Characterization and Biointensive Management of Fungal Fruit Rots of Cucurbits

by

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THESIS

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DECLARATION

I, hereby declare that the thesis entitled "Characterization and biointensive management of fungal fruit rots of cucurbits" 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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CONTENTS

CHAPTER	TITLE	PAGE NO.
1.	INTRODUCTION	1
2.	REVIEW OF LITERATURE	3
3.	MATERIALS AND METHODS	28
4.	RESULTS	43
5.	DISCUSSION	101
6.	SUMMARY	120
7.	REFERENCES	125
8	APPENDICES	143
9	ABSTRACT	144

LIST OF TABLES

Table No.	Title	Page No.
1	Locations of purposive sampling survey in various districts	44
2	Fungicides tested against fruit rot pathogens (*in vitro*)	37
3	Botanicals tested against fruit rot pathogens (*in vitro*)	38
4	Biocontrol agents evaluated against fruit rot pathogens (*in vitro*)	38
5	Occurrence of fungal fruit rot diseases of cucurbits in different districts of Northern Kerala	44
6	Details of isolates obtained during the survey	45
7	Per cent disease incidence of fungal fruit rots of cucurbits in Kasargod, Kannur and Kozhikkode districts	46
8	Sequence homology observed for *Choanephora* sp. in BLASTn analysis as per BLAST results	62
9	Sequence homology observed for *Sclerotium* sp. in BLASTn analysis as per BLAST results	63
10	Sequence homology observed for *Rhizoctonia* sp. in BLASTn analysis as per BLAST results	64
11	Sequence homology observed for *Phytophthora* sp. in BLASTn analysis as per BLAST results	65
12	Sequence homology observed for *Pythium* sp. in BLASTn analysis as per BLAST results	66
13	Sequence homology observed for *Corynespora* sp. in BLASTn analysis as per BLAST results	67
14	Sequence homology observed for *Fusarium* sp. in BLASTn analysis as per BLAST results	68

15a	*In vitro* evaluation of fungicides on the inhibition of mycelial growth of *Choanephora cucurbitarum*	71
15b	*In vitro* evaluation of botanicals on the inhibition of mycelial growth of *Choanephora cucurbitarum*	78
15c	*In vitro* evaluation of biocontrol agents on the inhibition of mycelial growth of *Choanephora cucurbitarum*	85
16a	*In vitro* evaluation of fungicides on the inhibition of mycelial growth of *Sclerotium rolfsii*	72
16b	*In vitro* evaluation of botanicals on the inhibition of mycelial growth of *Sclerotium rolfsii*	79
16c	*In vitro* evaluation of biocontrol agents on the inhibition of mycelial growth of *Sclerotium rolfsii*	86
17a	In vitro evaluation of fungicides on the inhibition of mycelial growth of *Rhizoctonia solani*	73
17b	In vitro evaluation of botanicals on the inhibition of mycelial growth of *Rhizoctonia solani*	80
17c	In vitro evaluation of biocontrol agents on the inhibition of mycelial growth of *Rhizoctonia solani*	87
18a	*In vitro* evaluation of fungicides on the inhibition of mycelial growth of *Phytophthora nicotianae*	74
18b	*In vitro* evaluation of botanicals on the inhibition of mycelial growth of *Phytophthora nicotianae*	81
18c	*In vitro* evaluation of biocontrol agents on the inhibition of mycelial growth of *Phytophthora nicotianae*	88
19a	*Invitro* evaluation of fungicides on the inhibition of mycelial growth of *Corynespora cassiicola*	75
19b	*Invitro* evaluation of botanicals on the inhibition of mycelial growth of *Corynespora cassiicola*	82
19c	*Invitro* evaluation of biocontrol agents on the inhibition of mycelial growth of *Corynespora cassiicola*	89

20	fungicides, botanicals and biocontrol agents tested against *Choanephora cucurbitarum* fruit rot in pumpkin	94
21	*In vivo* evaluation of selected fungicides, botanicals and biocontrol agents for management of *Choanephora cucurbitarum* fruit rot in pumpkin	95
22	Effect of different treatments on growth parameter (number of leaves/plant and number of branches/plant) of pumpkin during the management studies of *Choanephora cucurbitarum* fruit rot	97
23	Effect of different treatments on yield parameter (weight of the fruits /plant and number of the fruits /plant)of pumpkin during the management studies of *Choanephora cucurbitarum* fruit rot	99

LIST OF PLATES

Plate No.	Title	After page No.
1	Different locations of survey and collection of samples	44
2	Symptomatology and pathogenicity test of *Choanephora cucurbitarum* fruit rot	49
3	Symptomatology and pathogenicity test of *Sclerotium rolfsii* fruit rot	49
4	Symptomatology and pathogenicity test of *Rhizoctonia solani* fruit rot	49
5	Symptomatology and pathogenicity test of *Phytophthora nicotianae* fruit rot	49
6	Symptomatology and pathogenicity test of *Pythium deliense* fruit rot	49
7	Symptomatology and pathogenicity test of *Corynespora cassiicola* fruit rot	49
8	Symptomatology and pathogenicity test of *Fusarium equiseti* fruit rot	49
9	Colony and spore morphology of *Choanephora cucurbitarum*	55
10	Colony and sclerotial morphology of *Sclerotium rolfsii*	55
11	Colony and hyphal morphology of *Rhizoctonia solani*	55
12	Colony and sporangial and morphology of *Phytophthora nicotianae*	55
13	Colony, sporangial and gamatangial morphology of *Pythium deliense*	55
14	Colony and conidial morphology of *Corynespora cassiicola*	55
15	Colony and conidial morphology of *Fusarium equiseti*	55

16	DNA amplification profile of selected pathogens	68
17a	Effect of different levels of fungicides on radial growth of *Choanephora cucurbitarum*	71
17b	Effect of different levels of botanicals on radial growth of *Choanephora cucurbitarum*	78
17c	Effect of different biocontrol agents on radial growth of *Choanephora cucurbitarum*	85
18a	Effect of different levels of fungicides on radial growth of *Sclerotium rolfsii*	72
18b	Effect of different levels of botanicals on radial growth of *Sclerotium rolfsii*	79
18c	Effect of different biocontrol agents on radial growth of *Sclerotium rolfsii*	86
19a	Effect of different levels of fungicides on radial growth of *Rhizoctonia solani*	73
19b	Effect of different levels of botanicals on radial growth of *Rhizoctonia solani*	80
19c	Effect of different biocontrol agents on radial growth of *Rhizoctonia solani*	87
20a	Effect of different levels of fungicides on radial growth of *Phytophthora nicotianae*	74
20b	Effect of different levels of botanicals on radial growth of *Phytophthora nicotianae*	81
20c	Effect of different biocontrol agents on radial growth of *Phytophthora nicotianae*	88
21a	Effect of different levels of fungicides on radial growth of *Corynespora cassiicola*	75
21b	Effect of different levels of botanicals on radial growth of *Corynespora cassiicola*	82

21c	Effect of different biocontrol agents on radial growth of *Corynespora cassiicola*	89
22	*In vivo* studies in sick plot	91

LIST OF FIGURES

Figure No.	Title	page No.
1	*In vivo* evaluation of fungicides, botanicals and biocontrol agents for management of *Choanephora cucurbitarum* fruit rot	96
2	Effect of different treatments on yield parameter (weight of the fruits /plant)of pumpkin during the management studies of *Choanephora cucurbitarum* fruit rot	98
3	Effect of different treatments on yield parameter (number of the fruits /plant)of pumpkin during the management studies of *Choanephora cucurbitarum* fruit rot	98
4	Effect of different treatments on growth parameter (number of leaves/plant) of pumpkin during the management studies of *Choanephora cucurbitarum* fruit rot	100
5	Effect of different treatments on growth parameter (number of branches/plant) of pumpkin during the management studies of *Choanephora cucurbitarum* fruit rot	100

LIST OF APPENDICES

Sl. No.	Title	Page No.
1	Composition of media used	143
2	Composition of stains used	143

Introduction

1. INTRODUCTION

Cucurbitaceous vegetables consist of 90 genera and 750 species, which are used in various ways as human food in different countries. India ranks second in the production of vegetables after China (Gopalakrishnan, 2007). Over the last few years, India has witnessed voluminous increase in vegetable production. Improvement of production of vegetables from 58.5 million tonnes to 175 million tonnes was achieved within a time span of 1991-92 to 2016-17. During 2016-17, the area under vegetables was estimated as 10.3 million hectares with a production of 175 million tonnes (NHB, 2017). Most popular and widely cultivated vegetables in Kerala are seasonal and propagated by seeds. A very few are perennials, which are propagated vegitatively. Most of the vegetables are highly cross pollinated and extensively used for mixed cropping in Kerala.

Members of cucurbitaceae family keep plenty of variation like, salad crop (cucumber), dessert fruit (watermelon and muskmelon), candying (ash gourd) and cook crop (pumpkin, bitter gourd, bottle gourd etc.). Cucurbitaceous vegetables are the mine of nutrients; it contains almost all essential nutrients like, proteins, carbohydrates, minerals, vitamins and other nutrients and possesses antioxidant and anti-carcinogenic properties (Rahman, 2003; Duke, 1999). They are also used actively as traditional herbal remedies for a variety of diseases. They have anti-inflammatory, cardiovascular, antitumor and immunoregulatory activities (Saboo *et al.*, 2013; Dhiman *et al.,* 2012). Bitter gourd is rich in vitamin C (96 mg/100g), pumpkin contains high amount of carotene (1600 IU/100 g), spine gourd is high in protein (3.1 g/100 g) and chow-chow is rich in calcium (140 mg/100 g) (Anitha *et al.* 2013).

In Kerala, cucurbits are grown as irrigated crop (January-March, & September-December) or rainfed crops (May-August) and they require much longer growing period. Snake gourd, bitter gourd, pumpkin, ash gourd, cucumber, bottle gourd and ridge gourd are the important cucurbitaceous vegetables cultivated in Kerala (KAU, 2016). The varieties, seed rate and spacing are

17

different for each crop. Currently, availability of lot of tropical varieties of cucurbits suited to Kerala condition has led to a raise in the production. However, the cultivation of promising cultivars of cucurbits are under threat due to several constraints. One of the main constraints in the yield reduction of these crops is the fruit rot diseases. The warm humid tropical climatic condition of Kerala favours different fruit rots caused by fungi. Moreover fruit rots are wrongly diagnosed as secondary infection after fruit fly attack and not as a primary cause. No detailed systematic studies have been conducted in Kerala on the occurrence, symptomatology and management of fruit rots of cucurbits. Since fungicide application for fruit rots in cucurbits directly cause pesticide residue problem, we have to adopt good agricultural practices for effective and economic management of these pathogens.

In this situation, the current study was proposed to identify and characterize the pathogens causing fruit rots of cucurbits and to propose a biointensive management approach that can be successfully adopted by the farming community.

The study encompasses the following objectives:

- Survey on the occurrence of fruit rots of cucurbits
- Symptomatology of diseases
- Isolation of pathogens
- Characterization of pathogens
- *In vitro* evaluation of fungicides, botanicals and biocontrol agents
- *In vivo* evaluation of fungicides, botanicals and biocontrol agents

18

Review of Literature

2. REVIEW OF LITERATURE

Important cucurbitaceous vegetables grown in Kerala include pumpkin, cucumber, ridge gourd, bitter gourd, snake gourd, ash gourd, etc. A lot of work has been done by various researchers all over the globe on several crops of the family Cucurbitaceae, and their disease management strategies. The fruit rots cause serious loss in terms of quality and yield. They can be managed by adopting chemical, cultural and biological methods. Nowdays, consumers are quite careful about pesticide residues and organic cultivation. Systematic studies on the characterisation of different fruit rot causing fungi have not been carried out in Kerala. Most of the pathogen attacks are mistaken as secondary infection by saprophytic fungi after fruit fly attack. Therefore, the research on characterization and biointensive management of fungal fruit rots of cucurbits is the first attempt to document the fine points of these diseases in Kerala. A broad range of pathogens influence the productivity of cucurbitaceous vegetables, which constitute over 200 diseases (Babadoost and Zitter, 2009). The diseases may be caused by fungi, viruses, bacteria or phytoplasma. Even with the start of 20th century, researchers identified a number of fungi attacking fruits of cucurbits. Anjorin and Mohammed (2009), reported that these diseases might be soilborne, seedborne, transmitted by wind or insect vectors.

2.1. FUNGAL PATHOGENS

Various workers have reported different pathogens causing fruit rots of cucurbits *viz., Choanephora, Sclerotium, Rhizoctonia, Phytophthora, Pythium, Corynespora* and *Fusarium*. Drechsler (1925) identified the pathogen attacking cucumber as *Pythium aphanidermatum*. The pathogens cause seed abortion and rot, reduction or elimination of germination ability as well as seedling damage at later stages of growth, resulting in the advance of the disease as local or systemic infection (Khanzada *et al.*, 2001). Characterisation and management of fruit rot pathogens like *Alternaria alternata, Fusarium equiseti, Corynespora cassiicola*, of immature cucumbers under green house conditions was done by Al-Sadi *et al.*

20

(2011). Sharma (2013) reported the attack of different fungal pathogens like *Pythium, Phytophthora, Fusarium, Rhizoctonia* on the fruits of cucurbits.

Choanephora is an important soft rot causing fungal pathogen in cucurbits. *Choanephora cucurbitarum* cause soft rot mostly in trailing cucurbit mainly on floral parts and fruits. *Choanephora cucurbitarum* causing rot disease on various plants including cucumber, squash, okra, pepper and pea was documented by Yu and Ko (1997). Kwon and Jee (2005) reported *Choanephora cucurbitarum* causing soft rot in eggplants for the first time from South Korea. Kagiwada *et al.* (2010) isolated *Choanephora cucurbitarum* from *Mesembryanthemum crystallinum* (ice plant) having stem and leaf rot symptoms from hydroponic green-house in Japan. Saroj *et al.* (2012) reported wet rot in Aswagandha (*Withania somnifera*) caused by *Choanephora cucurbitarum* for the first time from India. Choudhary (2015) conducted research on *Choanephora cucurbitarum* wet rot management in cucumber. Gogoi *et al.* (2016) reported *Choanephora cucurbitarum* causing leaf rot on cauliflower for the first time in India. *Choanephora cucurbitarum* causes floral rot in ornamental plants and it was noticed in *Hibiscus syriacus* by Park *et al.* (2016). Pornsuriya *et al.* (2017) documented wet rot on the leaves of *Brassica chinensis* caused by *Choanephora cucurbitarum* in Thailand.

Sclerotium rolfsii was positioned in the form genus *Sclerotium* (Saccardo, 1913), as it forms differentiated sterile mycelia and sclerotia is a disturbing soil-borne fungus with a large host range (Aycock, 1966; Punja, 1988). However, the teleomorphic state was discovered later (Punja, 1988), verifying that the fungus was a basidiomycetes fungi. According to Kirk *et al.* (2008) it is now placed in the genera *Sclerotium* under anamorphic fungi. *Sclerotium rolfsii* commonly causes collar rot, but spotted leaf rot with a single small sclerotium in the middle has also been reported (Singh and Pavgi, 1965). The infection occurs on fruits that are in contact with the soil and the disease development is favored by warm humid conditions. Pumpkin fruits infected with *Sclerotium rolfsii* were lost because of the fruit rot (Zitter *et al.*, 2004).

Lewis and Papavizas (1980) gave the integrated management strategies for fruit rot caused by *Rhizoctonia solani* in cucumber. *Rhizoctonia solani* is a soil borne fungus found everywhere which damages large number of plant species around the world with diverse symptoms. Fruit rot (soil rot or belly rot) of cucumber caused by *Rhizoctonia solani* is the most severe disease in warm humid places of United States (Jones, 1961). Among the soil borne fungus that attacks numerous agriculturally significant crop plants *Rhizoctonia solani* is the most frequently occurring pathogen that causes huge loss to crop yields (Kumar and Singh, 2006a). It was also reported that, *R. solani* is one of the most significant, soil-borne fungus and the causative organism of root rot, crown rot, fruit rot and damping off in tomato producing areas (Taheri and Pourmahdi, 2013). 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.

Phytophthora nicotiana is a distressing plant pathogen worldwide, causing fruit, foliar, and crown rots (Erwin and Ribiero, 1996). *P. nicotianae* is also accountable for considerable damage on a number of other economically significant crops including fruits and vegetable crops (Erwin and Ribiero, 1996). Among the diseases caused by this pathogen, black shank of tobacco (Johnson *et al*. 2002) as well as gummosis and root rot of citrus species (Cacciola and Di San Lio, 2008) have been well studied. *P. nicotianae* has a wide host range, counting over 255 plant genera in 90 families (Mammella *et al.* 2013). Since 2011, fruit rot diseases caused by *Phytophthora nicotianae* have become progressively more prevalent in Southern zone of India, posing a major threat to vegetable crops like brinjal and ridge gourd production during the onset of southwest monsoon period (June to September) (Chowdappa *et al.,* 2016).

Soft rots in vegetables causes serious crop losses in cucurbitaceous crops and *Pythium* sp. are one among them. Drechsler (1925) conducted a study on soft rot caused by *Pythium* in cucumber. *Pythium* is a member of the family

Pythiaceae in the class Oomycota and order Peronosporales, which was positioned in the kingdom Chromista (Kirk *et al*., 2008). *P. deliense* was first mentioned by Meurs (1934) in Sumatra from tobacco plant affected with stem-burn disease. Since then it has been obtained from tropical countries and is recognized to be infectious to cucurbits, tomato and ginger (Plaats-Niterink, 1981). *Pythium* sp. are ubiquitous soil borne pathogens causing damping off, root rot and fruit rot diseases on many plant species such as cucumber, melon, carrot, cabbage and others (Abdelzaher, 2004). The plant diseases caused by *Pythium* sp. varies with the host plant, it is the causative agent of damping-off disease of different vegetable seedlings. It also causes cottony leak of fruits, seedling rots, etc. Damping-off and different types of rots caused by *Pythium aphanidermatum* were considered among the most troubling diseases of crop plants growing in polyhouse conditions (Parveen and Sharma, 2015).

Corynespora cassiicola is a prevalent fungal pathogen, linked with more than 70 hosts in different tropical and subtropical nations (Pollack and Stevenson 1973; Onesirosan *et al.* 1974). Castro (1979) reported leaf blight of cucumber (*Cucumis sativus*) caused by *C. cassiicola*, a new disease in the Valley of Culiacan, Sinaloa, Mexico, which had caused extensive damage in cucumber fields. *Corynespora cassiicola* infects 375 plant host species, from more than 76 nations globally, and shows symptoms of rot on the leaf, fruit and other parts also (Silva and Giordano, 2000). While, target leaf spot was reported in cucumber as a new ailment in China (Zou *et al*., 2002). It occurs in some ornamental plants like poinsettia, weeds like spiderwort and vegetables like cucumber, pumpkin, tomato and okra (Cutrim and Silva, 2003).

Losses due to fruit rots caused by the fungal pathogen *Fusarium* sp. are generally minor, but during favourable conditions they cause severe yield reduction in cucurbits. *Fusarium* sp. generally causes wilts and damping off in a number of vegetable crops. Dillard *et al.* (1998) observed Fusarium rot on cabbage heads. It is reported that, fruit rot is a severe problem in many vegetables producing polyhouses, being the second most key biotic issue to polyhouse

cucumber production in different parts of Oman after soil borne fungal diseases, especially under the warm humid climatic condition (Blancard *et al.*, 2005). Fungal fruit rot symptoms on immature salad cucumber is associated with infection by several plant pathogenic fungi including *Fusarium* sp., *Choanephora cucurbitarum*, *Phytophthora capsici* and others (Blancard *et al.*, 2005; Gevens *et al.*, 2006). Al-Sadi *et al.* (2011) reported *Fusarium equiseti* causing fruit rot in immature cucumber in the greenhouse. First report of *Fusarium equiseti* causing wilt of cumin, from India was in 2011 by Ramchandra and Bhatt (2011). 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

To confirm the pathogenicity of pathogens in cucurbits, isolated fungi were tested in their respective hosts (Granger and Horne, 1924). Johnson *et al.* (2014) demonstrated pathogenicity test of *Choanephora cucurbitarum* on different vegetables by mycelial bit inoculation. Johnson *et al.* (2014) observed the development of symptoms of soft rot and decay in cowpea pods after five days of artificial inoculation. (George, 2015) confirmed pathogenicity of the *Choanephora* sp. by proving Koch's postulates. Artificial inoculation was carried out on healthy, mature cowpea pods with 1cm diameter mycelial discs cut from 24 h old culture of *C. cucurbitarum* grown on PDA medium after giving definite number of pinpricks with sterile stainless steel needle. Gogoi *et al.* (2016) observed typical soft rot symptoms and signs of Choanephora rot after 5-7 days of inoculation by mycelial bit inoculation method.

For the pathogenicity test of *Sclerotium rolfsii*, fruit samples were used. Firstly the fruit samples were under washed tap water and surface sterilization was done with 0.1 per cent mercuric chloride and washed with sterile distilled water thrice. Inoculation by making pin prick was followed on the fruit surface using mycelium with a sterilized dissecting needle. Inoculated fruits were kept in a moist chamber. Control was inoculated with sterile distilled water. (Najera *et al.* 2018).

Wide host range of *Rhizoctonia* was reported earlier in 1996 by Tu *et al.* (1996). Zhang *et al.* (2009) carried out pathogenicity test of cabbage head rot caused by *Rhizoctonia solani* by mycelial bit inoculation method. Pathogenicity of *R. solani* causing stem blight was proved by stem inoculation method on tomato variety Arka vikas (Sumalatha *et al.*, 2017).

Pathogenicity test for *P. nicotinae* isolates from brinjal was conducted by adopting zoospore suspension method by Hong *et al.* (2002). Darine *et al.* (2007) isolated nine diverse isolates of *Phytophthora nicotianae* from infected black pepper and evaluated their pathogenicity in *C. annuum.* Kousik *et al.* (2015) conducted pathogenicity test of *Phytophthora capcisi* by mycelial agar bit inoculation method in bitter gourd.

The pathogenicity of three strains of *Pythium* sp. isolated from cucumber having cottony leak symptom was repeatedly demonstrated by inoculation into healthy cucumber fruits. Pieces of mycelium from pure cultures were inserted into aseptic incisions, which then were sealed with sterile moist cotton, and the cucumbers were placed in glass chamber. Cottony mycelial growth was covered the entire fruit after three days (Drechsler, 1925). 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.

In case of *Corynespora cassiicola,* the pathogenicity was tested with the isolated fungus on both the detached healthy whole fruits (*in vitro*) and the healthy fruits on the plants in the field (*in vivo*) as per the method suggested by Ash and Lanoiselet (2001). Pathogenicity tests were carried out by making pin pricks on the surfaces of fruits of okra, on which a mycelial bit was placed. In control plants a sterile PDA plug without fungal mycelium was placed on wound. Symptoms analogous to those revealed by naturally infected plant was developed on the wounded stem and fruits after 7 days of inoculation. The pathogen *Corynespora cassiicola* was reisolated from the inoculated plants, there by

confirming Koch's postulates (Ahmed and Khair, 2008). For *in vitro* assay, surface-sterilization of the fruit was done with 0.1% mercuric chloride for two minutes followed by washing with sterile water and drying with sterile filter papers. Injury was made to the fruits. Seven day old spore suspension of fungal pathogen was prepared in sterile water and applied on the injured portion using sterilized cotton. The fruits inoculated were securely positioned in a moist chamber at ordinary room temperature for a week (Pawar *et al.*, 2014). Pathogenicity test of *Corynespora cassiicola* in chilli was done by inoculating the isolated fungus through detached plant part technique (Chowdappa *et al.*, 2014).

Representative fungi isolated from diseased immature cucumber fruits including *Fusarium equiseti* were tested for pathogenicity using the following protocol. Immature salad cucumber (4–5 cm in length) obtained from 5–7 week old cucumber plants growing in the polyhouse were used. The detached fruits were surface sterilized using 70 per cent ethanol solution, and it was inoculated into Petri plates. Then the inoculation was done on the fruit with a mycelial disc obtained from fungal culture (Al-Sadi *et al.*, 2011). 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

Cultural and morphological studies are necessary for preliminary identification of different pathogens.

Similar reports on the morphology of *Choanephora cucurbitarum* isolated from pumpkin were given by Kirk (1964), Kwon *et al.* (2001a) and Kwon and Jee (2005) isolated from different crop plants such as petunia, eggplant, etc. *Choanephora cucurbitrum* isolate obtained from eggplant on PDA media appeared white to pale yellowish brown and produced pediculate, elliptic fusiform or ovate monosporous sporangiola with striations and measured 12-20 x 6-14 μm.

Sporangiospores were measured 14-22 x 7-10 µm 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 were light brown to dark brown colour. There are reports on morphology of *C. cucurbitarum* isolated from shoe flower (Kwon and Park, 2005) and *Boerhavia diffusa* (Singh *et al*., 2011). Gogoi *et al.* (2016) isolated *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 µm × 11–22 µm. Sporangia were subglobose with 35-85 µm diameter. Sporangiospores were brown coloured, with or without three or more thin appendages at both ends, which were elliptic, fusiform or ovoid and measured 7–10.5 µm × 10-27 µm. In a study by Pornsuriya *et al.* (2017) *Choanephora cucurbitarum* produced white creamy colonies which later changed into yellow with abundant sporangiola. Sporangiola were ellipsoid to ovoid with brown to dark brown colour measuring 9-22µm in length and 8-15µm in breadth. The fungus produced erect, non-septate, solitary and unbranched, sporangiophores having the dimension of 5-13µm length and 1-10 µm breadth.

Barnett and Hunter (1998) identified the fungus showing main hypha with 4.6 to 8 µm diameter, with ring-shape joints which allow secondary and tertiary branching and produced spherical sclerotium having 0.5 to 2. mm diameter. Sarma *et al*. (2002) considered the diferences in Indian isolates of *S. rolfsii* and observed that the variablity exists with respect to host plant as well as collected regions. Manu (2012) studied the morphological characters of twelve different isolates of *S. rolfsii* on PDA. He observed that the diameter of the colony varied from 4.1 to 8.0 cm at 28^{0}C after 72h of incubation period, sclerotia formed per plate varied from 261.7 to 1048.7, the colour of the sclerotia varied from light brown to dark brown with size of 1.10 to 2.10 mm with spherical shape. The fungal pathogen *Sclerotium rolfsii* was isolated from *Cucurbita maxima* on potato dextrose agar (PDA) medium and growth of milky white very fast-growing mycelia with abundant reddish brown sclerotial bodies were observed. The

sclerotia formed were initially white and later turned brown (Mahadevakumar *et al.*, 2016).

According to Abawi and Martin (1985) on potato dextrose agar *Rhizoctonia solani* from cabbage initially produced white mycelium, which later turned brown-grey with leathery texture. Rimmer *et al.* (2007) reviewed the morphology of *Rhizoctonia solani* from brassica vegetables and stated that mature hyphae were 5-15μm thick and branched at right angles, hyaline or brown. There was a septum formed with a slight constriction near the point of branching. *Rhizoctonia solani* produced multinucleate, irregular, moniliform and barrel-shaped cells of 250 μm in length. Zhang *et al.* (2009) isolated *Rhizoctonia solani* from cabbage infected with head rot. The colony was white coloured, which turned brown after 5 days. Hyphae branched at right or acute angles near the septa with a thickness ranging from 4.9 to 8.0 μm. The cells had six to eight nuclei with characteristic dolipore septum. The isolates produced dark brown sclerotia after 10 days of incubation with a diameter in the range of 0.5 to 5 mm. Shim *et al.* (2013) observed light to dark brown colonies of *Rhizoctonia* from Chinese cabbage with hyphae (5.01-11.12 μm diameter) and dark brown sclerotia with 0.38-1.28mm diameter.

The morphological characterization of 76 isolates of *Phytophthora* recieved from different crop plants including ridge gourd, brinjal and tomato in different localities of Southern zone of India harmonized the description of *Phytophthora nicotianae* based on different taxonomic keys (Stamps *et al.* 1990) and morphological characteristics observed such as ovoid sporangia. All isolates from ridge gourd, brinjal and tomato produced colonies without specific pattern and dense low spreading aerial mycelium in carrot agar medium. In carrot agar medium, the radial growth differed from 6 to 8 mm/day. All the isolates produced copious sporangium in liquid culture medium under light, produced rarely on carrot agar medium in light or dark. The sporangia were solitary, mostly spherical, ovoid with a papilla. The dimensions of sporangia varied from 26 to 53 × 23 to 38 μm with length: breadth ratio of 1.2 to 1.3. On carrot agar, all the isolates

produced numerous spherical chlamydospores that varied from 21 to 53 μm diameter within 16 days in dark and were either intercalary or terminal. Every isolates observed were heterothallic in nature. Antheridia produced were typically amphigynous, cylindrical and ranged from 9 to 14 × 8 to 12 μm. Oogonia ranged from 16 to 33 μm diameter and oospores produced were in the limit of 14 to 29 μm diameter. (Chowdappa *et al.*, 2016). According to Dos Santos *et al.* (2006) sporangia formed were papillate, persistent, and predominately ovoid, measuring 56.0 x 35.0 to 33.3x 24.5 μm (average: 42x 29μm) with a length-breadth ratio of 1.4:1. Chlamydospores were terminal or intercalary in the mycelium, with a diameter of 25.4 to 40.3 μm (average of 33.0 μm). *P. nicotianae* is heterothallic. Oospores measure 23-38 μm in diameter (average: 29 μm). The antheridia are amphygynous. Cultures of *P. nicotianae* on carrot agar medium were petaloid, with dense and cottony aerial mycelium and colonies had diffuse edges. The optimum temperature found for the mycelial growth is between 25 and 33°C.

Morphology of different structures *viz.*, oogonia, sporangia, and antheridia, the type and size of oospores produced, growth habit, and growth rate in different culture media are some of the general criteria used to distinguish *Pythium* sp. (Dick, 1990; Plaats-Niterink, 1981). According to Paul *et al.* (1995), morphology of *Pythium deliense* causing damping off in cucumber are follows. Hyphae are 7 - 8 μm diameter. Zoospores are formed at 25°C. The encysted one have the size of about 13 μm in diameter. Smooth walled, terminal oogonia measures 14- 30 μm diameter. Stalks of oogonia sometimes twisting towards antheridia. Antheridia observed are normally monoclinous. Oogonium formed are intercalary or terminal. Oospores formed were smooth walled, globose, aplerotic, one per oogonium, measures 13-25 μm in diameter, having 2 - 3 μm thick wall. Lodhi *et al.* (2004) studied morphological characteristics of *Pythium deliense* obtained from betel vine in Pakisthan. The study revealed that the main hyphae were 6 μm wide. Sporangia was most commonly terminal. Discharge of zoospores occurs at normal room temperature and it is released through discharge tubes of varying length. Encysted zoospore measured about 9-11 μm in diameter. globose,

smooth, terminal oogonia were observed and it measures 16-24 µm. Oospores observed were aplerotic and had 16-18 µm diameter. The ooplast ranges between 9-10 µm in diameter. Monoclinous, intercalary antheridia having 5-6 µm in width and 6-9 µm in length were seen. In most of the cases oogonial stalks showed curving towards the antheridium. Oospore wall had the thickness of 1-2 µm. Different colony characteristics of *Pythium deliense* were indefinite rosette pattern with profused aerial mycelium on PDA medium.

Gherbawy *et al.* (2005) isolated *Pythium. aphanidermatum* from soybean seedling and observed that colonies formed on cornmeal agar medium were cottony and on potato-carrot agar medium with aerial mycelium with no special pattern. Main hyphae were upto 10 mm in width. Zoosporangia showed complexes of swollen hyphal branches at terminal end having varying length. Zoospores formed at 15–30^{0}C. Zoospores which were encysted had 12 µm diameter. Oogonia are terminal, globose, smooth, and having 22–25 µm diameter. Antheridia produced in most of the cases intercalary, sometimes it was terminal, sac-shaped, it possess 11–15 µm length and 9–15 µm width. Oospores commonly aplerotic in nature, possess 20–23 µm in diameter, walls 1–2 µm 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 produced cottony white aerial mycelium without any special pattern. The main hypha measured 3.1-6.8 µm width. Sporangia composed of complexes of swollen hyphal branches having varying length terminally. Oogonia are globose, terminal, smooth, 19-21 µm diameter. Anthredia produced mostly intercalary, sac shaped, 9.3-12.6 µm in length, 8.5-11.4 µm in width, one per oogonium; 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. Rajalakshmi *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.

The mycelium of *Corynespora cassiicola* was white colored, pluffy and flocculent in potato dextrose agar medium, where with each ongoing day it became dark gray, forming a olive black colored triangle on the tomato fruit surface in later developmental stage as reviewed by Snow and Berggren (1989). The colony of *C. cassiicola* on potato dextrose agar medium emerged as effused, gray to olivaceous green at immature stage and later gradually turned into brown to blackish brown at mature stage, seldom velvety. Conidia produced solitary or in a chain of 2 - 8, they are variable in size, shape, and may be obclavate, straight or curved, olivaceous brown or brown, with 4 - 21 pseudosepta and it measured 42.2 - 220.7 µm length and 9.3 - 22.5 µm width. The potato dextrose agar medium (PDA) medium at room temperature (25°C) was found most suitable for the growth, development and sporulation of the fungus, which significantly corroborate with the observations of Tsay and Kuo (1991).

Ramchandra and Bhatt (2011) reported *Fusarium equiseti* from cumin plants suffered from wilt disease and when they were cultured on PDA medium produced macro and micro conidia. Macroconidia were 28.0-30.5µm in length and 3.5-5.25µm in width and slightly bended at the apex with 2-4 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 length. Chlamydospores observed were 7-13µm lenth and had globose shape. These were formed intercalary in solitary or in chains or clusters.

2.4. SYMPTOMATOLOGY OF FUNGAL DISEASES

Assessment of symptomatology is essential for the systematic study of an isolated pathogen. Detailed accounts on symptomatology of different fungal pathogens causing damges to cucurbits were reviewed by many workers across the world.

