Choanephora Pod Rot of Cowpea and its Ecofriendly Management

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by

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2015

DECLARATION

I hereby declare that this thesis entitled '**Choanephora Pod Rot of Cowpea and its Ecofriendly Management**' is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title of any University or Society.

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CERTIFICATE

Certified that this thesis entitled "**Choanephora Pod Rot of Cowpea and its Ecofriendly Management**" is a record of research work done independently by Ms.Milsha George (2013-11-148) under my guidance and supervision that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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Milsha George

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LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Per cent
@	At the rate of
°C	Degree Celsius
CD	Critical difference
cfu	Colony forming units
et al.	And other co-workers
h	Hours
sec	Seconds
min	Minutes
i.e.	that is
ha	Hectares
t/ha	Tonnes per hectare
l	Litre
ml	Millilitre
µl	Microlitre
cm	Centimeter
mm	Millimeter
µm	Micrometer
kg	Kilogram
g	Gram
mg	Milligram
µg	Microgram
ng	Nanogram

M	Molar
mM	Millimolar
rpm	Rotations per minute
sp. or spp.	Species (Singular and plural)
viz.	Namely
bp	Base pairs
kb	Kilobases
PCR	Polymerase Chain Reaction
ITS	Internal Transcribed Spacer
rDNA	Ribosomal DNA
ppm	Parts per million

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Introduction

1. INTRODUCTION

Cowpea (*Vigna unguiculata* L. *Walp.*), is an important leguminous vegetable crop in Kerala. It is a good source of protein, vitamins and minerals. The cultivation of this remunerative and nutritious crop is hampered by the incidence of several diseases. Recently, a wet pod rot disease has been noticed around the time of harvest, especially in the rainy season. The disease has been found to be more serious when the crop is raised for seed purpose. Wet rot or pod rot of cowpea caused by *Choanephora cucurbitarum* (Berk. & Ravenel) Thaxter had been reported to affect the yield significantly (Bashir *et al.,* 1985). The crop loss due to the disease was estimated to be 7% - 20% (Oladiran, 1980). The disease was found to be aggravated during the period of high temperature and humidity (Hussein and Ziedan, 2013).

C. cucurbitarum is a facultative saprophytic fungus belonging to sub division Zygomycotina, with wide host range encompassing families such as Amaranthaceae, Cucurbitaceae, Malvaceae, Solanaceae and Nyctaginaceae (Abel – Mortal *et al.,* 2010). The pathogen under favourable conditions is capable of reproducing sexually by zygospore and asexually by sporangia and conidia.

The pathogen has been reported to be managed by spraying fungicides such as mancozeb (Chahal and Grover, 1974) after pod set. Since the disease is noticed on the edible pods, the dependence on fungicides alone for management poses threat to human health and also enhances the risk of environmental pollution. These concerns along with the cost involved necessitate the disease to be managed by cheaper and ecofriendly strategies. Inadequacy of effective and economic crop protection strategies is one of the key factors limiting the expansion of organic farming in vegetables. Spraying non- sterilized actively aerated compost tea made from rice straw and empty fruit bunch of oil palm composts inhibited conidial germination of *C. cucurbitarum,* causal agent of wet rot of okra (Siddiqui *et al.,* 2009). Use of indigenous organic preparations such as panchagavya, jeevamruth, fish amino acid, vermiwash and biocontrol agents have also shown promise in

suppression of pest and disease incidence in several agricultural and horticultural crops.

Though noticed earlier, the cowpea pod rot by *C. cucurbitarum* is reported to be wide spread in recent times in Kerala. No systematic study has been undertaken for documenting the disease, its etiology and management. In this view, the present investigation "Choanephora pod rot of cowpea and its ecofriendly management" was taken up to study the symtomatology, etiology and to develop ecofriendly management tactics for pod rot of cowpea. The study was undertaken on the following lines:

- Symtomatology of pod rot of cowpea.
- Pathogenicity studies.
- Identification and characterization of the pathogen.
- Host range studies of the pathogen.
- Isolation and testing of antagonistic microorganisms.
- Evaluation of disease suppression using indigenous organic preparations.
- Studies on *in vitro* suppression of Choanephora pod rot of cowpea.
- Studies on *in vivo* suppression of Choanephora pod rot of cowpea.

Review of Literature

2. REVIEW OF LITERATURE

Cowpea is an important and widely grown leguminous crop in Kerala. An array of diseases affects the crop at various stages of its growth. Recently, Choanephora pod rot disease, seen as water soaking and decay of fruits, has been rampant on mature pods, especially, during the rainy season. Literature related to importance, yield loss, symptomatology, etiology and characterization of pathogen as well as ecofriendly management of pod rot of cowpea has been reviewed and detailed here. Since literature on these aspects pertaining to this disease on cowpea or other vegetable crops is scanty, similar works carried out with other fungal diseases of cowpea as well as other related crops have also being reviewed here to supplement the information.

2.1. IMPORTANCE, DISEASE SEVERITY AND YIELD LOSS

The cowpea pod rot has been reported to be wide spread in recent times in the cowpea growing tracts of Kerala and was found to be more serious when the crop is raised for seed purpose. The disease has not been accounted to be the cause of appreciable damage to crops annually and has been found to appear in epidemic form only under warm humid conditions.

Reports from Pakistan by Bashir *et al.* (1985) indicated that the disease severity due to *C. cucurbitarum* on cowpea ranged from 5 to 12%. Kwon *et al.* (2001) gave a detailed account on Choanephora pod rot of cowpea in Korea and reported that the infection rate ranged from 8.4 to 14.3%. Similarly, the reports from Korea by Kwon and Jee (2005) indicated that on eggplant the infection rate ranged from 2.5 to 3.0%.

Wilson and Jose (1965) estimated the yield loss to be 20% in cowpea. Losses due to Choanephora pod rot of cowpea from Southern Nigeria had been reported by Oladiran (1980) and the yield loss was estimated to be 7.5% to 20%. Adebanjo (2008) from Nigeria reported that the yield of *Amaranthus* spp. was reduced to half due to a devastating inflorescence disease associated with *C. cucurbitarum*. Wet rot caused by *C. cucurbitarum* in bhindi was reported to

result in yield loss of 5.0 - 50 per cent in tropical and subtropical countries (Rai *et al*., 2014).

2.2. ETIOLOGY

C. cucurbitarum is a facultative saprophyte that belongs to the sub division: Zygomycotina, order: Mucorales and representative of the family Choanephoraceae. Choanephora rot had been first reported in United States, by Berkeley (1875) as *Rhopalomyces cucurbitarum* from decaying squashes. Thaxter (1903) further identified the pathogen causing decay of squashes as *Aspergillus cucurbiteus.* The pathogen was observed by Moller (1903) on the petals of hibiscus and other malvaceous plants and described it as *Choanephora americana.* Wolf (1916) detailed the morphology of pathogen associated with the decay of squash and identified it as *Choanephora cucurbitarum.* He also found that the fungus also infected several plants belonging to Malvaceae such as*,* althea (*Hibiscus syriacus*)*,* scarlet hibiscus (*Hibiscus coccineus*)*,* okra (*Hibiscus esculentus*)*,* and cotton (*Gossypium herbaceum*) as well as the fading flowers of *Cucumis sativus*.

Two other species had been described from India, one *C. infundibulifera* (Curr.) *Cunn.* on the flowers of *Hibiscus rosa - sinensis* by Currey (1873) and the other, *C. simsonii* Cunn. on *Ipomoea rubro - coerulea* and *Zinnia elegans* by Cunningham (1879). Wilson and Jose (1965) established the pathogenicity of *C. cucurbitarum* with the pod rot of cowpea in Kerala. Singh and Allen (1979) reported that 'lamb's tail' disease of cowpea was caused by two species *C. cucurbitarum* and *C. infundibulifera.*

C. cucurbitarum is air borne, seed borne as well as soil borne and is more aggressive under humid conditions and thrives best at a temperature of 25°C and relative humidity of about 100%. A temperature of about 31°C has been found to stimulate the production of large sporangia but found to be unfavourable for conidia formation (Umana and Ikotun, 2000). For conidial formation a temperature of 25°C has been found to be optimum (Webster and Webster, 2007).

Kwon and Park (2005) reported that *Choanephora* spp. could be considered as weak pathogens that predominantly penetrated through wounds to cause infection. *Choanephora* spp. was reported to enter through the feeding damage and oviposition puncture made by the insects cowpea curculio (*Chalcoderis aevious*) in cowpea (Cutherbert and Ferry, 1975) or *Maruca testulasis* in beans (Irvine, 1957 and Karel and Matary, 1983).

2.3. SYMPTOMATOLOGY

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), millet, rice, sorghum and amaranthus *etc.* and further invade the fruits causing wet rots. The general appearance of Choanephora blight was similar to that of diseases caused by other Mucoralean fungi *viz., Mucor* and *Rhizopus*.

The symptoms caused by *C. cucurbitarum* on cowpea pods were described in detail by Wilson and Jose (1965) as water soaked lesions on the pods, which further developed into a 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. Similar symptoms had been reported earlier from India (Singh and Allen, 1979), Sri Lanka (Kangatharalingam, 1979), South Nigeria (Oladiran, 1980) and Korea (Kwon *et al*., 2001).

Wilson and Jose (1965) reported that under high humid conditions the infections were also noticed in flowers, flower stalks and stems. Turkensteen (1979) gave a detailed account on the expression of symptom by *C. cucurbitarum* on leaves and stem. The symptom on leaves has been reported to start at the leaf margins as water soaked lesions which later dried up and turned olive green to light brown. During dry conditions, sporangiophores developed, on which black pin headed sporangia containing sporangiospores were visible. On the petioles and stem, the initial symptoms appeared as small, light brown, elongated spots

which increased in size and coalesced to cause extensive rotting. From the affected petioles, the fungus entered into leaflets and stem where the affected tissues turned dark brown followed by shrinkage and disintegration causing the toppling down of the affected petioles and stem. In more advanced stages, the plants became completely necrotic (Gunasekara and Liyanage, 1985).

The fungus has wide host range encompassing chilli, pepper, bhindi, brinjal, amaranthus, millets, cereals *etc.* The symptoms caused by *C. cucurbitarum* varied considerably among different crops (Wolf, 1916).

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. The disease was severe under high temperature and humid conditions that favoured the disease development. It was often observed in the fields that the fruit surface was covered by the fungal hyphae and abundant sporangia and sporangiospores. The symptoms were similar to the soft rot caused by *Rhizopus* spp. or *Mucor* spp.

Adebanjo (2008) described the symptoms caused by *C. cucurbitarum* on amaranthus as extensive blighting of the inflorescence head which was accompanied by inflorescence dieback and breaking of the floral head. In case of severe infection the inflorescence heads were completely cut off and the plants did not produce any seeds, thereby causing severe reduction on yield. He also observed that young green inflorescence were more susceptible to infection.

According to Jana *et al.* (2011), Choanephora blight affected the chillies at the active growing stages. Water soaked lesion appeared at the point of each individual branch above which ultimate branches were produced. The lesions encircled the branch causing the death of the young branch above. The pathogen

also infected the fruits and caused rotting and death. Prominent hairy growth of the pathogen was seen in the affected parts of the plant.

