurrence of *Fusarium* spp. on Vegetable Crops and Assessment of Their Pathogenicity. *Pertanika J. Trop. Agric. Sci*, *37*(4).

Sharma, M. and Kulshrestha, S. 2015. *Colletotrichum gloeosporioides*: an anthracnose causing pathogen of fruits and vegetables. *Biosciences Biotechnol. Res. Asia*, *12*(2), pp.1233-1246.

Sharma, M., Deep, S., Bhati, D.S., Chowdappa, P., Selvamani, R. and Sharma, P. 2013. Morphological, cultural, pathogenic and molecular studies of *Alternaria brassicae* infecting cauliflower and mustard in India. *Afr. J. Microbiol. Res*, *7*(26), pp.3351-3363.

Sharma, N. and Sharma, S., 2008. Control of foliar diseases of mustard by *Bacillus* from reclaimed soil. *Microbiological Res*, *163*(4), pp.408-413.

Sharma, P., Deep, S., Bhati, D.S., Sharma, M. and Chowdappa, P. 2014. Penetration and infection processes of *Alternaria brassicicola* on cauliflower leaf and

Alternaria brassicae on mustard leaf: a histopathological study. *Plant Pathol. J, 13*(2), p.100.

Shim, C.K., Kim, M.J., Kim, Y.K., Jee, H.J., Hong, S.J., Park, J.H., Han, E.J. and Yun, J.C. 2013. Leaf rot and leaf ring spot caused by *Rhizoctonia solani* in Chinese cabbage. *Research in Plant Diseases*, 19 (4), pp.300-307

Sidlauskiene, A., Rasinskiene, A. and Surviliene, E. 2003. Effect of various protection means on *Alternaria* diseases of tomato, cucumber and cabbage seed plants. *Sodininkyste-ir-Darzininkyste*, *22*(3), pp.388-394.

Singh, K. and Rai, M. 2003. Evaluation of chemicals against *Alternaria* leaf spot of brinjal. *Ann. Plant Prot. Sci*, *11*(2), pp.394-395.

Singh, K., Lal, A.A., Kumar, D. and Meena, N.K. 2017. Evaluation of selected bio bgents, plant extracts and fungicides for the management of *Alternaria* leaf blight of Indian mustard. *Int. J. Curr. Microbiol. App. Sci*, *6*(4), pp.26-31.

Singh, S.L. and Pavgi, M.S. 1978. *Pythium* root rot of crucifers. *Mycopathologia, 63*(3), pp.155-159.

Singh,D. and Singh,A. 2000. Biocontrol of *Sclerorium rolfsii* by *Trichoderma* spp. in brinjal. In: *Proc. of Indian Phytopathological Society- Golden Jubilee. International conference on Integrated plant Disease Management for Sustainable Agriculture.* Indian Phytopathological Society, Indian Agricultural Research Institute, New Delhi, pp.322-323

Sinha, J.N. and Singh, A.P. 1995. *Cercospora brassicicola* leaf spot of *Brassica juncea* in Bihar. *J. Appl. Biol*, *5*(1/2).

Skidmore, A.M. and Dickinson, C.H. 1976. Colony interactions and hyphal interference between Septoria nodorum and phylloplane fungi. *Trans. Br. Mycological Soc*, *66*(1), pp.57-64.

Skidmore, A.M. and Dickinson, C.H., 1976. Colony interactions and hyphal interference between Septoria nodorum and phylloplane fungi. *Transactions of the British Mycological Society*, *66*(1), pp.57-64.

Sneh, B., Jabaji-Hare, S., Neate, S.M. and Dijst, G. eds. 2013. *Rhizoctonia species: taxonomy, molecular biology, ecology, pathology and disease control*. Springer Science & Business Media.

Sriraj, P.P., Sundravadana, S. and Alice, D. 2014. Efficacy of fungicides, botanicals and bioagents against *Rhizoctonia solani* inciting leaf blight on turmeric (*Curcuma longa* L.). *Afr. J. Microbiol. Res*, *8*(36), pp.3284-3294.

Srivastava, M., Gupta, S. K., Sexena, A. P., Shittu, L. A. J. and Gupta, S. K.2011. A review of occurrence of fungal pathogens on significant *Brassicaceous* vegetable crops and their control measures. *Asian J. Agic. Sci.* **3**(2):70-79

Srivastava, S. and Nelson, S. 2012. *Cercospora* leaf spot of eggplant. Plant Disease 82: pp.1-5.

Sundravadana, S., Alice, D., Kuttalam, S. and Samiyappan, R. 2007. Azoxystrobin activity on *Rhizoctonia solani* and its efficacy against rice sheath blight. *Tunisian J. Plant Prot*, *2*(2), p.79.