The symptoms caused by *Choanephora cucurbitarum* on cowpea pods were described in detail by Wilson and Jose (1965) as water soaked lesions on the pods, which further developed into wet rot of the affected tissues. In the advanced stages of disease development, the invaded portions became covered with a luxuriant whitish growth of the fungus, consisting of the conidial and sporangial fructifications which appeared as minute black pin head like structures. *C. cucurbitarum* had been reported to attack predominantly the floral parts of many plants such as squash, pumpkin, pepper, pea and bean (Kacharek *et al.*, 2003). The general appearance of *Choanephora* blight was similar to that of diseases caused by other Mucoralean fungi *viz., Mucor* and *Rhizopus*. On cucurbits, the fruits started rotting rapidly and white fungal mycelium appeared on the infected parts followed by development of fructifications (Kacharek *et al.,* 2003). Typical symptoms of soft rot on eggplant fruit caused by *C. cucurbitarum* were described in detail by Kwon and Jee (2005). The pathogen penetrated mainly through wounds on the fruit and the symptom initiated as water soaking and dark-green lesions. Under favourable environmental conditions, the diseased tissues showed complete rotting rapidly and eventually produced white mycelia and monosporous sporangiola on the lesions. In the final stages, the fruits were appeared like black spore ball covered with abundant tiny, black pin head like fruitification. Initially, the pin heads were white to brown in colour but later turned into purplish black colour within few days. The affected flowers and immature fruits became water soaked and very soft, finally watery soft wet rot was developed. The rotting was completed in 24 - 48 h. Symptoms were usually noticed to begin from the blossom end of the fruit. According to Park *et al.* (2014), the initial symptoms on Hibiscus exhibited as reddish purple coloured spots at the tip of the corolla of the flowers and it spread the entire flowers. Infected lesions came as water soaked lesion, which was followed by quick soft rotting of infected fruit tissues. In the developmental stage, white profuse growth of mycelia having black pin headed sporangia at the tip of the sporangiophore was observed. 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 *Choanephora cucurbitarum* attack young and expanded leaves. Symptom appeared as water-soaked lesion with dark mass of sporangiophores.

Zitter *et al.* (2004) observed that the infection of *Sclerotium rolfsii* occurs in fruits in contact with the soil and the disease development was favoured by warm and humid conditions. White mycelial mat was emerges out on the surface of basal fruits. The symptoms were initiated as small water soaked lesions and spread into the whole surface of the fruit. The fungus produced copious small globular or globoid sclerotia of similar size, which were initially white in colour but later turned into brown later this was observed both on potato dextrose agar medium and on the host fruit surface under field condition. The seriously infected fruits became soft wet and ultimately rotted (Kwon *et al.*, 2009). Symptoms started on where the fruits come in contact with the soil surface. When the disease advances, numerous small round sclerotial bodies develop in the fungal mycelial mat. Initially the sclerotia were white in colour; later becoming brownih in colour. Each sclerotia has the size of a mustard seed. The fungal pathogen also attaches the stem and crown, resulting in rapid wilting of the leaves. (Shankar *et al.*, 2014).

Warm humid condition, resulting from dense canopy favours the infection of *Rhizoctonia solani*. Symptom initially appeared as lesions starts as a water soaked areas which subsequently collapse and forms sunken irregular brown cankers on the fruit (Lewis and Papavizas, 1980). 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 cabbages affected with *Rhizoctonia solani* had a dark, sometimes soft decay at the base of superficial leaves and on rising cabbage heads. A brown coloured mycelium was seen on affected parts during the damp weather condition with infrequent small brown coloured sclerotia on the head of the cabbage. Yellowish brown coloured surface discoloration was seen on Very

small cucumbers. Large fruit which are in contact with the soil showed dark brown coloured water-soaked lesions and decay most frequently on the side of the fruit. The water soaked areas turn scabby and cracked if the lesion is allowed to dry (Shankar *et al.*, 2014).

Phytophthora sp. infection in tomato resulted in severe economic loss. Green stage of the fruit is the most susceptible stage for the infection. Inside the tomato fruit mucilaginous substances turn brown to black and finally decompose to a watery consistency in disease developmental stages (Sharma and Sohi, 1975). The infection to fruit of the tomato has been observed to occur at various stages of maturity. However, under warm humid climatic conditions it was generally covered with a white coloured downy mycelial growth. During the initial stage, the fruit remains as firm, there was no rotting symptom on the skin but ultimately decay progresses quickly and complete rotting of internal flesh upto the core was observed. The fruits shrink and become mummified (Singh, 1999; Gupta and Paul, 2001). *Phytophthora sp.* can infect most parts of the plants and can cause variety of symptoms including fruit rots in cucurbits (Housebeck and Lamour 2004; Granke *et al.* 2012). *Phytophthora nicotiana* is a hemibiotrophic pathogen. It establishes close contact with living cells of the host during the primary period of infection (biotrophy), before it induces death of host cell and grows in dead portion of the tissues (necrotrophy). Initial symptoms were seen as small water-soaked lesions spread rapidly until the entire fruit become necrotic and soft. Profuse white fungal cottony mycelial growth can be seen on rotten areas of the fruit when the humidity is high (Shankar *et al.*, 2014). Different isolates of *Phytophthora nicotianae* showed characteristic symptoms with white mycelial growth and lemoniform sporangia formation six days after inoculation. Detached fruits of brinjal cultivar Arka Kusumakar, tomato cultivar Arka Vikas, and ridge gourd cultivar Arka Sujata were used for the experiment. (Chowdappa *et al.* 2016). *Phytophthora nicotianae*, normally considered as a root rot causing pathogen, it possesses a broad host range including woody and herbaceous plants,

and causes crown decay, and may infect the aerial parts also including leaves, stems, and fruits (Panabieres *et al.*, 2016).

Drechsler (1925) described cucumber rot symptom caused by *Pythium aphanidermatum* as a 'cottony leak'. Tanina *et al.* (2004) observed primary symptoms on leaves and stem of chingensai (*Brassica campestris* L. *chinensis*) as small water soaked lesions at the bottom of the midrib. The diseased parts softened and changed into dark brown colour which progressed to stem portion of the plants leaving a fluffy white growth of mycelium. In cucurbits, *Pythium* sp. mostly finds on fruit portion in contact with soil. Small, water soaked lesions spread quickly until a great area of the fruit is necrotic and soft watery. Profuse, white coloured growth similar to tufts of white cotton can be found on rotten areas during the warm humid conditions (Shankar *et al.*, 2014).

The symptoms of *Corynespora cassiicola* initially appears on younger leaves of chilli as small brown circular spots later turning to circular with white papery centres surrounded by dark brown or black coloured borders. Yellow halos mostly appeared around the spots on the fruit surface. As the disease advanced, some spots progressively enlarged and coalesced. Sometimes cracking was also observed at the center of the fruit during the severe situation. Defoliation of infected leaves was observed in severely infected chilli plants. Dark brown spots also found on fruit and black lesions on stem (Kwon *et al.*, 2001). The lesions caused by *C. cassiicola* are mainly found on fruits and leaves of tomato fruit, causing rotting of the fruit tissue. Flocculent and confluent lesions of size 26.5 x 26.7 mm diameter, with deep depressions varies from 9 to 22 mm diameter, were found on the fruit surface 14 days after inoculation of the pathogen. The occurrence of a greyish brown colour or black halo in the center of the fruit and light gray colour on the peripheral area was observed. The damage was seen as a whitish in an advanced stage of development (Caetano *et al.*, 2018). Okra crop plants infected with *C. cassiicola* showed varied size of necrotic leaf spots, stem necrosis and severe fruit rot symptoms. Infected leaves showed typical symptom, which consists of lesions with pale yellow colour to brown coloured centres

having irregular outer ring bounderies of grey to dark brown coloured margin. Sometimes the cracks were seen at the center of the lesion. The infected stem showed initial symptom as dark, water soaked circular spots that finally coalesced and formed a large necrotic circular to irregular region having gray to black colour. Initial symptom found on the surface of the infected fruits, in the form of water soaked lesions having elliptical to irregular shape. Later on the lesions coalesced and produced outsized irregular necrotic areas on the fruit surface. (Ahmed and Khair, 2008).

Typical symptoms of *Fusarium* fruit rot on young fruits are in the form of yellowing which normally starts from the blossom end of each immature cucumber fruit, followed by browning and rotting. It develops mostly on warm humid climatic condition (Blancard *et al.*, 2005). Symptomatology of fruit rot of pumpkin caused by various fungal pathogens can vary considerably, depend on the stage of development of the lesion. Symptoms vary from small, corky spots to large, sunken areas covered with a white or grey coloured mold (Elmer *et al.*, 2007). 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*.

2.5. CHEMICAL CONTROL OF FUNGAL PATHOGENS

Fungicides are often a vital part of disease management as they control many diseases satisfactorily. Chahal and Grover (1974) reported that zineb, mancozeb, ziram and thiram were the most effective in controlling *Choanephora cucurbitarum* on chilli. Hammounda (2008) reported that mancozeb, dinocap and thiabendazole were found effective in reducing infection against *Choanephora*

cucurbitarum in the southern region of Oman (Dhofar). George (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 oxychloride, captan + hexaconazole, carboxin, carbendazim + mancozeb and propiconazole.

Yaqub and Shahzad (2006) reported that six fungicides *viz.* benomyl, mancozeb, thiovit, dithane M-45, carbendazim, and topsin-M were effective against *Sclerotium rolfsii.* Khan and Javaid (2015) reported that four fungicides tegula (tebuconazole), thiophanate methyl, ridomil gold (metalaxyl+mancozeb) and mancozeb significantly inhibit the radial growth of *Sclerotium rolfsii* under *in vitro* condition. The study concluded that tegula, mancozeb and thiophanate methyl can control *in vitro* growth of *Sclerotium rolfsii.* Thiophanate methyl followed by mencozeb could be used successfully to control collar rot of chickpea *in vivo.*

Diseases caused by *Rhizoctonia* are restricted by seed or soil treatments or both, or foliar applications of a variety of fungicides of various chemical groups, *viz.*benzimidazoles (thiabendazole, carbendazim and thiophanate-methyl), triazoles (propiconazole, hexaconazole, flusilazole, tebuconazole, triadimefon and triadimenol) (Kataria and Gisi, 1996). 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 mancozeb 75WP (0.2 %) was found to be the best. According to Sriraj *et al.* (2014), even at the lowest concentration of 10 ppm trifloxystrobin 25% + tebuconazole 50% and carbendazim totally inhibited the mycelial growth and sclerotia formation in *Rhizoctonia* blight in turmeric plant.

Sensitivity of *Phytophthora cryptogea* with contact, systemic and combination fungicides *viz.*, Bordeaux mixture, copper hydroxide 77WP (Kocide), propineb 70WP (Antracol), cymoxanil (8%) + mancozeb (64%) (Curzate M-8), carbendazim (12%) + mancozeb (63%) (Saaf), tebuconazole 5EC

(Folicur) revealed cent per cent inhibition with all the three concentrations tested (Praveen *et al.*, 2017). Black shank caused by *Phytophthora nicotianae* is a major disease of tobacco resulting in extensive loss of the crop. A study was carried out to screen the diverse fungicides to restrain the growth of black shank causing pathogen. Among eight fungicides tested at various concentrations, 0.1 per cent of trifloxystrobin + tebuconazole, fenamidone + mancozeb and metalaxyl + mancozeb exhibitted cent per cent inhibition and they found efficient over all the tested chemical fungicides. Among them, combination product fenamidone 10% + mancozeb 50 WG (0.1 per cent) considerably reduced black shank in the field (Jayalakshmi *et al.*, 2017). Buckeye rot disease caused by *Phytophthora nicotianae* var. *parasitica* tested against different chemical fungicides revealed that metaalaxyl+macozeb and cymoxanil+mancozeb are most effective and mancozeb and copper oxychloride are least effective (Gupta and Bharath, 2008).

Kanaiyalal (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 promising in mycelial growth inhibition. *Pythium ultimum* treated with carbendazim + mancozeb showed mean growth inhibition of 62.88% (Gholve *et al.*, 2014).

Sinnulingga and Suwarto (1996) reported that chemical control of *Corynespora* leaf fall disease in rubber plantation was advocated as a component of integrated disease management strategy. The fungicides recommended for disease control are carbendazim (0.2%), mancozeb (0.25%), carbendazim (0.2%) + mancozeb (0.2%), propineb (0.25), chlorothalonil(0.2%) and captafol (0.95%).

Al-sadi *et al.* (2011) stated that foliar application of carbendazim significantly reduced fruit rots of cucumber caused by *Fusarium equiseti* and *Corynespora cassiicola*. Sharma (2013) mentioned about the importance of seed treatment with thiram (0.25%) against fruit rot of cucurbitaceous vegetables caused by different fungal pathogens. 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 in tomato.

2.6. BOTANICALS

The use of organic preparations in agriculture has been targeted to produce healthy, nutritive, and pollution free food, thereby shielding the entire ecosystem, environment and human health. The utilization of organic preparations for controlling plant pathogens has often been considered as the best option for ecofriendly management of plant disease without upsetting the sustainability of environment or health of humans (Nene, 2012).

Olufolaji (1999) reported that the fruit and bark extracts of *Azadiracta indica* (neem) inhibits growth and sporulation of *Choanephora cucurbitarum*, which causes wet rot disease of *Amaranthus*. Soft rot disease of *Abelmoschus esculentus*, *Amaranthus hybridus* and *Vigna unguiculata* were observed as a result of *C. cucurbitarum* infection. The extracts derived from *Chromolaena odorata, Zingiber officinale, Gmelina arborea* and *Azadirachta indica* at different concentrations (0%, 10%, 20%, 30%, 40% and 50%) were used. With alcohol (ethanol) extract, there was total inhibition of fungal growth by all plant extracts used at all concentrations and a little extent of growth in aqueous extracts while neem (*Azadirachta indica*) shows the maximum inhibitory effect (Umana *et al*, 2015).

Kiran *et al*. (2006) reported that garlic extract gives maximum inhibition of *Sclerotium rolfsii* having an average of 65% at 2.5%, 5%, 10% concentarations while that of neem gives 12% inhibition only. Butt *et al*. (2016) reported that two important indigenous plants like *Alstonia scholaris* and *Azadirachta indica* leaf extract were more effective against *S. rolfsii* under *in vitro* condition at different concentrations (1%, 2%, 3%, 4% and 5%).

Banded leaf and sheath bligh of maize caused by *Rhizoctonia solani* is one of the most severe soil-borne disease of maize in various parts of India.

Evaluation of different botanicals and biocontrol agents lead to better management of the pathogen. Seven plants extracts namely: *Jatropha curcas, Azadirachta indica, Annona reticulata, Pongamia pinnata, Curcuma longa, Allium cepa* and *Datura stramonium* were tested in the lab using poison food method. Maximum mycelial growth inhibition was recorded in neem at both five (46.68%) and ten (51.12%) per cent concentrations (Rajput, 2013). Sheath blight disease of rice caused by the pathogen *Rhizoctonia solani* was isolated and tested against six botanicals and maximum per cent inhibition of radial fungal mycelial growth over control was showed by garlic extract (97.50%), neem extract (96.75%) and biskatali (94.25%) (Sifat and Monjil, 2017).

Pente *et al.* (2015) evaluated five botanicals *viz.*, neem, tulsi, onion, chrysanthemum and garlic at 5% concentration in the lab against *Phytophthora parasitica* by poisoned food method. Among five botanicals tested, 100 per cent inhibition of mycelial growth of *Phytophthora parasitica* var. *nicotianae* was observed in garlic extract which contains diallyl disulphide, allyl propyl disulphide, diallyl sulphide, diallyl trisulphide, allylalcohol, dimethyl trisulphide and allycin in its aromatic oils and the above mentioned compounds have antipathogenic effects (Akgul, 1993; Schwartz and Mohan,1995). Nagadze (2014) found that the water extracts of *Azadirachta indica* and *Allium sativum* are effective against *Phythopthora infestans*.

Neem extract is also capable of controlling pathogenic microorganisms. Neem extract has a potential for antifungal activities against fungi like *Pythium aphanidermatum, Alternaria alternata* and *Fusarium oxysporum* (Sukanya *et al.*, 2009; Al-Hazmi, 2013; Jain *et al.*, 2013). Brinjal damping off caused by *Pythium ultimum* is one of the key and critical diseases of brinjal. Ten different plant extracts (each at 10, 15 and 20 %) were studied. Among the plant extracts evaluated, garlic was found most efficient and recorded appreciably highest radial mycelial growth inhibition (94.83%) (Gholve *et al.*, 2014).

Gupta *et al.* (1982) reported that conidiospore germination of *Colletotrichum capsici* was inhibited by phytonoids of *Azardiracta indica, Allium sativum, Ocimum basilicum* and *Leucas* sp. Lakshmanan *et al.* (1990) evaluated antifungal property of ten plant extracts/botanicals in the laboratory condition against the cotton boll rot disease caused by *Corynespora cassiicola*. Among different botanicals tested garlic extract and clove extract were most successful in inhibiting radial mycelial growth by 96.8% and sporulation by 79.6 %.

Shivpuri *et al.* (1997) found that leaf extract of *Azadirachta indica* to be highly poisonous to *Fusarium oxysporum* f. sp. *ciceris*. Bansal and Gupta (2000) found leaf extract obtained from *Azadirachta indica* shows highly toxic against *Fusarium oxysporum*. Verma and Dhoroo (2003) reported that garlic and clove extract show total restriction of growth of *Fusarium oxysporum* f. sp *pisi*. Antifungal effect of neemazal against *Alternaria* and *Fusarium* was proved during the studies of Mishra *et al.* (2009).

2.7. BIOLOGICAL CONTROL OF FUNGAL PATHOGENS

The environmental problems and health concerns emerging out of irrational use of fungicides have prompted researchers to concentrate effort in developing alternative approaches for plant disease management with greater emphasis on organic disease control. Biological control measures involving the intelligent use of antagonistic microbes have gained strength in recent years.

Emoghene and Okigbo (2001) observed a considerable zone of inhibition between the pathogen *Choanephora cucurbitarum* and the isolates of *Xanthomonas campestris*, *Erwinia herbicola* and *Bacillus subtilis* on PDA plates. 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, which was analogous to the conventional fungicide Dithane M-45. Choudhary (2015) reported that among biocontrol agents *Trichoderma harzianum* + *Pseudomonas fluorescens*

treatment by seed-cum soil application was most effective followed by *Trichoderma harzianum* alone against *Choanephora cucurbitarum* in cucumber.

Karthikeyan *et al.* (2006) reported that *Trichoderma viride, Tricoderma harzianum* and *Pseudomonas fluorescens* were inhibitory bioagents against the growth of *Sclerotium rolfsii*. Ramzan *et al.* (2016) studied 15 bioagents against *Sclerotium rolfsii* responsible for root rot of mungbean in which *Trichoderma harzianum, B. cereus, B. subtilis, Trichoderma virens* and *Pseudomonas fluorescens* were efficient biocontrol agents but *Bacillus subtilis* and *Trichoderma harzianum* were the most effective.

Rehman *et al.* (2012) studied the comparative effect of different biological control agents against the pathogen *Rhizoctonia solani* causing damping off disease on cauliflower seedlings. They observed 85.5 and 83.0 per cent mycelial inhibition by *Trichoderma harzianum* and *T. viride.* Ashwini and Srividya (2014) isolated a strain of *Bacillus subtilis* obtained from the rhizosphere of chilli which showed broad spectrum antagonism against many pathogens. In dual culture, it inhibited *Alternaria* spp. (55%), *Colletotrichum gloeosporioides* (57%), *Phytophthora capsici* (55 %), *Fusarium solani* (42 %), *Rhizoctonia solani* (42 %), *F. oxysporum* (40 %) and *Verticillium* sp. (36 %).

The fungal antagonist *Trichoderma harzianum, Trichoderma hamatum* and *Trichoderma koningii* were found to wind around the hyphae and pierce the oospores of *Phytophthora meadii* and caused lyses (Vanitha *et al.*, 1994). Mushrif *et al.* (2005) isolated five bacterial antagonists (two *Bacillus* spp. and three *Pseudomonas* spp.) from the rhizosphere and phyllosphere of rubber, which were found antagonistic to *Phytophthora* and were well matched with fungicides mancozeb, carbendazim and metalaxyl. *Bacillus* spp. revealed higher efficacy in disease control when tested under laboratory and field conditions. According to Sharma *et al.* (2018) *Phytophthora nicotianae* is a serious hazard to the crop production, which affects mainly the fruits. In their investigation, six biolocontrol agents were studied for the effectiveness against *Phytophthora nicotianae* from

tomato. Out of the six biocontrol agents tested, *Trichoderma virens* gave the maximum mycelial growth inhibition (77.67%) followed by *Trichoderma hamatum* (69.40 %), *Trichoderma harzianum* (68.52 %) and *Trichoderma viride* (67.43 %). *Pseudomonas fluorescens* was least effectual with 53.67 per cent radial mycelial growth inhibition. The study revealed *T. harzianum* (CKT isolate) as the most abiotic stress tolerant isolate to promote growth and *Phytophthora* management in pepper nursery under gereenhouse conditions (elevated temperature upto 41°C) (Vithya *et al.* 2018)

Abdelzaher (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. El-Mohamedy (2012) identified the effects of biocontrol agents isolated from rhizosphere of broccoli plants. Biocontrol agents *T. harzianum, T. viride, Pseudomans fluorescens* and *Bacillus subtilis* totally inhibited the growth of *Pythium ultimum* on potato dextrose agar medium. Incorporating the soil with biocontrol agents *ie*, *Trichoderma harzianum, Pseudomans fluorescens* ($8x10^7$ spores/ml), *T. virde* ($5x10^6$ spores/ml) and *Bacillus subtilis* and dipping the seedling roots in the same preparation gave the highest result against *Pythium* root rot. In an experiment Jain *et al.* (2013) revealed the effectiveness of talc based formulation on damping off 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 maximum mycelial growth inhibition of 69.45% was given by *Trichoderma viride. Trichoderma koningii* and *Trichoderma hamatum* were found to be promising antagonists with 67.32 per cent and 63.99 per cent inhibition respectively. Bacterial biocontrol agents, *Bacillus subtilis* and *Pseudomonas*

fluorescens gave the inhibition of 59.72 and 56.37 per cent, respectively. Kipngeno *et al.* (2015) evaluated the effect of *Trichoderma asperellum* and *Bacillus subtilis* on *Pythium* damping off on Ethiopian kales (*Brassica carinata*). The incidence of post emergence damping off reduced to the range of 11-25.4 per cent on seeds coated with biocontrol agents whereas control recorded 64.8 per cent incidence. They suggested that the use of *B. subtilis* and *T. asperellum* mixture can provide a potential control against damping off.

Application of *P. fluorescens* as seedling dip and foliar application methods successfully controlled stem blight disease in *Phyllanthus amarus* caused by *Corynespora cassicola* (Mathiyazhagam *et al.*, 2004). Philip *et al.* (2005) isolated endophytic bacteria from roots, stem, petiole, leaves and flowers of rubber and the selected isolates showed systemic resistance on challenge inoculation with *C. cassiicola* under glasshouse condition.

Sahar *et al.* (2013) observed moderate inhibition of *Fusarium oxysporum* f.sp. *melongenae* causing Fusarium wilt disease in eggplant by *Bacillus subtilis*. At the 6th and 9th day of inoculation *Bacillus subtilis* recorded 50 and 48 % inhibition. Bana *et al.* (2017) conducted lab and field experiments for the control of root rot disease of fennel infected with *Fusarium oxysporum*. Under *in vitro* condition, *Trichoderma harzianum* recorded 79.44% 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 21 per cent disease incidence, while *Trichoderma viride* and *Pseudomonas fluorescens* recorded 21.65 and 25.27 per cent disease incidence, respectively.

Materials & Methods

3. MATERIALS AND METHODS

The study entitled 'Characterization and bio intensive management of fungal fruit rots of cucurbits' was conducted in the Department of Plant Pathology, College of Agriculture, Padannakkad during the period 2017-19. 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 FRUIT ROTS OF CUCURBITS

Purposive sampling surveys were carried out on the incidence of fungal fruit rots of cucurbits in Kasargod, Kannur and Kozhikkode districts of Kerala, grown as rainfed (May-August) and irrigated (January-March and September-October) crops. The infected fruit samples were collected for the isolation of associated pathogens from different locations of above three districts. The details regarding the locations surveyed are given in Table.1

3.2. ASSESSMENT OF PER CENT DISEASE INCIDENCE AND PER CENT DISEASE SEVERITY

The disease incidence 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 severity of disease were assessed by recording the number of fruits infected using the following formula (Al-Sadi *et al*., 2011)

$$\text{Per cent disease severity (PDS)} = \frac{\text{Number of fruits affected /plant}}{\text{Total number of fruits/plant}} \times 100$$

3.3. ISOLATION OF PATHOGENS

Fruits of cucurbits which were naturally infected, showing characteristic symptoms were collected as samples from diverse locations as mentioned in Table 1. These samples were washed under tap water and cut into small bits consisting of both healthy and infected parts using a sterilized blade and were disinfected with mercuric chloride (0.1 per cent) for one to two minutes. After three consecutive washings in sterile distilled water, the washed samples were located aseptically in sterile Petri plates containing solidified Potato Dextrose Agar (PDA) medium. The inoculated Petri plates were incubated at normal room temperature (28± 2^0C). The fungal mycelial growth obtained from second to sixth day of incubation was then subcultured to solidified PDA medium in sterile Petri plates. Purification of these isolates was done by single hyphal tip method. Maintenance was done by periodic subculturing and the different isolates were maintained in PDA slants under refrigerated condition at 4^0C for further studies.

3.4. SYMPTOMATOLOGY OF DISEASES

During the survey, symptom developments of fruit rots of cucurbits under natural conditions were studied. The symptoms developed during artificial conditions were also recorded. For this study, the fungal pathogens causing fruit rot diseases in cucurbits were artificially inoculated by Mycelial Bit Inoculation Method (MBIM) (Rocha *et al*., 1998). For this, detached, fresh, healthy un

47

infected fruits were collected, brought to the lab and washed under tap water followed by surface sterilization using 70 per cent ethanol. Injury was given by pinprick method on fruit surface using sterile needle. From two to seven day old culture of test pathogen, mycelial discs of size 8 mm diameter was taken and inoculated in inverted position on injured fruit surface. The spot of inoculation was then covered with moistened cotton. After inoculating the mycelial bit, the fruits were kept in incubation chamber under high humidity at normal room temperature till the symptoms appeared on inoculated portions of the fruit. The healthy fruit with injury without inoculation with the fungal mycelial bit kept as control.

3.5. PATHOGENICITY OF ISOLATES

The isolates obtained from the infected fruits of cucurbits during the survey in three northern districts of Kerala were selected. Artificial inoculation of cultures on healthy fruits was done to test pathogenicity by following Koch's postulates. This study was carried out on detached fruits under *in vitro* conditions as per standard procedure as mentioned in 3.4.

3.6. CHARACTERISATION AND IDENTIFICATION OF PATHOGENS

The isolates were numbered by giving code numbers. Characterisations of fungal pathogens isolated were done based on their morphological and cultural characters.

3.6.1. Cultural and morphological characters

Visual observations on the growth and development of the respective pathogens were recorded. For this, mycelial discs of the pathogenic fungus on PDA with a diameter of 5mm was inoculated at the center of PDA plates and incubated at 28 ± 2^0C. Variations in colony characteristics, pigmentation, and growth pattern of each isolate 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,

morphological characterisation was done. With the help of ZEN imaging software, photomicrographs and measurements of fungal structures were taken. For further confirmation of the identity of each pathogen by ITS sequencing, the isolates were sent to Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram.

Isolated fungal pathogens based on their morphological and cultural characters were temporarily identified upto their genus level. Species level identification was done at Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram by ITS sequencing method.

3.6.2. Molecular characterisation

Isolated pathogens were subjected to molecular characterisation at Rajiv Gandhi Centre for Biotechnology (RGCB) 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).

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

About 100 mg of the tissue/mycelium was homogenized using liquid nitrogen and the powdered tissue was transferred into a microcentrifuge tube. Four hundred microlitres of buffer PL1 was added and vortexed for 1 minute. Ten microlitres of RNase A solution was added and inverted to mix. The homogenate was incubated at 65^0C for 10 minutes. The lysate was transferred to a Nucleospin filter and centrifuged at 11000 x g for 2 minutes. The flow through liquid was collected and the filter was discarded. Four hundred and fifty microlitres of buffer PC was added and mixed well. The solution was transferred to a Nucleospin Plant II column, centrifuged for 1 minute and the flow through liquid was discarded. Four hundred microlitre buffer PW1 was added to the column, centrifuged at 11000 x g for 1 minute and flow though liquid was discarded. Then 700 µl PW2 was added, centrifuged at 11000 x g and flow through liquid was discarded. Finally 200 µl of PW2 was added and centrifuged at 11000 x g for 2 minutes to

dry the silica membrane. The column was transferred to a new 1.7 ml tube and 50 µl of buffer PE was added and incubated at 65°C for 5 minutes. The column was then centrifuged at 11000 x g for 1 minute to elute the DNA. The eluted DNA was stored at 4°C.

B. Agarose Gel Electrophoresis for DNA Quality check

The quality of the isolated DNA was analysed by means of agarose gel electrophoresis. 1µl of 6X gel-loading buffer (0.25% bromophenol blue, 30% sucrose in TE buffer pH-8.0) was supplemented to 5µl of DNA. The samples were loaded to 0.8% agarose gel made in 0.5X TBE (Tris-Borate-EDTA) buffer consisting 0.5 µg/ml ethidium bromide dye. Electrophoresis process was carried out with 0.5X TBE as electrophoresis buffer at 75 volt until bromophenol dye front has moved to the bottom side of the gel. The gels were observed in an ultraviolet transilluminator (Genei) and the image was captured under ultraviolet light using equipment called gel documentation system (Bio-Rad).

C. PCR Analysis

PCR (polymerase chain reaction) amplification reactions were carried out in 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 done in a 20 µl reaction volume having 1X Phire PCR buffer (contains enzyme, 0.1 mg/ml BSA and 3% DMSO, 0.5M Betaine, 5pM of forward primer 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 done in PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems).

PCR amplification profile

ITS & LSU

98 °C	-	30 sec	
98 °C	-	5 sec	40 cycles
60 °C	-	10 sec	
72 °C	-	15 sec	
72 °C	-	60 sec	
4 °C	-	∞	

D .Agarose Gel electrophoresis of PCR products

The PCR products were checked in 1.2 per cent agarose gels prepared in 0.5 X TBE buffer solution containing 0.5 µg/ml ethidium bromide dye. 1 µl of 6X loading dye was incorporated with 5 µl of PCR products obtained and was loaded and electrophoresis was carried out at 75 volt with 0.5X TBE as electrophoresis buffer for the time period of about 1-2 hours, until the bromophenol blue front had moved to almost the bottom end of the gel. The molecular standard used here was 2-log DNA ladder (NEB). The gels were observed in an ultraviolet transilluminator (Genei) and the image was captured under ultra violet light using gel documentation system (Bio-Rad).

E. 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 was mixed with 2 µl of ExoSAP-IT and incubated at 37^0C for 15 minutes followed by enzyme inactivation at 80^0C for 15 minutes.

F. Sequencing using Big Dye 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 ^{0}C for 2 minutes followed by 30 cycles at 96 ^{0}C for 30 sec, 50^{0}C for 40 sec and 60^{0}C for 4 minutes for all the primers.

G. Post Sequencing PCR Clean up

1. Maked master mix I of 10µl milli Q and 2 µl 125mM EDTA per reaction
2. Added 12µl of master mix I to each reaction containing 10µl of reaction contents and are properly mixed.
3. Maked master mix II of 2 µl of 3M sodium acetate pH 4.6 and 50 µl of ethanol per reaction.
4. Added 52 µl of master mix II to each reaction.
5. Contents are mixed by inverting.
6. Incubated at room temperature for 30 minutes
7. Spun 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. Decanted the supernatant and repeated 70% ethanol wash
11. Decanted the supernatant and air dried the pellet.

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

H. 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 v 5.1 (Drummond *et al.*, 2010).

3.7. EFFICACY OF FUNGICIDES, BOTANICALS AND BIOCONTROL AGENTS AGAINST PATHOGENS UNDER *IN VITRO* CONDITIONS

The present study was carried out to evaluate the effectiveness of fungicides, botanicals and biocontrol agents as depicted in Table 3, Table 4 and Table 5, respectively against fungal fruit rot causing pathogens of cucurbits.