Hussein and Ziedan (2013) reported the symptoms of Choanephora pod blight on bhindi. On newly opened blooms splitting and collapse were recorded. Fruits became infected and covered with dense white mycelium bearing black pin headed sporangia at its tip, which eventually resulted in early detachment and failure of the affected pods to develop further. The affected parts were reported to show soft rotting. Pods invaded by *C. cucurbitarum* were stunted as compared to the healthy pods. The fungus causing wet/bud rot in bhindi has been reported to enter into the fruits from blossom end and developed feathery mass of black spores (Rai *et al*., 2014). Further, wet rot on leaves and stem were also noticed. On the affected parts, silky hair like structures was also observed. In severe cases, heavy defoliation was also reported.

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). In the final stages, the fruits were reported to look like a pin cushion with numerous small, black headed pins tucked in it. Initially, the heads were white to brown but turned purplish black within a few days. The affected flowers, pedicels, and immature fruits became water soaked and a soft, wet rot developed. The rotting was completed in 24 - 48 h. Symptoms were usually noticed to begin from the blossom end of the fruit.

There are also reports of the incidence of *C. cucurbitarum* on medicinal plants. The symptoms of twig blight caused by *C. cucurbitarum* on *Boerhaavia diffusa* (Punarnava) was described in detail by Singh *et al*. (2011). The disease began as foliar infections which then expanded and killed tissues of twigs. The fungus invaded young twig tissue as a result of the terminals and branches distal to the point of infection became light green and finally reddish brown. The small branches were found girdled by the disease. Leaves in between the infected twigs were also infected, giving a blighted appearance. Severe infection on the main

shoot led to the death of the plants. The main roots of dead plants were completely devoid of secondary roots. The disease caused significant reduction in weight and volume of roots which have medicinal value. Saroj *et al.* (2012) noticed symptoms of water soaking and rotting due to this pathogen in *Withania somnifera* (Ashwagandha), a medicinal herb native to India and commercially cultivated for the production of root withanolides that have anticarcinogenic properties. Symptoms first appeared as water soaked lesions on leaves and stems that progressed to a wet rot.

According to Park and Jo (2014), the initial symptoms on *Hibiscus rosa - sinensis* appeared as reddish purple spots at the tip of flowers and expanded to encompass the entire flowers. Infected lesions appeared to be water soaked and reddish brown, which was followed by rapid rotting of infected tissues. In the advanced stage, white luxuriant growth of mycelia bearing black headed sporangia at the tip was observed.

2.4. IDENTIFICATION OF THE PATHOGEN

2.4.1. Cultural and Morphological Identification

Abel - Mortal *et al.* (2010) reported that *C. cucurbitarum* cultured in petri plates containing PDA when incubated at 25°C, the fungal mycelia covered the medium within three days. Initially, the fungal colony was white but on ageing, it became yellowish brown in colour.

Wilson and Jose (1965) gave a detailed description of sporangia and conidia produced by the pathogen in culture. The sporangium was terminal and pendant on the curved end of an erect sporangiophore. Sporangia measured 38.5 – 152.0 μ in diameter. Sporangiospores were light brown, ovoid, and smooth, and provided with tufts of hair like appendages at both ends that measured 14.5 – 23.5 μ in size. The conidiophores were erect, ending in capitate vesicles from which short stalked secondary vesicles were produced on which, short sterigmata bearing conidia were produced profusely. Conidia were light red to reddish brown, ovoid

in shape with hyaline appendages at the ends. These measured 13.5- 20.5 µ × 8.0 -12.5 µ in size.

Kagiwada *et al.* (2010) reported that the monosporous sporangiophores were aseptate, smooth-walled, and hyaline, arising from the medium and bearing monosporous sporangiola at end. The monosporous sporangiola were brownish black, oval, with striations, 8–13 × 9.12–20 µm in size, and readily detached from the sporangiophore. Sporangia possessed columellae and were brownish - black, sub-globose, splitting into two pieces, and 31–69 µm in diameter. Sporangiophores were aseptate, smooth-walled, hyaline, non - branching and bearing sporangia. Sporangiospores were oval, with striations visible at high magnification, smooth walled, hyaline, and 7–12 × 9–26 µm in size, with 10 or more appendages at both ends. The zygospores formed at or below the surface of the medium, were dark reddish-brown to almost black, smooth, sub-globose, and 42–65 µm in diameter, with a large, central oil droplet. The suspensors were tong - shaped, arising from twisted and distorted hyphae, and nearly equal in size. Similar reports on the morphology of *C. cucurbitarum* on cowpea were given by Kirk (1964) and Kwon *et al.* (2001), from petunia by Kwon *et al.* (2001), from eggplant by Kwon and Jee (2005), from shoe flower by Kwon and Park (2005) and from *B. diffusa* by Singh *et al.* (2011).

2.4.2. ITS – Sequence Based Molecular Identification of *Choanephora* spp.

The ribosomal RNA genes (rDNA) possessed characteristics that were suitable for the detection of pathogens at the species level. The rDNA repeat unit contains genic and nongenic or spacer regions. Each repeat unit consisted of a copy of 18S, 5.8S and 28S like rDNA and two spacers, the internal transcribed spacer (ITS) and intergenic spacer (IGS) (O'Donnell, 1992). The rDNA genes had been employed to analyze major evolutionary events because it highly is conserved, whereas, the rDNA internal transcribed spacer (ITS 1 and ITS 2) is more variable so that it had been used for the investigation of the species level

relationship (Bruns *et al.,* 1991) and had been used in classifying fungal species due to its systemic and taxonomic usefulness.

Kagiwada *et al.* (2010) reported that the ITS – sequence based molecular identification of the isolate obtained from ice plant showing the signs and symptoms of Choanephora rot when compared with the rDNA ITS region sequences of *C. curcurbitarum, C. infundibulifera* and *Blakeslea trispora* showed 100% sequence identity with *C. cucurbitarum*. Abel - Mortal *et al.* (2010) conducted the sequence analysis of the isolate causing the rot of floral tops of *Hyoscyamus muticus* and observed that the rDNA of the isolate had maximum similarity (96%) with those of Choanephoraceae species (EF060399), while the 28S rDNA of the isolate had maximum similarity (95%) with that of *C. cucurbitarum.* Sequence analysis of 28 S rDNA was found to be in consonance with the morphological identification of the isolate.

2.5. *IN VITRO* MANAGEMENT OF THE DISEASE

2.5.1. Biological Control

2.5.1.1. Biocontrol Agents

Biological control is a safe method to reduce incidence or severity of plant diseases incidence without collateral damages to the environment as well as to human health induced by application of fungicides (Tucci *et al*., 2011). Antagonistic fungi belonging to the genus *Trichoderma* as well as several bacteria like *Pseudomonas fluorescens*, are used as biocontrol agents (BCAs) to suppress plant pathogens through a series of mechanisms including competition for nutrients and space, fungistasis, antibiosis and modification of the rhizosphere (Benı´tez *et al*., 2004).

Zhou *et al.,* 1999 observed that commercially ripe peaches when wounded and co-inoculated with phylloplane isolates of *Pseudomonas syringae* (MA-4 and NSA-6) or *P. fluorescens* (BAP-3) at a concentration of 1 x 10^7 cfu/ml in combination with sporangiospores of *Rhizopus stolonifer* , reduced Rhizopus rot

to 5% and 8%, respectively from 53% in the inoculated check after 5 days incubation.

Salman and Ahmad (2005) reported that *Trichoderma harzianum* formulated as invert emulsion (water - in- oil formulation) was effective in reducing the lesion size of soft rot caused by *Rhizopus* spp. in apple, pear and peach.

Siddiqui *et al.* (2008) reported that suspension of *T. harzianum* suppressed the wet rot of okra/bhindi caused by *C. cucurbitarum* up to 47% and rice straw fortified with *Trichoderma* could reduce the disease to 89.75%. The mode of inhibition in mycelial growth of *C. cucurbitarum* was through lytic activity and competition. Hyphal coiling around the pathogen or attachment at the point of contact with hook like structures by the mycoparasite was also observed. After these interactions, the mycoparasite penetrated the host mycelium through partial degradation of the cell wall of the pathogen. The radial mycelial growth of *C. cucurbitarum* was restricted within the contact area or interaction zone, resulting in lysis and disintegration of the cell wall of the pathogen.

Motesharrei and Salimi (2014) reported that *T. harzianum* obtained from soils showed different mechanisms such as mycoparasitism, production of volatile compounds and secretion of secondary metabolites that prevented the growth of plant pathogens partly. Anil *et al.* (2015) observed that the isolates of *Trichoderma* were able to release inorganic phosphorus from tri calcium phosphate and showed consistent ability to produce siderophores and indole-3-acetic acid (IAA). They also opined that the cell wall degrading enzymes produced during the interaction were responsible for the antagonistic activity of the *Trichoderma* towards *Sclerotium rolfsii* and *Rhizoctonia solani.* While working with the antagonistic potential of mycoflora of composts, Arathy (2014) found that *Trichoderma virens* caused significant suppression of cowpea collar rot and web blight caused by *R. solani.*

Antagonistic bacteria are useful in controlling many fungal and bacterial diseases caused by phytopathogens through the production of enzymes, toxins and inhibitors. Among the biocontrol bacteria, *P. fluorescens* gained importance over years because of its inherent beneficial properties for crop growth promotion and disease suppression through an array of mechanisms (Jubina, 1997).

Kar *et al.* (2014) reported that *P. fluorescens* inhibited the growth of some of the seed borne fungi of groundnut such as *Aspergillus niger, Aspergillus flavus* and *Fusarium* spp. to 42.2%, 13.3% and 45.7% respectively.

Pandey and Chandel (2014) tested the antagonistic effect of *P. fluorescens* against *Pyricularia oryzae, Fusarium oxysporum*, *A. niger*, *A. flavus*, *Alternaria alternata* and *Erysiphe cruciferarum*. They reported that the maximum inhibition in colony diameter was observed in *P. oryzae* (89%) followed by *A. niger* (80%), *A. alternata* (77%), *F. oxysporum* (76%) and *A. flavus* (71%) and *E. cruciferarum* (64%) *in vitro.* The mechanism of antagonism was reported as competition, parasitism, antibiosis, and induction of host resistance.

Abou - Aly *et al.* (2015) observed that *P. fluorescens* isolated from the rhizosphere of the plants could be effectively used as a biocontrol agents against some soil borne fungi such as *F. oxysporum*, *R. solani* and *S. rolfsii*.

2.5.1.2. Organic Preparations

The utilization of organic amendments for controlling soil-borne 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. Vedic literature has outlined systematized agricultural practices insisting the use of panchagavya and kunapajala to enhance disease management of crops (Nene *et al.,* 2012).

Sugha (2005) reported that the indigenous organic preparation, panchagavya, prepared from five products of cow showed antifungal potential against *R. solani, S. rolfsii, F. solani*, *S. sclerotiorum* and *Phytphthora colocasiae.*

Panchagavya resulted in 40-100 per cent inhibition of mycelial growth of all tested pathogen.

Sebastian and Lourduraj (2007) reported that panchagavya enhanced the biological efficiency of crop plants and the quality of fruits and vegetables and also had the properties of both fertilizer and bio - pesticide resulting in increase in the economic yield of crops such as rice, green gram, sunflower, turmeric, moringa, and coleus. Sumangala and Patil (2009) reported that the use of panchagavya resulted in 86.30 per cent inhibition of mycelial growth and 95.90 per cent inhibition of spore germination of *Curvularia lunata in vitro*. They also reported that seed treatment with panchagavya further enhanced the seed germination by 90.70% and gave a seedling vigor index of 1036.36.