Tanina, K., Tojo, M., Date, H., Nasu, H. and Kasuyama, S. 2004. *Pythium* rot of chingensai (*Brassica campestris* L. chinensis group) caused by *Pythium ultimum* var. *ultimum* and *Pythium aphanidermatum*. *J. Gen.l Plant Pathol*, *70*(3), pp.188-191.

Thakur, S. 2015. Epidemiology and management of *Alternaria* leaf spot of cabbage. M.Sc. (Ag) thesis, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan.80p.

TU, D.V., Somasekhara, Y.M. and Govindaraju, C. 2015. *In vitro* and *in vivo* evaluation of new molecules of fungicides against leaf spot (*Alternaria brassicicola* (Schw.)

Wiltshire) of cabbage (*Brassica oleracea* var. *capitata* L.). *ecialise Sp*, *49*(2), pp.359-363.

Tu,C.C., Hsieh, T.F, Chang-Chang, Y.1996. *Rhizoctonia* species-Taxonomy, Molecular biology, Ecology, Pathologyand disease control. B. Sneh *et al.* (eds.) Kluwer Academic Publishers. 369-377

Vincent, J.M.1927. Distrotion of Fungl hyphae in the presence of certain inhibitors. *Nature* 159:800

Warkentin, T. D., Rashid, K. Y., and Zimmer, R. C. 1995. Effectivesness of a detached leaf assay for determination of the reaction of pea plants to powerdy mildew. *Can. J. Plant Pathol.* 17:87-89

Wellman, F.L. 1932. *Rhizoctonia* bottom rot and head rot of cabbage. *J. agric. Res*, *45*, pp.461-469.

Wheeler, B. E. J. 1969. *An Introduction of Plant Disease.* John Wiley and Sons Ltd., London, 301p

White, T. J., T. Bruns, S. Lee, and J. W. Taylor (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315-322 In: PCR Protocols: A Guide to Methods and Applications, eds. Innis, M. A., D. H. Gelfand, J. J. Sninsky, and T. J. White. Academic Press, Inc., New York.

Yadav, C. L., Kumar, N., and Kumar, R. 2014. Effect of seed treatments with fungicides, bioagents and botanicals against *Alternaria* leaf spot in cabbage (*Brassica oleracea* var. *capitata* L.). *Trends in Biosci.* 7(23):3823-3827

Yang, G.H., Chen, J.Y. and Pu, W.Q. 2007. First report of head rot of cabbage and web blight of snap bean caused by *Rhizoctonia solani* AG-4 HGI. *Plant pathol*, *56*(2), pp.351-351. Doi: 10.1111/j.1365-3059.2007.01543.x

Yu, M.Q. and Ko, W.H. 1997. Factors affecting germination and the mode of germination of zygospores of *Choanephora cucurbitarum*. *J. Phytopathol*, 145 (8-9), pp.357-361.

Zhang, L., Zheng, L., Hsiang, T., Lv, R. and Huang, J. 2009. An outbreak of head rot of cabbage caused by *Rhizoctonia solani* AG2-1 in central China. *Plant Disease*, *93*(1), pp.109-109.

Appendices

APPENDIX- I

COMPOSITION OF MEDIA USED

1. **Potato Dextrose Agar**

   Peeled and sliced potatoes - 200g

   Dextrose ($C_6H_{12}O_6$) - 20g

   Agar - agar - 20g

   Distilled water - 1000 ml

Potatoes were boiled in 500 ml of distilled water and the extract was collected by filtering through a muslin cloth. Agar –agar was dissolved separately in 500 ml of distilled water. The potato extract was mixed in the molten agar and 20 g of dextrose was dissolved in the mixture. The volume made upto 1000 ml with distilled water and sterilized at 15 psi and 121°C for 15 min.

APPENDIX-II

COMPOSITION OF STAIN USED

1. **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 and 3 ml glycerol.

Abstract

Characterization and management of fungal pathogens of cabbage (*Brassica oleracea* var. *capitata* L) and cauliflower (*Brassica oleracea* var. *botrytis* L)

by

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ABSTRACT OF THE THESIS
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ABSTRACT

Characterization and management of fungal pathogens of cabbage (*Brassica oleracea* var.*capitata* L.) and cauliflower (*Brassica oleracea* var. *botrytis* L.)