3.7.1. *In vitro* evaluation of chemical fungicides

Poisoned food technique (Nene and Thapliyal, 1993) was used for the *in vitro* evaluation of fungicides. The three concentrations used were that of the recommended dosage as per Package of Practices by KAU (KAU, 2016) and a lower and higher concentrations from the recommended dosage were also used. The experimental design used was Completely Randomized Design (CRD) with four treatments and three replications. The fungal isolates were grown on PDA medium for one week prior to the experiment. The fungicides, each at three different concentrations, were added to 100 ml molten PDA medium to obtain the required concentration (Table 3 and Table 4). About 20ml of poisoned medium was poured in each sterilized Petri dishes and thoroughly mixed. The mycelial disc of 5 mm diameter was taken from periphery of one weak old colony and placed at the center of Petri plates and incubated at 28 $\pm 1^{0}$C. 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: Per cent inhibition

C: Mycelial growth in control (mm)

T: Mycelial growth in treatment (mm)

3.7.2. *In vitro* evaluation of botanicals

Poisoned food technique (Nene and Thapliyal, 1993) was used for the *in vitro* evaluation of botanicals. Three concentrations were selected and the experimental design was Completely Randomized Design (CRD) with three treatments and three replications. The fungal pathogens were grown on PDA medium for one week prior to the experiment. The botanicals *viz.,* azadirachtin, garlic extract and ready to use neem oil garlic soap (first botanical product of Kerala Agricultural University) each at three different concentrations were added to 100ml molten PDA medium to obtain the required concentration (Table 4). Ready to use neem oil garlic soap is the first botanical product in ready to use form from Kerala Agricultural University which was targeted against sucking pests of different crops. Antifungal action of this product was evaluated in this study. About 20 ml 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 one weak old colony and placed at the center of Petri plates and incubated at 28 $\pm 1^0$C. 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: Per cent inhibition

C: Mycelial growth in control (mm)

T: Mycelial growth in treatment (mm)

Table.3 Fungicides evaluated against fruit rot pathogens (*in vitro*)

Sl. No	Chemical Fungicides	Trade Name	Manufacturer	Concentrations (%)		
				Low	Recommended	High
1	Mancozeb 75% WP	Mega-M 45	K.P.R Fertilisers Ltd, Andhra Pradesh	0.1	0.2	0.3
2	Mancozeb 63%+Carbendazim 12%	Saaf	UPL	0.1	0.2	0.3
3	Mancozeb 64%+Cymoxanil 8%	Curzate	DUPONT	0.1	0.2	0.3
4	Tebuconazole 5% EC	Folicur	Bayer Crop Science Ltd. Thane	0.05	0.1	0.2

Table.4 Botanicals evaluated against fruit rot pathogens (*in vitro*)

Sl.No	Botanicals	Trade Name	Manufacturer	Concentrations (%)		
				Low	Recommended	High
1	Azadirachtin 0.1%	Neemazal	Parry's Bio Pvt. Ltd.	0.1	0.2	0.3
2	Garlic extract	-	-	0.5	1.0	2.0
3	Ready to Use neem oil garlic soap	-	Kerala Agricultural University	0.1	0.6	1

Table 5. Biocontrol agents evaluated against fruit rot pathogens (*in vitro*)

Sl.No	Biocontrol agents	Concentration (%)
1	*Trichoderma viride*	2
2	*Pseudomonas fluorescens*	$1x10^6$cfu/ml
3	*Bacillus subtilis*	$1x10^6$cfu/ml
4	PGPR (Plant Growth Promoting Rhizobacteria) Mix-II	2
5	PGPM (Plant Growth Promoting Microbes)	2

3.7.3. *In vitro* evaluation of biocontrol agents

The reference cultures of KAU *viz., Trichoderma viride, Pseudomonas fluorescens, Bacillus subtilis,* PGPR (Plant Growth Promoting Rhizobacteria) Mix-II from College of Agriculture, Vellayani and PGPM (Plant Growth Promoting Microorganisms) from College of Horticulture, Vellanikkara were tested against isolated fungal fruit rot pathogens. Dual culture technique (Skidmore and Dickinson, 1976) was used for evaluation of *Trichoderma viride, Pseudomonas fluorescens* and *Bacillus subtilis*. Poisoned food technique (Nene and Thapliyal, 1993) was used for evaluation of PGPR Mix-II and PGPM. The experimental design was Completely Randomized Design (CRD) with five treatments and three replications. The per cent inhibition of PGPR Mix-II and PGPM was calculated using the formula given by Vincent (1927) as mentioned in 3.7.1

3.7.3.1. *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 (Skidmore and Dickinson, 1976). Agar discs of 5mm diameter were cut from the edge of the vigorously growing seven day 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 Petridish of 90 mm diameter and incubated at room temperature (28± 1°C). Three replications were maintained for each treatment. The nature of parasitism of *Trichoderma viride* on the pathogen was studied at regular intervals. Petriplates inoculated with 5mm agar discs of respective pathogens served as control. Measurements of growth were taken when control plates reached full growth. The percentage inhibition of mycelial growth was calculated as mentioned in 3.7.1.

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

Assessment of nature of parasitism against the pathogens was done by categorisation in four groups using the method of Purakayastha 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

3.7.3.2. *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 (Skidmore and Dickinson, 1976) by simultaneous antagonism. The bacterial antagonists were streaked on both the ends of Petri dish in the PDA medium two centimetres away from the edge of the plate prior to pathogen inoculation. Then 5mm sized culture disc of pathogen was cut from the edge of pure culture and was placed on the center of the Petri dish. Plates were incubated at room temperature (28± 1°C) till control plate attained full growth. Three replications were maintained for each isolate. Petri dishes inoculated with pathogen alone served as control. Observations on growth of the pathogens 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 3.8.1.

3.7.3.3. *In vitro* evaluation of consortium of microbes PGPR Mix-II and PGPM

Poison food technique (Nene and Thapliyal, 1993) was used for the *in vitro* evaluation. The fungal pathogens were grown on PDA medium for one week prior to the experiment. PGPR Mix-II and PGPM each at recommended concentrations were added to 100ml molten PDA medium to obtain the required concentration (Table 5). 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 one weak old colony and was placed in the centre of Petri plates and incubated at 28 $\pm 1^0$C. 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 formula given by Vincent (1927).

3.8. EVALUATION OF CHEMICAL FUNGICIDES, BOTANICALS AND BIOCONTROL AGENTS UNDER *IN VIVO* CONDITIONS

A field experiment was laid out for the management of the most severe and predominant fungal pathogen using the promising treatments obtained under *in vitro* studies. Selection of fungal pathogen and the crop 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, botanicals and biocontrol agents as carried out in 3.7. All the agronomic practices were adopted as per Package of Practices Recommendations, Kerala Agricultural University (KAU, 2016). Observations on disease incidence and percent disease reduction were recorded. Effect of different treatments on the growth parameters of the cucurbit crop during the management of the major pathogen was also evaluated and biometric observations were taken.

The details of the experiment are as follows:

Design	: RBD
Number of treatments	: 7
Replications	: 3

3.9. 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).

60

Results

4. RESULTS

The present investigations on the 'Characterization and bio intensive management of fungal fruit rots of cucurbits' was carried out to identify various pathogens infecting fruits of cucurbits and to study the symptomatology, characterization and management of fruit rot of cucurbits. The results of the investigation carried out during 2017-19 are presented below.

4.1 SURVEY AND COLLECTION OF DISEASED SAMPLES

A purposive sampling survey was conducted in three districts *viz.*, Kasargod, Kannur and Kozhikode for the collection of diseased samples of fruits of cucurbits and the disease incidence and severity was recorded during the cropping season of 2017-18 (Plate 1). The diseased specimens were collected from these locations during different seasons (Table 1), and the pathogens were isolated. The details of the survey conducted are presented in Tables 5 and 6.

4.2. ASSESSMENT OF PER CENT DISEASE INCIDENCE AND PERCENT DISEASE SEVERITY

The disease incidence and severity of fungal fruit rots of cucurbits in seven different locations of three districts were recorded and the diseased fruits were collected. Disease incidence was calculated as percentage of the proportion of number of infected and healthy plants. Disease severity was calculated as a percentage of the proportion of number of infected and healthy fruits per plant Data obtained is given in the (Table 7).

The data revealed that the incidence of different fungal fruit rots of cucurbits varied within a range of 6 to 51 per cent. The disease incidence (51%) and severity (58.8%) was maximum in fruit rot-1 (FR 1) of pumpkin obtained from COA, Padannakkad. Fruit rot FR-4 was the second severe disease with incidence 47% and 43.75% severity. The results of survey showed that FR-7 and FR-5 were causing minor damage to the crop (10% and 6%, respectively) (Table 7).

Table.1. Locations of purposive sampling survey in various districts

District	Location
Kasaragod	College of Agriculture, Padannakkad (Polyhouse)
	College of Agriculture, Padannakkad (Open field)
	Periya
	Karakode
	Kurunthoor
Kozhikkode	Nadapuram
Kannur	Panoor

Table 5. Occurrence of fungal fruit rot diseases of cucurbits in different districts of Northern Kerala

Sl. No.	District	Location	Type of cultivation	Season
1	Kasaragod	COA, Padannakkad	Open field	January-March
		COA, Padannakkad	Polyhouse	September-December
		Periya	Open field	May-August
		Karakode	Open field	September-December
		Kurunthoor	Open field	January-March
2	Kannur	Panoor	Open field	January-March
3	Kozhikode	Nadapuram	Open field	September-December

CoA, Padannakkad, (Poly house)

CoA, Padannakkad (Instructional Farm)

Periya

Karakode

Kurunthoor

Panoor

Plate 1. Different locations of survey and collection of samples

Table 6. Details of isolates obtained during the survey

Sl. No.	Disease	Host	Isolate code
1	Fruit rot- 1	Pumpkin	FR 1
2	Fruit rot- 2	Pumpkin	FR 2
3	Fruit rot- 3	Cucumber	FR 3
4	Fruit rot- 4	Ridge gourd	FR 4
5	Fruit rot- 5	Cucumber	FR 5
6	Fruit rot- 6	Snake gourd	FR 6
7	Fruit rot- 7	Salad cucumber	FR 7

Table 7. Per cent disease incidence and severity of fungal fruit rots of cucurbits in Kasargod, Kannur and Kozhikode districts

Sl. No	District	Location	Isolate code	PDI	PDS
1	Kasaragod	COA, Padannakkad (open field)	FR 1	51	58.8
		COA, Padannakkad (Polyhouse)	FR 7	10	14.49
		Periya (open field)	FR 4	47	43.75
		Karakode (open field)	FR 3	22	18.7
		Kurunthoor (open field)	FR 6	24	25
2	Kannur	Panoor (open field)	FR 2	37	37.5
3	Kozhikode	Nadapuram (open field)	FR 5	6	8.3

4.3. ISOLATION OF PATHOGENS

Seven different pathogens were isolated from naturally infected samples of fruits of cucurbitaceous vegetables collected from various locations of three districts in northern Kerala (Table 7). The pathogens were isolated from fruits, purified by single hyphal tip method and maintained on PDA by periodic subculturing.

4.4. SYMPTOMATOLOGY OF DISEASES

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

4.4.1 Symptomatology in natural condition

Survey was conducted in seven locations in three districts of northern Kerala and different symptoms of fungal fruit rot diseases of cucurbits and their variations were noted. The two main types of rotting observed were soft rots and wet rots.

4.4.1.1. Fruit rot- 1

Initial symptom started as small water soaked lesions at the tip of the corolla of the female flower of pumpkin after the closure of corolla. Later fruitification of the fungus was spread along the corolla and reached the blossom end of the fruit within a day. Later, this water soaked lesions with profuse fruitificatins covered the entire fruit and finally soft wet decay of the fruit occurred causing shedding of the fruits (Plate 2).

4.4.1.2. Fruit rot- 2

The symptom of fruit rot-2 was mainly seen on fruits of pumpkin in contact with soil surface. Initial symptom was appearance of small water soaked lesions on the fruits which caused rotting of the whole fruit. White mycelial mat and sclerotial bodies developed within ten days. Initially the sclerotial bodies were

67

white and later the colour changed to brown. Discolouration was found in the internal tissues also (Plate 3).

4.4.1.3. Fruit rot- 3

Initially symptom appeared irregular water soaked lesions on the mature fruits of cucumber, which subsequently turned into sunken dark brown cankers and finally these cankers coalesced and entire fruit decayed within a week. Internal tissues disintegrated and cracks were formed on the cankers during drying (Plate 4).

4.4.1.4. Fruit rot- 4

Fruit rot- 4 on immature and mature fruits of ridge gourd produced initial symptoms as dark green water soaked lesions with white mycelial growth on the surface. Later lesions changed colour to light brown. Lesions with white mycelia enlarged and resulted in soft watery rot. Finally, fruit drop occurred within two weeks. Disease severity was high in warm humid weather (Plate 5).

4.4.1.5. Fruit rot- 5

Pathogen produced watery soft rot with white puffy cottony mycelium on the fruits of cucumber. Symptom started as water soaked lesions. Entire fruit was covered with fungus and complete collapse of the fruits occurred within one week of infection due to the fast growing nature of the pathogen. This wet rot produced offensive smell also (Plate 6).

4.4.1.6. Fruit rot- 6

Fruit rot- 6 was observed on mature snake gourd fruits. Symptoms initially appeared as water soaked sunken spots with black concentric rings. Later, complete rotting occurred, causing internal discoloration with sporulation on the surface (Plate 7).

4.4.1.7. Fruit rot- 7

Fruit rot- 7 was observed on immature salad cucumber in the polyhouse. Initially symptom started as yellowing of the tissue at the blossom end of the fruits. Yellow portion gradually turned into brown and spread on entire fruit and dry rotting was observed after seven days of infection (Plate 8).

4.4.2. Symptomatology under artificial conditions

Under artificial conditions, developments of symptoms on the fruits were studied. The symptoms noticed were similar to that observed in field under natural conditions.

4.5. PATHOGENICITY OF ISOLATES

The pathogenicity of different isolates from fruits of cucurbits was proved in detached fruits by artificial inoculation using Mycelial Bit Inoculation Method. Details of the symptoms observed after inoculation of each pathogen are described below.

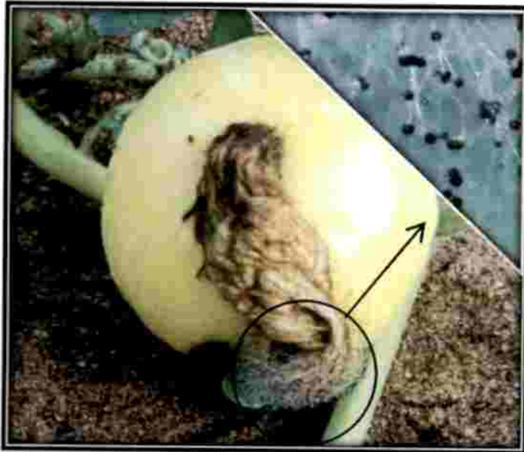
4.5.1. Fruit rot- 1

The pathogenicity test of fruit rot-1 showed that the pathogen could infect the fruits of pumpkin and could produce typical symptoms of the disease after two days of inoculation. Initial symptom started as small water soaked lesion, which later enlarged with profuse fructifications. Eventually, it spread to entire fruit and later appeared as soft wet decay. Symptoms produced on detached fruit were similar to that in the field (Plate 2).

4.5.2. Fruit rot- 2

Small water soaked lesions were developed initially on the fruits of pumpkin which later produced rotting of the whole area along with the production of white mycelial mat and brown sclerotial bodies. Initial symptoms were

69

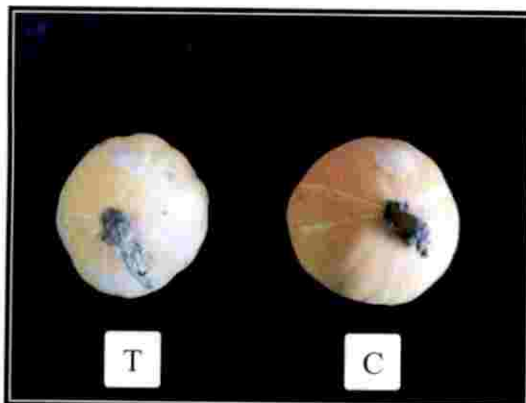


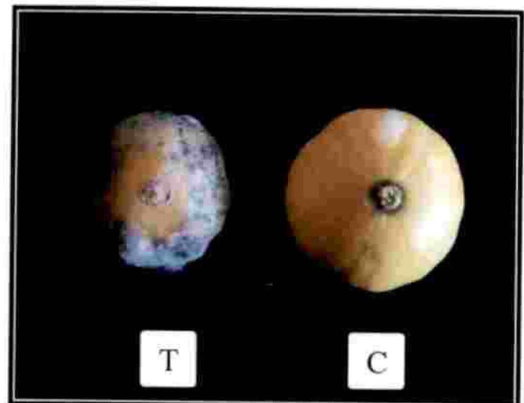
Sporulation at the tip of the flower

Spread along the flower

Spread downward into the fruit

Cover entire fruit and results in soft rot





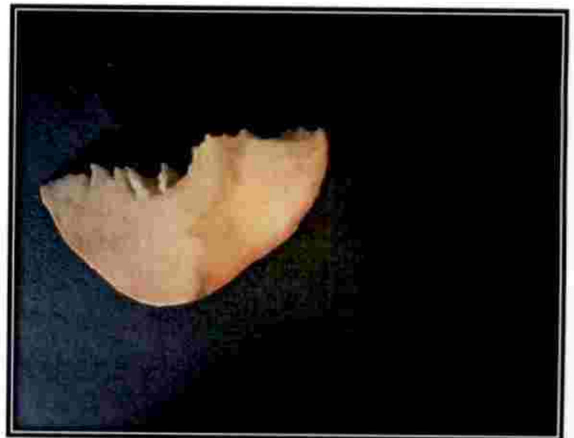
Pathogenicity test

Plate 2. Symptomatology and pathogenicity test of *Choanephora cucurbitarum* fruit rot

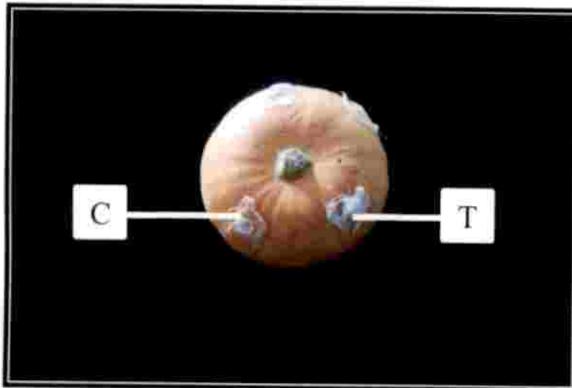
White mycelial mat

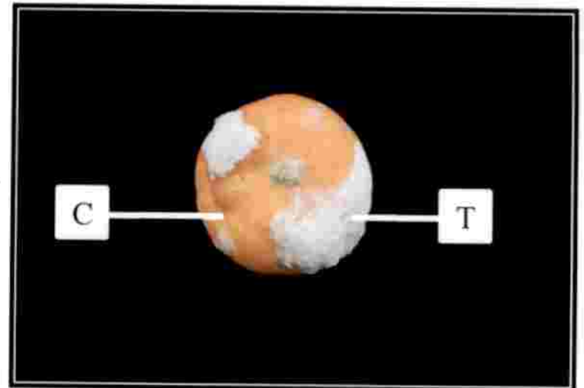
Sclerotia production

Internal growth of mycelium



Internal discoloration

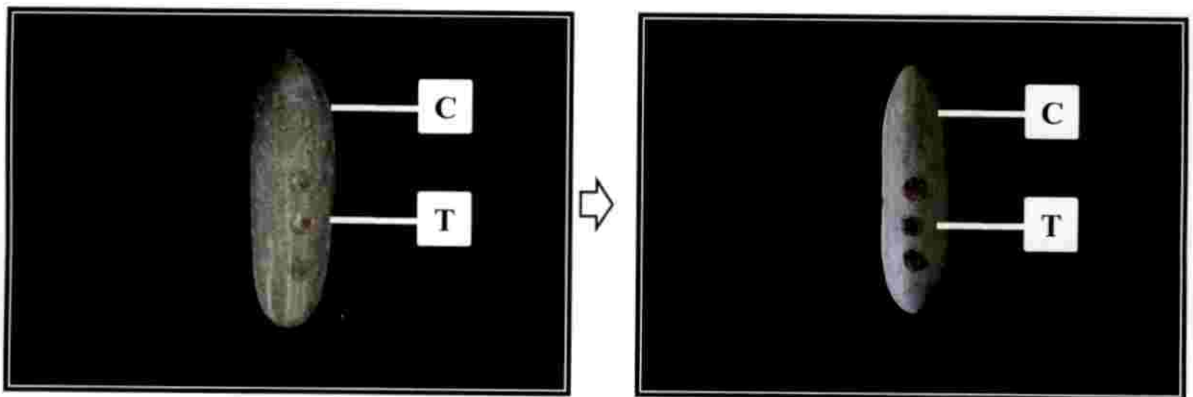




Pathogenicity test

Plate 3. Symptomatology and pathogenicity test of *Sclerotium rolfsii* fruit rot

Irregular water soaked lesions with sunken dark brown cankers

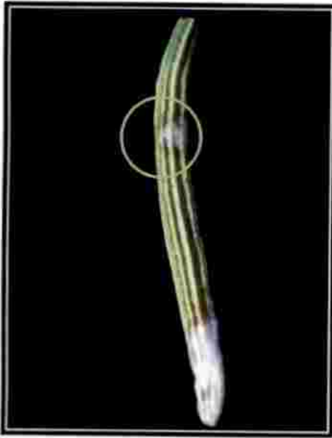


Pathogenicity test

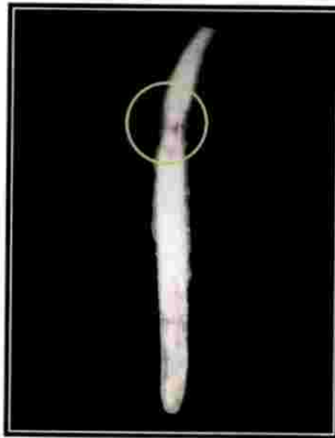
Plate 4. Symptomatology and pathogenicity test of *Rhizoctonia solani* fruit rot

Dark water soaked lesions

White mycelial growth

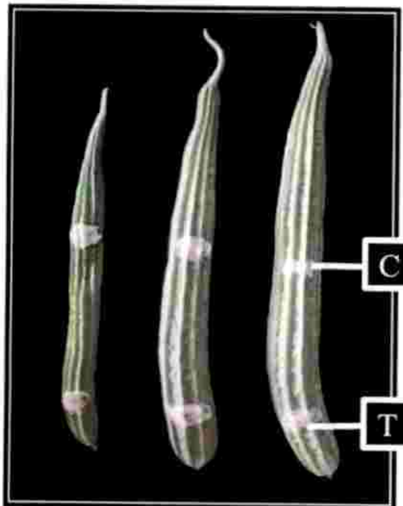


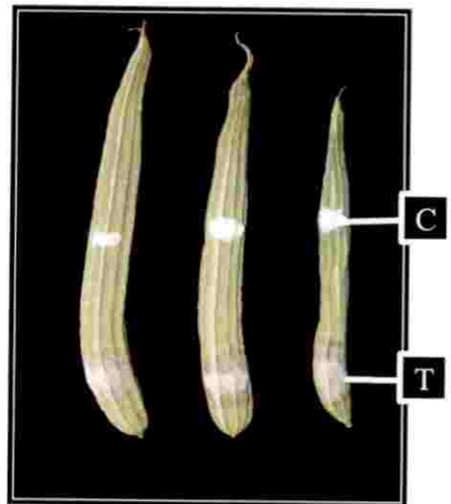
Infected whole fruit



Longitudinal section

transverse section

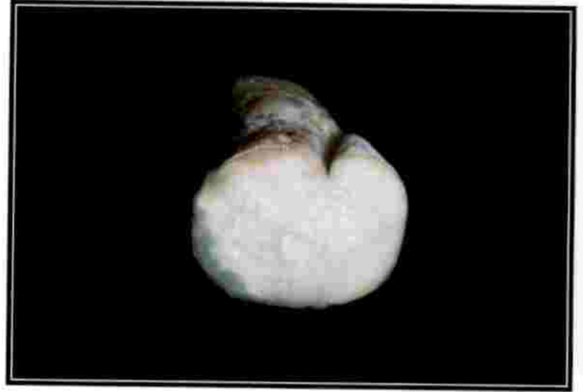




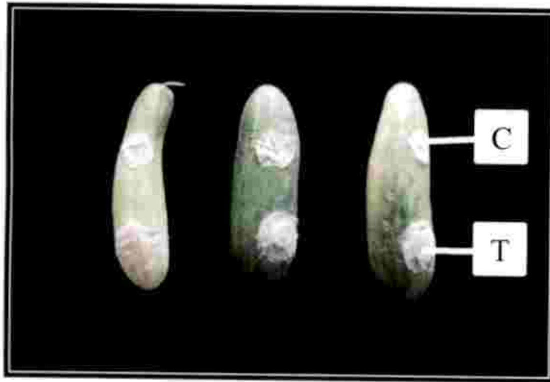
Pathogenicity test

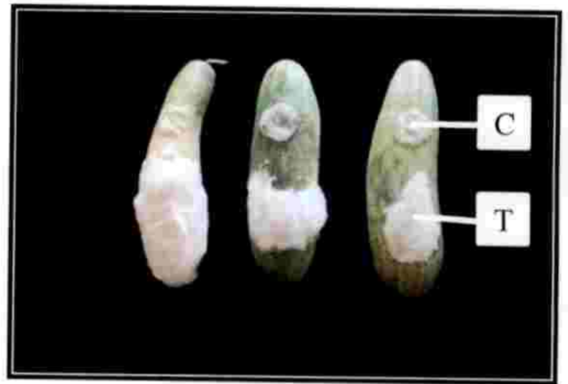
Plate 5. Symptomatology and pathogenicity test of *Phytophthora nicotianae* fruit rot

Water soaked lesions with cottony mycelial growth



Internal discoloration and watery soft rot

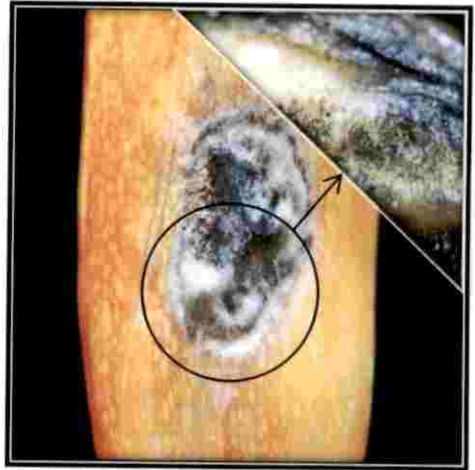




Pathogenicity test

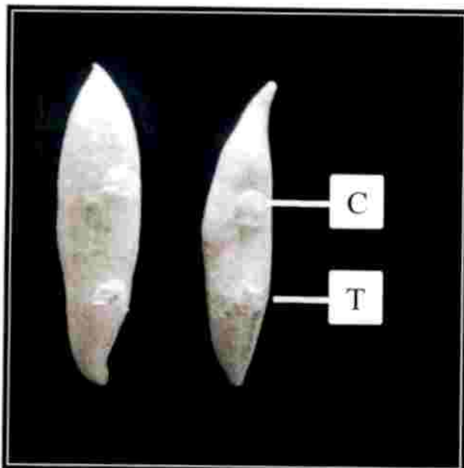
Plate 6. Symptomatology and pathogenicity test of *Pythium deliense* fruit rot

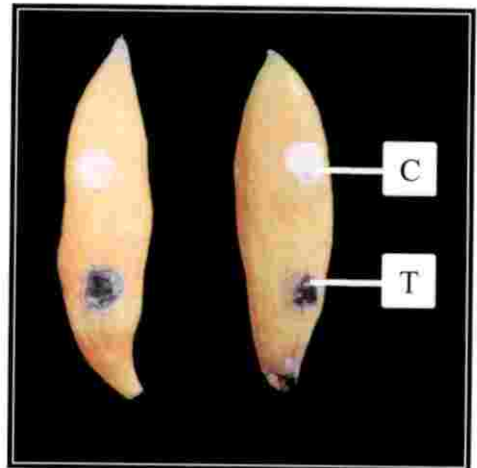
Water soaked sunken spots with black concentric rings


Expansion and sporulation

Flesh- soft rot

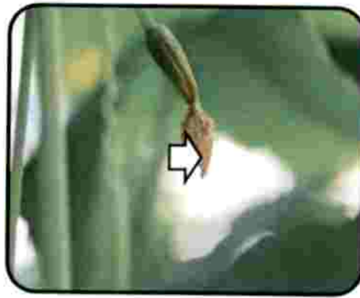
Tip-dry rot





Pathogenicity test

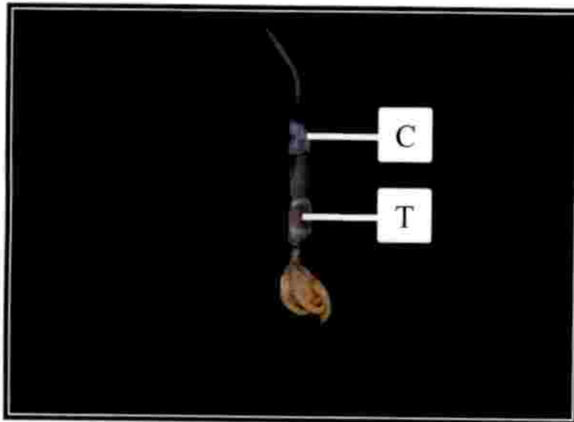
Plate 7. Symptomatology and pathogenicity test of *Corynespora cassiicola* fruit rot

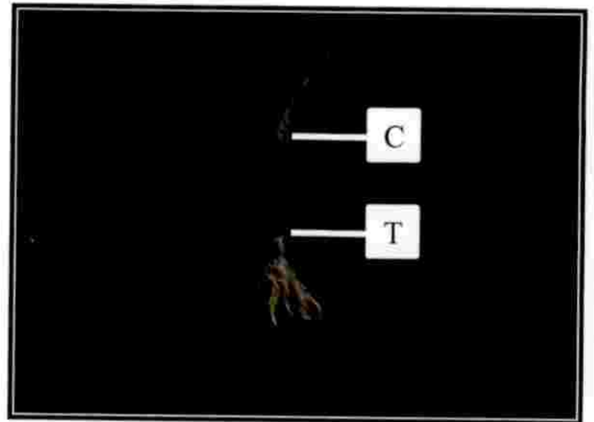


Starts from blossom end

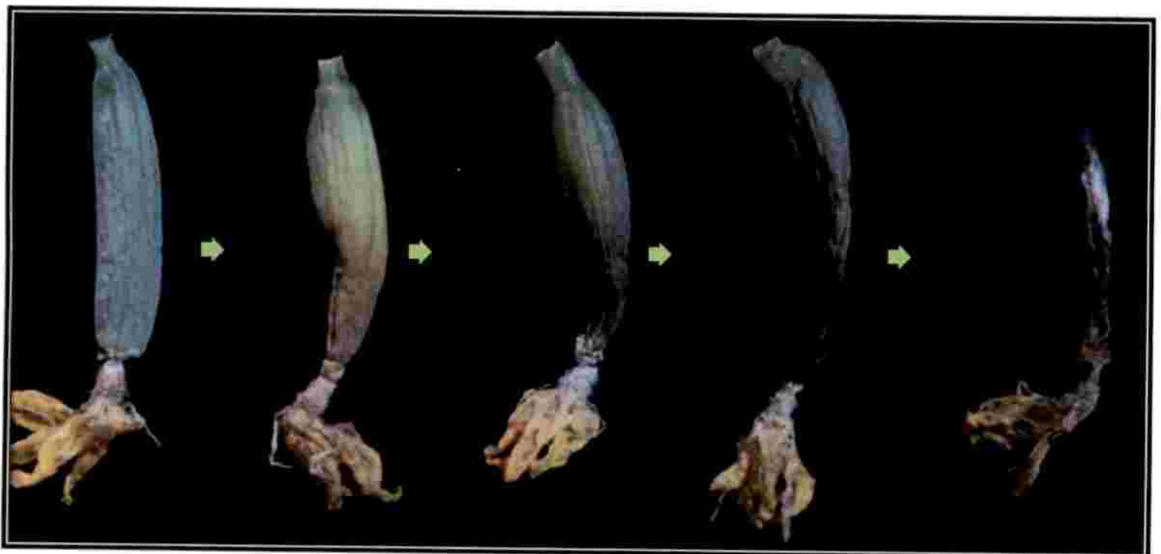
Yellowing of the tissue

Browning and dry rot





Pathogenicity test



Symptom progression

Plate 8. Symptomatology and pathogenicity test of *Fusarium equiseti* fruit rot

produced within five to six days of inoculation. Later internal discolouration was also observed (Plate 3).

4.5.3. Fruit rot- 3

The pathogen causing fruit rot- 3 produced irregular water soaked lesions on third day of inoculation in cucumber which subsequently turned into sunken dark brown cankers. Cracks were formed on these cankers during drying. (Plate 4).

4.5.4. Fruit rot- 4

Fruit rot- 4 was observed in ridge gourd where symptoms appeared on the third day of inoculation as dark water soaked lesion with white mycelial growth on the surface of the fruits. Later these lesions started to enlarge and resulted in soft watery rot (Plate 5).

4.5.5. Fruit rot- 5

Fruit rot- 5 was observed in cucumber. After second day of inoculation, symptom started as a water soaked lesion with white cottony mycelial growth. The pathogen was fast growing and covered the entire fruit within five days, finally causing wet rot (Plate 6).

4.5.6. Fruit rot- 6

Fruit rot- 6 was observed on snake gourd fruits in which symptoms initially appeared as water soaked sunken spots. Black concentric rings were developed, a week after inoculation. Later spots expanded and complete rotting was observed on which sporulation of fungus was also noticed (Plate 7).

4.5.7. Fruit rot- 7

Fruit rot- 7 was observed in immature salad cucumber in the polyhouse. Pathogenicity was confirmed by observing the symptom produced on the fruits

three days after inoculation. Initially, symptom started as yellowing of the tissue followed by browning and later dry rotting was also observed (Plate 8).

4.6. CHARACTERISATION AND IDENTIFICATION OF PATHOGEN

The pathogen causing fruit rots of cucurbits isolated from different diseased samples from seven locations in three districts of northern Kerala were subjected to cultural and morphological studies for characterisation and thereby identification of the isolates. A detailed description regarding each isolate is presented below.

4.6.1 Cultural and morphological characters

4.6.1.1. Fruit rot-1

Cultural characters

The pathogen was observed on pumpkin from the instructional farm, COA, Padannakkad. The pathogen covered a radial growth of 90 mm in 24 hours after inoculation on PDA with cottony white aerial mycelium. Sporulation was observed on the periphery of the Petri plate. The under surface of colony was creamy white in colour (plate 9).

Morphological characters

Sporangiophores having vesicles bearing apical sporangiola were observed. Sporangiola were formed at the apex of 14.12-16.32 μm wide sporangiophores. Monosporous sporangiola were ovoid with brown to dark brown colour and striations on the surface. They measured 12.12-14.65μm x 6.33-8.52 μm in size. Sporangia were globose to sub-globose with 33.70-73.61μm diameter. Sporangiospores were brown coloured, elliptic to ovoid and measured 12.68-18.38μm x 6.48-7.99μm (Plate 9). Based on these cultural and morphological characters the pathogen was tentatively identified as *Choanephora* sp.

4.6.1.2. Fruit rot-2

Cultural characters

The pathogen was isolated from pumpkin, collected from the vegetable fields of Panoor in Kannur district during the survey. On PDA medium, the pathogen causing fruit rot-2 produced milky white mycelia which were fast growing. Similar colour was observed on the underside of colony. Colour changed to creamy white or light brown after ten days. It took 72 hours to 96 hours for full growth in a 90 mm Petri dish. Spherical sclerotia (0.5 to 2.0 mm) were produced after 10 days of inoculation. Initially sclerotia were white in colour and later turner to reddish brown (Plate10).

Morphological characters

Main hyphae had 4.5 to 8.0 µm diameter, with ring-shape joints which produced secondary and tertiary hyphae with 2.0 to 4.5 µm diameter (Plate10). The pathogen based on its cultural and morphological characters was tentatively identified as *Sclerotium* sp.

4.6.1.3 Fruit rot- 3

Cultural Characters

The fungal pathogen causing fruit rot- 3 was isolated from cucumber fruit during the survey at Karakkode in Kasargod district. The creamy white colony of the fungus later turned into brown with feathery aerial mycelium. The underside of the colony appeared dark brown at the center and light brown in the periphery. After seven days of inoculation, sclerotial bodies were produced with light brown to dark brown colour, globular to irregular shaped, with a size of 1-3 mm diameter mostly on the center of the Petri dish. It took 48 hours to 72 hours for full growth in a 90 mm Petri dish (Plate 11).