Among the samples from panchagavya, the 1000 µl dilution alone showed 100% antifungal activity while 500 µl and 100 µl showed moderate antifungal activity, but at lower dilutions (10 µl) no antifungal activity was observed (Joseph and Sankarganesh, 2011). Adhao (2013) reported that panchagavya at 4% suppressed the growth of soil borne pathogen, *F. oxysporum* under *in vitro* conditions. Anees (2014) reported that panchagavya at 2.5%, 5% and 10% and fish amino acid at 10% completely inhibited the mycelial growth of *Pythium aphanidermatum,* the casual agent of stem rot of cowpea.

Application of vermicompost extract on tomato increased plant germination, growth and effectively suppressed a range of plant diseases. The beneficial response was due to plant growth regulators or hormones produced by high microbial activity in vermicompost (Edward *et al.,* 2006). Kamble *et al.* (2009) opined that vermiwash (30%) was effective in inhibiting the growth and sporulation of *A. solani in vitro.*

2.5.1.3. Compost Tea

Compost tea had been increasingly used as an alternative plant disease control measure in organic agriculture. Brinton *et al.* (1996) reported that

principal active agents in compost tea mycoflora are fungi belonging to the genera *Penicillium* and *Trichoderma* that contributed to disease suppression.

Dianez *et al.* (2007) reported that compost tea suppressed diseases by promoting the proliferation of beneficial microbes, which then exerted biological control of pathogens. Siddiqui *et al.* (2009) reported that the use of non sterilized actively aerated compost tea made from rice straw and empty fruit bunch of oil palm completely inhibited the mycelial growth of *C. cucurbitarum in vitro.* They also observed that compost tea application caused alterations in mycelial morphology and lysis leading to inhibition of mycelial growth. Souleymane *et al.* (2010) reported that compost tea significantly inhibited mycelial growth of *Botrytis cinerea* by 57-75 % and *Phytophthora infestans* causing foliar diseases in tomato by 100%, as compared to the control under *in vitro*. Haruna *et al.* (2011) reported the *in vitro* efficacy of poultry manure based compost extract, cowdung based compost extract and neem leaf based compost extract on *F. oxysporum f.sp. lycopersici,* the casual agent of tomato wilt disease. They observed that neem leaf based compost extract inhibited the radial mycelial growth of the fungus by 46.5 %.

2.5.2. Fungicides

Fungicides are often a vital part of disease management as they control many diseases satisfactorily. Contact fungicides are prophylactic in nature, present on the plant as a protective barrier before the pathogen arrives or begins to develop so that it prevents infection, whereas systemic ones are absorbed into plant tissue and cure the disease after infection has established.

The literature citing the effectiveness of fungicides in controlling the growth of zygomycetous fungi are scanty but, El-Helaly *et al.* (1968) observed that complete inhibition of the growth of *C. cucurbitarum*, the incitant of blossom end rot of vegetable marrow in Egypt, under *in vitro* conditions by incorporation of Cupravit, Dithane M-22, Miltox in the medium, followed by Karathane, Dithane M - 45, Cuprosan, Dithane Z - 78 and Orthocide 50 while Thiovit was

ineffective. Meah and Mian (1981) found that incorporation of Vitavax [carboxin] 200 at 5000 ppm into growth medium completely inhibited growth of *C. cucurbitarum* pathogenic on chilli, while Brassicol [quintozene] at 1000 ppm caused suppression. Gunasekera *et al*. (1989) while testing the efficacy of eight systemic fungicides for *in vitro* suppression of *C. cucurbitarum*, the incitant of flower blight of winged bean , observed that triadimenol, vinclozolin and bitertanol were the most effective ones in inhibiting spore germination and mycelial growth. Hammouda (2008) reported that mancozeb, dinocap and thiabendazole at the rate of 3g/kg of seed were effective in reducing Choanephora fruit rot.

Laboratory evaluation of eleven fungicides against *Collectotrichum capsici,* the incitant of brown blotch of cowpea (*Vigna unguiculata* (L.) Walp) revealed that Benlate, Delsene M, Aldrex T and Fernasan D prevented growth of the fungus at all concentrations tested while only high doses of Macuprax and Panoctine inhibited the growth of the fungus (Alabi *et al.,* 1986). Bhuiyan and Fakir (1993) reported that the fungicides Granosan M, Captan, Homai 80 per cent w.p., Topsin M and Vitavax-200, tested under *in vitro* conditions, significantly reduced the seed-borne infection of *Collectotrichum dematium* var. *truncate* on soyabean and increased germination compared to that of the untreated control. The strobilurin fungicide, azoxystrobin was found to significantly reduce mycelial growth, spore production of *Rhizopus oryzae.* The germination of spores was completely inhibited within the first 48 h after inocultation of *R. oryzae,* the incitant of post harvest rot of flue-cured tobacco under *in vitro* conditions (Kortekamp, 2007). Shovan *et al.* (2008) evaluated the efficacy of Tilt-250 EC, Vitavax-200, Rovral 50 WP, Dithane M-45 and Cupravit at 100, 200 and 400 ppm against *C. dematium* and concluded that Tilt-250 EC at all the concentrations completely inhibited the colony growth of the fungus. Vitavax-200 inhibited the mycelial growth upto 77.41% whereas the other fungicides were ineffective in controlling the mycelial growth of the pathogen.

Shugha *et al.* (1995) evaluated the efficacy of twelve fungicides against *F. oxysporum* f. sp. *ciceri* causing wilt of chickpea, and found that carbendazim and thiram alone, or, in combination were effective in inhibiting the mycelial growth. Carbendazim, copper oxy chloride and mancozeb when used at 500, 1000, 1500 and 2000 ppm resulted in 100% inhibition of the growth of pathogen *F. oxysporum* f.sp. *pisi* under *in vitro* conditions (Verma and Dohroo, 2002). The chickpea seed treated with thiram (0.15%) and captan (0.1%) effectively inhibited the growth *F. oxysporum* f.sp. *ciceris* (90%) under *in vitro* conditions (Nikam *et al.,* 2007).

Thaware *et al.* (2011) observed that under *in vitro* conditions, mancozeb (0.2 per cent) and propiconazole (0.05 per cent) completely inhibited the growth of the test fungus *A. alternata* causing leaf blight of cowpea (*Vigna unguiculata*). Yadav and Anandi (2013) reported that the combination of carbendazim 50 WP + mancozeb 70 WP could completely control the growth of *F. oxysporum* f.sp. *ciceri,* pathogen causing fusarial wilt of chickpea under laboratory conditions.

2.6. *IN VIVO* MANAGEMENT STUDIES

2.6.1. Studies on Biological Control

The environmental issues and health concerns arising out of irrational use of fungicides have prompted researchers to focus their effort in developing alternative strategies for plant disease management with greater emphasis on organic disease control. Biological control involving the use of antagonistic microorganisms has gained momentum in recent years. *Trichoderma* spp., a soil – borne fungi, has been demonstrated as an effective antagonist to several plant pathogenic fungi and is widely exploited as biocontrol agents against many plant diseases.

Ahamed *et al.* (1999) observed that there was a reduction in the pathogen population of *P. capsici* and root rot to the extent of 24-76% when plants were grown in substrate inoculated with *T. harzianum*. Similarly, observed that application of *T.viride* was found to improve the emergence and growth of

seedlings as well as resulted in reduction in disease severity (Abu-Taleb and Al-Mousa, 2008).

Akrami *et al.* (2011) reported that *Trichoderma* spp. and its secondary metabolites had showed the potential to control plant pathogenic fungi causing various diseases such as Fusarium wilt of tomato (Cotxarrera *et al.,* 2002), Rhizoctonia disease in *Brassica rapa* (Ibrahim, 2005), Sclerotium foot rot of chilli (Jinantana and Sariah, 1998), damping off disease of *Pythium* spp. (Martin and Loper, 1999) and *S. sclerotiorum* (Singh, 1991).

Pant (2011) reported that the antagonistic fungi *T. viride* obtained from rhizosphere showed antagonistic potential against *F. oxysporum* f.sp. *lycopersici* causing wilt of tomato. He also reported that *T. viride* not only reduced the disease incidence but also improved the seed germination and plant health.

Andrabi *et al.* (2011) reported that seed coating with *T. viride* resulted in minimum disease incidence (9.24%) of wilt of chickpea caused by *F. oxysporum* f. sp. *ciceri*, *F. solani* and *R. solani.* Madhavi and Bhattiprolu (2011) reported that the application *T. viride* in soil was found to be effective in managing fusarium wilt in chilli caused by *F. solani.*

Antagonistic biocontrol bacteria like *P. fluorescens* have been reported to be effective against a broad spectrum of plant pathogens, including fungi, bacteria and viruses in many plant species and due to their ability to colonise the host roots and to induce ISR, these fluorescent pseudomonads have gained importance and widely used as important tool for biological control of many phytopathogens. Couillerot *et al.* (2008) highlighted the potential of *P. fluorescens* in the biological control of phytopathogens.

Wilson *et al.* (1987) observed that the Rhizopus rot of peach could be inhibited by fruit dip with the biocontrol bacterium, *Enterobacterium cloacae* (isolate D-3). Treatments of stone fruits such as apricot, peach and nectarine with the antagonistic bacterium *Pantoea agglomerans* strain EPS 125 decreased incidence and intensity of soft rot caused by *R. stolonifer* and significant reduction

of conidial and mycelial germination of *R. stolonifer* was achieved when the pathogen as well as the bacteria were co-cultivated on peach leachate or nectarine juice. The biocontrol efficacy was positively correlated with concentration of the antagonistic bacterium. Bonaterra *et al.* (2003) proposed pre-emptive pathogen exclusion by wound colonization and direct interaction with the pathogen as mechanism of biocontrol.

A study was carried out by Sumana *et al.* (2012) to find out the possibility of using ecofriendly measures to manage *Fusarium* wilt and root knot complex disease of tobacco and observed that application of *T. viride* and *P. fluorescens* in talc formulations gave dual benefit of crop protection and growth promotion. The *Fusarium* wilt disease was suppressed to the extent of 58.46% and 60.15% by application of *T. viride* and *P.fluorescens*, respectively and gave a consonant increase yield of 29.97% and 26.88% respectively.

2.6.2. Organic Preparations

The use of organic preparations in agriculture has been targeted to produce nutritive, healthy and pollution free food, thereby protecting the entire ecosystem and human health.

Siddiqui *et al.* (2009) reported that the application of compost tea in field conditions resulted in increase of inducible enzymes peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) until 8^{th} day, which were significantly higher in okra plants pre-treated with non-sterilized tea in view of the high nutritive and microbiological properties. There was a further increase in inducible compounds when the pre-treated okra plants were challenged with *C. cucurbitarum*. It was also observed that this resistance decreased over time probably due to the highly stressed environment of the leaf surfaces which could have negatively affected the survival of the antagonists. The consequent reduction in efficacy of compost tea necessitated the plants to be sprayed at short intervals with the compost tea to derive sustained suppressive effect.