Cabbage *(Brassica oleracea* var. *capitata* L.) and cauliflower (*Brassica oleracea* var. *botrytis* L.) are the most popular and widely cultivated cruciferous vegetables in Kerala. One of the main constraints in the production of these crops is the occurrence of fungal diseases, on which no detailed systematic studies have been conducted in Kerala. The study was carried out during 2015-2017 at College of Agriculture, Padannakkad with the objective to identify and characterize the fungal diseases of cabbage and cauliflower occurring in the selected districts of Kerala and to study the management of most severe and predominant disease under *in vitro* and *in vivo* conditions. Purposive sampling surveys were conducted for the occurrence of fungal diseases in cabbage and cauliflower in Thrissur, Wayanad, Idukki and Kasargod districts and diseased plant samples were collected. Results of survey showed prevalence of eight different fungal diseases with a range of 5.4 - 69.3 per cent disease incidence and 8.1 - 68.3 per cent disease severity in case of cabbage. In cauliflower, PDI and PDS were with a range of 3.1 - 52.2 per cent and 4.9 - 44.2 per cent respectively.

Isolations done from the infected specimens collected during the survey yielded eight genera of fungal pathogens. For selecting the most potent isolate, virulence test was conducted, and used for further studies. Characterisation of the selected pathogens were carried out based on the cultural and morphological characters and identified up to generic level. Further identification of species of each genus was done by molecular characterisation by sequencing the ITS region of each fungus by *in silico* analysis and confirmed as *Alternaria brassicicola, Rhizoctonia solani, Colletotrichum gloeosporioides, Curvularia lunata, Choanephora cucurbitarum, Pythium aphanidermatum* and *Fusarium equiseti*. Symptomatology of these fungal diseases were studied in detail both under natural and artificial conditions. *In vitro* evaluation of fungicides and biocontrol agents

was done against the selected seven pathogens. Ten fungicides at three concentrations and three bio control agents were selected for the studies.

In vitro studies showed that against *A. brassicicola* the most effective fungicides at the recommended concentration were, trifloxystrobin 25% + tebuconazole 50%, tebuconazole 5EC and Bordeaux mixture with 100 per cent inhibition. Against *R. solani* and *C. lunata* six fungicides *viz.*, tebuconzole 5EC, copper oxy chloride 50 WP, trifloxystrobin 25% + tebuconazole 50%, propineb 70 WP and Bordeaux mixture produced 100 per cent inhibition. Trifloxystrobin 25% + tebuconazole 50%, tebuconazole 5EC and Bordeaux mixture were the three effective fungicides against *C. gloeosporioides*, *F. equiseti* and *C. cucurbitarum* which recorded 100 per cent inhibition over control. But against *P. aphanidermatum* from cauliflower, copper fungicides showed a lead in the inhibition *viz.*, copper oxychloride 50WP, copper hydroxide 77WP, Bordeaux mixture followed by mancozeb 75WP.

In vitro evaluation of biocontrol agents showed that *T. viride* was the most effective for controlling *P. aphanidermatum* with 75 per cent inhibition followed by *A. brassicicola* with 67 per cent inhibition. Antagonistic reaction of *T. asperellum* was mostly overgrowth on test pathogen. Similarly *P. fluorescens* also showed maximum inhibition against *P. aphanidermatum* (50%). Effectiveness of the bacterial bioagent, *B. subtilis* showed maximum inhibition against *R. solani*.

Three major pathogens *viz.*, *A. brassicicola, R. solani* in cabbage and *P. aphanidermatum* in cauliflower were selected for the *in vivo* studies. Three biocontrol agents and fungicides which showed inhibition above 60 per cent were selected for *in vivo* evaluation.

In vivo evaluation of fungicides for the management of *Alternaria* leaf blight of cabbage showed that trifloxystrobin 25% + tebuconazole 50% (0.03%) and tebuconazole 5EC (0.1%) were the best two fungicides showed 50 per cent disease reduction over control followed by Bordeaux Mixture(1%). Yield of cabbage was also highest for these three treatments. Among biocontrol agents, *T. viride* was most effective in controlling the disease with a higher yield. For the management of *Rhizoctonia* leaf blight of cabbage, same two fungicides *viz.*, trifloxystrobin

25% + tebuconazole 50% (0.03%) and tebuconazole 5EC (0.1%) were found to be most effective. *B. subtilis* was more effective than other two biocontrol agents which was significantly higher than control treatment. *In vivo* studies for the management of *Pythium* curd rot of cauliflower showed that trifloxystrobin 25% + tebuconazole 50% could produce 79 per cent of disease reduction over control with higher yield. Among the bioagents, *T. viride* produced higher yield with 62 per cent disease reduction.

The present work resulted a detailed systematic study on the fungal pathogens of cabbage and cauliflower in selected districts of Kerala and emphasizes that trifloxystrobin 25% + tebuconazole 50% (0.03%) is the best chemical and *T. viride* is the effective biocontrol agent for field application for the management of these fungal diseases. Future line work should be concentrated on the residue analysis of these fungicides and formulation of a bio-intensive management strategy.