Morphological characters

The pathogen produced mycelia with right angled branching and at the point of origin of the right angle, a characteristic septum was observed. At the branching point hyphae showed a constriction. Light brown hyphal cells were barrel shaped with a thickness ranging from 5.2-8.5μm (Plate 11). Based on above mentioned characters, the pathogen was identified as *Rhizoctonia* sp.

4.6.1.4 Fruit rot- 4

Cultural characters

The colony of the pathogen causing fruit rot- 4 from ridge gourd appeared as white coloured colonies on PDA with non specific pattern and dense or low spreading aerial mycelium. Similar colour was noticed on back side of the colony with irregular borders. The fungus took nine to eleven days for full growth in a 90 mm Petri dish (Plate 12).

Morphological characters

Morphological characteristics of the fungus was noted and found that sporangia were non caducous, ovoid or lemon shaped and papillate in structure. The dimensions of sporangia ranged from 25 to 52 × 23 to 36 μm. On PDA, isolate produced spherical chlamydospores that ranged from 20 to 50 μm diameter; it may be either terminal or intercalary (Plate 12). The pathogen based on its cultural and morphological characters was tentatively identified as *Phytophthora* sp.

4.6.1.5 Fruit rot- 5

Cultural characters

The colony of the pathogen had cottony white fluffy mycelium without any special pattern (Plate 13). The pathogen was fast growing which covered the 9 cm diameter Petri dish within 48 hours.

Morphological characters

It produced hyaline hypha with 0.81-3.25µm width. Sporangia were lobbed consisting of terminal complexes of swollen hyphal branches of varying length and upto 6.5µm wide oogonia which bear terminal, globose, smooth, and 14.0-20.8 µm in diameter. Most of the anthredia were intercalary, broadly sac shaped, 7.2-10.92 µm long and 6.5-8.62 µm wide, one per oogonium, and homothallic. Oospores were aplerotic 12.42-3.12 µm in diameter with wall thickness of 1.6-3.21µm. Based on the characters the pathogen was tentatively identified as *Pythium* sp. (Plate 13).

4.6.1.6 Fruit rot -6

Cultural characters

The pathogen on snake gourd was found to be a slow growing fungus and covered 9 cm diameter after 12-14 days of inoculation. Colony had effuse, gray to light olivaceous green at immature stage and turned brown to dark blackish brown at maturity, often hairy or velvety (Plate 14).

Morphological characters

Conidia formed were solitary or in chains of 2 - 6, variable in shape, cylindrical, straight or curved. The colour was pale olivaceous brown or brown, and smooth, with 4 - 20 pseudosepta and measured 41.2 - 219.7µm long and 9.2 - 21.5µm wide (Plate 14). Based on the characters the pathogen isolated from snake gourd was tentatively identified as *Corynespora* sp.

4.6.1.7. Fruit rot -7

Cultural characters

The colony of the fungus causing fruit rot of salad cucumber produced dense white mycelium with irregular borders, which later turned to brownish orange colour. Underside of the colony appeared light orange or pink colour. It took seven days for full growth in 90 mm Petri dish (Plate 15).

81

Morphological characters

Hyphae were hyaline with 2.3-4.2µm thickness. Both micro and macro conidia were observed. Where, macroconidia were elongated with pronounced curved foot cell which measured 14.3-28.2 µm x 2.45-4.24 µm in size with 2-5 septa. Microconidia elongated, oval shaped with one to two cells and measured 4.2-4.6 µm in length and 2.1-3.4 µm width (Plate 15). Based on these cultural and morphological characters the pathogen isolated from immature cucumber was tentatively identified as *Fusarium* sp.

4.6.2 Molecular characterisation

Preliminary identification of the pathogen upto 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 pathogens were analysed through the online BLASTn programme of NCBI. Details of the result of sequence comparison of these isolates are presented in Tables 8-14. The gel electrophotresis of genomic DNA and PCR product are given in Plate 16.

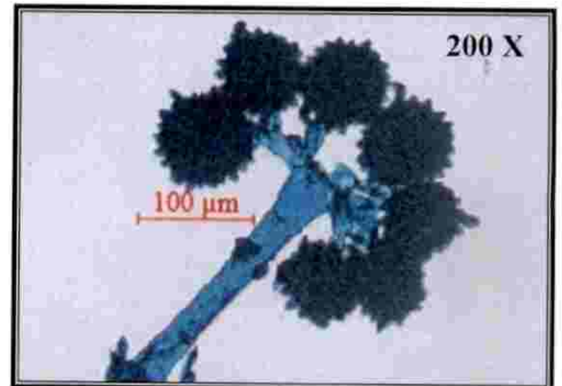
The ITS sequence of pathogens are as follows

1. *Choanephora* sp. (F1)

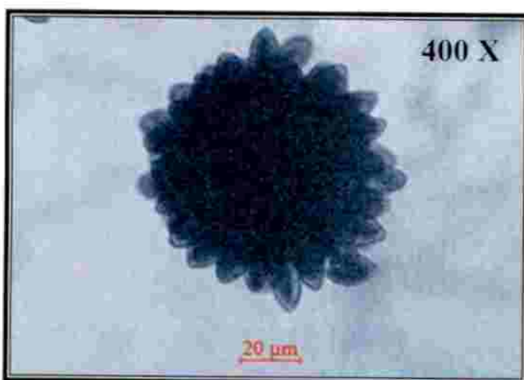
CCTTGGATGTCCAACTGATTTCAGATCAAGATGAAGAGTTTTATTTGG
GAGGCCCCAACAAAGTCCAAGTCGCAAGAGCTTTCCTTTATATTAAAA
AAAAGTTCAGGCTAGATGAACAGATTCAGGCCTTACTCAATTTAAAAG
GTCGCCATTACTAGCTTCCTTCATGACCATTCAAAAAAAAAATTTGAA
TGAGGGTTGTTTTTGATACTGAAACAGGCGTGCTCACTGGAATACCAA
TGAGCGCAAGATGCGTTCAAAGACTCGATGATTCACTGAATTTGCAAT
TCACACTAGTTATCGCAATTTGCTACGTTCTTCATCGATGCAAAAGCCA
AGAAATCCATTGTTAAAAGTTGTTTTATAGATATTACTACCTATGTTAC

a. Culture plates of *Choanephora cucurbitarum*

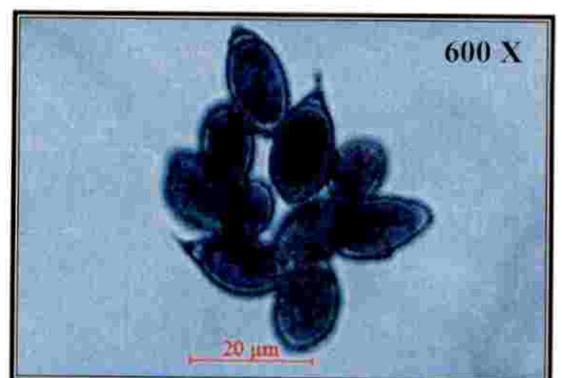
b. Black pinhead like fruitifications



c. Sporangiophore with vesicles bearing sporangiola

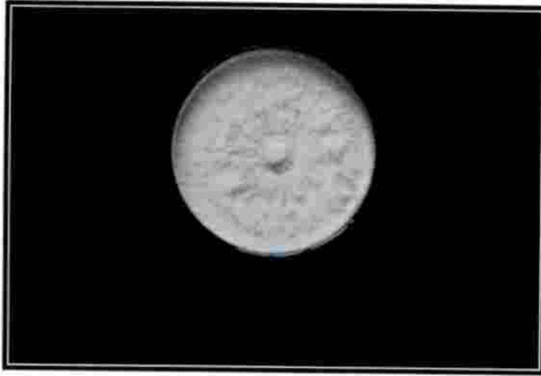


d. Individual vesicle

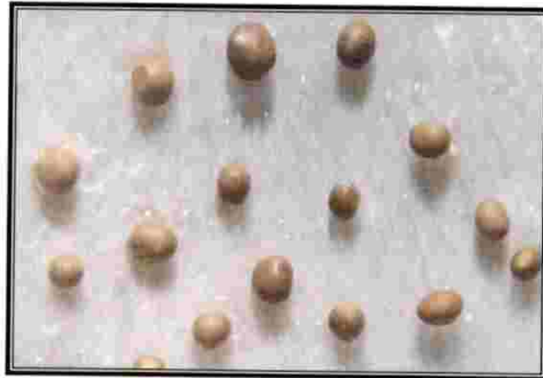


e. Monosporous sporangiola

Plate 9. Colony and spore morphology of FR-1



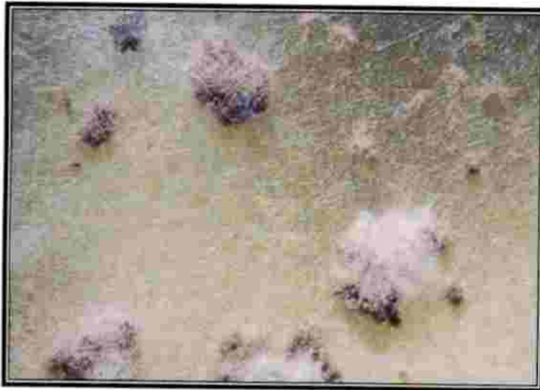
a. Culture plates of *Sclerotium rolfsii*



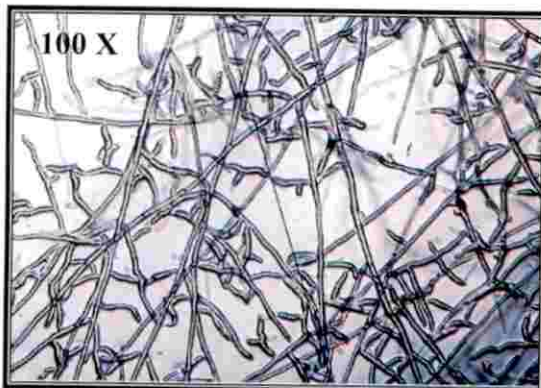
b. Sclerotia

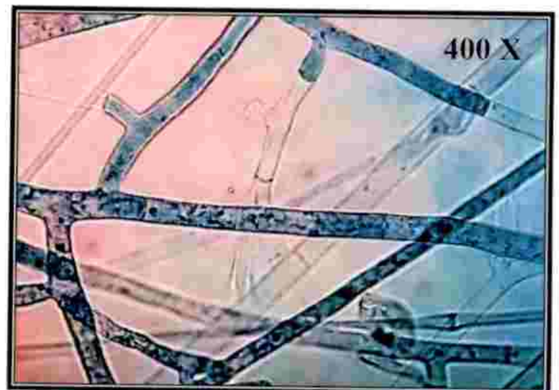
Plate 10. Colony and sclerotial morphology of FR-2

a. Culture plates of *Rhizoctonia solani*



b. Sclerotia

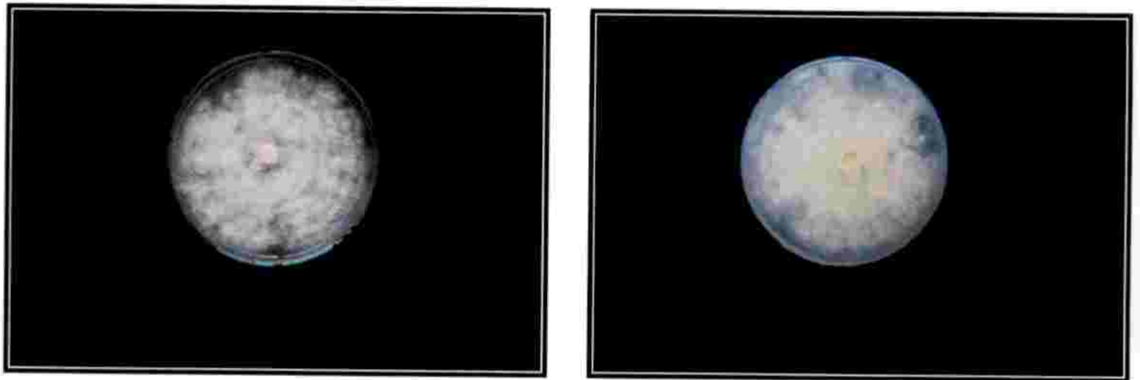




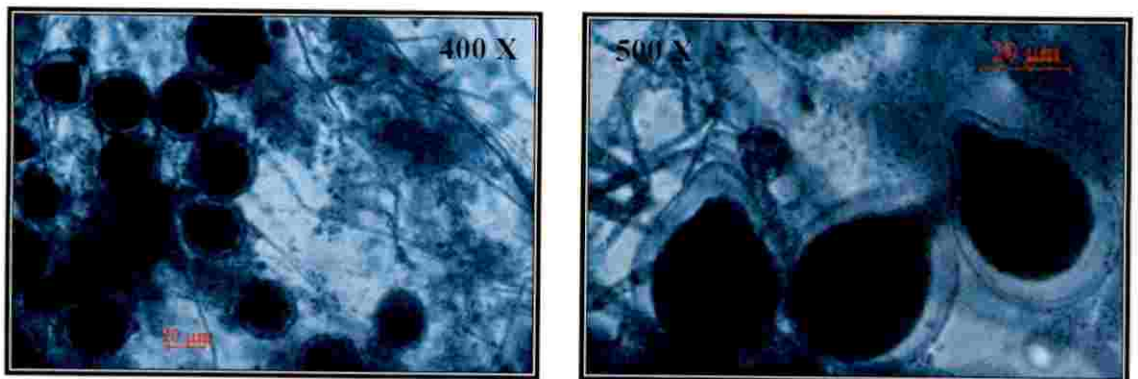
c. Hyphal anastomosis

d. Branching characters of hypha

Plate 11. Colony, sclerotial and hyphal morphology of FR-3



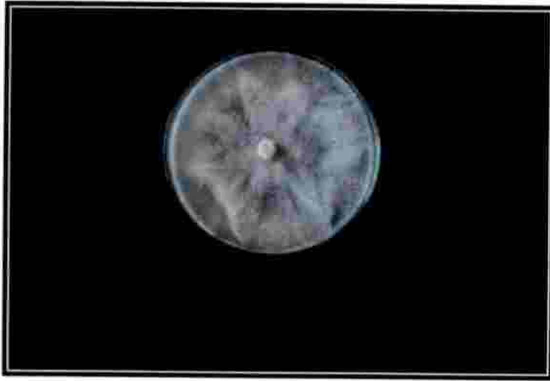
a. Culture plates of *Phytophthora nicotianae*

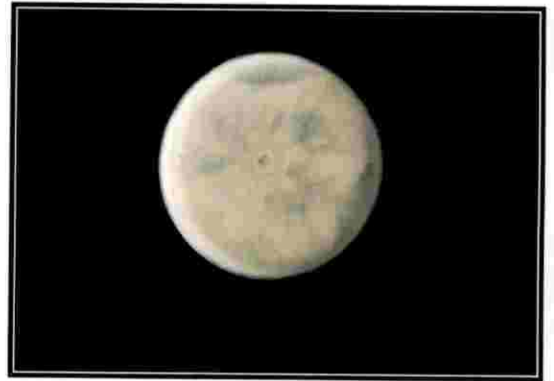


b. Papillate lemoniform sporangia

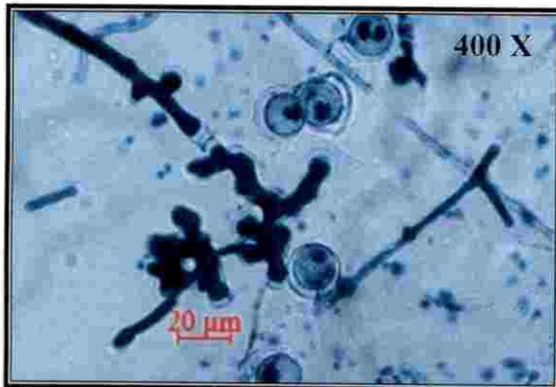
c. Chlamydospore

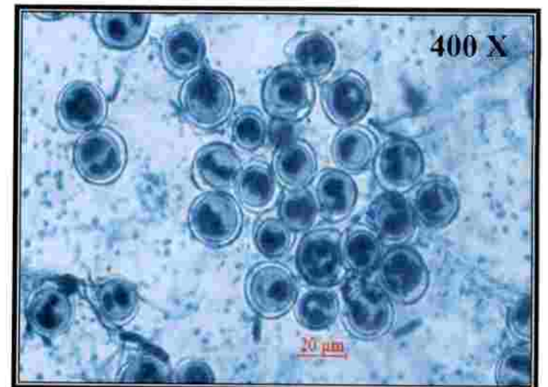
Plate 12. Colony and sporangial morphology of FR-4





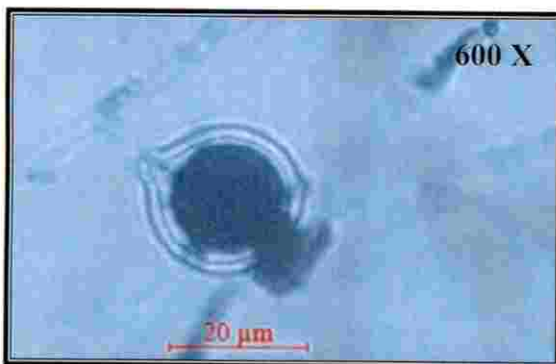
a. Culture plates of *Pythium deliense*

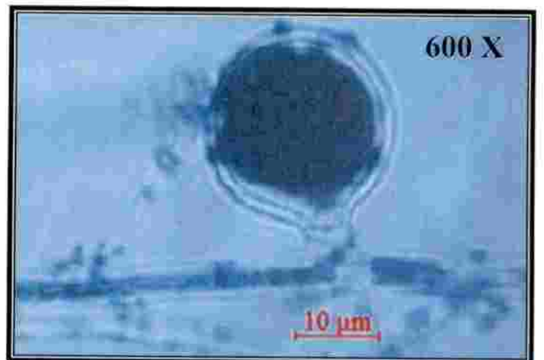




b. Lobbed sporangia

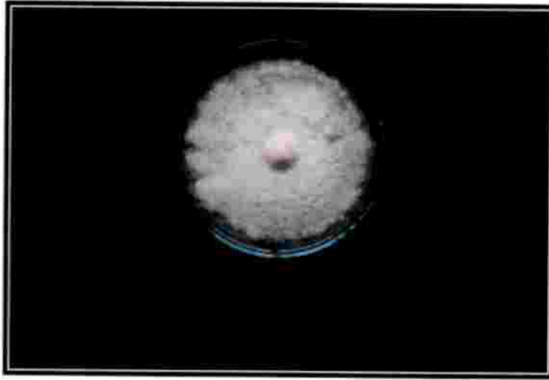
c. Oospores

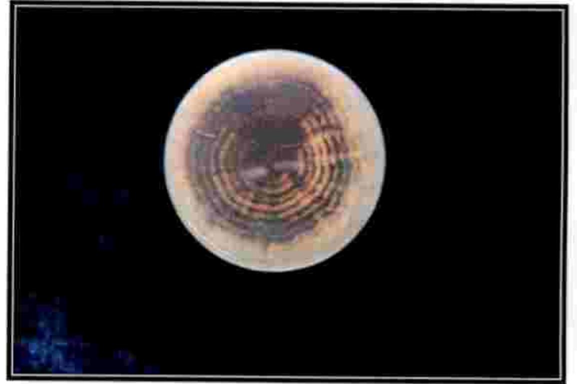




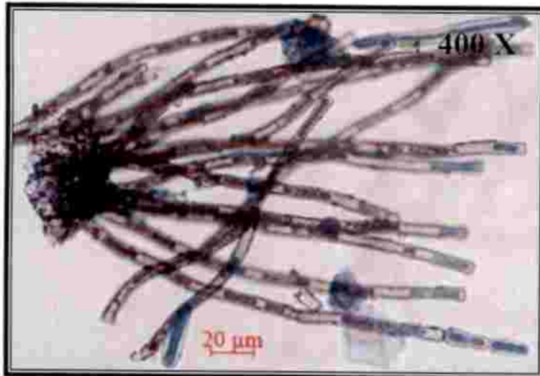
d. Antheridium and Oogonium (Gamatangial contact)

Plate 13. Colony, sporangial and gamatangial morphology of FR-5

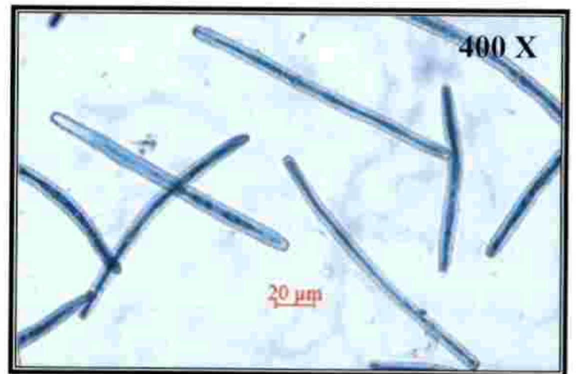




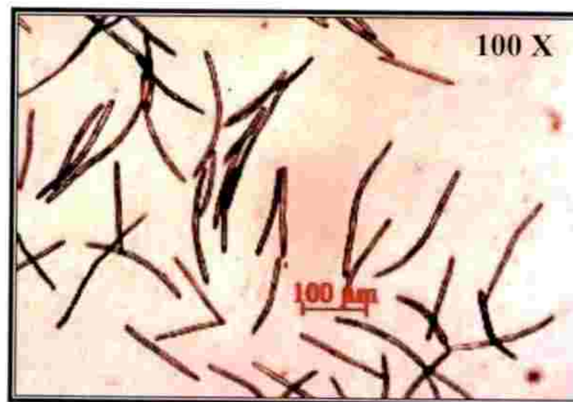
Culture plates of *Corynespora cassiicola*



Conidiophores



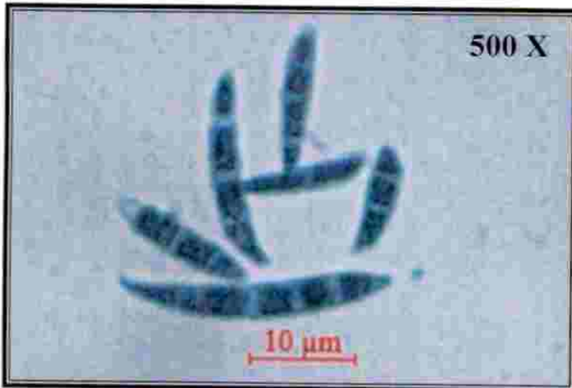
Conidia

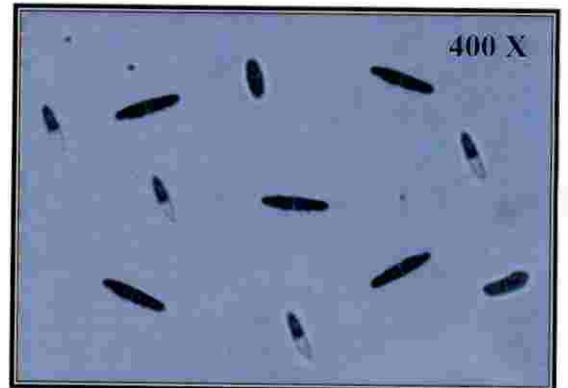


Conidia

Plate 14. Colony and conidial morphology of FR-6

Culture plates of *Fusarium equseti*





Macro conidia

Micro conidia

Plate 15. Colony and conidial morphology of FR-7

ATTTTATAATCTGATCAATTGAAAGTATATAAACAGGGTACCAAGCCT
AAGCTTGACCATAGCTCGGTTAACATTTCTCATGCCTACCCATATAGC
ACAAGAACATCCCTCAAACGCCATGAAATAAAACAGTTCACAGTAAA
ATAGATAGTAGATCAGGACAAGCCCGAAATACTTCTTATTTATTTAAT
GATCCTTCCGCAGGTTCACCCTACGGAAGATAGGATCATTAAATAAAT
TAAAGTATTTCGGGCTGTGTCTGATCTACATCTTGTACTGTGAACTGTT
TTATTTCAGGCGTTTGAGGGATGTTCTTGTGCTATATGGGTGGGCATGA
GAAATGTTTAACGAGCTATGGTCAAGCTAGCTGGTACCTGTTTATATT
CTTTCATTGATCAATATAAGTAACAAGTGGTATATCTATAACACTTAA
CAATGACTTGGCCTGCATTCGATAAGAAGTACAATTGCCATACGAGGA
GTGCATCGTAAACACGGCCTTCTGTAGACGACACG

2. *Sclerotium* sp. (F2)

ATRTATGCACTTATATGCTAGATATATGCGCATGRTTTTATAAGTAGTR
CATACAAGCTAGAATCCCCTTTGGTATAGGCGTAGACATATTATCACA
CCAACCGCAGGCATTTCTAYATGTCCTACTAATARTTTTAAAGAGAGC
CAGTCAGATATTCTTAACTAGCAACTCTCACATCCAAGCCTTGACAAA
TACAAAAATTTGTAAGGTTGAGAATTTAATGACTCTCAAACAGGCATG
CCCCTCGGAATACCAAAGGGCGCAAGGTGCGTTCAAAGATTCGATGAT
TCACTGGATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTT
CATCGATGCAAGAGCCAAGAGATCCGTTGTTGAAAGTTGTRTATTTTT
CAATTAAGAGAAACTTGTAAAAAAAAACAAAGCTTCAATATGAGATC
GTTCTATGTAACATACAATAAGAGTTA

3. *Rhizoctonia* sp. (F3)

CATAAAATAGAATATTGTCCAAGTCAATGGACTATTAGAAGCGGTTCA
TCTGCATTTACCTTGGCCACCTTTTTTACGGGTGTCCTCAGCGATAGAT
AACTTATCACGCCGAGTGGAACCAAGCATAACACTGAGATCCAGCTA
ATGCACAAAGAGGAGCAGGTGTGAAGCTGCAATAAGATCCTCCAAAA
CCAAAGTAAAAAAGACCAATTGAATTAACAAAAGGTTTACTTTGAAG
ATTTCATGATACTCAAACAGGCATGCTCCAAGGAATACCAAGGAGCGC

AAGGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACAT
TACTTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGA
TCCGTTGTTGAAACTTAGTATTAGATGCGTTACATCAATTACATTCAGT
TTAAATTAAGTARAGTGTGTGTAAATTAAGTAGACAGCAAATGGATGA
TGGAATTAAATCC

4. *Phytophthora* sp. (F4)

GATGCATACCGAAGTACACATTAAGTTCCCAAATGGATCGACCCTCGA
CAACCGAAGCTGCCACCCTACTTCGCAACAGCAAAGCCAATTCAAAA
GCCAAGCCACCGAGCTATGGTTCACCAGTCCATCACGCCACAGCAGGA
AAAACGTCCAATAAGTGCATTGTTCAGCCGAAGCCAACCATACCACGA
ATCGAACACTCTTCCATTAAYGCCGCAGCAGACAAACCAGTCGCCAAT
TTGCCACAATAGCAGCCTTCACAACCAGCAACGCCACCACTTTTCGAG
CAAAGAGAAGTTCAGTTTAGTACATTTAAAAGGACTCGCAGCCGGTCC
GAAGACCAATCGCAAGACACTTCACATCTGACATCTCCTCCACCGACT
ACACGGAAGGAAGAAAGTCAAGTTTAATGTACGGACACTGATACAGG
CATACTTCCAGGACTAACCCGGAAGTGCAATATGCGTTCAAAATTTCG
ATGACTCACTGAATCCTGCAATTCGCATTACGTATCGCAGTTCGCAGC
GTTCTTCATCGATGTGCGAGCCTAGACATCCACTGCTGAAAGTTGCTA
TCTAGTTAAAAGCAGAGACTTTCGTCCCCACAGTATATTCAGTATTAA
GGAATGGGTTTAAAAGAAAAAAAGACTACTAAATCAGGCCGAAG

5. *Pythium* sp. (F5)

TTGTCTGCTAAAAAGCAAACAGTCCCAAATTGGTGTTGCCTTCTTTACC
CTATCCGAAAACAGGGCGCCCAAGAGCAGCAAAACCCTACTACACAG
CAACCATTTGCCCAGACCATTGCCTCACACAAATCAGGTACACCTCAA
GGAAAGAACAGAAAACCACACTCCGTCAGCTGCAACGTCGGGCCGAA
GCCTAACATACCGCCAATAGAGGTTGCTTCCTTTAATGTCCTAACCGA
AGTCGCCCAAATGCGCAAAGCGATCCAAACAGATCACTGCGATTCGA

GAATCACACTTCATCTTAGACACAAGAAAGAGCGAACGTACATTTAAA
GGGACTCGCGTCTTCTCCTCCGAAAAGGGAGTCAACGAGACACCTCAC
ATTCTGCCATCTCTCTCCCTGACTACACAGAAAAAGAAAGGCAAGTTT
GATGTACGGACACTGATACAGGCATACTTCCAGGCATAACCCGAAAGT
GCAATGTGCGTTCAAAATTTCGATGACTCACTGAATTCTGCAATTCGC
ATTACGTATCGCAGTTCGCAGCGTTCTTCATCGATGTGCGAGCCTAGA
CATCCACTGCTGAAAGTTGTTATGGAT

6. *Corynespora* sp. (F6)

1TTGGAATCCTACCTGATCCGAGGTCAAATTCTGGTGTGGGGGGGCTC
ATGGTGCGCCGACCCGCAGCCACTTCAGCGCTTGCTCTGCTGCGCTCG
TGGCCTGCTGGGAACCGGCCGCCAATGCTTTTGAGGCGAGTCCAGGCG
CGCGGAGGCGGGACAGACGCCCAACACCAAGCTAGGCTTGAGGGTTG
AAATGACGCTCGAACAGGCATGCCCTAAGGAATACCAAAGGGCGCAA
TGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTAC
TTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCC
GTTGTTGAAAGTTGTAAATAGTTTGTTTTGTTTTGTTCAGACGTGTACT
TCTTGTACTGCAATGGGTTTGTGGTGGGTCCCCGTTGGCAGGCGAGCC
TGCCGAGGAAACGAGAGGTGCTCATAAACAAAGGGTGCTATCTCGAA
GGGGGCGAGGCCCCTACAATAATGATCCCTTCCGCAGAATCACGCAAC
CCAGGGGATCATTATCGTAGGGGCCTCGCCCCTTTCGAGATAGCACCT
TTGTTTATGAGCACTCTGTTTCTCGGCAGGCTGCCGCGACGGGGGAC
ACCAAACCATGTGCGGTACAGAAGTTACACTCTGAACAAACAAAAAA
CATTTACACTTTTCAGCGAGCTTGGGTTTGGCTGCATAAAAAGCACAA
TGCATTAATATGTGATTGCTAATCGTGATCACAACTTGAAGCCATGGC
TTGGTATCTAGGCAGCCGTTGCAGGCTATTACCTAACATGGTTGGGGA
TAGTCGACGGGACCTAAAATGGCGACG

7. *Fusarium* sp. (F7)

CGGGAGGGTTCCACATCCCAAAACCCCTGTGACATACCTATACGTTGC
CTCGGCGGATCAGCCCGCGCCCCGTAAAACGGGACGGCCCGCCCGAG

GACCCCTAAACTCTGTTTTTAGTGGAACTTCTGAGTAAAACAAACAAA
TAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAA
GAACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGA
ATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGG
CATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCAGCTTGGTGTTGG
GACTCGCGGTAACCCGCGTTCCCCAAATCGATTGGCGGTCACGTCGAG
CTTCCATAGCGTAGTAATCATACACCTCGTTACTGGTAATCGTCGCGG
CCACGCCGTTAAACCCCAACTTCTGAATGTTGACCTCGGATCAGGTAG
GAATACCCGCTGAACTTAAGCATATCAATAAGCGGAAGGAA

4.6.2.1. Sequence comparison of *Choanephora* sp.

Sequence homology search for *Choanephora* culture revealed 99.16 per cent identity with *Choanephora cucurbitarum* strain JPC2 (Acccession MH041503.1) and *Choanephora cucurbitarum* strain JPC1 (Accession MH041502.1), 99.30 per cent identity with *Choanephora cucurbitarum* isolate CP-5 (Accession KX790359.1), 99.12 per cent identity with *Choanephora cucurbitarum* strain CBS 150.51 (Accession MH856791.1) and 99.12 per cent identity with *Choanephora cucurbitarum* isolate C.B-18 (Accession KX462163.1). Sequence analysis of the *Choanephora* culture showed homology with *C. cucurbitarum* with more than 99 per cent identity and 89-94 per cent query coverage, so confirmed the isolate as *Choanephora cucurbitarum*, which tentatively identified earlier as *Choanephora* sp. through cultural and morphological characterization.

4.2.2.2 Sequence comparison of *Athelia* sp.

Sequence homology of *Athelia* culture showed 98. 7 per cent identity with *Athelia rolfsii* isolate AR-02 (Accession MK288124.1), *Athelia rolfsii* strain AR-02 (Accession MH858139.1), *Athelia rolfsii* isolate DG92 (Accession MF776034.1) and *Athelia rolfsii* isolate SrCAMKS16 (Accession KY640627.1). Hence, the isolate which was earlier identified as *Athelia* sp. by cultural and morphological characters was further confirmed with the result of molecular characterization.

4.6.2. 3.Sequence comparison of *Rhizoctonia* sp.

Comparison of nucleotide sequence of *Rhizoctonia* culture revealed that it showed 99.8 per cent identity with *Rhizoctonia solani* isolate Ds1 (Accession MK587771.1), *Rhizoctonia solani* isolate Dm1 (Accession MK587770.1), *Rhizoctonia solani* stain AG 1-IA isolate YJWC2-01 (Accession MG397065.1), *Rhizoctonia solani* strain AG 1-IA isolate XS3-01 (Accession MG397063.1) and *Rhizoctonia solani* strain AG-1 IA XS3-01 (Accession MG397062.1). Hence sequence analysis of the *Rhizoctonia* culture showed homology with *R. solani* with more than 99 per cent identity and cent per cent query coverage, isolate was confirmed as *R. solani*.