Patil (2009) reported that three sprays of vermiwash @ 50% and panchagavya @ 3% were the most effective indigenous technology knowledge for controlling the soyabean rust and for increasing the grain yield. Anuja (2010) opined that application of panchagavya could suppress foliar blight of amaranthus caused by *R. solani* by 47.29% when compared with the control. Mansour and Sayed (2011) observed that in green house trials, soil amended with compost tea showed significant reduction of root rot of beans caused by *Fusarium solani, R. solani* and *Macrophomina phaseolina.* They also recommeneded compost tea as a substitute for chemical fungicides for controlling the soil borne pathogens of beans.

Ashlesha and Paul (2012) reported that vermiwash at 4% inhibited the growth of *Ralstonia solanacearum,* the causal agent of bacterial wilt of capsicum, and that it also enhanced the survival of seedlings upto 14 days. Chadha *et al.* (2012) observed that application of vermiwash in crops was effective in enhancing the productivity and for suppressing the growth of various plant pathogens by producing anti - bacterial and anti - fungal compounds, hormones and siderophores. They also noticed that application of vermiwash gave 65%, 10%, 26% and 27% higher yields in knoll- khol, onion, French bean and paddy, respectively.

Sarkar *et al.* (2014) evaluated the potential of panchagavya and kunapajala in growth promotion as well as the enhancement of defense mechanisms in the seedlings of tomato, chilli and cowpea. Application of the panchagavya in all the three vegetables significantly induced defense related enzymes that contributed to ISR and reduction in disease incidence. They also reported that foliar spraying of jeevamruth was effective in enhancing the productivity of various crops and in managing diverse plant pathogens.

Fish amino acid is a fermented organic preparation based on fish or fish waste and jaggery. Hebsybai (2014) recorded a significant reduction of 70.53% in leaf blight caused by *R. solani* when the amaranthus plants were sprayed with fish

amino acid (5 ml/ litre). Anees (2014) observed that, application of fish amino acid @ 5% reduced stem rot of cowpea caused by *P. aphanidernatum.* In the plants sprayed with this organic preparation, the percentage of disease incidence was low (9.5%).

Al- Dahmani *et al.* (2003) found that compost teas produced using a range of materials and methods varied in their ability to control bacterial spot on tomato. Foliar application of compost tea produced with composted cow manure significantly reduced the infection rate in many vegetable crops. Dianez *et al.* (2006) reported that production of siderophores by the microflora of compost tea was responsible for disease suppression of soil borne pathogens.

Application of compost tea significantly reduced the pre and post-emergence damping-off disease caused by *R. solani* and also increased the survival of peanut plants (El-Shinnawi *et al.*, 2011). Martin and Brathwaite (2012) reported that compost tea was effective in suppression of various soil-borne diseases including damping-off and root rots caused by *Pythium ultimum*, *R. solani* and *Phytophthora* spp. and wilts caused by *F. oxysporum* and *Verticillium dahliae*. Arathy (2014) observed that foliar spraying and soil drenching of compost teas @ 1 litre / pot at fornightly intervals effectively suppressed collar and web blight disease of cowpea caused by *R. solani.* Spraying and drenching cowpea plants with neem leaf compost tea enriched with *Trichoderma* gave the lowest web blight index and collar rot incidence. Martin (2014) reported that compost tea and compost-water extract, could suppress phytopathogens and plant diseases and was an important disease control measure to organic producers who have limited disease control options.

2.6.3. Chemical Control

Fungicides have played a decisive role in averting or bringing down plant diseases which has helped to reduce major crop losses all over the world. A perusal of literature revealed that very little work has been done in managing Choanephora induced rots in crops using chemicals. However, El-Helaly *et al.*

(1968) observed that application of 0.1% Karathane, 0.5% Miltox and 0.4% Cupravit gave the best control of Choanephora blossom blight and fruit rot of vegetable marrow, followed by 0.3% Dithane M-22 and 0.3% Dithane M-45. Pre-inoculation spray of zineb, mancozeb, ziram and thiram on ripe chilli fruits could not completely eradicate Choanephora rot but afforded 80% suppression (Chahal and Grover, 1974). Tripathi *et al*. (1978) evaluated fungicides for the control of leaf blight and tip rot of sun hemp (*Crotalaria juncea* L.) caused by *C. cucurbitarum*. They reported that the disease was effectively controlled by Thiram, Ferbam, Blitox-50 and Ziram. The field trials conducted by Gunasekera and Shanthichandra (1990) in Sri Lanka revealed that the fungicides triadimenol, bitertanol and vinclozolin were effective in reducing the incidence of *Choanephora* blight on winged bean. Pod yield was significantly higher following treatment with triadimenol or bitertanol for all accessions of winged bean except UPS 45. Jana *et al.* (2011) reported that copper oxy chloride + metalaxyl formulation gave excellent control of twig blight caused by *C. cucurbitarum* in chilli. They also observed that when copper oxy chloride (4%), thiram (3%) and mancozeb + metalaxyl (4%) were sprayed on chilli, the number of infected twigs was found to be less as compared to control, while carbendazim was found ineffective in controlling the disease.

Fungicides have been tested for their effect on by post harvest disease caused various zygomycetous fungi such as *Rhizopus* spp., and *Mucor* spp., known to cause post harvest rots in several fruits, vegetables, tubers, flowers *etc*. McMillan (1974) observed that spraying the young flowers and immature fruits of jackfruit with 2 to 3 lbs per 100 gallons of 53% of copper hydroxide controlled the soft rot caused by *Rhizopus atrocarpi.* Pre-emergence and post- emergence mortality caused by *R. stolonifer* in marigold were reduced by seed treatment with thiram, Blitox (copper oxychloride), Brassicol (quintozene) and Bavistin (carbendazim) (Narayanappa and Sohi, 1985). Akhtar *et al.* (1994) reported that application of thiabendazole (Tecto-60) gave the best control of the post harvest decay of tomato caused by *R. stolonifer.* Majumdar and Pathak (1997) reported that guava fruit rot caused by *Rhizopus arrhizus* was controlled by application of

copper oxy chloride and Planofix (NAA) on guava fruit. Aggarwal *et al.* (2003) while studying the effect of fungicides on mycorrhizae and rhizosphere microbes of sunflower found that *Mucor racemosus* was susceptible to Bavistin up to 20 days after treatment but after 40th day it was present in nearly all concentration. Dithane M-45 also caused similar deleterious effect on *M. racemosus* like Bavistin. *Mucor* sp. was not affected much at the lower concentrations *i.e.,* up to 0.5% but the higher concentrations of Dithane M-45 restricted the growth of *Mucor* sp. They also observed that colonization of zygomycetous mycorrhizal fungi showed a decreasing trend with increasing concentrations of Bavistin, with minimum mycorrhizal root colonization occurring at 1% concentration. Pathak and Zaidi (2013) studied the effect of seed treatment by the fungicides and observed that mancozeb @ 2g per kg seed increased the germination percentage and reduced *Mucor* spp., *Rhizopus oryzae*, *Rhizopus* spp. and seed mycoflora of wheat. Etebu and Benjamin (2014) showed that a triazole fungicide, ketoconazole at 5mg/ml significantly inhibited *Mucor* sp.

Materials and Methods

3. MATERIALS AND METHODS

3.1.SYMPTOMATOLOGY

The symptoms of Choanephora pod of cowpea based on the descriptions given by Bashir *et al.*(1985) were observed in the field conditions. The symptoms are described and documented.

3.2. ISOLATION AND PROVING PATHOGENICITY OF PATHOGEN ASSOCIATED WITH Choanephora POD ROT OF COWPEA

3.2.1. Isolation of Pathogen and Purification of the Isolates

Cowpea pods infected by *Choanephora cucurbitarum* were collected from various fields of Instructional Farm, College of Agriculture, Vellayani, Thiruvananthapuram district, Kerala. The diseased pods were washed under running water and pods were cut into bits containing diseased portion along with the healthy portion. The bits were then surface sterilized using 1.0% sodium hypochlorite for 1 min followed by washing in three changes of sterile water. The bits were transferred into a sterile filter paper for the absorption of water and were cultured on potato dextrose agar (PDA) (Appendix 1) medium poured into sterile petri dishes under aseptic condition. The petri plates were then sealed using parafilm, incubated at room temperature (27°C - 30°C) for 24 - 48 h and observed for fungal growth. The fungal growth observed on the petri dishes was transferred to PDA slant (Aneja, 2003).

The fungal isolates obtained were purified by single spore technique (Dhingra and Sinclair, 1985). Serially diluted spore suspension of each isolate prepared from three day old culture was plated on sterilized plain agar (2.0%) in petri dishes under aseptic conditions. The plates were incubated at room temperature for 24 h. Single spores located under microscope were marked with a fine tip marker and then transferred into PDA slants for further growth and stored for further studies.

3.2.2. Pathogenicity Studies

The pathogenicity studies were carried out on excised healthy cowpea pods (var. Bhagyalakshmi) collected from the fields. The pods were washed under running water followed by surface sterilization using 70% ethanol. Artificial inoculation of the pathogen was carried out prior to which the pods were wounded using needle followed by the deposition of 1cm diameter mycelial discs cut out from 24h old culture of *C. cucurbitarum* at the site of injury. The site of inoculation was covered with wet cotton. After inoculation, the pods were kept in plastic covers in order to maintain humidity and incubated at room temperature for 24 - 48h till the appearance of symptom on the inoculated pods. Pin pricked pods covered with moist cotton swab alone served as the control.

3.2.3. Virulence Rating

The isolates were subjected to virulence rating based on the time taken for the complete rotting of the inoculated pods and the most virulent isolate was selected.

3.3. CHARACTERIZATION OF PATHOGEN

3.3.1. Cultural Characterization of Pathogen

The cultural characteristics of the pathogen were studied by growing them on different solid media such as Potato Dextrose Agar (PDA), Potato Sucrose Agar (PSA), Oat Meal Agar (OMA), Corn Meal Agar (CMA), Carrot Agar (CA), Malt Extract Agar (MEA), Czapeck's Dox Agar (CDA), Yeast Extract Agar (YEA), Nutrient Agar (NA) (Appendix 1) for selecting the best medium for the growth of the pathogen and then observing the nature of growth and sporulation of the pathogen on the best medium.

The sterile petri plates were poured with 20-25 ml of the media under aseptic condition and was allowed to solidify. Culture disc of 7 mm was cut out using a cork borer from three day old culture which was placed at the centre of the

petri plates and incubated at room temperature till complete growth was attained in any one of the media tested. Three replications of each of the treatment were maintained.

3.3.2. Morphological Characterization of Pathogen

The morphological characters were studied by observing the mycelial characters, sporulation, characteristics of the sporangiophore, and by measuring the size of sporangia, sporangiola and sporangiospore by preparing slides stained with cotton blue (Appendix II) and observing under 45X and 100X magnification of Leica DM750.

3.3.3. Molecular Characterization of the Pathogen by Partial Sequencing of Internal Transcribed Spacer (ITS) Region of rDNA.

The molecular characterization of different isolates obtained were done by comparison of the ITS sequences of the isolates. The procedure for molecular characterization was as follows:

3.3.3.1. DNA Isolation Using NucleoSpin® Plant II Kit (Macherey-Nagel)

About 25 mg of the tissue/mycelium was homogenized using liquid nitrogen and the powdered tissue was transferred to a microcentrifuge tube. Four hundred µl of buffer PL1 was added and vortexed for 1 min. Ten µl of RNAase A solution was added and inverted to mix. The homogenate was incubated at 65ºC for 10 min. The lysate was transferred to a Nucleospin filter and centrifuged at 11000 x g for 2 min. The flow through liquid was collected and the filter was discarded. Four hundred and fifty µl of buffer PC was added and mixed well. The solution was transferred to a Nucleospin Plant II column, centrifuged for 1 min and the flow through liquid was discarded. Four hundred µl buffer PW1 was added to the column, centrifuged at 11000 x g for 1 min 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 min 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 65ºC for 5 min. The column was then centrifuged at 11000 x g for 1 min to elute the DNA. The eluted DNA was stored at 4ºC.

3.3.3.2. Agarose Gel Electrophoresis for DNA Quality Check

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

3.3.3.3. PCR Analysis

PCR amplification reactions were carried out in a 20 µl reaction volume which contained 1X Phire PCR buffer (contains 1.5 mM $MgCl_2$), 0.2 mM each dNTPs (dATP, dGTP, dCTP and dTTP), 1 µl DNA, 0.2 µl Phire Hotstart II DNA polymerase enzyme, 0.1 mg/ml BSA and 3% DMSO, 0.5 M Betaine, 5 pM of forward and reverse primers.

Primers used

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

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

3.3.3.4. PCR Amplification Profile

ITS

98 ºC	-	30 sec	
98 ºC	-	5 sec	} 40 cycles
62 ºC	-	10 sec	
72 ºC	-	15 sec	
72 ºC	-	60 sec	
4 ºC	-	∞	

3.3.3.5. Agarose Gel Electrophoresis of PCR products

The PCR products were checked in 1.2% agarose gels prepared in 0.5X TBE buffer containing 0.5 µg/ml ethidium bromide. One µl of 6X loading dye was mixed with 5 µl of PCR products and was loaded and electrophoresis was performed at 75V power supply with 0.5X TBE as electrophoresis buffer for about 1-2 h, until the bromophenol blue front had migrated to almost the bottom of the gel. The molecular standard used was 2-log DNA ladder (NEB). The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad).

3.3.3.6. ExoSAP-IT Treatment

ExoSAP-IT (GE Healthcare) consisted 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^{o}C for 15 min followed by enzyme inactivation at 80^{o}C for 15 min.

3.3.3.7. Sequencing Using BigDye Terminator v3.1

Sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle sequencing kit (Applied Biosystems, USA) following manufactures protocol. The PCR mix consisted of the following components:

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

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

3.3.3.8. Post Sequencing PCR Clean up

A master mix I of 10 µl milli Q and 2 µl 125 mM EDTA per reaction was made. 12 µl of master mix I to each reaction containing 10 µl of reaction contents was added and were properly mixed. A master mix II of 2 µl of 3M sodium acetate pH 4.6 and 50 µl of ethanol per reaction was made. 52 µl of master mix II was added to each reaction. The contents were mixed by inverting. Incubated at room temperature for 30 min. Further it was centrifuged at 14,000 rpm for 30 min. The supernatant was decanted and add 100 µl of 70% ethanol was added. It was then spinned at 14,000 rpm for 20 min. The supernatant was decanted and 70% ethanol wash was repeated. The supernatant was decanted and the pellet was air dried. The cleaned up air dried product was sequenced in ABI 3500 DNA Analyzer (Applied Biosystems).

3.3.3.9. Sequence Analysis and Submission of Sequences in NCBI

The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 (Drummond *et al.,* 2010). The identity of ITS - rDNA conserved region of the pathogen associated with Choanephora pod rot of cowpea was established by performing a similarity search using Basic Local Alignment Search Tool (BLAST) in the National Centre for Biotechnology Information (NCBI) database and the sequences were matched with existing available database for species confirmation. Based on the sequence matching results, the rDNA sequences were bankitted in the NCBI database and accession number were obtained.

3.3.3.10. Phylogenetic Analysis

The data set based on the ITS - rDNA region of the pathogen associated with Choanephora pod rot of cowpea and other *Choanephora* reference sequences were retrieved from NCBI Genbank database (USA) and compared. Multiple sequence alignment was done using ClustalW2 and phylogenetic analysis through TreeView software. A phylogenetic tree was constructed using neighbour-joining (NJ) method (Kagiwada *et al.,* 2010).

3.4. HOST RANGE OF THE PATHOGEN

Surveys were conducted during 2014 - 2015 in various vegetable growing areas at College of Agriculture, Vellayani to study the occurrence of *Choanephora* induced rots in other vegetable crops. The plant part showing similar symptom of Choanephora rot were noted and the samples were brought to the laboratory for isolation as described in the 3.1.

In order to carry out the host range of the pathogen in weeds, the commonly occurring weeds in and around the cowpea fields were uprooted and brought to the laboratory. It was then potted separately in small pots filled with potting

mixture. After establishing the growth, *in vitro* inoculation of the pathogen was done as described under 3.3. The weeds collected included *Amaranthus viridis* L., *Cynodon dactylon* (L.) Pers, *Chromolaena odorata* (L.) King & H.E. Robins, *Boerhaavia diffusa* L. Nom. Cons and *Cyperus rotundus* L.

3.4.1.Comparision of *Choanephora* Isolate From Different Host

The cultural, morphological and molecular characterization of isolates obtained from different hosts was studied using the methods described in 3.3.1, 3.3.2 and 3.3.3.

3.5. ISOLATION AND TESTING OF ANTAGONISTIC MICROORGANISMS

3.5.1. Isolation of the Antagonistic Micro flora From the Healthy Cowpea Pods

The saprophytic micro flora present on the healthy pods collected from the diseased field was studied. The serial dilution technique described by Johnson and Curl (1972) was used for the isolation of antagonist micro flora from the healthy cowpea pods. One gram of healthy cowpea pod was weighed out and was transferred into 99 ml of sterile water contained in a 250 ml conical flask and was shaken for 20 min in a rotary shaker to get 10^{-2} dilution. From this, two fold serial dilution yielded 10^{-4} dilution which was used for enumeration of fungi. Two fold dilution from this was done further to obtain10^{-6} dilution used for isolation and enumeration of bacteria.

One ml aliquots from 10^{-4} dilution was pipette out into sterile petri plates under aseptic condition and melted and cooled Martin's Rose Bengal Agar medium (Appendix 1) was poured at the rate of 20 ml per plate and rotated gently for thorough mixing. Similarly one ml from 10^{-6} dilution was transferred into sterile petri plates and plated using nutrient agar medium (Appendix 1). The plates were sealed using parafilm and incubated at room temperature for three to four days. The plates were examined for the growth of fungal and bacterial colonies. Observations were recorded on colony counts in petri plates and

expressed as number of colony forming units (cfu) per gram of sample. The colonies were observed for colour and shape and transferred to PDA slant / nutrient agar slants. The single colonies of fungus were purified by hyphal tip method. Purification of bacteria was done by streaking on nutrient agar plates and the single colonies of the bacteria transferred were then stored under refrigerated condition for identification and subsequent studies of antagonism.

3.5.2. *In vitro* Evaluation of the Antagonistic Fungi and Bacteria for Suppression of the Pathogen by Dual Culture Technique

3.5.2.1. Antagonistic Fungi

The fungal isolate obtained through serial dilution technique was evaluated for suppression of the pathogen *C. cucurbitarum* by dual culture method described by Skidmore and Dickinson (1976). The mycelial disc of 7 mm diameter cut from seven day old culture of the saprophytic fungus and the pathogen were placed on two opposite sides of petri plates containing sterilized PDA and incubated at room temperature. Three replications were maintained for the experiment. Control plates contained only the pathogen. Observations were recorded on the radial growth.

The percentage inhibition of the pathogen over the control was calculated by the formula (Vincent, 1927),

$$I (\%) = R1- R2/ R1 \times 100$$

I - Percentage growth inhibition

R1 - Growth of pathogen in control

R2 – Growth of pathogen in treatment

3.5.2.2. Antagonistic Bacteria

The bacterial antagonists obtained through serial dilution were tested for antagonism to *C. cucurbitarum* by dual culture technique (Utkhede and Rahe, 1983). The nutrient agar medium was melted and poured into sterile petri plates.

After solidification, culture bits of 7 mm size of the pathogen was placed at the centre of each dish. The respective bacterial isolate was then streaked 2.5 cm away on the both sides perpendicular to the pathogen placed at the centre. The percentage inhibition was calculated using the formula given in 3.5.2.1.

3.5.3. Identification and Morphological Characterization of Selected Fungal Antagonist

The selected fungal and bacterial isolates were subjected to identification and morphological characterization. The fungal antagonist selected was subjected to single spore isolation technique for purification and slide culture for morphological identification.

3.5.3.1. Single Spore Isolation Technique

The single spore isolation technique by Dhingra and Sinclair (1985) was used for the purification of the selected fungal antagonist. A small bit of mycelium containing the conidia was transferred to a test tube containing sterile water and made into a spore suspension. From this, a loopful of the spore suspension was the taken and streaked on water agar in zig-zag manner. The plates were sealed using paraffin film and were incubated at room temperature for 24 h. The colonies developed from the single conidia were sub cultured into PDA slants.

3.5.3.2. Slide Culture Technique

The method described by Riddell (1950) was used for the identification of the fungal isolate. The slide culture unit which consisted of petri plates containing a filter paper, two pieces of glass rods, two coverslips and one microscopic slides were autoclaved in a hot air oven. A five mm plain agar block was cut out using a sterile blade and using a sterilised inoculation needle, it was placed on the microscopic slides kept on glass rods. The mycelium of the fungal isolate was then inoculated at four corners of the agar block and coverslip was placed over the agar block. The filter paper was moistened with sterile water. The slide culture

units were then sealed and were incubated at room temperature. The slides were examined under low and medium power objectives of a Leika DM750 microscope and micro - morphological characters of mycelia and conidia were observed.

3.5.4. Identification of Bacteria

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

Endospore staining was done based on the method described by Schaeffer and Fulton, 1933. The smear of the bacteria was prepared and was heat fixed. The slide was placed on the rim of a beaker containing boiling water with the smear on the upper side. The smear was flooded with malachite green (Appendix II) when the water in the beaker started boiling. The slide was left for 3 - 4 min. The slide was taken and excess stain was drained by washing under tap water. The smear was counter stained using safranin and washed after 30 sec. A drop of cedar oil was dropped into the slide and observed under oil immersion objective.

The bacterial isolate that showed maximum inhibition of the mycelia of *C. cucurbitarum* was identified by biochemical characters by sending the culture at Cashew Export and Promotion Council of India (CEPC), Kollam.

3.6. *IN VITRO* EVALUATION OF DISEASE SUPPRESSION USING INDIGENOUS ORGANIC PREPARATIONS

3.6.1. Preparation of Panchagavya

Panchagavya was prepared by following the steps described in organic POP, KAU (2009). Cow dung (7 kg) and cow ghee (1kg) were mixed in a clean plastic bucket thoroughly both in the morning and evening hours and kept aside for three days. After three days, cow urine (10 l) and water (10 l) were added. The mixture was kept for 15 days with regular mixing both in the morning and evening hours. After 15 days, 3 l of cow milk, cow curd (2 l), tender coconut water (3 l), jaggery (3 kg) and well ripened poovan banana (12 nos.) were added in to this. The bucket was then kept under shade and the mouth of the bucket was covered with mosquito proof net. The content was stirred twice a day in the morning and evening. The stock solution was used for disease management studies after 30 days.