4.6.2.4. Sequence comparison of *Phytophthora* sp.

Nucleotide of *Phytophthora* culture recorded 100 per cent identity with *Phytophthora nicotianae* TARI p212061 (Accession MG584512.1), 99.85 per cent identity with *Phytophthora nicotianae* isolate TARI p20181 (Accession MG570058.1), *Phytophthora nicotianae* isolate TARI p213102 (Accession MG570056.1), *Phytophthora nicotianae* isolate TARI p209212 (Accession MG570056.1) and *Phytophthora nicotianae* isolate TARI p212061 (Accession MG584512.1). The pathogen showed more than 99 per cent identity and query coverage with *Phytophthora nicotianae*, therefore the cultured pathogen is identified to be the same.

4.6.2.5. Sequence comparison of *Pythium* sp.

Comparison of nucleotide sequence of *Pythium* culture revealed that the fungus showed 99.83 per cent identity with *Pythium deliense* NMR11 (Accesssion LC332030.1), *Pythium deliense* RYG9 (Accession LC332029.1), *Pythium deliense* isolate 43503 (Accession KX260337.1), *Pythium deliense* Pp-45 (Accession KP183964.1) and *Pythium deliense* NAH-3 (Accession KM597162.1). Hence sequence analysis of the *Pythium* culture showed homology with *Pythium*

deliense with 99.83 percent identity and 100 percent query, isolate was confirmed as *Pythium deliense.*

4.6.2.6. Sequence comparison of *Corynespora* sp.

Nucleotide of *Corynespora* culture recorded 97.5 per cent identity with *Corynespora cassiicola* strain UM591 (Accession KU990882.1) and *Corynespora cassiicola* strain 6M (Accession JX087444.1), 90 percent with *Corynespora* sp. FZJ-1 (Accession JN853778.1), and *Corynespora cassiicola* isolate XQ3-1 (Accession MH569606.1) and 99.4 with *Corynespora cassiicola* isolate ACC72 (Accession KP748289.1). Hence, the pathogen showed more than 97 per cent identity with *Corynespora cassiicola* and therefore the cultured pathogen was identified to be the same.

4.6.2.7. Sequence comparison of *Fusarium* sp.

Nucleotide analysis of *Fusarium* culture gave 99.41 per cent identity with *Fusarium equiseti* isolate FUS-18 (Accession MH879583.1) and 99.60 per cent identity with *Fusarium equiseti* strain YD-RF1 (Accession MK530522.1), *Fusarium equiseti* strain FMT (Accession MH015345.1), *Fusarium equiseti* isolate (Accession LS479414.1) and *Fusarium equiseti* isolate (Accession LS479413.1). As it showed more than 99 per cent identity *Fusarium equiseti*, the pathogen confirmed to be the same.

Table 8. 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 cucurbitarum* starin JPC2	1072	94%	0.0	99.16	MH041503.1
2.	*Choanephora cucurbitarum* strain JPC1	1072	94%	0.0	99.16	MH041502.1
3.	*Choanephora cucurbitarum* isolate CP-5	1031	90%	0.0	99.30	KX790359.1
4.	*Choanephora cucurbitarum* strain CBS 150.51	1014	90%	0.0	99.12	MH856791.1
5.	*Choanephora cucurbitarum* Isolate C.B-18	1014	89%	0.0	99.64	KX462163.1

Table 9. Sequence homology observed for *Athelia* (*Sclerotium*) sp. in BLASTn analysis as per BLAST results

Sl. No.	Description	Max. score	Query coverage (%)	E value	Identity (%)	Accession
1.	*Athelia rolfsii* isolate AR-02	828	100%	0.0	98.70	MK288124.1
2.	*Athelia rolfsii* strain AR-02	828	100%	0.0	98.70	MH858139.1
3.	*Athelia rolfsii* isolate DG92	828	100%	0.0	98.70	MF776034.1
4.	*Athelia rolfsii* isolate SrCAMKS16	828	100%	0.0	98.70	KY640627.1
5.	*Athelia rolfsii* isolate SrCAMKS15	828	100%	0.0	98.70	KY640626.1

Table 10. 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* isolate Ds1	905	99.80	0.0	100%	MK587771.1
2.	*Rhizoctonia solani* isolate Dm1	905	99.80	0.0	100%	MK587770.1
3.	*Rhizoctonia solani* stain AG 1-IA isolate YJWC2-01	905	99.80	0.0	99%	MG397065.1
4.	*Rhizoctonia solani* strain AG 1-IA isolate XS3-01	905	99.80	0.0	99%	MG397063.1
5.	*Rhizoctonia solani* strain AG-1 IA XS3-01	905	99.80	0.0	99%	MG397062.1

Table 11. Sequence homology observed for *Phytophthora* sp. in BLASTn analysis as per BLAST results

Sl. No.	Description	Max. score	Query coverage (%)	E value	Identity (%)	Accession
1.	*Phytophthora nicotianae* isolate TARI p20181	1227	100%	0.0	99.85	MG570058.1
2.	*Phytophthora nicotianae* isolate TARI p213102	1227	100%	0.0	99.85	MG570057.1
3.	*Phytophthora nicotianae* isolate TARI p209212	1227	100%	0.0	99.85	MG570056.1
4.	*Phytophthora nicotianae* isolate TARI p208297	1227	100%	0.0	99.85	MG570054.1
5.	*Phytophthora nicotianae* isolate TARI p212061	1227	100%	0.0	100	MG584512.1

98

Table 12. Sequence homology observed for *Pythium* sp. in BLASTn analysis as per BLAST results

Sl. No.	Description	Max. score	Query coverage (%)	E value	Identity (%)	Accession
1.	*Pythium deliense* NMR11	1103	100%	0.0	99.83	LC332030.1
2.	*Pythium deliense* RYG9	1103	100%	0.0	99.83	LC332029.1
3.	*Pythium deliense* isolate43503	1103	100%	0.0	99.83	KX260337.1
4.	*Pythium deliense* Pp-45	1103	100%	0.0	99.83	KP183964.1
5.	*Pythium deliense* NAH-3	1103	100%	0.0	99.83	KM597162.1

99

Table 13. Sequence homology observed for *Corynespora* sp. in BLASTn analysis as per BLAST results

Sl. No.	Description	Max. score	Query coverage (%)	E value	Identity (%)	Accession
1.	*Corynespora cassiicola* strain UM591	1005	80%	0.0	97.5	KU990882.1
2.	*Corynespora cassiicola* strain 6M	1003	70%	0.0	97.5	JX087444.1
3.	*Corynespora* sp. FZJ-1	929	61%	0.0	99.0	JN853778.1
4.	*Corynespora cassiicola* isolate XQ3-1	926	61%	0.0	99.0	MH569606.1
5.	*Corynespora cassiicola* isolate ACC72	924	60%	0.0	99.4	KP748289.1

100

Table 14. 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* isolate FUS-18	915	96%	0.0	99.41	MH879583.1
2.	*Fusarium equiseti* stain YD-RF1	911	95%	0.0	99.60	MK530522.1
3.	*Fusarium equiseti* strain FMT	911	95%	0.0	99.60	MH015345.1
4.	*Fusarium equiseti* isolate	911	95%	0.0	99.60	LS479414.1
5.	*Fusarium equiseti* isolate	911	95%	0.0	99.60	LS479413.1

101

Plate 16. DNA amplification profile of selected pathogens

4.7. EFFICACY OF FUNGICIDES, BOTANICALS AND BIOCONTROL AGENTS AGAINST PATHOGENS UNDER *IN VITRO* CONDITIONS

In vitro studies were conducted for the evaluation of fungicides, botanicals and biocontrol agents against the selected five pathogens obtained from fruits of cucurbits. Selections of pathogens were done based on the severity of occurrence during the survey (Table 7). Fungicides, botanicals, PGPR-II and PGPM mix were tested by poisoned food technique and biocontrol agents *viz*., *Trichoderma viride, Pseudomonas fluorescens* were evaluated by dual culture technique as described in 3.8.1 and 3.8.2.

4.7.1. *In vitro* evaluation of fungicides against major fungal pathogens causing fruit rot in cucurbits

Four fungicides and three botanicals at three concentrations and five biocontrol agents at recommended concentration were selected for *in vitro* evaluation against the five most severe pathogens *viz*., *Choanephora cucurbitarum, Sclerotium rolfsii, Rhizoctonia solani, Phytophthora nicotianae* and *Corynespora cassiicola.*

4.7.1.1. *Choanephora cucurbitarum*

In vitro evaluation studies of fungicides against *Choanephora cucurbitarum* showed that among the four fungicides tested, tebuconazole 5EC showed 100 per cent inhibition in all the three concentrations tested (Table 15a and Plate 17a). The second best fungicide was mancozeb 64% + cymoxanil 8% with 91.11 per cent inhibition. The above two fungicides were significantly effective from the other two fungicides *viz*.,. mancozeb 63% + carbendazim 12% and mancozeb 75WP (84.44 per cent and 85.55 per cent inhibition, respectively).

4.7.1.2. *Sclerotium rolfsii*

The data revealed that the fungicide tebuconzole 5EC was significantly superior because it showed 100 per cent inhibition at all the three concentrations against *Sclerotium rolfsii* (Table 16a and Plate 18a). Mancozeb 64% + cymoxanil

103

8% also showed a similar trend of 100 per cent inhibition at recommended and higher concentrations. Other two fungicides *viz.*, mancozeb 63% + carbendazim 12% and mancozeb 75WP showed significantly lower inhibition effect even at higher dosage than recommended (72.21 and 83.33 per cent, respectively).

4.7.1.3. *Rhizoctonia solani*

Evaluation of effectiveness of fungicides on inhibiting the growth of *Rhizoctonia solani* showed 100 per cent inhibition against all fungicides at all concentrations (Table 17a and Plate 19b).

4.7.1.4. *Phytophthora nicotianae*

Against *Phytophthora nicotianae,* all four fungicides showed 100 per cent inhibition at all concentrations (Table 18a and Plate 20a).

4.7.1.5. *Corynespora cassiicola*

Among the four fungicides tested against *Corynespora cassiicola* all the three concentrations of mancozeb 64% + cymoxanil 8% gave 100 per cent inhibition and proved to be superior. Effect of mancozeb 64% + carbendazim 12% and (88.88 per cent inhibition), tebuconzole 5EC (83.32 per cent inhibition) were on par with mancozeb 64% + cymoxanil 8%. Mancozeb 75WP (66.66) was the least effective with 66.66 per cent inhibition. At recommended and higher concentrations mancozeb 64% + cymoxanil 8% and mancozeb 64% + carbendazim 12% showed cent per cent inhibition of the growth of *Corynespora cassiicola*. But at recommended dosage, tebuconzole 5EC and mancozeb 75WP found to be least effective. At the higher concentration, the three fungicides, tebuconzole 5EC, mancozeb 64% + cymoxanil 8% and mancozeb 63% + carbendazim 12% showed 100 per cent inhibition and they were at par with each other. Here also mancozeb 75WP was least effective (Table 19a and Plate 21a).

104

Table 15a. ***In vitro*** **evaluation of fungicides on the inhibition of mycelial growth of *Choanephora cucurbitarum***

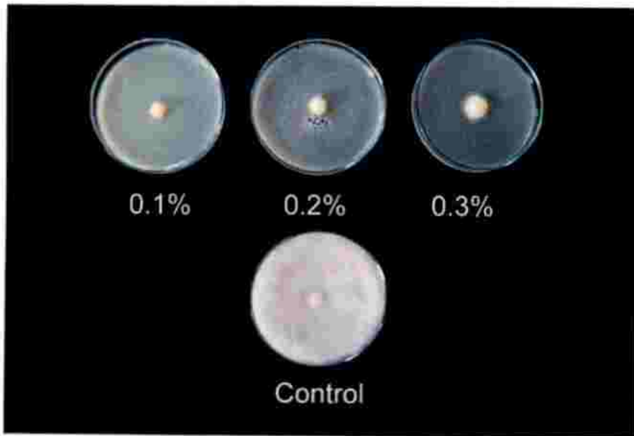
Treatments	Fungicides	Inhibition of mycelial growth of*Choanephora cucurbitarum* (%)*		
		C-1	C-2	C-3
T1	Mancozeb 75% WP (0.1%, 0.2%, 0.3)	72.22* (58.17)**	82.22 (65.04)	84.44 (66.74)
T2	Mancozeb63%+Carbendazim 12%(0.1%, 0.2%,0.3%)	73.33 (58.88)	83.33 (68.88)	85.55 (67.64)
T3	Mancozeb 64%+Cymoxanil8% (0.1%, 0.2%,0.3%)	84.44 (66.74)	86.66 (68.56)	91.11 (72.64)
T4	Tebuconazole 5% EC (0.05%. 0.1%, 0.2%)	100 (90.00)	100 (90.00)	100 (90.00)
CD		1.27	1.44	1.37
SE		0.38	0.43	0.41

* Mean of three values

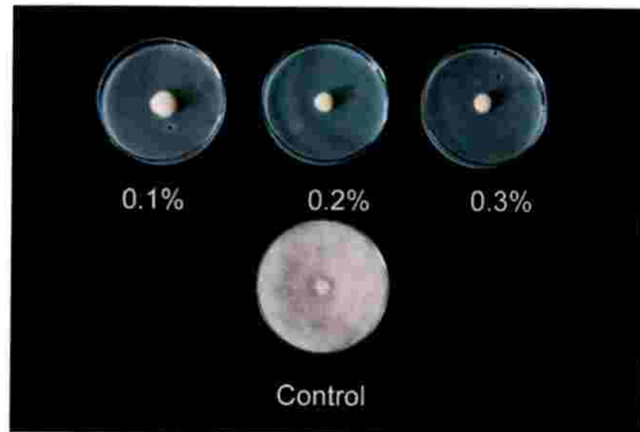
** Values in parenthesis are angular transformed values

C- Concentration

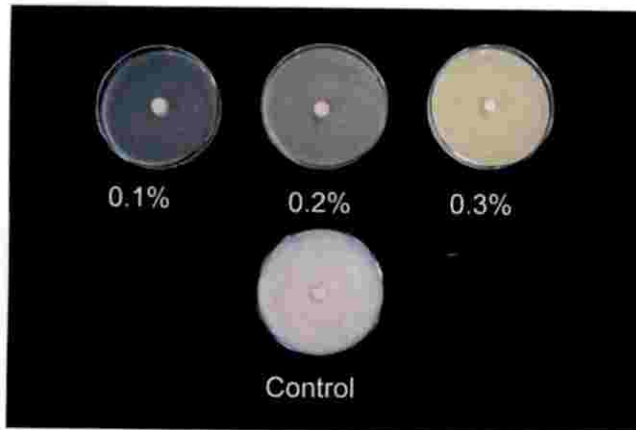
105



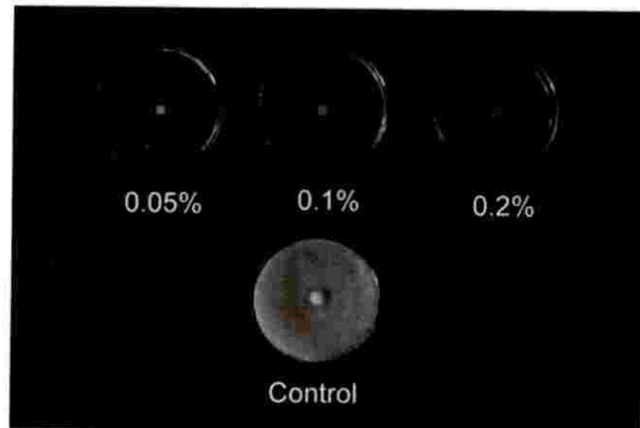
Mancozeb 75 WP



Mancozeb 63% + Carbendazim 12%



Mancozeb 64% + Cymoxanil 8%



Tebuconazole

Plate 17a. Effect of different *fungicides* on radial growth of *Choanephora cucurbitarum*

Table 16a. ***In vitro*** **evaluation of fungicides on the inhibition of mycelial growth of** ***Sclerotium rolfsii***

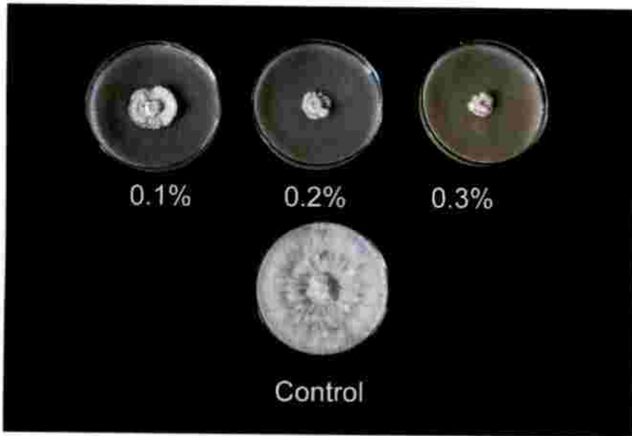
Treatments	Fungicides	Inhibition of mycelial growth of *Sclerotium rolfsii* (%)*		
		C-1	**C-2**	**C-3**
T1	Mancozeb 75% WP (0.1%, 0.2%, 0.3)	61.10* (51.42)**	72.21 (58.24)	83.32 (66.07)
T2	Mancozeb63%+Carbendazim 12%(0.1%, 0.2%,0.3%)	49.99 (44.98)	66.66 (54.75)	72.21 (58.24)
T3	Mancozeb 64%+Cymoxanil8% (0.1%, 0.2%,0.3%)	83.32 (66.07)	100 (90.00)	100 (90.00)
T4	Tebuconazole 5% EC (0.05%. 0.1%, 0.2%)	100 (90.00)	100 (90.00)	100 (90.00)
CD		6.01	4.70	5.36
SE		1.81	1.42	1.61

* Mean of three values

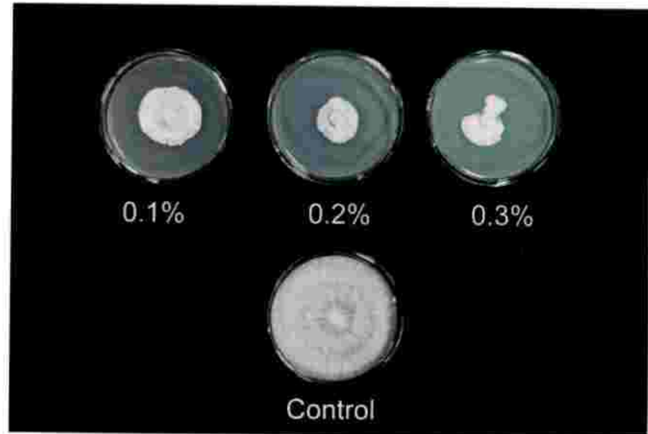
** Values in parenthesis are angular transformed values

C- Concentration

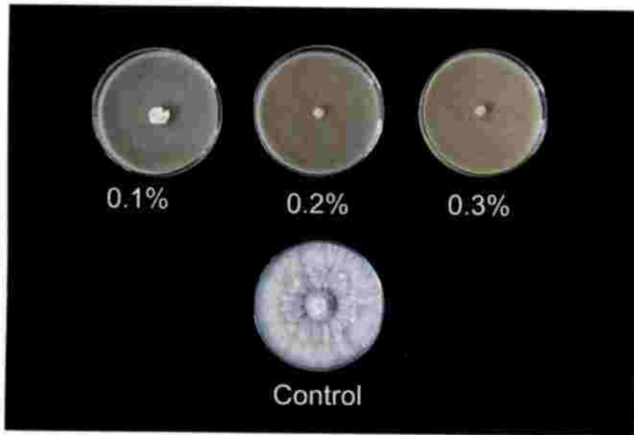
107



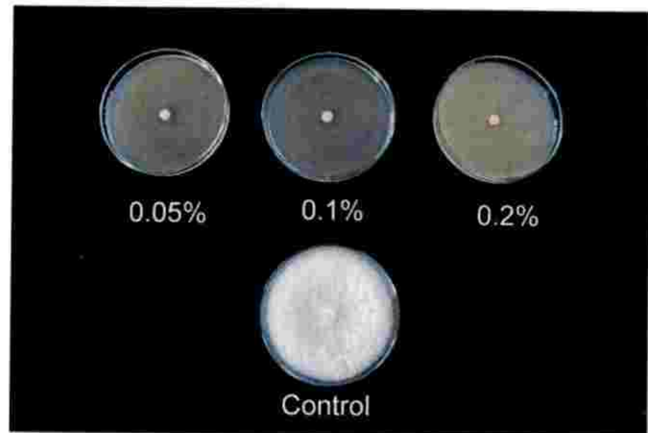
Mancozeb 75 wp



Mancozeb 63% + Carbendazim 12%



Mancozeb 64% + Cymoxanil 8%



Tebuconazole

Plate 18a. Effect of different *fungicides* on radial growth of *Sclerotium rolfisii*

Table 17a. ***In vitro*** **evaluation of fungicides on the inhibition of mycelial growth of** ***Rhizoctonia solani***

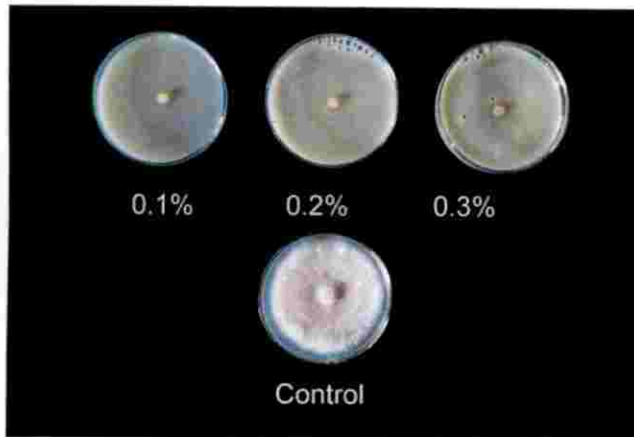
Treatments	Fungicides	Inhibition of mycelial growth of *Rhizoctonia solani* (%)		
		C-1	**C-2**	**C-3**
T1	Mancozeb 75% WP (0.1%, 0.2%, 0.3)	100* (90.00)**	100 (90.00)	100 (90.00)
T2	Mancozeb63%+Carbendazim 12%(0.1%, 0.2%,0.3%)	100 (90.00)	100 (90.00)	100 (90.00)
T3	Mancozeb 64%+Cymoxanil8% (0.1%, 0.2%,0.3%)	100 (90.00)	100 (90.00)	100 (90.00)
T4	Tebuconazole 5% EC (0.05%. 0.1%, 0.2%)	100 (90.00)	100 (90.00)	100 (90.00)
CD		-	-	-
SE		-	-	-

* Mean of three values

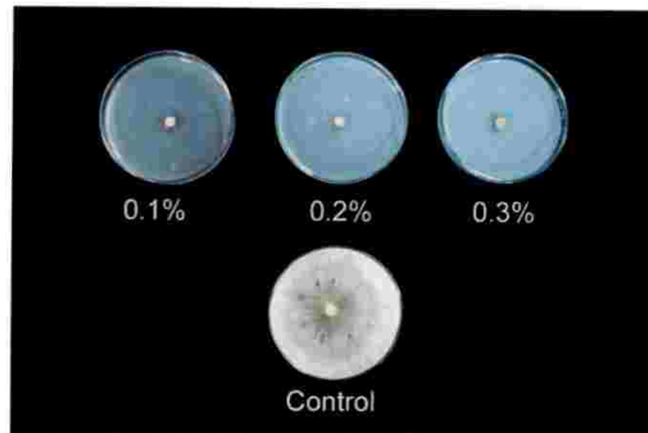
** Values in parenthesis are angular transformed values

C- Concentration

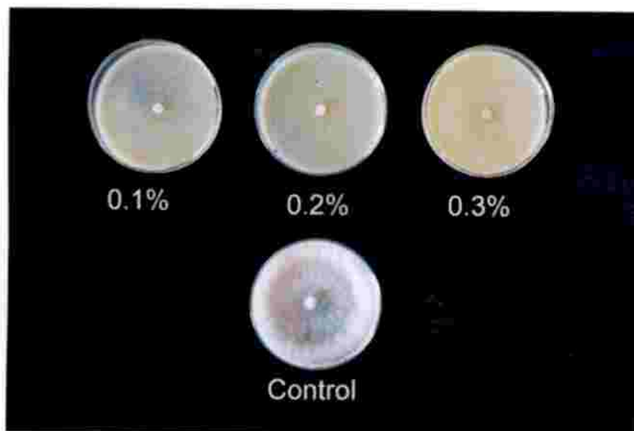
109



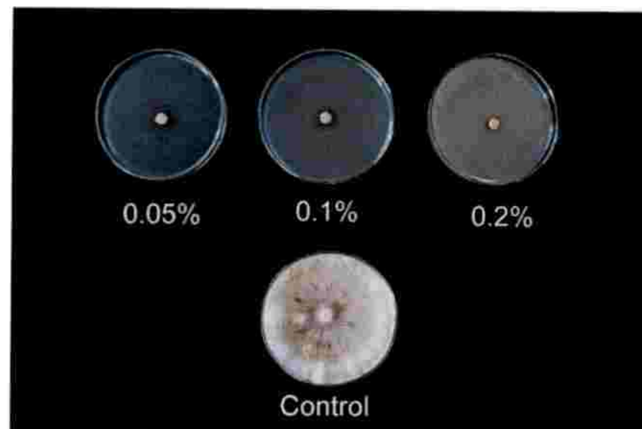
Mancozeb 75 wp



Mancozeb 63% + Carbendazim 12%



Mancozeb 64% + Cymoxanil 8%



Tebuconazole

Plate 19a. Effect of different *fungicides* on radial growth of *Rhizoctonia solani*

Table 18a. ***In vitro*** **evaluation of fungicides on the inhibition of mycelial growth of** ***Phytophthora nicotianae***

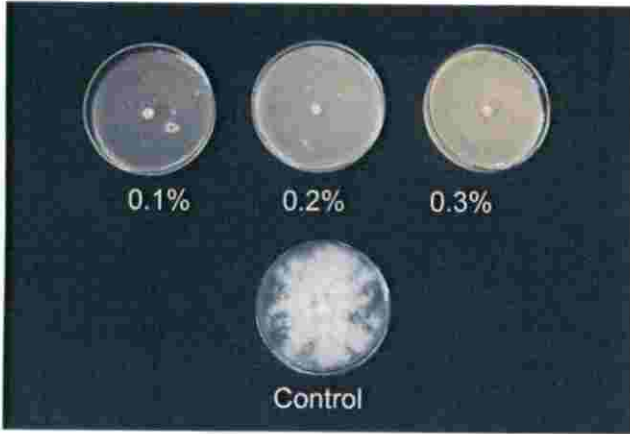
Treatments	Fungicides	Inhibition of mycelial growth of *Phytophthora nicotianae* (%)*		
		C-1	**C-2**	**C-3**
T1	Mancozeb 75% WP (0.1%, 0.2%, 0.3)	100* (90.00)**	100 (90.00)	100 (90.00)
T2	Mancozeb63%+Carbendazim 12%(0.1%, 0.2%,0.3%)	100 (90.00)	100 (90.00)	100 (90.00)
T3	Mancozeb 64%+Cymoxanil8% (0.1%, 0.2%,0.3%)	100 (90.00)	100 (90.00)	100 (90.00)
T4	Tebuconazole 5% EC (0.05%. 0.1%, 0.2%)	100 (90.00)	100 (90.00)	100 (90.00)
CD		-	-	-
SE		-	-	-

* Mean of three values

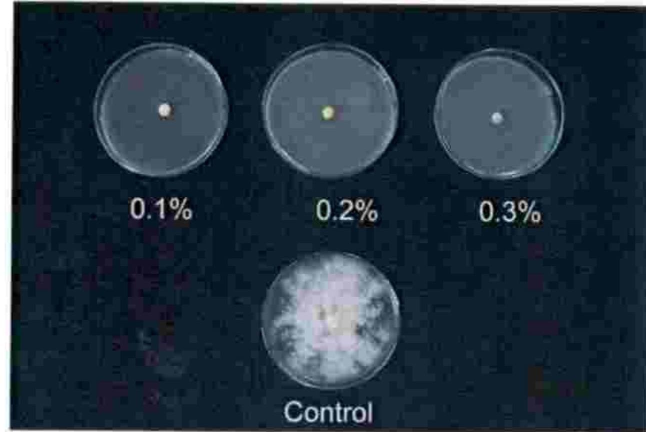
** Values in parenthesis are angular transformed values

C- Concentration

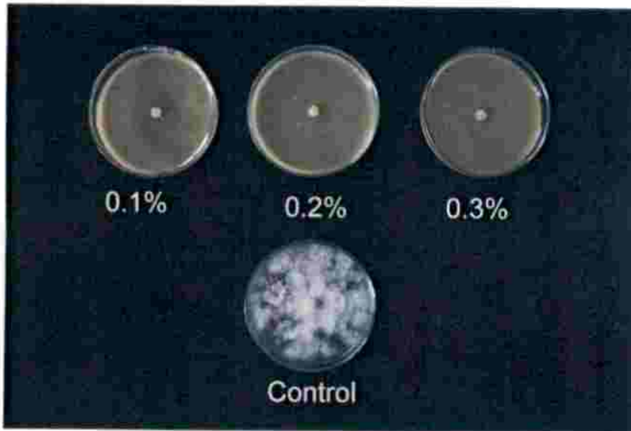
111



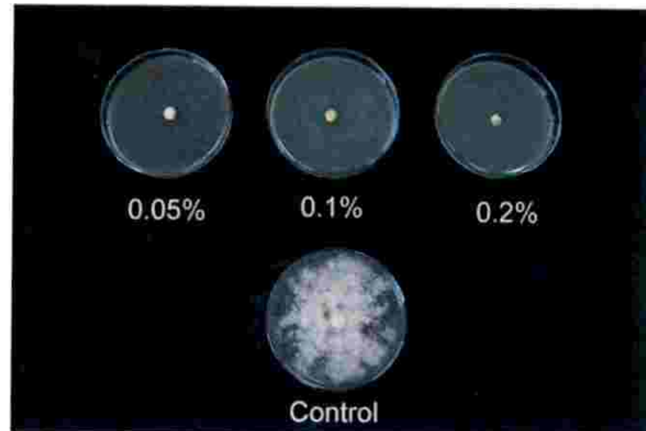
Mancozeb 75 WP



Mancozeb 63% + Carbendazim 12%



Mancozeb 64% + Cymoxanil 8%



Tebuconazole

Plate 20a. Effect of different *fungicides* on radial growth of *Phytophthora nicotianae*

Table 19a. ***In vitro*** **evaluation of fungicides on the inhibition of mycelial growth of** ***Corynespora cassiicola***

Treatments	Fungicides	Inhibition of mycelial growth of *Corynespora cassiicola* (%)*		
		C-1	**C-2**	**C-3**
T1	Mancozeb 75% WP (0.1%, 0.2%, 0.3)	66.66* (54.75)**	77.77 (61.96)	88.88 (70.49)
T2	Mancozeb63%+Carbendazim 12%(0.1%, 0.2%,0.3%)	88.88 (70.49)	100 (90.00)	100 (90.00)
T3	Mancozeb 64%+Cymoxanil8% (0.1%, 0.2%,0.3%)	100 (90.00)	100 (90.00)	100 (90.00)
T4	Tebuconazole 5% EC (0.05%. 0.1%, 0.2%)	83.32 (66.07)	88.88 (70.49)	100 (90.00)
CD		5.25	3.68	-
SE		1.58	1.11	-

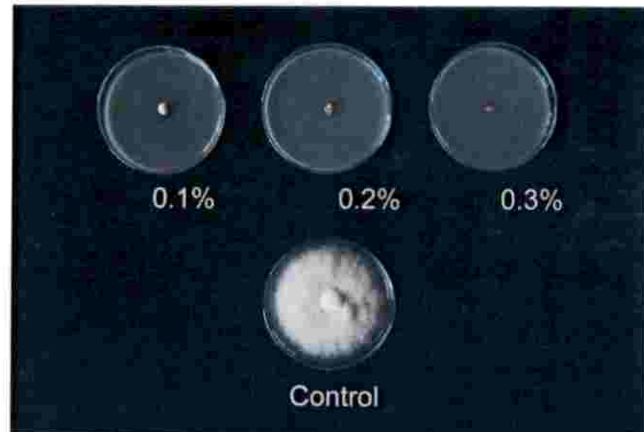
* Mean of three values

** Values in parenthesis are angular transformed values

C- Concentration

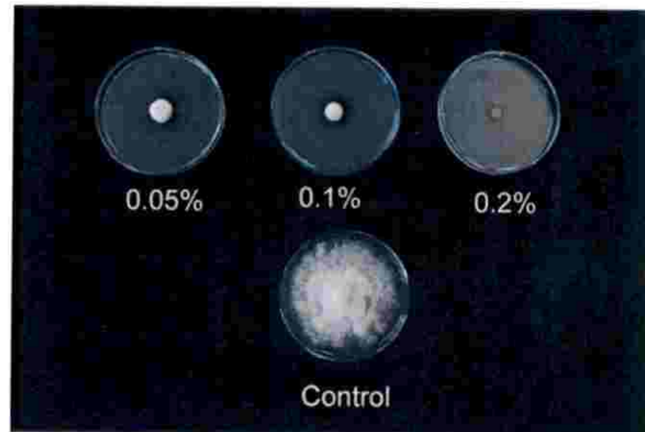
113

Mancozeb 75 wp



Mancozeb 63% + Carbendazim 12%

Mancozeb 64% + Cymoxanil 8%



Tebuconazole

Plate 21a. Effect of different *fungicides* on radial growth of *Corynespora cassiicola*

4.7.2. *In vitro* evaluation of botanicals against major fungal pathogens causing fruit rot in cucurbits

In vitro evaluation studies of botanicals against *Choanephora cucurbitarum* showed that garlic extract at 1 and 2 per cent concentrations (100 per cent inhibition) and azadirachtin 0.1% (64.44 per cent inhibition) at lower concentration as the best treatments. Next best botanical was azadirachtin at 0.2 and 0.3 per cent concentrations (Table 15b and Plate 17b).

In the case of inhibition effect of botanicals against *Sclerotium rolfsii*, garlic extract at 2 per cent showed 100 per cent inhibition. Next best botanicals were garlic extract at 1% and azadirechtin at 0.3% with 77.77 per cent inhibition. Neem oil garlic soap was least effective with 44.44 percent inhibition at highest dose of 1%. (Table 16b and Plate 18b).

In the *in vitro* evaluation of botanicals against *Rhizoctonia solani,* garlic extract found to be the most effective at 1% and 2% concentrations. Different from other two pathogens, azadirachtin 0.1% and ready to use neem oil garlic soap were effective in inhibiting *Rhizoctonia solani* with 83.32 and 72.21 per cent inhibition. Antifungal action of ready to use neem oil garlic soap was on par with commercial neem product azadirachtin. (Table 17b and Plate 19b).