3.6.2. Preparation of Fish Amino Acid

Fish amino acid was prepared by following the steps described by Weinert *et al.* (2014) with slight modifications, mixing one kg of sardine fish (*Sardina pilchardus*) with one kg of jaggery in a plastic can and kept the plastic can under shade condition. The mouth of the can was covered with paper and tied with the string and kept undisturbed for 25 days. After 25days, the content was filtered through muslin cloth and stored in the same can. The filtered content was used in the field by mixing with water (5 ml/ l of water).

3.6.3. Preparation of Jeevamruth

Jeevamruth was prepared by the method described by Chadha *et al.* (2012). It was prepared by mixing 10 kg cow dung, 10 l cow urine, 2 kg jaggary, 2 kg pulse flour, a handful of fertile soil with 200 l water and kept for one week incubation.

3.6.4. Preparation of Aerated Compost Tea

Aerated compost tea was prepared by the modified Bucket - Bubbler technique (Arathy, 2014). In a 20 l bucket, 10 l of water was taken with an air bubbler which was then attached to an aquarium type aeration pump. Air was blown into it from the air bubbler. 2 kg of the compost was then taken in a muslin cloth, tied and then suspended into the water from a glass rod. The aerator provided continuous flow of air and created enough turbulence to mix the brew. To harvest the brew, the aerator was turned off for half an hour to allow the solids to settle down at the bottom of the bucket.

3.6.5. *In vitro* Evaluation of Organic Preparations on Fungal Growth

The organic preparations like compost tea, panchagavya, jeevamruth, vermiwash and fish amino acid were evaluated for *in vitro* suppression of the pathogen *C. cucurbitarum* by poisoned food technique described by Nene and Thapliyal (1993). The concentrations tested were 2.5%, 5% and 10% for all the organic preparations. For carrying out the study, 47.5 ml, 45 ml and 40 ml of distilled water was taken in three 250 ml conical flask. Similarly in another three 250 ml conical flask, double strength PDA were taken and sterilized by autoclaving. The organic preparations were initially filtered through Whatman No.1 filter paper. It was then further sterilized by passing through bacterial proof filter before adding to the media. The measured quantity *i.e.* 2.5 ml, 5 ml and 10 ml of the organic preparations were then added into 47.5 ml, 45 ml and 40 ml of the distilled water under aseptic condition and mixed thoroughly. This mixture of different concentrations was then added to 50 ml of molten double strength PDA contained in three separate conical flasks to get desired concentration. The amended medium (20 ml) was then poured into the sterile petri plates and was allowed to solidify. The 7 mm mycelial disc from three day old *C. cucurbitarum* culture was inoculated at the centre of the amended medium under aseptic conditions. The plates were sealed using parafilm and were incubated at room temperature.

In another method, the organic preparations of desired concentrations were directly poured into desired quantity of distilled water without passing through Whatman No.1 filter paper and bacterial proof filter. This was then added to 50 ml of autoclaved PDA medium making it an amended medium. The medium was then poured into sterile petri plates and the pathogen was inoculated at the centre.

Similarly, another procedure carried out was pouring the organic preparations of desired concentrations *i.e.* 2.5 ml, 5 ml and 10 ml was added to 97.5 ml, 95 ml and 90 ml of the PDA medium and were subjected to autoclaving. The medium were then poured into sterile petri plates under aseptic conditions and the pathogen was inoculated at the centre.

In all the above methods, unamended PDA medium inoculated with the pathogen at the centre served as the control. Observations were taken when the mycelium of the pathogen attained full growth in the control petri plates. Percentage inhibition of growth over control was calculated using the formula (Vincent, 1927).

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

Where,

I – Percentage inhibition

C - Growth of *C. cucurbitarum* in unamended medium

T - Growth of *C. cucurbitarum* in amended medium

3.7. *IN VITRO* EVALUATION OF FUNGICIDES AGAINST *C. cucurbitarum*

The *in vitro* suppression of the *C. cucurbitarum* using fungicides was done by using poisoned food technique described by Nene and Thapliyal (1993). Nine commercially available fungicides were used for evaluation. The desired concentration of the fungicide (Table 1) was weighed out. Three conical flasks containing 50 ml of distilled water and another containing 50 ml of double strength PDA were taken and were sterilized by autoclaving at 1.1 kg/ cm^2 for 20

min. The concentration of the fungicide which was weighed out was added into the distilled water and was shaken thoroughly. This was then added into 50 ml of molten double strength PDA to get the desired concentrations. The amended medium was then poured into sterile petri plates under aseptic conditions and was allowed to solidify. The same procedure was repeated for all the fungicides. Each plate was inoculated in the centre with the 7 mm mycelial disc cut out from three day old of *C. cucurbitarum* under aseptic condition. The plates were sealed using parafilm and were incubated at room temperature.

Unamended PDA medium inoculated with the pathogen at the centre served as the control. Observations were taken when the mycelium of the pathogen attained full growth in the control petri plates. The percentage inhibition was calculated using the formula mentioned under 3.6.5.

3.8. *IN VITRO* SUPPRESSION OF Choanephora POD ROT OF COWPEA USING ORGANIC PREPARATIONS AND SELECTED ANTAGONISTS.

Fresh and healthy cowpea pods were collected from the field. The pods were washed under running water and then the pods were surface sterilized using 70% ethanol. The desired concentration (the concentration of the organic preparation which showed suppression of pathogen *in vitro*) of indigenous organic preparations and selected antagonists (Table 2) were sprayed on excised pods. The pods were then kept in a polythene cover and were incubated at room temperature for 24 h. The pods without any treatment served as the control. Artificial inoculation of the pathogen were carried out after giving definite number of pin pricks on all the pods using needle followed by the deposition of the fungal mycelium at the site of injury. The pods were then incubated at room temperature till infection starts appearing in the control pods. Three replications of each treatment were maintained. The percentage suppression of the disease by each treatment was worked out based on the lesion size and using the formula given in 3.6.5.

3.9. STUDIES ON *IN VIVO* SUPPRESSION OF CHOANEPHORA POD ROT OF COWPEA.

A pot culture experiment was conducted in CRD to evaluate the efficacy of the selected treatments (Table 3) in order to study the suppression of Choanephora pod rot under field conditions. The pot culture experiment was initiated during November 2014 at College of Agriculture, Vellayani. The susceptible bush type cowpea variety Bhagyalakshmi was used for evaluation. The seeds were sown in pro - trays filled with potting mixture consisting of vermicompost and coirpith in 1:1 ratio. The cowpea seedlings were transplanted to the pot filled with potting mixture (sand, soil and cow dung in 1:1:1 ratio) after the emergence of first true leaves. Each pot contained one plant and three replication of each treatment were maintained. The treatments were given as foliar application after the pods attained maturity followed by challenge inoculation of the pathogen at 10^6 cfu/ml.

The occurrence of pod rot was recorded at weekly intervals. Scoring of the disease was done using 0-4 disease scale (Ziedan *et al.,* 2012) (Plate 1).

Grade	Description
0	Healthy
1	0 - 25 % of the pod infected
2	26 - 50 % of the pod infected
3	51 – 75 % of the pod infected
4	> 75 % of the pod infected

The percentage disease incidence were calculated by using the formula

$$\text{Percentage Disease Incidence} = \frac{\text{No. of pods affected}}{\text{Total no. of pods}} \times 100$$

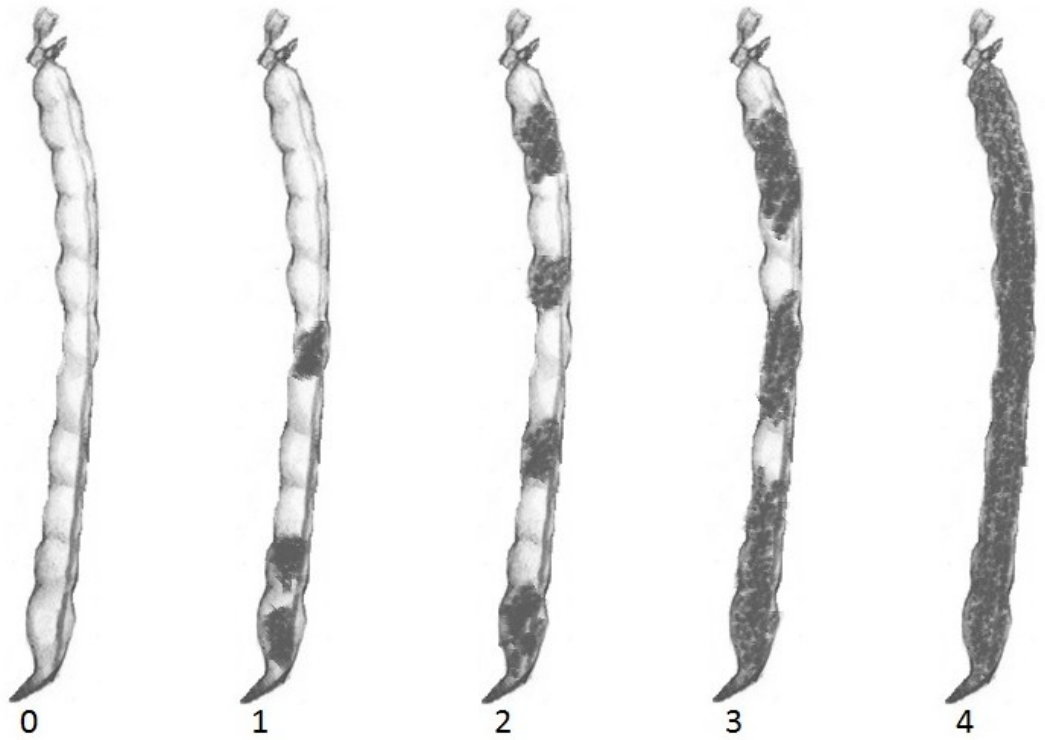


Plate 1: Score chart for assessment of intensity of Choanephora pod rot of cowpea

0: Healthy, 1 : 0 - 25 % of the pod infected, 2: 26 - 50 % of the pod infected,

3: 51 – 75 % of the pod infected, 4 : > 75 % of the pod infected

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

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

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

Table 1: Different concentrations of the fungicides tested against *C. cucurbitarum* (*in vitro*)

Chemical name	Trade name	Concentrations used (g/100ml)
Mancozeb	Indofil M-45	0.1, 0.2, 0.3
Copper oxy chloride	Fyter	0.1,0.2, 0.3
Captan + Hexaconazole	Taqat	0.15, 0.2, 0.25
Azoxystrobin	Amistar	0.05, 0.1, 0.15
Carbendazim	Bavistin	0.05, 0.10, 0.15
Carboxin	Vitavax	0.2, 0.25, 0.30
Carbendazim+ mancozeb	Cosuit	0.15, 0.2, 0.25
Propiconazole	Tilt	0.05, 0.1, 0.15
Copper hydroxide	Kocide	0.1, 0.2, 0.3

Table 2: Organic preparations and antagonists tested for suppression of *C.cucurbitarum*

Serial no:	Organic preparations and selected antagonist	Concentration
1	Panchagavya	10%
2	Jeevamruth	10%
3	Vermiwash	10%
4	Fish amino acid	10%
5	Compost tea	10%
6	*Trichoderma virens*	10^6cfu/ml
7	*Pseudomonas fluorescens*	10^6cfu/ml

Table 3: List of selected *in vivo* treatments

Treatment no:	Treatments
T1	Foliar spray of selected *Trichoderma virens*
T2	Foliar spray of selected *Pseudomonas fluorescens*
T3	Foliar spray of compost tea
T4	Foliar spray of panchagavya
T5	Foliar spray of fish amino acid
T6	Foliar spray of jeevamruth
T7	Foliar spray of copper hydroxide (0.2%)
T8	Foliar spray of mancozeb (0.3%)
T9	Foliar spray of propiconazole (0.1%)
T10	Foliar spray of KAU released talc based formulations of *Trichoderma* (2%)
T11	Foliar spray of KAU released talc based formulations of *Pseudomonas* (2%)
T12	Untreated control
T13	Inoculated check

Results

4. RESULTS

Experiments were conducted under *in vitro* and *in vivo* conditions to study the symptomatology, morphological and cultural characters of *Choanephora cucurbitarum,* pathogen causing cowpea pod rot, and to evolve various ecofriendly strategies to manage the pathogen. The experiments were conducted during 2013-2015 at the Department of Plant Pathology, College of Agriculture, Vellayani, Thiruvananthapuram. The results obtained from the laboratory and pot culture experiments are summarized below:

4.1. SYMPTOMATOLOGY

Symptomatology of Choanephora pod rot of cowpea was studied by collecting infected samples from various fields at College of Agriculture, Vellayani. The symptoms were observed in mature cowpea pods maintained for seed purpose as well as for vegetable purpose. Apart from the pods, the symptoms were also observed in other parts of the plant such as leaves, stem and flowers. The initial symptoms appeared as water soaked lesions on the pods (Plate 2a), which further expanded and caused wet rot of infected tissues (Plate 2b). In the advanced stages of disease development, the invaded portions became covered with a luxuriant fluffy white growth of the fungus, consisting of mycelium and sporangiophores along with sporangia (Plate 2c) which appeared as minute black headed pin like structures around the pods, giving 'lamb's tail' like appearance and finally resulting in complete decay of pods (Plate 2d).

The pods which were attacked by pod borer (*Maruca vitrata)* were more susceptible to the disease (Plate 2e). The per cent of disease in pod borer infested pods was found to range from 25 to 100 (Figure 1). Out of 56 pods examined, percentage of injured pods with *Choanephora* was 77.89 and that of percentage of uninjured pods with *Choanephora* were 15.83.

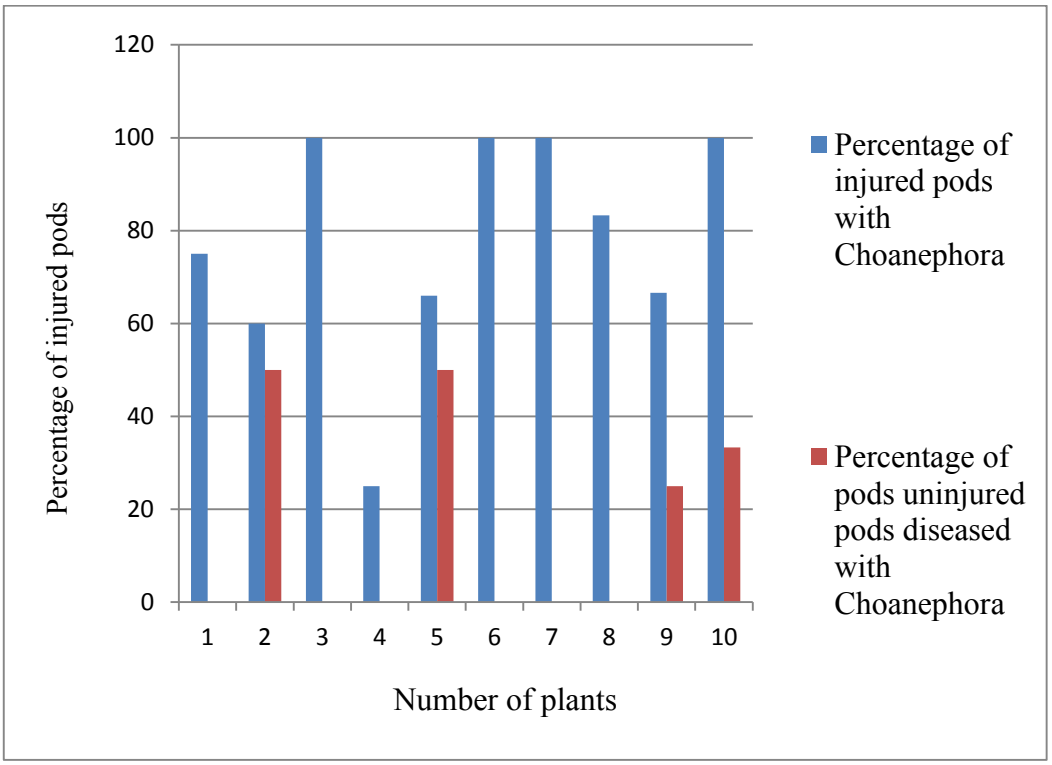


Figure 1: Influence of pod borer infestation on incidence of Choanephora pod rot of cowpea

On leaves, symptom started at the leaf margin as small water soaked lesions later on became dry and turned necrotic (Plate 2f). During dry conditions,sporangiophores developed black pin headed sporangia containing spores inside (Plate 2g). On peduncle (Plate 2h), stem (Plate 2i) and flowers (Plate 2j), initial symptoms appeared as small, light brown, elongated spots which increased in size and coalesced to cause wet rotting. From the affected peduncle, the fungus entered into leaflets and further invaded the stems. The affected tissue turned dark brown and became shrunken and disintegrated, causing toppling down of peduncle and stem. In advanced stages, the plant became completely necrotic.

The details of onset and development of symptoms under field conditions are given in the Table 4 (Plate 2k). Water soaked lesion, rotting, white mycelial growth and development of sporangia was observed in all the samples while necrosis was observed in leaf and flower samples.

4.2. ISOLATION AND PROVING PATHOGENICITY OF PATHOGEN ASSOCIATED WITH Choanephora POD ROT OF COWPEA

4.2.1. Isolation and Purification of Pathogen

The pathogen causing Choanephora pod rot of cowpea was isolated from the infected pods and leaves of cowpea plants collected from the cowpea growing field at College of Agriculture, Vellayani. On PDA medium, the pathogen produced white mycelia which on maturity showed black pin heads indicating sporulation. The fungal cultures were maintained on PDA slants by periodic sub culturing. Seven isolates of the pathogen were obtained of which four were isolated from cowpea pods, one from leaf peduncle, one from leaf and one from flower of cowpea. The fungal isolates from cowpea pods were numbered from C1 – C4, isolate from the pedicel as C5, isolate from the leaf as C6 and isolate from the flower as C7. The isolates obtained from cowpea pods (C1 – C4) were used for pathogenicity studies.

a. Water soaked lesion

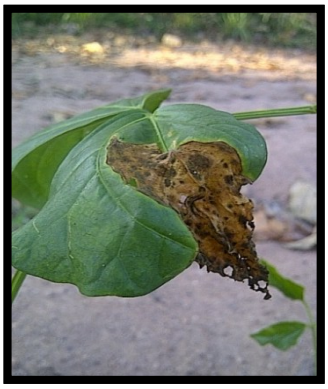
b. Wet rotting

c. Pods with mycelium and fruiting bodies (lamb's tail like symptom)

d. Completely rotted pods with mycelial growth

e. Pod borer attacked pods

Plate 2 a-e: Symptoms of Choanephora pod rot of cowpea under natural conditions



f. Necrotic leaf

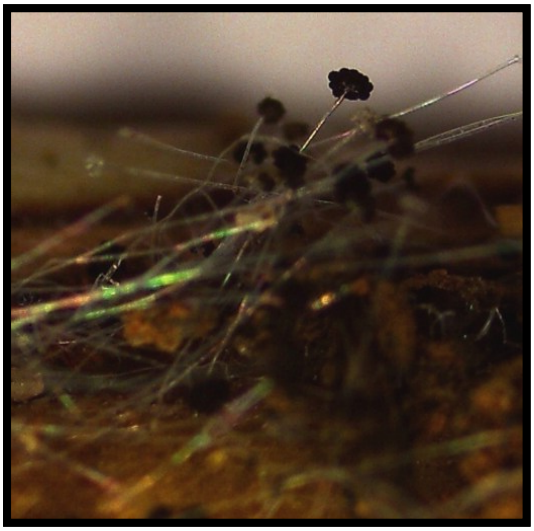
g. Advanced lesions with mycelial growth and sporulation

h. Peduncle with mycelial growth and fructifications

i.Stem with mycelia and fruiting bodies

j. Flower with mycelia and fruiting bodies

Plate 2 f-j: Symptoms of Choanephora pod rot of cowpea under natural conditions (continued)



k. Stereomicroscopic view revealing slender mycelium bearing at the tip a cluster of monosporous sporangiola

Plate 2 k: Symptoms of Choanephorapod rot of cowpea under natural conditions (continued)

Table 4. Onset and development of Choanephora pod rot under natural field conditions

Sl. No.	Isolate no:	Time of collection	Parts affected	Lesion size (cm×cm)	Nature of symptom				
					W	R	N	WMG	S
1.	C1	June	Mature pod	7.5 × 0.5	+	+	-	+	+
2.	C2	July	Mature pod	10.2 × 0.8	+	+	-	+	+
3.	C3	November	Mature pods	11.2 × 0 .7	+	+	-	+	+
4.	C4	October	Mature pods	8.3 × 0.5	+	+	-	+	+
5.	C5	September	Pedicel	5.0× 1.0	+	+	-	+	+
6.	C6	August	Leaf	4.0× 1.3	+	+	+	+	+
7.	C7	December	Flower	3.5 × 1.4	+	+	+	+	+

W : Water soaked lesion

R : Rotting of pods

N : Necrosis

WMG: White mycelial growth

S : Sporangial production

4.2.2. Pathogenicity Test

The pathogenicity of the different isolates of *Choanephora* sp. was confirmed 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 a sterile stainless steel needle. The cowpea pods showed wet rot within 24 h of inoculation and further showed development of white mycelia studded with black sporangial heads within 72h (Plate 3a- 3d). Re- isolation of the pathogen from the artificially inoculated pods yielded *C. cucurbitarum* identical to the original culture. Development and progression of symptoms on inoculation of *Choanephora* isolate on cowpea pods is given in Table 5.

4.2.3. Virulence Rating

The isolates C1, C2, C3 and C4 were subjected to virulence rating (Table 6) based on the time taken for complete rotting of the pods (Figure 2). The time taken for complete rotting of the pods varied between isolates, the isolate C3 took 72 h while C1 and C4 took 96 h and C2 took 120 h for complete rotting of the pods. Among the four isolates, C3 was therefore selected as the most virulent isolate for use in further studies.