While testing the effectiveness of botanicals against *Phytophthora nicotianae*, same result was obtained as in the case of above mentioned pathogens. The garlic extract was the best botanical with 100 per cent inhibition at 2% concentration followed by 1% and 0.5% (77.77 and 66.66 per cent respectively). The effects of treatments azadirachtin 0.1% and ready to use neem oil garlic soap were significantly not different (Table 18b and Plate 20b).

While testing the effectiveness of botanicals against *Corynespora cassiicola*, same result was obtained as in the case of above mentioned fungal pathogens. The garlic extract was the best botanical with 100 per cent inhibition at 2% concentration followed by 1% and 0.5% (77.77 and 66.66 per cent, respectively). The effects of treatments azadirachtin 0.1% and ready to use neem oil garlic soap were significantly not different (Table 19b and Plate 21b).

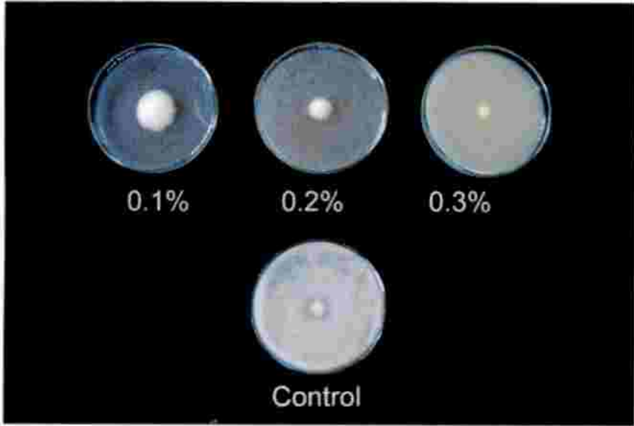
Table 15b. ***In vitro*** **evaluation of botanicals on the inhibition of mycelial growth of** ***Choanephora cucurbitarum***

Treatments	Botanicals	Inhibition of mycelial growth of *Choanephora cucurbitarum* (%)		
		C-1	**C-2**	**C-3**
T1	Azadiractin 0.1% (0.1%, 0.2%, 0.3%)	64.44* (53.37)**	82.22 (65.06)	89.99 (71.55)
T2	Garlic extract (0.5%, 1%, 2%)	22.22 (27.60)	100 (90.00)	100 (90.00)
T3	Ready to use neem oil garlic soap (0.1%, 0.6%, 1%)	0 (0.00)	0 (0.00)	11.07 (18.87)
CD		9.32	1.95	7.42
SE		2.64	0.55	2.10

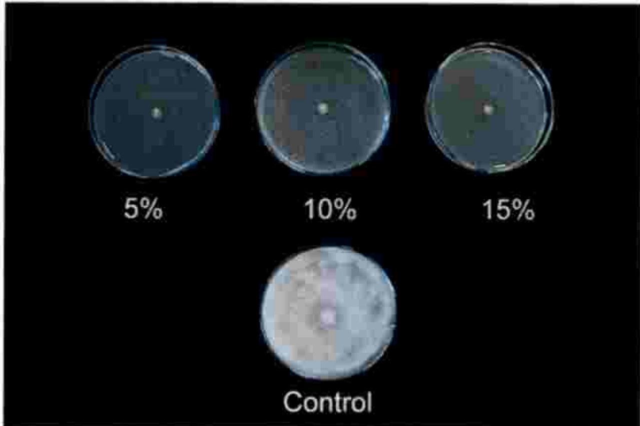
* Mean of three values

** Values in parenthesis are angular transformed values

C- Concentration

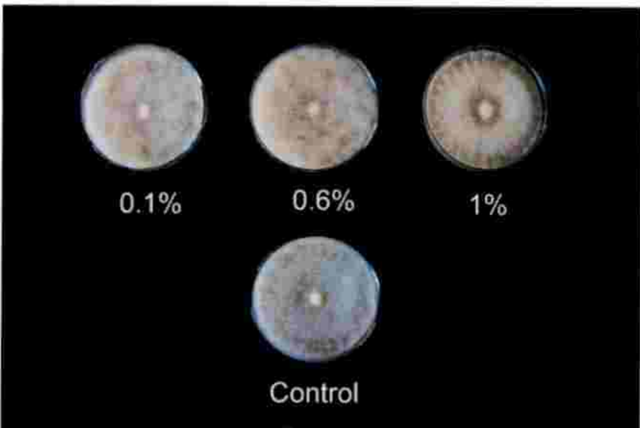


Azadirachtin 0.1% EC



Garlic extract (higher concetrations)

Garlic extract (lower concentrations)



Neem oil garlic soap

Plate 17b. Effect of different *botanicals* on radial growth of *Choanephora cucurbitarum*

Table 16b. ***In vitro*** **evaluation of botanicals on the inhibition of mycelial growth of** ***Sclerotium rolfsii***

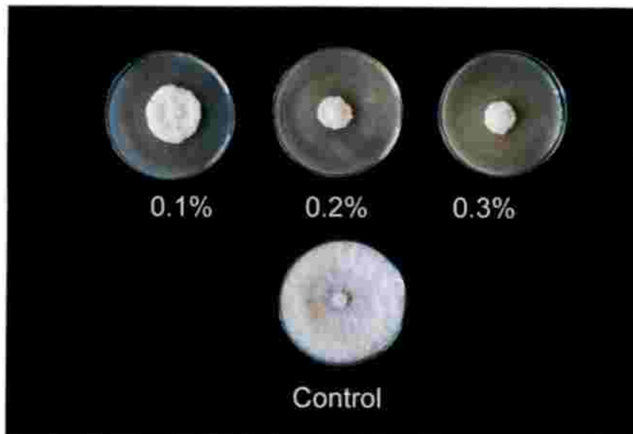
Treatments	Botanicals	Inhibition of mycelial growth of *Sclerotium rolfsii* (%)		
		C-1	**C-2**	**C-3**
T1	Azadiractin 0.1% (0.1%, 0.2%, 0.3%)	55.55* (48.18)**	72.21 (58.24)	77.77 (61.84)
T2	Garlic extract (0.5%, 1%, 2%)	61.10 (51.42)	77.77 (61.84)	100 (90.00)
T3	Ready to use neem oil garlic soap (0.1%, 0.6%, 1%)	33.32 (35.19)	38.88 (38.53)	44.44 (41.79)
CD		6.69	5.69	4.53
SE		1.89	1.61	1.28

* Mean of three values

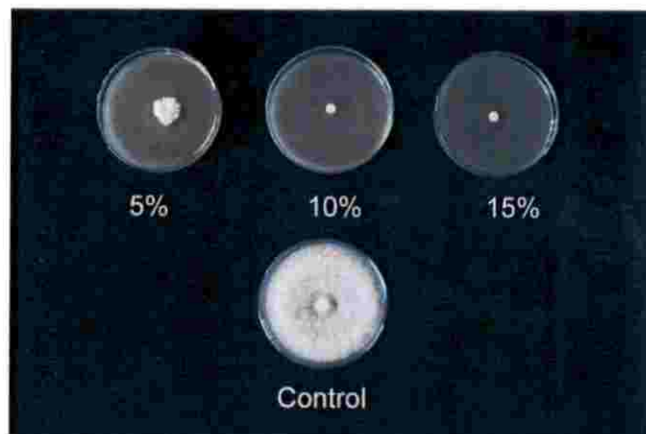
** Values in parenthesis are angular transformed values

C- Concentration

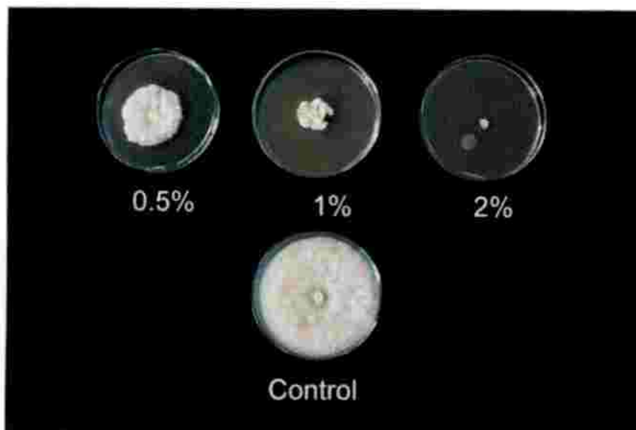
118



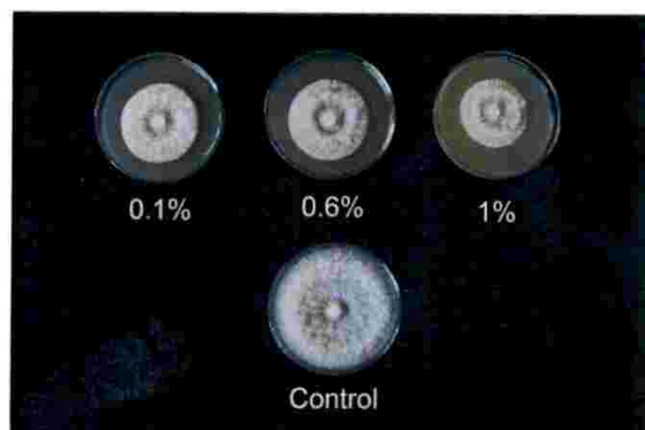
Azadirachtin 0.1% EC



Garlic extract (higher concetrations)



Garlic extract (lower concentrations)



Neem oil garlic soap

Plate 18 b. Effect of different *botanicals* on radial growth of *Sclerotium rolfsii*

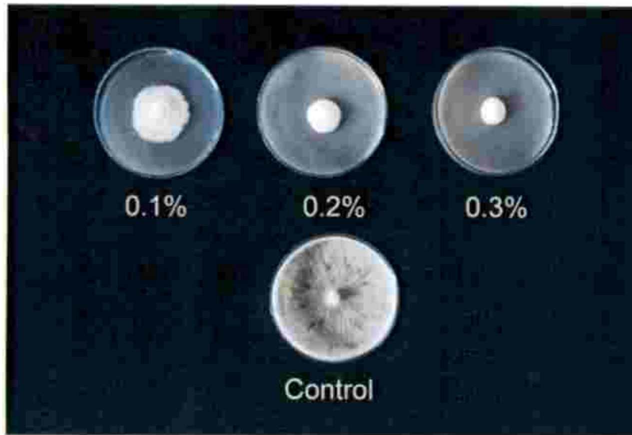
Table 17b. ***In vitro*** **evaluation of botanicals on the inhibition of mycelial growth of** ***Rhizoctonia solani***

Treatments	**Botanicals**	**Inhibition of mycelial growth of *Rhizoctonia solani* (%)**		
		C-1	**C-2**	**C-3**
T1	Azadiractin 0.1% (0.1%,0.2%, 0.3%)	50* (44.98)**	72.21 (58.24)	83.32 (66.07)
T2	Garlic extract (0.5%, 1%, 2%)	77.77 (61.84)	88.88 (70.49)	100 (90.00)
T3	Ready to use neem oil garlic soap (0.1%, 0.6%, 1%)	44.44 (41.77)	66.66 (54.75)	72.21 (58.24)
CD		3.77	5.78	6.59
SE		1.07	1.64	1.87

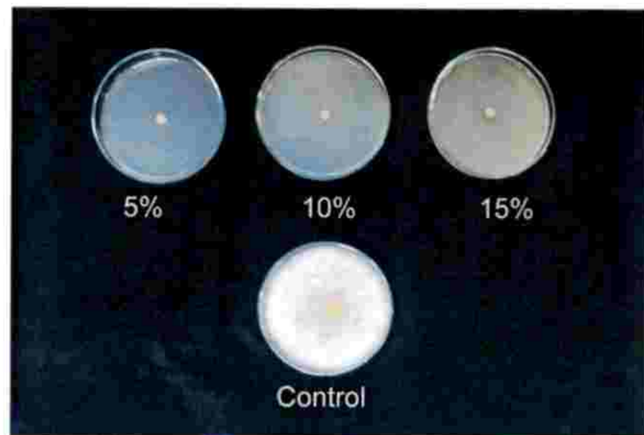
* Mean of three values

** Values in parenthesis are angular transformed values

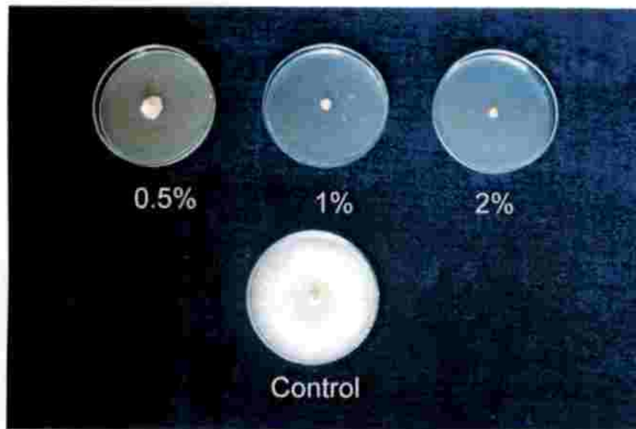
C- Concentration



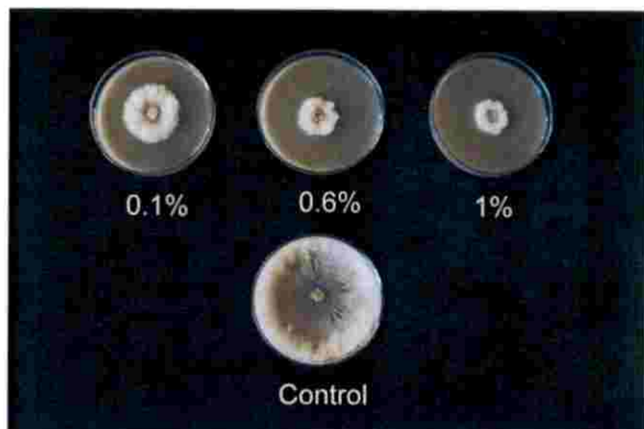
Azadirachtin 0.1% EC



Garlic extract (higher concetrations)



Garlic extract (lower concentrations)



Neem oil garlic soap

Plate 19b. Effect of different *botanicals* on radial growth of *Rhizoctonia solani*

Table 18b. ***In vitro*** **evaluation of botanicals on the inhibition of mycelial growth of** ***Phytophthora nicotianae***

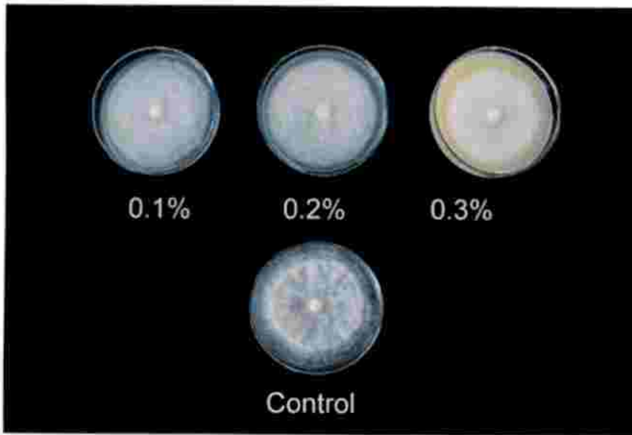
Treatments	Botanicals	Inhibition of mycelial growth of *Phytophthora nicotianae* (%)		
		C-1	**C-2**	**C-3**
T1	Azadiractin 0.1% (0.1%,0.2%, 0.3%)	5.55* (11.02)**	22.22 (28.11)	33.32 (35.19)
T2	Garlic extract (0.5%, 1%, 2%)	66.66 (54.71)	77.77 (61.96)	100 (90.00)
T3	Ready to use neem oil garlic soap (0.1%, 0.6%, 1%)	0 (0.00)	16.66 (23.88)	27.77 (31.71)
CD		11.74	6.81	5.78
SE		3.32	1.93	1.64

* Mean of three values

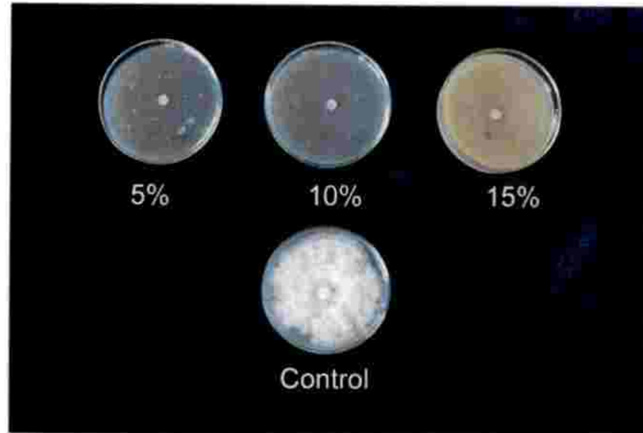
** Values in parenthesis are angular transformed values

C- Concentration

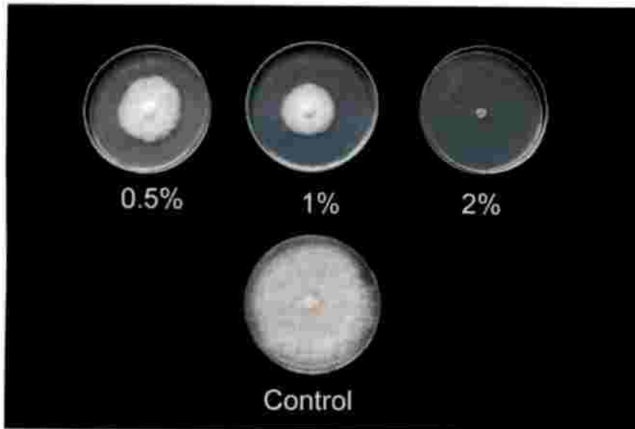
122



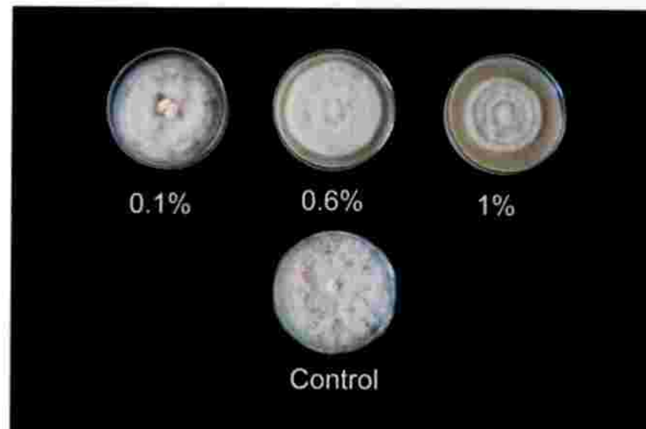
Azadirachtin 0.1% EC



Garlic extract (higher concetrations)



Garlic extract (lower concentrations)



Neem oil garlic soap

Plate 20b. Effect of different *botanicals* on radial growth of *Phytophthora nicotianae*

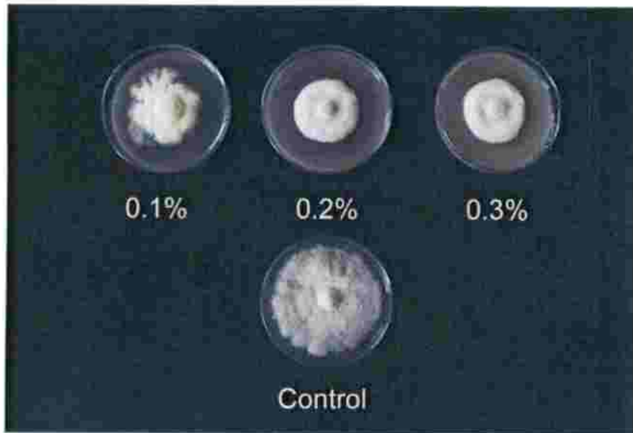
Table 19b. ***In vitro*** **evaluation of botanicals on the inhibition of mycelial growth of** ***Corynespora cassiicola***

Treatments	Botanicals	Inhibition of mycelial growth of *Corynespora cassiicola* (%)		
		C-1	**C-2**	**C-3**
T1	Azadiractin 0.1% (0.1%,0.2%, 0.3%)	33.31* (35.19)**	44.44 (41.77)	49.99 (44.98)
T2	Garlic extract (0.5%, 1%, 2%)	66.66 (54.71)	77.77 (61.84)	100 (90.00)
T3	Ready to use neem oil garlic soap (0.1%, 0.6%, 1%)	5.53 (11.00)	16.66 (23.88)	33.33 (35.05)
CD		12.39	6.33	8.87
SE		3.51	1.79	2.51

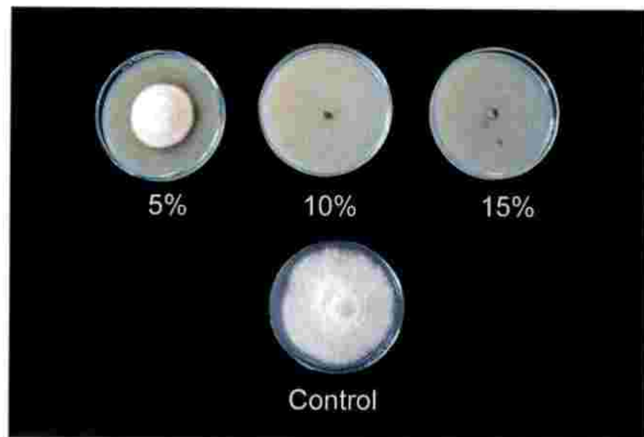
* Mean of three values

** Values in parenthesis are angular transformed values

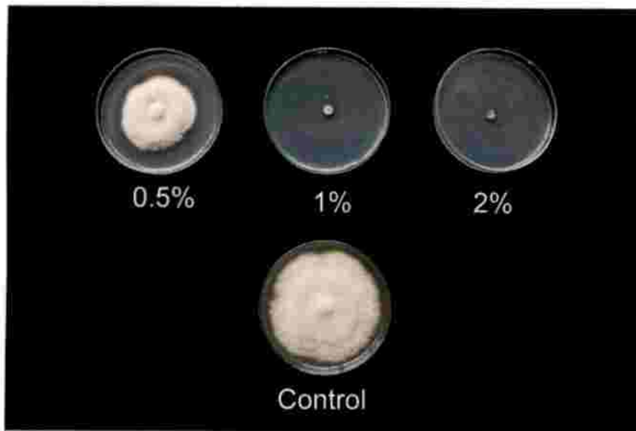
C- Concentration



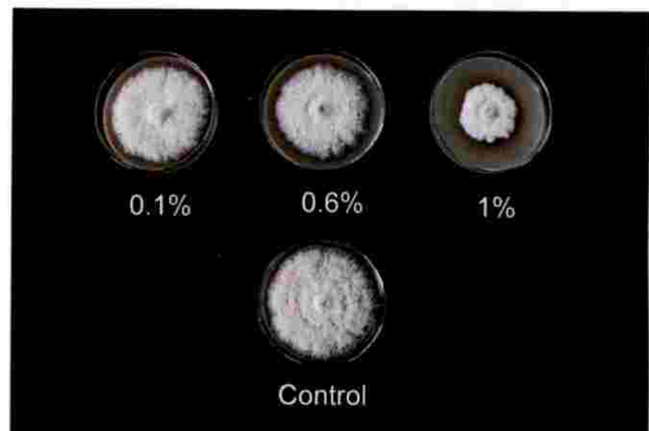
Azadirachtin 0.1% EC



Garlic extract (higher concetrations)



Garlic extract (lower concentrations)



Neam oil garlic soap

Plate 21b. Effect of different *botanicals* on radial growth of *Corynespora cassiicola*

4.7.3. *In vitro* evaluation of biocontrol agents against major fungal pathogens causing fruit rot in cucurbits

In vitro evaluation studies of biocontrol agents revealed that, among five biocontrol agents tested *Trichoderma viride* significantly inhibited *Choanephora cucurbitarum with* 77.77 per cent inhibition. On par effect was noticed for *Bacillus subtilis* with 61 per cent inhibition. PGPM and PGPR Mix-II were having least effectiveness with 27.77 and 22.22 per cent inhibition, respectively. (Table 15c and Plate 17c).

Against *Sclerotium rolfsii the* most effective biocontrol agents were *Bacillus subtilis* (83.32 per cent inhibition) and PGPM (77.77 per cent inhibition) which were at par with each other. PGPR Mix-II had at par effect with PGPM with 66.66% inhibition, but *Pseudomonas fluorescens* could not be considered as effective against *Sclerotium rolfsii* because, it did not inhibit the growth of *Sclerotium rolfsii* (Table 16c and Plate 18c).

Against *Rhizoctonia solani*, *Trichoderma viride* was found to be the most effective with 100 per cent efficiency. Next effective biocontrol agents were *Bacillus subtilis* (61.10 per cent inhibition) and PGPM (44.44 per cent inhibition). The bacterial antagonist *Pseudomonas fluorescens* was not effective in inhibiting *Rhizoctonia solani* (Table 17c and Plate 19c).

Trichoderma viride and PGPM were the best biocontrol agents against *Phytophthora nicotianae* since they showed 100 per cent inhibition. The bacterial bio control agents *P. fluorescens* and *B. subtilis* showed 61.1 and 50 per cent inhibition. PGPR Mix-II was least effective against *Phytophthora nicotianae.* (Table 18c and Plate 20c).

Against *Corynespora cassiicola* PGPM was the best inhibitor with 100 per cent inhibition. PGPR Mix-II (66.66 per cent inhibition) and bacterial antagonist *B. subtilis* (61.10 per cent inhibition) were having on par effect. While *T. viride* (44.44) and *P. fluorescens* (49.99) were least effective in inhibiting *Corynespora cassiicola* (Table 19c and Plate 21c).

127

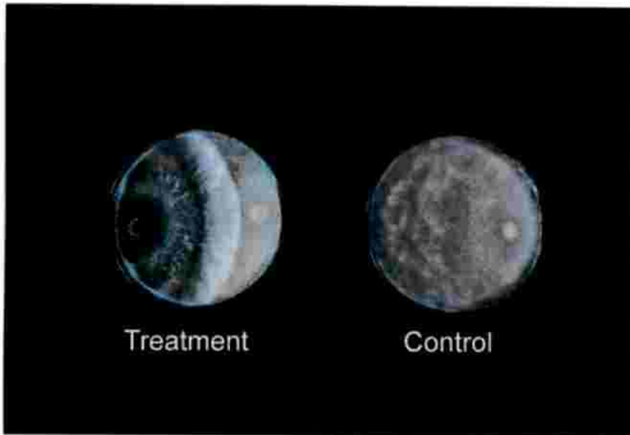
Table 15c. *In vitro* evaluation of biocontrol agents on the inhibition of mycelial growth of *Choanephora cucurbitarum*

Treatments	Biocontrol agents	Inhibition of mycelial growth of *Choanephora cucurbitarum* (%)
T1	*Trichoderma viride*	77.77* (61.84)**
T2	*Pseudomonas fluorescens*	11.10 (19.05)
T3	*Bacillus subtilis*	61.10 (51.42)
T4	PGPR Mix-II	22.22 (27.60)
T5	PGPM	27.77 (31.47)
CD		10.19
SE		3.19

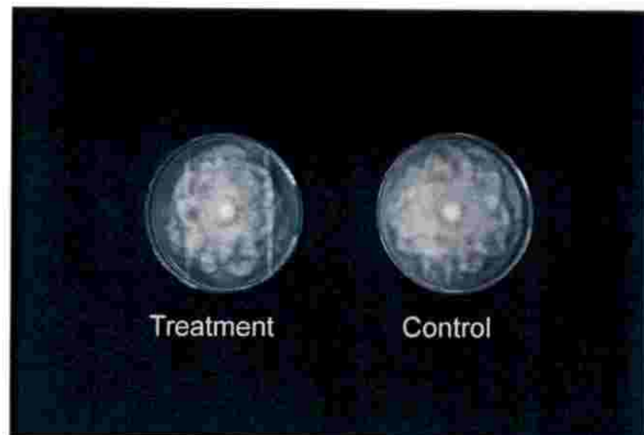
* Mean of three values

** Values in parenthesis are angular transformed values

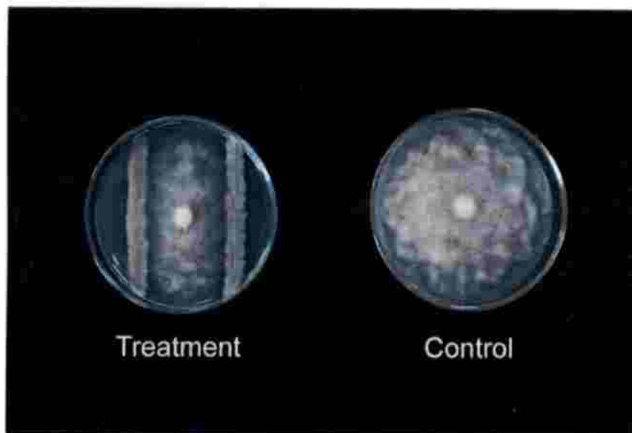
C- Concentration



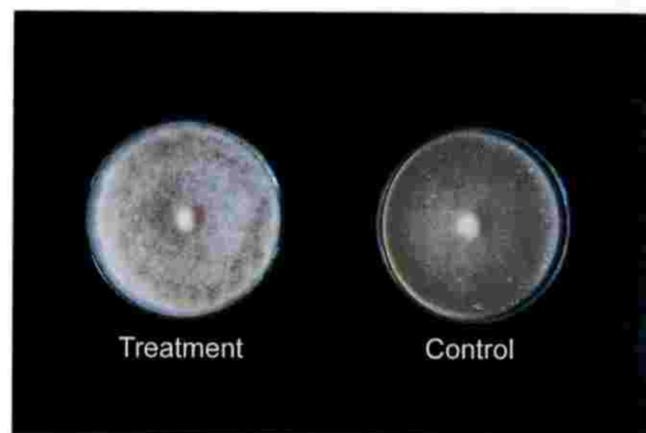
Trichoderma viride



Pseudomonas fluorescens



Bacillus subtilis



PGRP Mix-II

PGPM

Plate 17c. Effect of different *biocontrol agents* on radial growth of *Choanephora cucurbitarum*

Table 16c. *In vitro* evaluation of biocontrol agents on the inhibition of mycelial growth of *Sclerotium rolfsii*

Treatments	Biocontrol agents	Inhibition of mycelial growth of *Sclerotium rolfsii* (%)
T1	*Trichoderma viride*	49.99* (44.98)**
T2	*Pseudomonas fluorescens*	0 (0.00)
T3	*Bacillus subtilis*	83.32 (66.07)
T4	PGPR Mix-II	66.66 (54.75)
T5	PGPM	77.77 (61.96)
CD		6.12
SE		1.91

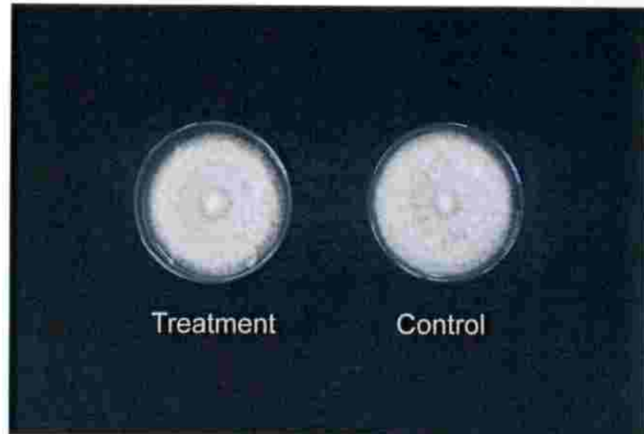
* Mean of three values

** Values in parenthesis are angular transformed values

C- Concentration

130

Trichoderma viride



Pseudomonas fluorescens

Bacillus subtilis

PGRP Mix-II

PGPM

Plate 18c. Effect of different *biocontrol agents* on radial growth of *Sclerotium rolfisii*

Table 17c. *In vitro* evaluation of biocontrol agents on the inhibition of mycelial growth of *Rhizoctonia solani*

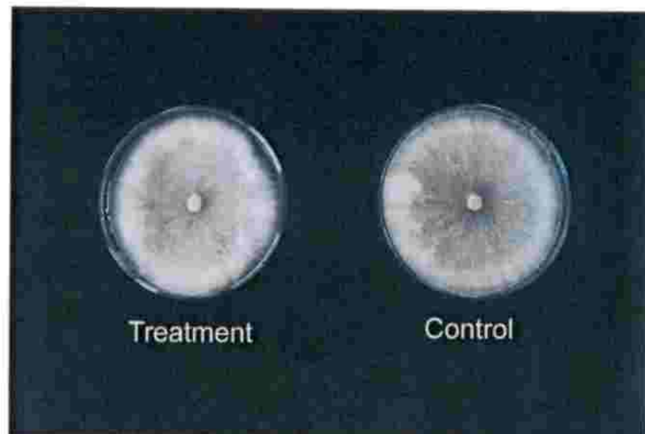
Treatments	Biocontrol agents	Inhibition of mycelial growth of *Rhizoctonia solani* (%)
T1	*Trichoderma viride*	100* (90.00)**
T2	*Pseudomonas fluorescens*	0 (0.00)
T3	*Bacillus subtilis*	61.10 (51.42)
T4	PGPR Mix-II	33.32 (35.19)
T5	PGPM	44.44 (41.77)
CD		4.69
SE		1.47

* Mean of three values

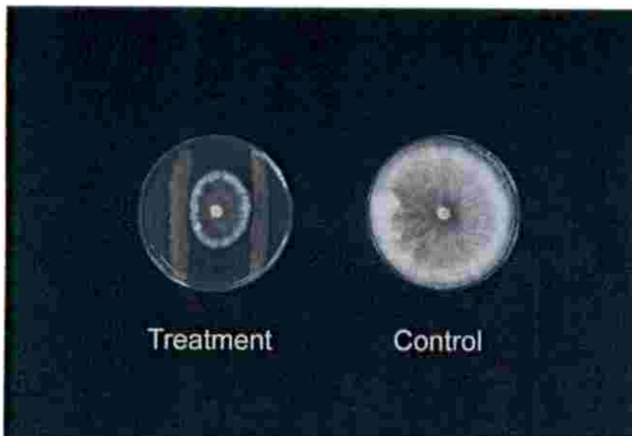
** Values in parenthesis are angular transformed values

C- Concentration

Trichoderma viride



Pseudomonas fluorescens



Bacillus subtilis

PGRP Mix-II

PGPM

Plate 19c. Effect of different *biocontrol agents* on radial growth of *Rhizoctonia solani*

Table 18c. *In vitro* evaluation of biocontrol agents on the inhibition of mycelial growth of *Phytophthora nicotianae*

Treatments	Biocontrol agents	Inhibition of mycelial growth of *Phytophthora nicotianae* (%)
T1	*Trichoderma viride*	100* (90.00)**
T2	*Pseudomonas fluorescens*	61.10 (51.42)
T3	*Bacillus subtilis*	50 (44.98)
T4	PGPR Mix-II	16.66 (23.88)
T5	PGPM	100 (90.00)
CD		4.71
SE		1.40

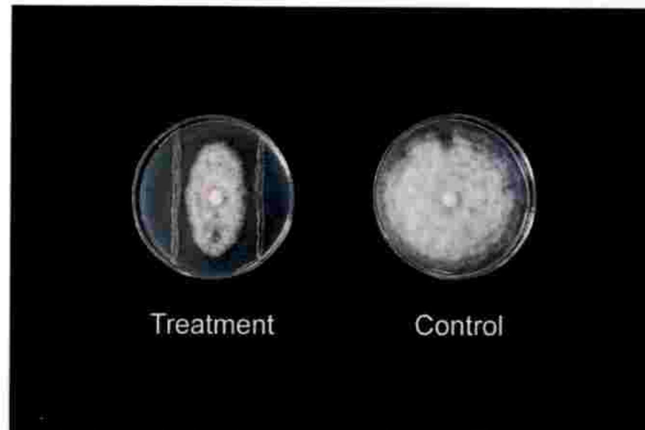
* Mean of three values

** Values in parenthesis are angular transformed values

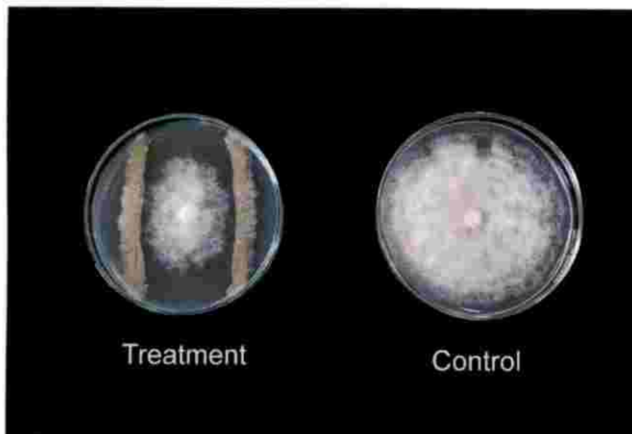
C- Concentration

133

Trichoderma viride



Pseudomonas fluorescens



Bacillus subtilis

PGRP Mix-II

PGPM

Plate 20c. Effect of different *biocontrol agents* on radial growth of *Phytophthora nicotianae*

Table 19c. *In vitro* evaluation of biocontrol agents on the inhibition of mycelial growth of *Corynespora cassiicola*

Treatments	Biocontrol agents	Inhibition of mycelial growth of *Corynespora cassiicola* (%)
T1	*Trichoderma viride*	44.44* (41.73)**
T2	*Pseudomonas fluorescens*	49.99 (44.98)
T3	*Bacillus subtilis*	61.10 (51.42)
T4	PGPR Mix-II	66.66 (54.75)
T5	PGPM	100 (90.00)
CD		7.09
SE		2.22

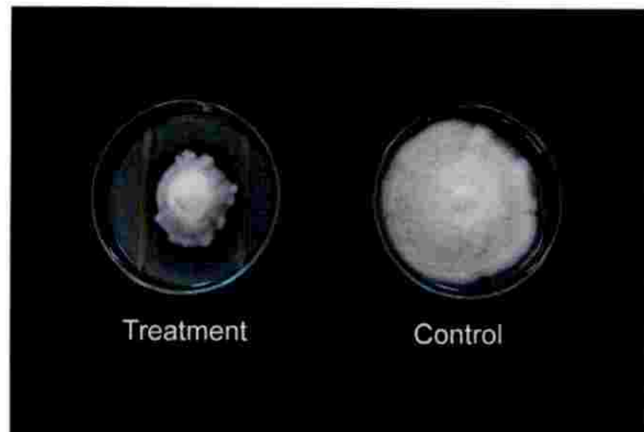
* Mean of three values

** Values in parenthesis are angular transformed values

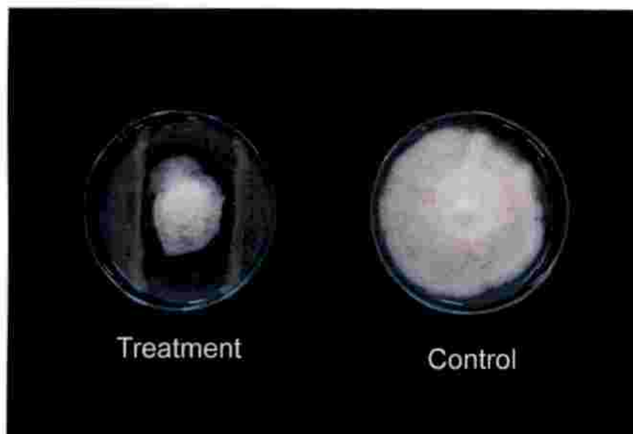
C- Concentration

135

Trichoderma viride



Pseudomonas fluorescens



Bacillus subtilis

PGRP Mix-II

PGPM

Plate 21c. Effect of different *biocontrol agents* on radial growth of *Corynespora cassiicola*

4.8. EVALUATION OF FUNGICIDES, BOTANICALS AND BIOCONTROL AGENTS AGAINST FRUIT ROT OF PUMPKIN UNDER *IN VIVO* CONDITIONS

Based on the severity of disease and estimation of per cent disease incidence (PDI), during the purposive survey in seven locations of three districts, the most severe pathogen, *Choanephora cucurbitarum* in pumpkin was selected for the *in vivo* studies. The best two treatments from *in vitro* evaluation of fungicides, botanicals and biocontrol agents were selected based on the efficacy in inhibiting the pathogen and was applied as described in materials and methods. Effects of different treatments on *in vivo* management of the fruit rot caused by *Choanephora cucurbitarum* and the growth parameters of pumpkin during the management were also evaluated. Details of the experiments and results are furnished below.

In vivo experiments were carried out in an area of 4.75 cents which was observed as a sick plot in the previous two seasons, having enormous amount of pathogenic inoculum. Pumpkin variety Arka Suryamukhi (released from IIHR, Bengaluru) was selected for the study. Land was prepared and pits were made with a size of 60 cm diameter and 30-45 cm depth with a spacing of 4.5x2 m. Seeds were sown directly at the rate of 4-5 seeds per pit. Farm yard manure was applied @ 10.8 kg/pit and 123.3 rock phosphate as basal dose. Unhealthy plants were removed after two weeks and only three plants were maintained per pit. Manures were applied in two split doses (3.6 kg/pit) at winding and flowering stages. All the cultural operations were carried out as per the Package of Practices Recommendations (organic) of KAU (KAU, 2016).

Periodical observations were made for the development of symptoms on the fruits. The details of different treatments given are shown in Table 20. Fungicide treatment was given as spray application on the fruits a day after anthesis and observations were taken 10 days after each spray. The biocontrol

agents *Trichoderma viride* and *Bacillus subtilis* were also applied prophylactically @ 20g per litre of water (talc based formulation) 10 days after sprouting as soil drench.

Blossoming of female flowers started 35 days (5 weeks) after sowing and anthesis continued for two weeks. The fruit rot caused by *Choanephora cucurbitarum* was observed within one week after anthesis of the female flower. Therefore applications of different treatments as spray on fruits were given whenever female flowers blossomed. Tagging of fruits was continued for two weeks to complete spraying as per schedule. Observations on PDS on fruit rot were recorded ten days after each spray. Growth parameters were also observed after specified intervals for comparison.

4.8.1. *In vivo* evaluation of fungicides, botanicals and biocontrol agents for management of *Choanephora* fruit rot of pumpkin

The results showed that chemical control was the most effective method for management of *Choanephora* fruit rot in pumpkin. Tebuconazole 5EC was the best chemical tested where none of the fruits were infected by *Choanephora* (Table 21). Mancozeb 64% + cymoxanil 8% (PDS 6.66 per cent) were recorded as second best treatment which is statistically at par with tebuconazole 5EC. Botanicals were also having effect on managing Choanephora fruit rot. Azadirachtin 0.1% showed (PDS of 16.66 per cent) 73.69 per cent disease reduction over control, and garlic extract (PDS of 20 per cent) 68.41 per cent reduction over control. Among the two biocontrol agents, the fungal agent *Tricoderma viride* was having lesser effect on managing *Choanephora* fruit rot than all the above treatments (PDS of 30 per cent with 52.62 per cent reduction over control). Effect of bacterial biocontrol agent *Bacillus subtilis* (PDS of 36.66 per cent with 42.11 per cent reduction over control) was least compared to other treatments.

Plate 22. *In vivo* studies in the sick plot

4.8.2. Effect of different treatments on growth parameters during the management of *Choanephora* fruit rot of pumpkin

Biometric observations were taken to assess the effect of different fungicides, botanicals and biocontrol agents in the *in vivo* studies on management of *Choanephora* fruit rot of pumpkin. Number of leaves and branches were noted. The number of leaves on 60 DAS revealed no significant effect among the treatments except biocontrol agents. Plants treated with the biocontrol agents (*Bacillus subtilis* and *Trichoderma viride*) showed comparatively higher number of leaves than others. As per observation, average number of leaves produced by the plants treated with *Bacillus subtilis* and *Trichoderma viride* were 98.33 and 94.33 and they were significantly higher than all other treatments. The same trend was also observed in the case of branching of the vines as shown in the (Table 22, Figures 2&3). The plants treated with *Bacillus subtilis* and *Trichoderma viride* were having significantly higher number of branches per vine (10.66 and 10.33 respectively) and it is significantly higher than all other treatments.

4.8.3. Effect of different treatments on yield parameters during the management of *Choanephora* fruit rot of pumpkin

The effect of different fungicides, botanicals and biocontrol agents on yield of pumpkin during the management of Choanephora fruit rot was studied. When result data was analyzed, weight of fruits had a significant effect among the treatments., the highest yield of 9 kg/plant, was for T_2 (tebuconazole 5EC) and T_1 (mancozeb 64%+cymoxanil 8%) (8.33 kg/plant), which were statistically at par. Azadirachtin 0.1% (7.33 kg/plant) and garlic extract (7 kg/plant) were at par with their effect on managing fruit rot. But these botanicals were significantly superior than the chemicals. For both the biocontrol agents *viz.*, *Trichoderma* and *Bacillus*, there was are no significant influence on the yield parameters of pumpkin (6 kg and 5.33 kg/plant). The same trend was also observed in the case of fruit number as shown Table 23(Figures 4&5). The highest number of fruits, 7 /plant, was for T_2 (tebuconazole 5EC) and T_1 (mancozeb 64%+cymoxanil 8%)

(6.33 /plant), which were statistically at par. Azadirachtin 0.1% (5.33 /plant) and garlic extract (5 /plant) were stastically at par with their effect on managing fruit rot. But these botanicals were significantly lower in effect than chemicals. Both the biocontrol agents, *Trichoderma* and *Bacillus* are not significantly influencing the yield parameters of pumpkin (4 kg and 3.33 /plant).

144

Table 20. fungicides, botanicals and biocontrol agents tested against *Choanephora cucurbitarum* fruit rot in pumpkin

Treatment No.	Treatments	Method of application	Time of application	Quantity applied
T_1	Mancozeb 64%+cymoxanil 8%)	Spray application on fruit(SAF)	Day after anthesis	0.2%
T_2	Tebuconazole5 EC	Spray application on fruit(SAF)	Day after anthesis	0.1%
T_3	Azadirachtin 0.1%	Spray application on fruit(SAF)	Day after anthesis	0.2%
T_3	Garlic extract	Spray application on fruit(SAF)	Day after anthesis	1%
T_5	*Trichodema viride*	Soil drenching (SD)	10 days after sprouting	20 g of talc based formulation / 1Lof water
		Spray application on fruit(SAF)	Day after anthesis	20 g of talc based formulation / 1Lof water
T_6	*Bacillus subtilis*	Soil drenching (SD)	10 days after sprouting	20 g of talc based formulation / 1Lof water
		Spray application on fruit(SAF)	Day after anthesis	20 g of talc based formulation / 1Lof water

Table 21. ***In vivo*** **evaluation of selected fungicides, botanicals and biocontrol agents for the management of *Choanephora cucurbitarum* fruit rot in pumpkin**

Treatment no.	Treatments (Foliar spray)	Conc. (%)	PDI 10 days after spray	Per cent disease reduction (PDR) over control
T_1	Mancozeb 64%+Cymoxanil 8%	0.2	6.66 (12.28)	89.48
T_2	Tebuconazole 5EC	0.1	0 (0.00)	100
T_3	Azadiractin 0.1%	0.2	16.66 (23.84)	73.69
T_4	Garlic extract	1	20 (26.55)	68.41
T_5	*Trichoderma viride*	2	30 (32.98)	52.62
T_6	*Bacillus subtilis*	2	36.66 (37.21)	42.11
T_7	Control	–	63.33 (52.75)	–
CD			8.22	
SE			2.63	

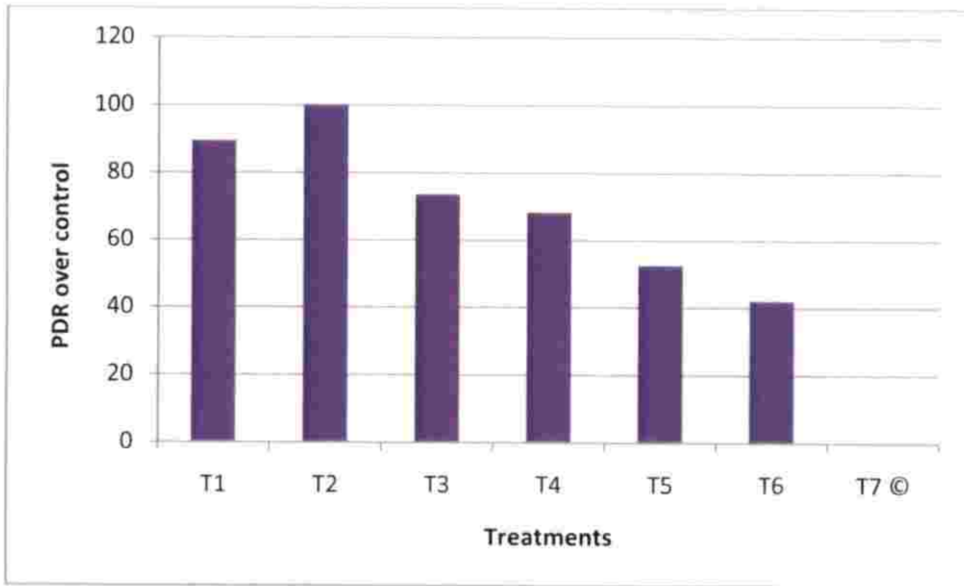


Fig.1. *In vivo* evaluation of fungicides, botanicals and biocontrol agents for management of *Choanephora cucurbitarum* fruit rot

Table 22.Effect of different treatments on growth parameters during the management of *Choanephora* fruit rot of pumpkin

Treatment No.	Treatments	Conc. (%)	Average number of branches per plant	Average number of leaves per plant 60 DAS
T_1	Mancozeb 64%+Cymoxanil 8%	0.2	8.00	81.00
T_2	Tebuconazole 5EC	0.1	7.66	78.66
T_3	Azadiractin 0.1%	0.2	7.66	82.00
T_4	Garlic extract	1	7.00	78.00
T_5	*Trichoderma viride*	2	10.33	94.33
T_6	*Bacillus subtilis*	2	10.66	98.33
T_7	Control	–	7.33	82.00
CD			2.25	6.08

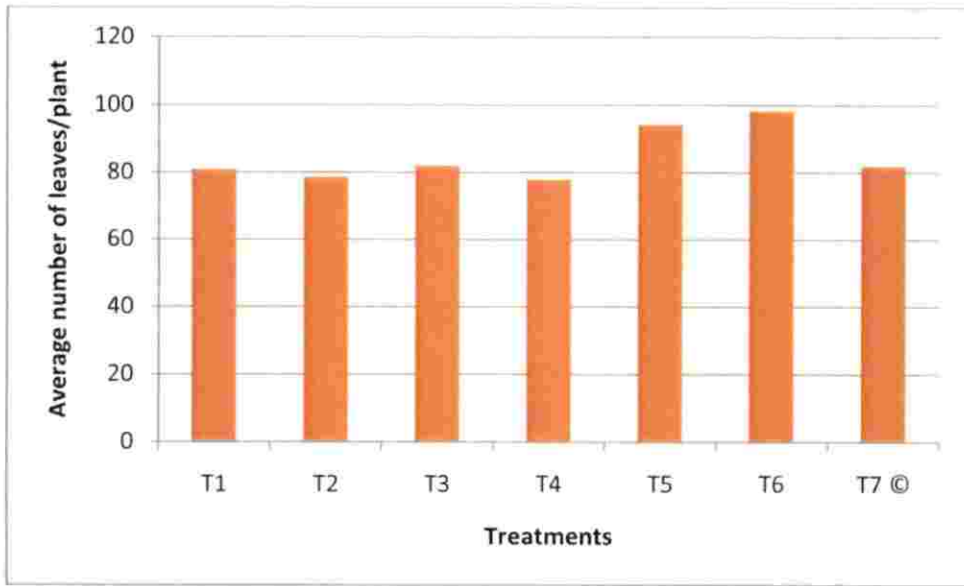


Fig.4. Effect of different treatments on growth parameter (number of leaves/plant) of pumpkin during the management studies of *Choanephora cucurbitarum* fruit rot

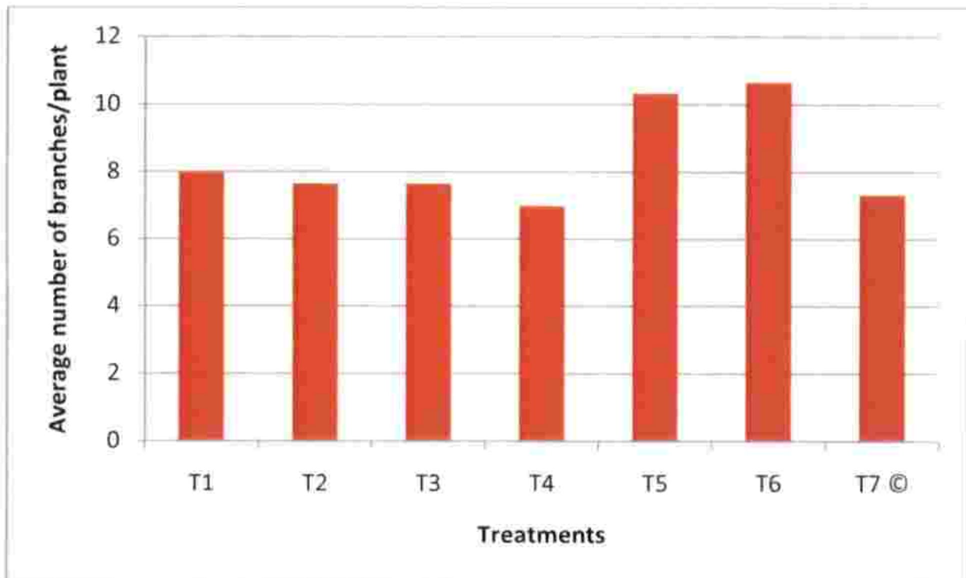


Fig.5. Effect of different treatments on growth parameter (number of branches/plant) of pumpkin during the management studies of *Choanephora cucurbitarum* fruit rot

146

Table 23. Effect of different treatments on yield parameters during the management studies of *Choanephora* fruit rot in pumpkin

Treatment No.	Treatments	Conc. (%)	Average number of fruits per plant	Average yield per plant (weight in kg)
T_1	Mancozeb 64%+Cymoxanil 8%	0.2	8.33	6.33
T_2	Tebuconazole 5EC	0.1	9.00	7.00
T_3	Azadiractin 0.1%	0.2	7.33	5.33
T_4	Garlic extract	1	7.00	5.00
T_5	*Trichoderma viride*	2	6.00	4.00
T_6	*Bacillus subtilis*	2	5.33	3.33
T_7	Control	–	3.33	1.33
CD			0.86	0.86

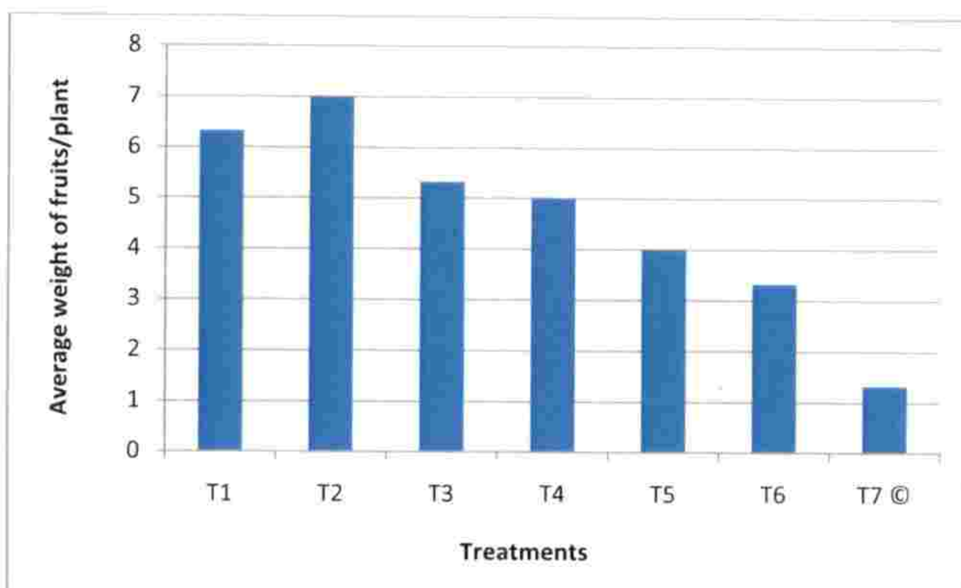


Fig.2. Effect of different treatments on yield parameter (weight of the fruits/plant) of pumpkin during the management studies of *Choanephora cucurbitarum* fruit rot

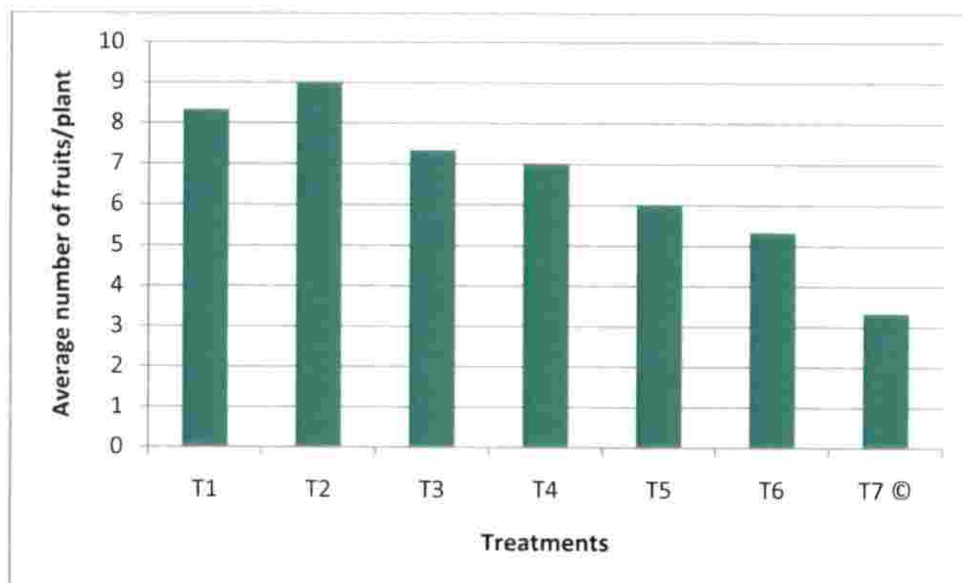


Fig.2. Effect of different treatments on yield parameter (number of the fruits/plant) of pumpkin during the management studies of *Choanephora cucurbitarum* fruit rot

Discussion

5. DISCUSSION

Cucurbits are the most widely cultivated vegetables in Kerala. One of the main constraints in the production of these crops is the incidence of fungal diseases, including fungal fruit rots. Detailed systematic studies on the identification and characterization of fungal fruit rots of cucurbits were not undertaken in Kerala. In this context, the present study on the characterization and biointensive management of fungal fruit rots of cucurbits was undertaken. The studies were carried out as per the technical programme during the period 2017-2019 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

Purposive sampling surveys were conducted in nine locations of three districts *viz.*, Kasargod, Kannur and Kozhikode for the collection of diseased samples of the fruits and recorded the disease incidence and severity during crop season of 2017-19. The diseased specimens were collected from these locations and the pathogens were isolated. The survey revealed that, the maximum disease incidence was in fruit rot-1 in College of Agriculture (CoA), Padannakkad (instructional farm) of Kasargod district (51 per cent) in the open field. Out of the seven types of fruit rots in cucurbits Fruit rot-1 (caused by *Choanephora cucurbitarum*) and Fruit rot-4 (caused by *Phytophthora nicotianae*) were the most destructive. In both the places, favourable environmental condition might be the reason for severe occurrence of the fungal fruit rots. 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 fruit samples and

174585

150

microscopic examinations the different isolates were categorized into seven categories *viz*., FR 1, FR 2, FR3, FR4, FR5, FR6 and FR7.

Fruit rot-1 was the major pathogen during the survey and was identified as *Choanephora cucurbitarum* (FR1) obtained from pumpkin (Arka Suryamukhi) cultivated in instructional farm of COA, Padannakkad in the Kasargod district of Kerala. Yu and Ko (1997) documented wet rot of *Choanephora cucurbitarum* on different crop plants including cucurbits. These results are also in accordance with the studies conducted by Choudhary (2015) on wet rot caused by *Choanephora cucurbitarum* in cucurbits and their management.

The pathogen (FR2) suspected as *Sclerotium rolfsii,* was isolated from pumpkin. Zitter *et al*. (2004) recorded *Sclerotium rolfsii* fruit rot from pumpkin.

The pathogen (FR3) obtained from cucumber during the survey at Karakkode was suspected as *Rhizoctonia solani*. Jones (1961) reported fruit rot (soil rot, belly rot) of cucumber caused by *Rhizoctonia solani* is the most severe disease limiting the production in warm humid places of United States. Lewis and Papavizas, (1980) observed fruit rot caused by *Rhizoctonia solani* in cucumber and studied the integrated management strategies.

The pathogen (FR4) caused fruit rot which was suspected as *Phytophthora nicotianae* was noticed on ridge gourd and Chowdappa *et al*. (2016) isolated *Phytophthora nicotianae* from ridge gourd in South India. Erwin and Ribiero in 1996 found that *Phytophthora nicotiana* is a devastating plant pathogen on a global scale, causing fruit rot in vegetable crops.

Fruit rot-5, which was suspected as *Pythium* sp. was observed on the cucumber during the survey. According to Abdelzaher 2004, *Pythium* sp. are ubiquitous soil-borne pathogens causing fruit rot diseases on many plants such as cucumber and melon. Drechsler (1925) conducted a study on soft rot caused by *Pythium* in cucumber.

The pathogen FR6 isolated from snake gourd was suspected as *Corynespora cassicola.* which was obtained from Kurunthur , Kasargod. Silva and Giordano in 2000 found that the fungi *Corynespora cassiicola* infects 375 plant species, from more than 76 countries worldwide, and present symptoms of decay both on the leaf and on the fruit. Castro (1979) reported that *C. cassiicola* attacked cucurbits in Mexico.

The fungal pathogen FR7 isolated from immature salad cucumber in the polyhouse was suspected as *Fusarium equiseti.* Blancard *et al.* (2005) reported the incidence of *Fusarium* fruit rot in green house cucumber. Al-sadi *et al.* (2011) reported *Fusarium equisetii* causing the fruit rot in immature cucumber in the greenhouse in Oman. These studies are in conformity to the present observations.

5.2. PATHOGENICITY OF ISOLATES

Artificial inoculation of suspected pathogens *viz., Choanephora, Sclerotium, Rhizoctonia, Phytophthora, Pythium, Corynespora, Fusarium,* on detached fruits were done and pathogenicity was proved for each isolate. The symptoms produced by fruit rot pathogens during pathogenicity tests were similar to those in the survey fields.

There are a number of studies to prove pathogenicity of each of these fungi by different researchers. Johnson *et al.* (2014) experimentally proved pathogenicity of *Choanephora cucurbitarum* on different vegetables by mycelial bit inoculation method. Johnson *et al.* (2014) observed the development of symptoms of soft rot and decay in cowpea pods after five days of artificial inoculation. George in 2015 confirmed the pathogenicity of the *Choanephora* sp. by proving Koch's postulates through mycelial disc inoculation method. Gogoi *et al.* (2016) observed typical soft rot symptoms and signs of *Choanephora rot* after 5-7 days of inoculation by mycelial bit inoculation method.

The results of the present study are in line with the findings of Najera *et al.* (2018) where they could prove pathogenicity of *Sclerotium rolfsii* in detached fruit sample of *Cucurbita argyrosperma*.

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 in the field 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 Cucurbitaceae family. Nik and Yapi (1979) studied pathogenicity of *R. solani* and proved pathogenicity by brushing the inoculum on the French bean pod. Zhang *et al.* (2009) carried out pathogenicity test of cabbage head rot by *Rhizoctonia solani* by mycelial bit inoculation method. In the upcoming years, *Rhizoctonia* will become a serious threat to crops by emerging as a universal pathogen. Also pathogenicity of *R. solani* causing blight symptoms on stems was proved by stem inoculation technique on a tomato cultivar Arka vikas (Sumalatha *et al*., 2017).

Fruit rot- 4 was observed with more severity and was suspected as *Phytophthora*. Different workers proved pathogenicity of *Phytophthora*. Pathogenicity test for *P. nicotinae* isolates from brinjal was conducted by adopting zoospore suspensions method of Hong *et al*. (2002). Kousik *et al.* (2015) conducted pathogenicity test of *Phytophthora capcisi* by mycelial agar bit inoculation method in bitter gourd. Choudappa *et al*. (2016) observed *Phytophthora* attack on ridge gourd. Darine *et al.* (2017) isolated 9 different isolates of *Phytophthora nicotianae* from infected pepper plants and studied their pathogenicity in *Capsicum annuum.*

Likewise, the Koch postulates as pathogenicity test for fungal pathogen *Pythium* is in correspondence with the results of Drechsler (1925). He demonstrated the pathogenicity of three strains of *Pythium* sp. isolated from cucumber having cottony leak symptom. Pieces of mycelium from pure cultures were inserted into

aseptic incisions and cottony mycelial growth covered the fruit after three days. 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.

Ahmed and Khair (2008) carried out the pathogenicity test of *Corynespora cassiicola* with the isolated fungus on both the detached healthy whole fruits (*in vitro*) and the healthy fruits on the plants in the field itself (*in vivo*) as per the method suggested by Ash and Lanoiselet (2001). Pawar *et al.* (2014) and Chowdappa *et al.* (2014) waere also followed the same method.

Representative fungi isolated from diseased immature cucumber fruits including *Fusarium equiseti* were tested for pathogenicity by mycelial disc inoculation on the detached fruits (Al-Sadi *et al.*, 2011). 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 cucurbits reproducing distinct symptoms observed as in naturally infected plants.

5.3. SYMPTOMATOLOGY OF THE PATHOGENS

Assessment of symptomatology is essential for the systematic study of a isolated pathogen. Detailed accounts on symptomatology of different fungal pathogens causing fruit rots of cucurbits were studied in the present work and many of the effort done by different workers are in accordance with the present study.

Typical soft rot symptoms were noticed on immature fruits of pumpkin. Symptoms of *C. cucurbitarum* infection were characterized by water soaked lesions followed by fruitification and complete wet rotting of the fruits. Kacharek *et al.* (2003) observed rapid rotting and white fungal mycelium on the infected parts followed by development of fructifications in cucurbits as a result of *C. cucurbitarum*

154

infection. Many authors reported similar symptoms by *C. cucurbitarum* on different vegetables (Wilson and Jose, 1965; Kwon and Jee, 2005; Park *et al*., 2014; Gogoi *et al*., 2016).

Among the fruit rot diseases, symptoms of *Sclerotium rolfsii* appeared as white mycelial mats with brown colored sclerotia, discoloured internal tissue due to internal growth of mycelium. Otther workers observed similar symptoms of *Sclerotium rolfsii* on the fruits in *Cucurbita agyrosperma* (Zitter *et al*., 2004), *Cucurbita melo* (Kwon *et al*., 2009) and pumkin (Shankar *et al*., 2014).

Symptoms of the *Rhizoctonia solani* infection on cucurbits was seen as water soaked brown lesions on fruits which resulted in decay of fruits. Lewis and Papavizas, (1980) observed that warm humid condition results from dense canopy favour infection of *Rhizoctonia solani*. Symptom initially appeared with lesions as water soaked areas which subsequently collapse and it forms sunken irregular brown cankers on the fruit. Similar disease symptoms were also reported by Abawi and Martin (1985), Yang *et al*. (2007) and Shankar *et al*. (2014) in cabbage and cucumber.

Fruit rot by *Phytophthora* appeared as dark water soaked lesions on the fruits with white mycelial growth, eventually resulting in watery rot of the fruits. Occurrence of this pathogen in vegetables is common during the warm humid climate. Similar to above symptoms were reported by Sharma *et al*. (1975), Sharma and Sohi (1989), Singh (1999), Gupta and Paul (2001) Housebeck and Lamour (2004), Granke (2012) Shankar *et al*. (2014), and Panabieres *et al*. (2016) on various vegetable crops. Choudappa *et al*. (2016) observed symptoms with white mycelial growth and sporangia after six days of inoculation on detached fruits of tomato cultivar Arka Vikas, brinjal cultivar Arka Kusumakar, and ridge gourd cultivar Arka Sujata.

The pathogen associated with fruit rot in cucumber was suspected as *Pythium* sp. The pathogen caused water soaked lesions on the fruits. Affected regions get softened. A fluffy white mycelium was visible on the surface of rotted areas. The occurrence of *Pythium deliense* on cucumber has not been reported so far, however similar species, *Pythium aphanidermatum* was reported with above same symptom (Drechsler, 1925). Similar symptoms on crucifers (Tanina *et al.*, 2004) and in cucurbits (Shankar *et al.*, 2014) were also repored.

Water soaked sunken spots with black concentric rings with sporulation and final soft rotting were observed in snake gourd affected with *C. cassiicola* during this study. Kwon *et al.* (2001) observed the disease symptoms of *C. cassiicola* in chilli fruits as small brown circular spots, later turned to circular or irregular shape with white papery centres delimited by dark brown concentric ringed borders. Ahmed and Khair (2008) observed water soaked lesions with pale yellow to brownish centres having irregular outer rings of gray to dark brown margin in okra. Sometime the centre of the lesion was found cracked. Caetano *et al.* (2018) observed the sunken spot with profused sporulation on tomato.

The pathogen obtained from immature cucumber was suspected as *Fusarium* and yellowing of blossom end of the fruit and gradual browning and dry rot was noticed as symptoms which lead to complete rotting of the fruit. Similar observation in the form of yellowing at the flower end of each immature fruit, followed by browning and rotting was made by Blancard *et al.* (2005) on cucumber and Rimmer *et al.* (2007) on crucifers.

5.4. CHARACTERISATION AND IDENTIFICATION OF PATHOGEN

5.4.1. Cultural and morphological characters

Cultural and morphological characters are important tools in identification and classification of fungi. The fungal pathogens were temporarily identified up to

159

the genus level based on the studies of symptomatology, cultural and morphological characters. The molecular characterization of seven selected pathogens was carried out at Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram by ITS sequencing to identify at species level. Fruit rot-1, Fruit rot-2, Fruit rot-3, Fruit rot-4, Fruit rot-5, Fruit rot-6, and Fruit rot-7 were identified as *Choanephora cucurbitarum, Sclerotium rolfsii, Rhizoctonia solani, Phytophthora nicotianae, Pythium deliense,Corynespora cassiicola* and *Fusarium equiseti,* respectively.

Study on wet rot pathogen *Choanephora* obtained from pumpkin 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 sporangial vesicles were as described by Kirk (1964), and Kwon *et al.* (2001), Kwon and Jee (2005) and Gogoi *et al.* (2016) during their studies on *Choanephora* in various crops. In a study by Pornsuriya *et al.* (2017) in *Brassica chinensis* affected with *Choanephora cucurbitarum* produced white colonies which 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.

The pathogen *Sclerotium* produced whitish fast-growing mycelia with numerous reddish brown sclerotia on potato dextrose agar (PDA) medium and development of sclerotia were observed which were white at first and later turned into brown. Colony and sclerotial characters studied were comparable with the reports of Manu (2012) and Mahadevakumar *et al.* (2016). Mahadevakumar *et al.* (2016) observed the development of whitish fast-growing mycelia with numerous reddish brown sclerotia in potato dextrose agar (PDA) medium inoculated with *Sclerotium rolfsii* obtained from *Cucurbita maxima*.

Cultural and morphological characters of the fruit rot causing pathogen in cucumber, *Rhizoctonia,* were also studied and these results are in accordance with the description given by Rimmer *et al*. (2007) and Zhang *et al.* (2009) on crucifers. Shim *et al.* (2013) obtained light to dark brown colonies of *Rhizoctonia* from chinese cabbage, which consist of hyphae with 5.01-11.12 µm diameter and dark brown sclerotia with 0.38-1.28 mm diameter.

The fruit rot- 4 was obtained from ridge gourd. The pathogen was *Phytophthora*, identified based on sporangial characters. All the characters including papillate sporangia, dense low spreading mycelium and chlamydospores were in accordance with those reported by Chowdappa *et al.* (2016), according to his observation in ridge gourd all isolates of *Phytophthora nicotianae* produced abundant sporangia on CA in light or dark. Sporangia were borne singly on sympodium and noncaducous. Sporangia were predominately spherical, ovoid, obturbinate with a prominent papilla and all isolates produced abundant spherical chlamydospores and similar observations were made by Dos Santos *et al*. (2006) in black wattle.

Paul *et al*. (1995) and Lodhi *et al*. (2004), studied the characters of oogonia, antheridia and oospore of the fungus *Pythium deliense*. These characters are analogous to the findings obtained in the present study. Similar observations were also made by Gherbawy *et al.* (2005) on soya beans. Parveen and Sharma (2015) also reviewed the morphology of *Pythium aphanidermatum*, from crucifers and found that the pathogen produced aplerotic oospores, which was exactly the 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 morphological and cultural characters of causal agent of fruit rot-6, *Corynespora* were also studied in detail. Characterization of *Corynespora cassiicola* was done by Tsay and Kuo (1991) and Snow and Berggren (1989). They reported the mycelium as white, flocculent and the characters of conidia as solitary or in chains of 2 - 6, very variable in shape, obclavate to cylindrical, straight or curved, pale olivaceous brown or brown, smooth, with 4 - 20 pseudosepta, and measured 41.2 - 219.7μ long and 9.2 - 21.5μ wide, which was similar to the characters noted in this study.

The dry fruit rot 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). Lazreg *et al*. (2014) observed *Fusarium equiseti* from wilted aleppo pine plants with loosely floccose whitish aerial mycelium on PDA, where it produced microconidia and macroconidia having a pronounced curvature with elongated apical cell.

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

The five selected pathogens were used for *in vitro* evaluation of fungicides, botanicals and biocontrol agents. Efficacy of four fungicides, three botanicals and five biocontrol agents were tested against these pathogens.

5.5.1. *In vitro* evaluation of chemical fungicides

In vitro evaluation of chemical fungicides on the inhibition of the pathogens provides preliminary information and serves as a guide for field testing. *In vitro* evaluation studies of fungicides showed that, among the four fungicides tested, tebuconazole 5EC and mancozeb 64% + cymoxanil 8% were the two fungicides effective against *Choanephora cucurbitarum*. Chahal and Grover (1974) reported that

Mancozeb was very effective (80% suppression) in controlling *Choanephora cucurbitarum* in chilli. Hammounda (2008) reported that mancozeb is effective in reducing infection against *Choanephora cucurbitarum* in the southern region of Oman (Dhofar). George (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 including mancozeb, carbendazim + mancozeb and propiconazole which comes under the tebuconazole containing triazole group. These results are in agreement with the present studies.

The data revealed that the fungicide tebuconzole 5EC (Folicur), was significantly superior among all the four fungicides, because at lower concentration they showed 100 per cent inhibition of the pathogen (Table 21, Plate 21A and 21B). The fungicides tebuconzole 5EC and mancozeb 64% + cymoxanil 8% were on par and superior at recommended and higher concentration and both of them showed cent per cent inhibition. Khan and Javaid (2015) reported similar findings that four fungicides including tebuconazole and mancozeb significantly inhibiting the radial growth of *Sclerotium rolfsii* under *in vitro* condition (100 per cent suppression).

In vitro studies with *Rhizoctonia* revealed that the fungicides tebuconzole 5EC (Folicure), mancozeb 63% + carbendazim 12%, mancozeb 75WP and mancozeb 64% + cymoxanil 8% showed 100 per cent inhibition of the pathogen at all the three concentrations. Similar results were obtained for Kataria and Gisi (1996). According to them *Rhizoctonia* diseases could be controlled by seed or soil treatments or both, or foliar applications of a variety of fungicides of diverse chemical groups, *viz.*, benzimidazoles (carbendazim) and triazoles (tebuconazole).

Evaluation of fungicides against *Phytophthora nicotianae* showed cent per cent inhibition by all the fungicides at all concentrations ie., tebuconzole 5EC (Folicure), mancozeb 63% + carbendazim 12%, mancozeb 75WP and mancozeb

160

64% + cymoxanil 8% gave 100 per cent inhibition at all the three concentrations. These results are in accordance with the findings of Praveen *et al.* (2017). Fungicides *viz.*, cymoxanil(8%) + mancozeb (64%) (Curzate M-8), carbendazim (12%) + mancozeb (63%) (Saaf), tebuconazole 250EC (Folicur) showed cent percent inhibition against *Phytophthora* sp. at all the three concentrations. Also Gupta and Bharath (2008) treated Buckeye rot causing pathogen *P. nicotianae var. parasitica* with different fungicides and found that cymoxanil+mancozeb is the most effective fungicide and mancozeb and COC are least effective.

Evaluation of fungicides against *Corynespora cassiicola* revealed that at the lower concentration mancozeb 64% + cymoxanil 8% (100 per cent inhibition) were superior, followed by mancozeb 64% + carbendazim 12% (88.88 per cent inhibition) and tebuconzole 5EC (83.32 per cent inhibition). At recommended concentration mancozeb 64% + cymoxanil 8% and mancozeb 64% + carbendazim 12% (on par) showed cent per cent inhibition of pathogen. These results is in accordance with the observations of Sinnulingga *et al.* (1996), where fungicides like carbendazim (0.2%), mancozeb (0.25%), carbendazim (0.2%) + mancozeb (0.2%) were most effective for the control of *Corynespora casssilcola.*

Management strategies adopted during the study include, chemical control, control by using boatanicals and biological control. Among these methods, chemical fungicides are being widely used to control diseases in vegetables. But over dosage and inappropriate application of fungicides leave harmful residues causing environmental pollution and result in the development of resistant pathogenic strain through mutation. In order to manage these fruit rot pathogens in cucurbits most effectively, an ecofriendly, integrated disease management programmed should be implemented to avoid overuse of a single control method and fight against genetic resistance.

197

5.5.2. *In vitro* evaluation of botanicals

The utilization of organic preparations for controlling plant pathogens has often been considered as the best option for eco-friendly management of plant disease without upsetting the sustainability of environment or health of humans.

In vitro evaluation studies of botanicals recorded that among the three botanicals tested, *viz.,* azadirachtin, garlic extract and ready to use neem oil garlic soap. Garlic extract (100 per cent inhibition) at medium and higher concentration and azadirachtin 0.1% (64.44 per cent inhibition) at lower concentration were found to be effective against *Choanephora cucurbitarum*. Olufolaji (1999) reported that the fruit and bark extracts of *Azadirachta indica* (neem) inhibits (13.85%) growth and sporulation (82.5%) of *Choanephora cucurbitarum*, which causes wet rot disease of Amaranthus. For the soft rot infection of *Abelmoschus esculentus* due to *Choanephora cucurbitarum* infection, extract (5%) obtained from *Azadirachta indica* was found to be effective (55%) (Umana *et al*., 2015).

In the case of *Sclerotium rolfsii*, at the lower and recommended concentrations, garlic extract and azadirachtin 0.1% were found to be most effective (statistically on par) and ready to use neem oil garlic soap was least effective. At higher concentration, treatment efficiency order of efficacy was garlic extract (100%), azadirachtin 0.1% and ready to use neem oil garlic soap. Kiran *et al*., (2006) reported that garlic extract (5%) gives maximum inhibition (65 per cent) against *Sclerotium rolfsii*. Butt *et al*. (2016) reported that *Azadirachta indica* leaf extract at different concentrations (1%, 2%, 3%, 4% and 5%) were very effective against *S. rolfsii* under *in vitro* conditions.

Among the botanicals tested against *Rhizoctonia solani*, garlic was found to be most effective one at all concentrations. At lower and medium concentrations azadirachtin 0.1% and ready to use neem oil garlic soap were found to be next best botanicals. Antifungal action of ready to use neem oil garlic soap was at par with

commercial neem product azadirachtin. At higher concentration azadirachtin 0.1% found to be the next best botanical followed by ready to use neem oil garlic soap. Sifat and Monjil (2017) validated the above results, according to them garlic (10% concentration) (97.50%), neem (10% concentration) (96.75%) gives best results against *Rhizoctonia solani* in rice. Rajput (2013) also gave similar results of neem in maize. He evaluated the antifungal action of different botanicals *viz., Azadirachta indica, Jatropha curcas, Annona raticulata, Curcuma longa, Allium cepa, Pongamia pinnata* and *Datura stramonium* against *R. solani* using poisoned food technique. Maximum inhibition of mycelial growth was recorded in azadirachta indica at both five (46.67%) and ten (51.11%) per cent concentration.

The data on the botanicals tested against *Phytophthora* revealed that garlic extract shows maximum inhibition at all concentrations. The next best treatments were azadirachtin 0.1% and ready to use neem oil garlic soap, which were significantly not different. Pente *et al.* (2015) evaluated antifungal effect of neem (5%) and garlic (5%) against *Phytophthora* obtained from citrus, he observed 69.9 and 100 per cent inhibition of radial mycelial growth. Nagadze (2014) found that the water extracts (0.78% and 3.13% concentration) of *Allium sativum* and *Azadirachta indica* were effective against *Phythopthora infestans*.

As in the case of *Corynespora cassiicola* garlic extract was found to be most effective at all concentrations. The next best alternative among the tested botanicals were azadirachtin 0.1% followed by ready to use neem oil garlic soap at all concentrations. Gupta *et al.* (1982) reported that conidial germination of *C. capsici* was inhibited by *Allium sativum* and *Azardiracta indica*. Lakshmanan *et al.* (1990) tested antifungal property of 10 plant extracts under *in vitro* against the cotton boll rot caused by *Corynespora cassiicola*. Among the different plant extracts, garlic (5% concentration) was the most effective (95.8%).

163

5.5.3. *In vitro* evaluation of biocontrol agents.

In the integrated disease management approach, bio control agents are identified as one of the important component as they are safer, cheaper and ecofriendly. In the present study, *in vitro* evaluation of fungal and bacterial bio control agents against fruit rot pathogens of cucurbits were conducted.

In vitro evaluation of fungal antagonist *Trichoderma viride* and bacterial antagonists *Pseudomonas fluorescens* and *Bacillus subtilis* and microbial consortium *viz.,* PGPR- Mix II and PGPM were tested against five fungal pathogens isolated from various cucurbits.

Among the five biocontrol agents tested against *Choanephora cucurbitarum, Trichoderma viride* and *Bacillus subtilis* (77.77 and 61.10, respectively) were the most effective biocontrol agents. Emoghene and Okigbo (2001) observed a considerable zone of inhibition between the pathogen *Choanephora cucurbitarum* and the isolates of *Bacillus subtilis* on PDA plates. 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. Choudhary (2015) reported that, among biocontrol agents *Trichoderma harzianum + Pseudomonas fluorescens* treatment as seed-cum soil application was most effective against *Choanephora cucurbitarum* in cucumber.

When the mechanism of mycoparasitism of *Trichoderma* was studied, the microscopic observations showed the coiling and disintegration of the hyphae of *Choanephora* by the *Trichoderma* (Plate 17c). This was in line with the observations made by Poornima (2007). Coiling and lysis of pathogenic hyphae by the *Trichoderma* spp. were reported by Poornima and Santhakumari (2008).

Bacillus subtilis (83.32%) and PGPM (77.77%) were the most effective biocontrol agents against *Sclerotium rolfsii* with on par effect. A next best biocontrol

164

agent against *Sclerotium* was *Trichoderma viride*. This result is in accordance with Karthikeyan *et al.* (2006), who reported that *Trichoderma viride* is an inhibitory bioagent against the growth of *Sclerotium rolfsii* in causing stem rot of ground nut. Ramzan *et al.* (2016) studied 15 bioagents against *Sclerotium rolfsii* responsible for root rot of mungbean in which *Bacillus subtilis* and *Trichoderma harzianum* were found to be more effective (89.2 per cent inhibition)

The best three biocontrol agets against *Rhizoctonia solani* were found to be *Trichoderma viride* (100), *Bacillus subtilis* (61.10) and PGPM (44.44). Rehman *et al.* (2012) studied the comparative effect of different biological control agents against *Rhizoctonia solani*. They observed 85.5 and 83.0 per cent mycelial inhibition by *Trichoderma harzianum and T. viride,* respectively. Ashwini and Srividya (2014) isolated a strain of *Bacillus subtilis* from the rhizosphere of chilli, showed broad spectrum antagonism against *Rhizoctonia solani*.

Trichoderma viridae and PGPM (on par) were the best treatments and they showed cent per cent inhibition of *Phytophthora nicotianae*. It was followed by *Pseudomonas fluorescens* and *Bacillus subtilis*. Mushrif *et al.* (2005) isolated five bacterial antagonists (two *Bacillus spp.* and three *Pseudomonas spp.*) from the rhizosphere and phyllosphere of rubber plants, which were antagonistic to *Phytophthora*. *Bacillus* spp. showed higher efficacy in disease control when tested under laboratory and field conditions. Sharma *et al.* (2018) found that *T. viride* (67.43 %) is very effective against *Phytophthora nicotianae var. parasitica* obtained from tomato, while *Pseudomonas fluorescens* was least effective with 53.67 per cent mycelial growth inhibition. The study of Vithya *et al.* (2018) revealed that *Trichoderma viridae* is very effective (67.3% inhibition) for the management *of Phytophthora* in black pepper nursery.

The best tested biocontrol agent was against *Corynespora cassiicola* was PGPM and it showed cent per cent inhibition. PGPR Mix-II (66.66) and bacterial

antagonist *Bacillus subtilis* (61.10) were the next best biocontrol agents. While *Trichoderma viride* and *Pseudomonas fluorescens* were the least effective as compared to others. Application of *P. fluorescence* effectively reduced stem blight disease incidence in *Phyllanthus amarus* caused by *C. cassicola* (Mathiyazhagam *et al.*, 2004). Philip *et al.* (2005) isolated endophytic bacteria, which showed systemic resistance on challenge inoculation with *C. cassiicola* under glasshouse condition.

5.6. EVALUATION OF FUNGICIDES, BOTANICALS AND BIOCONTROL AGENTS AGAINST FRUIT ROT OF PUMPKIN UNDER *IN VIVO* CONDITIONS

All the results obtained in the *in vitro* evaluation may not be in line with the field situations. Physiology of crop and micro and macro climate will influence the efficacy. Therefore evaluation of best two fungicides, botanicals and biocontrol agents were done under *in vivo* conditions against selected pathogen (*Choanephora cucurbitarum*) in selected crop (pumpkin, Arka Suryamukhi) based on per cent disease incidence.

In vitro evaluation of chemical fungicides on the inhibition of the pathogens provides preliminary information and serves as a guide for field testing (*in vivo* studies. During the *in vivo* studies on the management of *Choanephora cucurbitarum* fruit rot of pumpkin it was proved that the most effective fungicide against *C. cucurbitarum* was tebuconzole 5EC. It showed least per cent disease incidence (zero per cent) and cent per cent disease reduction over the control. George (2015) studied the effect of nine fungicides against *Choanephora cucurbitarum* in cowpea. Under *in vivo* condition, at recommended concentration, 100 per cent inhibition of growth was recorded by chemicals including mancozeb, carbendazim + mancozeb and propiconazole (triazole group). Chahal and Grover (1974) reported that mancozeb was very effective (80 per cent inhibition) in controlling *Choanephora cucurbitarum* on chilli. Hammouda (2008) reported that mancozeb was very effective in reducing infection against *Choanephora cucurbitarum* in the field condition in

166

southern region of Oman (Dhofar). These results are in accordance with the present studies, where, Mancozeb 64% + cymoxanil 8% (PDS was 6.66) was recorded as the second best treatment but statistically they were at par with tebuconazole. Third best treatment recorded were azadirachtin 0.1% and it showed PDI of 16.66, and garlic extract revealed almost similar reduction in PDS (20) and they were also significantly not different.

Restriction of the disease incidence by the treatment of *Bacillus subtilis* was very less ie., PDS was very high (36.66) as compared to other treatments. Data revealed that it was also on par with *Trichoderma viride* (30). 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. Choudhary (2015) reported that among biocontrol agents *Trichoderma harzianum* + *Pseudomonas fluorescens* treatment on seed-cum soil application was most effective against *Choanephora cucurbitarum* in cucumber.

Results of *in vivo* experiment can be summarized as follows. In the upcoming years, fruit rots may be the major threat to cucurbits cultivation in Kerala. Therefore an integrated disease management strategy must be followed in near future. For the management of *Choanephora cucurbitarum* fruit rot of pumpkin tebuconzole 5EC 0.1 per cent can be recommended as effective fungicide with a better yield eventhough it is a systemic fungicide. Mancozeb 64% + cymoxanil 8% also gave good results, therefore this chemical can also be recommended. Mancozeb 64% + cymoxanil 8% has both protective and curative action which offers not only disease control but also improves quality and yield of crop. The waiting periods of both the above chemicals are less than three weeks in different vegetables (DPPQS, 2012). Azadirachtin 0.1% can be suggested as an effective botanical for the management of *C. cucurbitarum*. Under *in vitro* conditions, garlic extract performed well, but *in vivo* conditions it came just next to the Azadirachtin 0.1%. Better performance of garlic extract under *in vivo* conditions may be due to the volatile chemicals present in it.

167

Application of *Trichoderma viride* as soil drench and spray is also an effective bio intensive method to manage the fruit rot in pumpkin, eventhough the effect of biocontrol agents is very less as compared to other treatments. *Trichoderma viride* and *Bacillus subtilis* are the better choices among them.

Regarding the biometric observations, there is no significant difference among the treatments in the number of leaves and branches compared to control, but plants treated with the biocontrol agents (*Bacillus subtilis* and *Trichoderma viride*) showed comparatively higher number of leaves than others. This may be due to the growth promoting effects of bacterial and fungal antagonists used. Podile and Dube (1988) and Yedidia *et al.* (2001) revealed the overall growth promoting activities of *Bacillus subtilis* and *Trichoderma viride* in cucurbits.

The present study revealed that field application of fungicides, botanicals and bio-control agents can be included in a biointensive management strategy, for the control of fungal fruit rots of cucurbits. 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 showed that field application of best combinations of plant products, bio-control agents and chemical fungicides can be effectively combined and a biointensive management strategy can be worked out for the control of fungal fruit rots of cucurbits. But use of eco-friendly techniques alone is not a complete remedy for the management of fruit rot diseases, integrated approach by optimizing the usage of pesticides and maximizing the usage of botanicals and biocontrol agents will surely pave the path for better, eco-friendly and sustainable management of the fungal fruit rots of cucurbits. In continuation of this project work, the future line of work should be concentrated on the quick and accurate diagnosis of the pathogen involved in fruit rot diseases of cucurbits and this will help in planning and implementing the control strategies with less chemical inputs, more effective and less costly for the grower and safer for the environment.

168

Summary

6. SUMMARY

Cucurbits are the most economically important vegetable crops. It belongs to the family Cucurbitaceae. The warm humid tropical climatic conditions of Kerala attract many fungal pathogens, especially in the intensively cultivated tracts. Moreover, detailed systematic studies for identification and characterization of fungal pathogens causing fungal fruit rots in cucurbits were not undertaken so far in Kerala. In this context, the present study is proposed to identify and characterize the fungal fruit rot diseases of cucurbits occurring in northern zone of Kerala and to study the biointensive management of five selected pathogens *in vitro* conditions and most severe and predominant disease under *in vivo* conditions.

1. A purposive sampling survey was conducted at various locations in three districts *viz.,* Kasargod, Kannur and Kozhikode of north Kerala, which was during the three seasons under open field and polyhouse conditions, which revealed the severe incidence of various fungal fruit rots in cucurbits.
    - Based on the distinct symptoms, Fruit rots were categorized into, fruit rot- 1, fruit rot- 2, fruit rot- 3, fruit rot- 4, fruit rot- 5, fruit rot- 6 and fruit rot- 7.
    - During the survey in Kasargod district, fruit rot- 7, fruit rot- 1, fruit rot- 4, fruit rot- 3 and fruit rot- 6 were observed on immature cucumber, pumpkin, ridgegourd, cucumber, snake gourd respectively with per cent disease incidence of 10 per cent, 51, 47 per cent , 22 per cent and 24 per cent respectively.
    - Survey was conducted in one location of Kozhikode district, where fruit rot- 5 was observed with per cent disease incidence of 6 per cent.
    - Fruit rot- 2 in pumpkin was obtained during the survey at Panoor in Kannur district with per cent disease incidence of 37 per cent.

2. Symptomatology studies were carried out both under natural and artificial conditions.

   - Fruit rot-1, under natural conditions, exhibited initial symptom as small water soaked lesions at the tip of the corolla of the female flower of pumpkin, then started fructification, which spread along the corolla and reached the blossom end of the fruit. Later this water soaked lesions with profused fructifications cover the entire fruit surface and finally fell down.
   - Fruit rot-2 showed small water soaked lesions on pumpkin fruits, which later produced rotting of the whole area along with the production of white mycelial mat and sclertial bodies.
   - Fruit rot-3 appeared as irregular water soaked lesions on the mature fruits of cucumber, subsequently it turned into sunken dark brown cankers and finally these cankers coalesced and entire fruits were rotted. When it dries some fruits formed cracks.
   - Fruit rot- 4 developed as dark green water soaked lesions with white mycelial growth on the surface of the mature or immature fruits of the ridge gourd. Later it changed to light brown lesions, these lesions with white mycelial cover started to enlarge and resulted in soft watery wet rot.
   - Fruit rot-5 produced watery soft rot with white puffy cottony mycelium on fruits of cucumber.
   - Fruit rot-6 started as water soaked sunken spots with black concentric rings on snake gourd. Later on further expansion, sporulation and complete rotting with internal discoloration were observed.
   - Fruit rot-7 was observed in immature salad cucumber in the polyhouse. Initially symptom started as yellowing of the tissue at the blossom end of the fruits. Yellowed portion gradually turned into brown, which spread to whole fruit and complete black dry rotting was observed.

171

3. Isolation of pathogens from diseased samples collected during the surveywas carried out. Seven isolates were obtained and allotted seven isolate codes *viz.,* FR-1, FR-2, FR-3, FR-4, FR-5, FR-6, and FR-7 for the isolates obtained from fruit rot- 1, fruit rot- 2, fruit rot- 3, fruit rot- 4, fruit rot- 5, fruit rot- 6 and fruit rot- 7, respectively.
4. Cultural and morphological characterization of pathogens was undertaken for genus level identification. Species level confirmation was done by molecular characterization, which was carried out at Rajive Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram.
5. FR-1 was identified as *Choanephora cucurbitarum,* FR-2 was identified as *Sclerotium rolfsii,* FR-3 was identified as *Rhizoctonia solani,* FR-4 was identified as *Phytophthora nicotianae*, FR-5 was identified as *Pythium deliense*, FR-6 was identified as *Corynespora cassiicola,* FR-7 was identified as *Fusarium equiseti.*
6. Pathogenicity was established by Mycelial Bit Inoculation Method (MBIM) on detached fruits.
7. *In vitro* evaluation of fungicides, botanicals and biocontrol agents against five selected fungal pathogens revealed that:
    - Against *Choanephora cucurbitarum* fruit rot, tebuconazole 5EC showed 100 per cent inhibition at all concentrations. Among the botanicals, garlic extract at medium and higher concentrations and azadirachtin 0.1% at lower concentration were the best. Among biocontrol agents, *Trichoderma viride* was the best.
    - Against *Sclerotium rolfsii* fruit rot, tebuconzole 5EC (Folicur) was significantly superior among all the four fungicides at lower concentration, which showed 100 per cent inhibition of the radial mycelial growth. At recommended and higher concentrations, tebuconzole 5EC and mancozeb 64% + cymoxanil 8% were superior and both of them showed cent per cent inhibition. Among botanicals, garlic extract (6.10 and 77.77) and azadirachtin 0.1% (55.55 and 72.21) (on par)

found to be most effective at the lower and recommended concentrations. At higher concentration, garlic extract (100%) was the best. Among biocontrol agents, *Bacillus subtilis* (83.32) and PGPM (77.77%) (on par) were the best treatments.

- Against *Rhizoctonia solani* fruit rot, all the chemical fungicides recorded cent per cent inhibition at all concentrations. Among the botanicals, garlic extract was the most effective treatment at all concentrations. *Trichoderma viride* (100 % inhibition) was the best biocontrol agets against *Rhizoctonia solani*.
- Against *Phytophthora nicotianae* fruit rot, all the fungicides at all concentrations showed cent per cent inhibition. Among the botanicals, garlic extract showed maximum inhibition (66.66%, 77.77%, 100%) at all concentrations. Among the biocontrol agents, *Trichoderma viridae* and PGPM (on par) were the best treatments, which showed cent per cent inhibition.
- Against *Corynespora cassiicola* fruit rot, mancozeb 64% + cymoxanil 8% were superior (100% inhibition) at the lower concentration. At recommended concentration mancozeb 64% + cymoxanil 8% and mancozeb 64% + carbendazim 12% (on par) showed cent per cent inhibition. At higher concentration tebuconzole 5EC, mancozeb 64% + cymoxanil 8% and mancozeb 63% + carbendazim 12% showed 100 pe cent inhibition and they were significantly not different. Among the botanicals, garlic extract was the most effective (66.66%, 77.77% and 100%) at all concentrations. The best biocontrol agent against *Corynespora cassiicola* was PGPM, which showed cent per cent inhibition.

8. *In vivo* evaluation of the best two fungicides, botanicals and biocontrol agents, which showed promising result under *in vitro* evaluation, was carried out against *Choanephora cucurbitarum* causing fruit rot in pumpkin.

    - Among the two chemical fungicide selected, tebuconzole 5EC showed least per cent disease incidence (zero per cent) and cent per cent disease reduction over control ie., it is the best treatment.
    - Among the botanicals best treatment recorded were Azadirachtin 0.1%, which showed 16.66% per cent disease incidence in the field condition.
    - Among the biocontrol agents *Trichoderma viride* was the most effective.
    - Statistically there was no significant difference within the treatments of chemicals, botanicals and biocontrol agents, but there was significant difference in between the chemicals, botanicals and biocontrol agents.
    - As a whole, efficacy order can be concluded as chemicals, botanicals and biocontrol agents, respectively.
    - Yiel parameters (number of fruits/plant and weight of the fruits/plant) evaluation also showed similar trend.
    - Growth parameters (Number of leaves and branches/plant) revealed significantly higher values for the biocontrol agents *viz., T. viride* and *B.subtilis,* which may be due to the growth promotion effect of *T. viride* and *B.subtilis*.

174585

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193

Appendices

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 up to 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 Biointensive Management of Fungal Fruit Rots of Cucurbits

by

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ABSTRACT OF THE THESIS

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ABSTRACT

Characterization and Biointensive Management of Fungal Fruit Rots of Cucurbits

Cucurbits are the most popular and widely cultivated vegetables in Kerala. One of the main constraints in the production of these crops is the occurrence of fungal fruit rots, on which no detailed and systematic studies have been conducted in Kerala. The study was carried out during 2017-2019 at College of Agriculture, Padannakkad with the objective to identify and characterize the fungal pathogens causing fruit rots in cucurbits, occurring in the northern zone of Kerala and to study the management of selected fruit rot diseases under *in vitro* and most severe and predominant disease *in vivo* conditions. Purposive sampling surveys were conducted for the occurrence of fungal fruit rots in cucurbits in Kasargod, Kannur and Kozhikkode districts. Diseased plant samples were collected. Results of survey showed prevalence of seven different fungal diseases with a range of 6- 51 per cent disease incidence.

Isolations done from the infected fruit samples collected during the survey yielded seven genera of fungal pathogens. Characterization 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 characterization by sequencing the ITS region of each fungus by *in silico* analysis and confirmed as *Choanephora cucurbitarum, Sclerotium rolfsii, Rhizoctonia solani, Phytophthora nicotianae, Pythium deliense, Corynespora cassiicola* and *Fusarium equiseti*. Symptomatologies of these fungal diseases were studied in detail both under natural and artificial conditions. *In vitro* evaluation of fungicides, botanicals and biocontrol agents was done against the selected five pathogens. Four fungicides and three botanicals at three concentrations and five biocontrol agents were selected for the studies.

198

In vitro studies showed that, against *Choanephora cucurbitarum* fruit rot, tebuconazole 5EC showed 100 per cent inhibition at all the three concentrations. Among botanicals, garlic extract at medium and higher concentrations and azadirachtin 0.1% at lower concentration were the best. Among biocontrol agents, *Trichoderma viride* was the best.

Against *Sclerotium rolfsii* fruit rot, tebuconzole 5EC (Folicur), were significantly superior than all other fungicides at lower concentration, which showed 100 per cent inhibition. The fungicides tebuconzole 5EC and mancozeb 64% + cymoxanil 8% were superior at recommended and higher concentration and both of them showed cent per cent inhibition. Among botanicals, garlic extract and azadirachtin 0.1% were the most effective at the lower and recommended concentrations. At higher concentration garlic extract was the best. Among biocontrol agents, *Bacillus subtilis* and PGPM were the most effective treatments and they were at par with each other.

Against *Rhizoctonia solani* fruit rot, all the chemical fugicides recorded cent per cent inhibition at all concentrations. Garlic extract was the best at all concentrations. The best biocontrol agets against *Rhizoctonia solani* was *Trichoderma viride.*

Against *Phytophthora nicotianae* fruit rot, all the fungicides at all concentrations showed cent per cent inhibition. The data on the botanicals revealed that, garlic extract shows maximum inhibition (66.66%, 77.77%, 100%) at all concentrations. Among biocontrol agents, *Trichoderma viridae* and PGPM were the best treatments and they showed cent per cent inhibition.

Against *Corynespora cassiicola* fruit rot, mancozeb 64% + cymoxanil 8% were superior (100% inhibition) at the lower concentration. At recommended concentrations, mancozeb 64% + cymoxanil 8% and mancozeb 64% + carbendazim 12% showed cent per cent inhibition. At higher concentrations, tebuconzole 5EC, mancozeb 64% + cymoxanil 8% and mancozeb 63% + carbendazim 12% were showed 100 per cent inhibition and they were significantly not different. Among the

199

botanicals garlic extract was the most effective at all concentrations. The best biocontrol agent against *Corynespora cassiicola* was PGPM, which showed cent per cent inhibition.

In vivo evaluation of management of *Choanephora cucurbitarum* fruit rot in pumpkin emphasized that tebuconazole 5 EC and mancozeb 64%+cymoxanil 8% are the best treatments. Botanicals viz., azadirachtin 0.1 % and garlic extract came as next best treatments and biocontrol agents, *T. viride* and *B.subtilis* were the least effective treatments. These can be recommended as effective antipathogenic agents for field application for the management of the fungal fruit rot disease in pumpkin. The present work resulted in a detailed and systematic study on the fungal fruit rots of cucurbits in northern zone of Kerala. Future line work should be concentrated on the field level studies of all the fruit rots of cucurbits in different districts of Kerala and on the recommendations of local specific management practices.

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