4. 3. IDENTIFICATION OF THE PATHOGEN

4.3.1. Cultural Characteristics

The mycelial growth of the pathogen on different solid media such as Czapek Dox Agar (Plate 4a), Potato Sucrose Agar (Plate 4b), Carrot Agar (Plate 4c), Malt Agar (Plate 4d), Oat Meal Agar (Plate 4e), Potato Dextrose Agar (Plate 4f), Corn meal Agar (Plate 4g) were studied using the isolate C3 and the best medium for culturing of the pathogen was determined. The growth of the pathogen *Choanephora* sp. (C3 isolate) was determined on different solid media. The results showed that 24 h after inoculation, full growth was obtained in PDA

a.C1

b. C2

c. C3

d. C4

Plate 3a-d: Pathogenicity testing of four isolates of *C.cucurbitarum* obtained from cowpea pods

Table 5: Development and progression of Choanephora pod rot on inoculated pods by *Choanephora cucurbitarum* isolates obtained from cowpea pods

Isolate No:	Source	*Time taken for development of symptom (h)		Lesion size (cm×cm)
		WR	WMS	
C1	Mature pod	21	48	7.8 × 0.6
C2	Mature pod	24	72	3.5 × 0.4
C3	Mature pod	20	48	8.2 × 0.7
C4	Mature pod	24	72	5.2 × 0.8

*mean of three replications

WR : Wet rot

WMS : White mycelia studded with black sporangial heads

Table 6: Virulence rating of isolates of *Choanephora* sp. obtained from cowpea pods

Isolate No:	24 h	48 h	72 h	96 h	120 h
C1	+	+ +	+ + +	+ + + +	+ + + +
C2	+	+ +	+ +	+ + +	+ + + +
C3	+	+ + +	+ + + +	+ + + +	+ + + +
C4	+	+ +	+ + +	+ + + +	+ + + +

+ : 25% rotting of pods

+ + : 50% rotting of pods

+ + + : 75% rotting of pods

+ + + + : complete rotting of pods

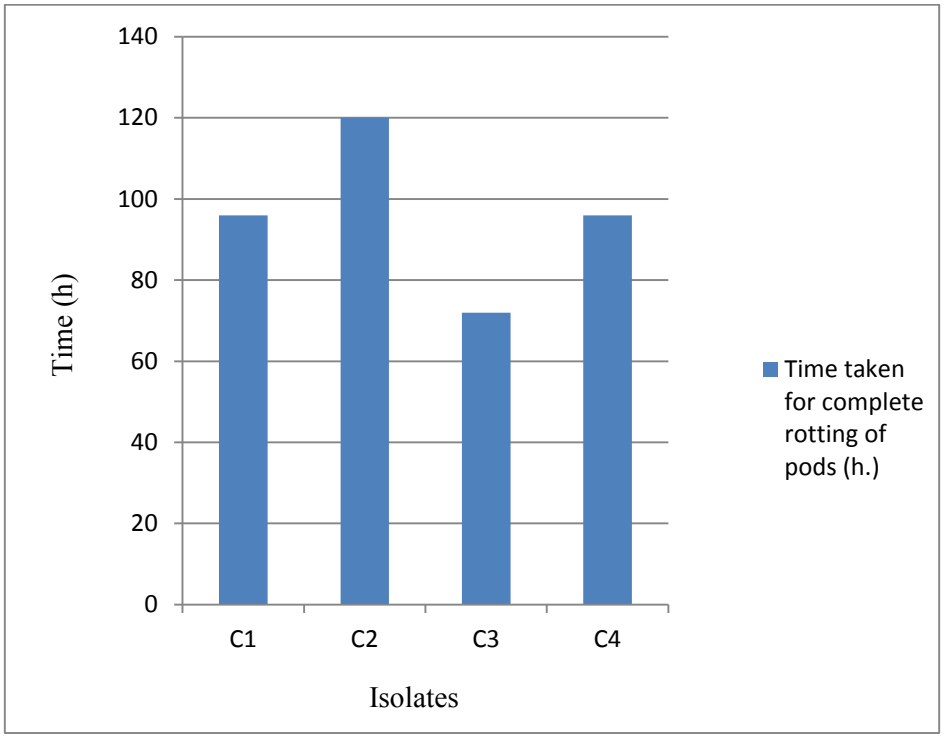
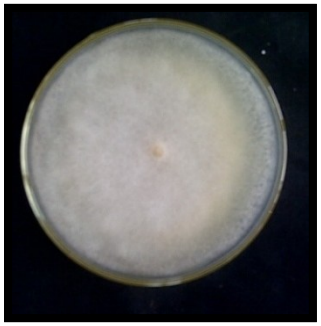


Figure 2: Time (h) taken for *Choanephora cucurbitarum* isolates obtained from cowpea pods for completely rotting of pods under *in vitro* conditions

a.Czapek-Dox Agar



b. PSA

c.Carrot Agar

d. Malt Agar

e. Oat Agar Meal

f.PDA

g. Corn Meal Agar

Plate 4 a-g: Growth of *C. cucurbitarum* on different solid media

medium (9.0 cm) which was significantly superior from all the media, followed by PSA (8.5 cm). The pathogen was found to have least growth in carrot agar media (Table 7).

On PDA medium, the isolate C3 grew rapidly to cover the whole petri dish (9 cm) within 24 h. The fungal colonies were white in colour on the upper surface (Plate 5a) and yellowish on the lower side of the petridish (Plate 5b). The white coloured mycelium on maturity produced black pin heads indicating onset of sporulation (Plate 5c).

4.3.2. Morphological Characteristics

The morphological characteristics of the virulent isolate C3 was studied by growing the isolate on PDA. The following observations were made on morphological characters:

- The mycelium was hyaline, unbranched and without any septations (Plate 6a).
- Sporangiophores were non-septate (Plate 6b)
- Two types of asexual structures were produced: drooping sporangia (Plate 6c) and monosporous sporangiola (Plate 6d).
- The drooping multisporous sporangia were sub-globose in shape and 90.75 - 110 µm in size.
- The sporangia were non – columellate and dehisced into two half releasing the spores (Plate 6e).
- Sporangiospores were elliptic, fusiform or ovoid in shape, light brown or dark brown in color and sized 16-22 × 8.23 -10 µm (Plate 6f).
- The sporangiophore (conidiophore) from which the monosporous sporangiola arose was long slender, branched at the apex with primary vesicle from which secondary vesicles (Plate 6g) were produced on the stalks which bears sporangiospores (conidia).

Table 7: Mycelial growth and sporulation of the virulent isolate of *Choanephora* sp., the incitant of pod rot of cowpea, on different solid media

Sl. No.	Treatments	Radial growth of pathogen after 24h. (cm) *	Sporulation
1.	Czapeck Dox Agar	5.90^{cd}	-
2.	Potato Sucrose Agar	8.50^{ab}	-
3.	Carrot Agar	2.90^{e}	-
4.	Malt Agar	6.16^{c}	-
5.	Oat Meal Agar	7.96^{b}	-
6.	Potato Dextrose Agar	9.00^{a}	+
7.	Corn Meal Agar	5.36^{d}	–
	CD (0.05)	0.55	

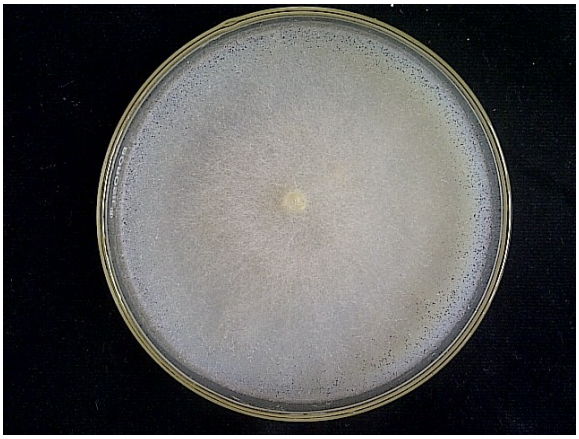
*Mean of three replications

Values on the parenthesis are arc- transformed

Treatments with same alphabets in the superscript, do not differ significantly

a. Mycelial growth: upper view

b. Mycelial growth: lower view



c.Sporulation

Plate 5a-c: Mycelial growth and sporulation of *Choanephora* sp.on PDA medium

- Monosporous sporangiola were elliptic, fusiform or ovoid, striate, and measured 12-20 × 6-14 µm (Plate 6h).

Based on the cultural and morphological characters the pathogen was identified as *Choanephora cucurbitarum* (Table 8).

4.3.3. Molecular Characterization of the Pathogen Isolate by Partial Sequencing of Internal Transcribed Spacer (ITS) Region of rDNA

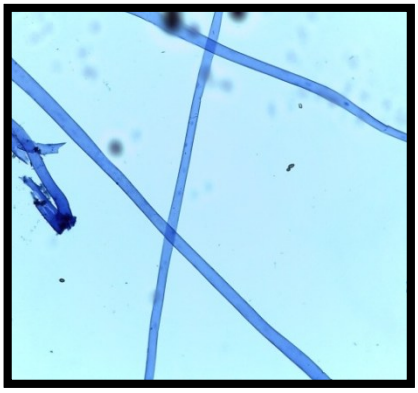
The ITS - rDNA region of virulent isolate (C3) of *Choanephora* spp. was sequenced for molecular characterization and identification of the pathogen. Amplification using the primers ITS 1 and ITS 4 resulted in amplicon of approximately 540 bp long (Plate 7a-b). Sequences of the virulent isolate (Appendix III) of *Choanephora* spp. were deposited in the GenBank and used for similar sequences in NCBI database using BLAST program. Alignment of the sequences of representative isolate collected for this study with other known sequences of *Choanephora* spp. obtained from the GenBank revealed that an identity of 83- 100% exist among the sequences.

The virulent isolate obtained from the cowpea pods (KR261840) used in the present study belonged to the first cluster where it showed 100% similarity with two isolates of *C. cucurbitarum* (KP406599.1 and KM200034.1) which was obtained from bhindi and *Phlox paniculata* reported from Korea (Figure 3).

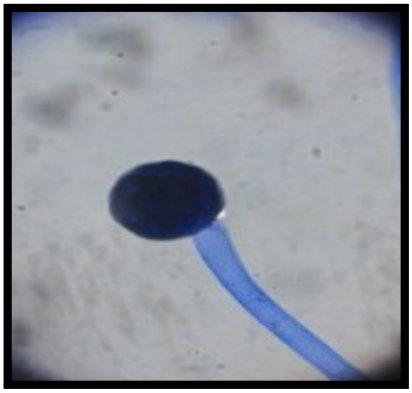
The cultural and morphological characteristics, of the fungus showed conformity for those described for *C. cucurbitarum* by Krik (1984). The isolate was further confirmed as *Choanephora cucurbitarum* (Berk. &Ravenel) Thaxt. by the Fungal Identification Service, Mycology and Plant Pathology Group, Agharkar Research Institute, Pune, India (Accession no: NFCCI- 3461).

4.4. HOST RANGE STUDIES

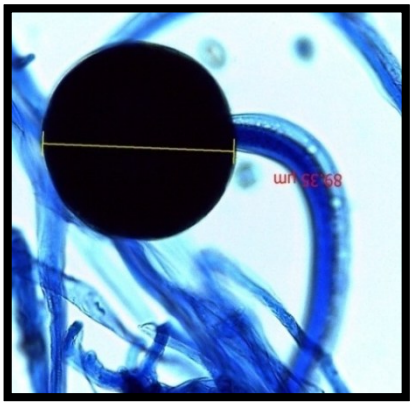
Attempts were made to isolate *C. cucurbitarum* from other vegetable crops and weeds other than cowpea, grown in and around the infected cowpea fields.



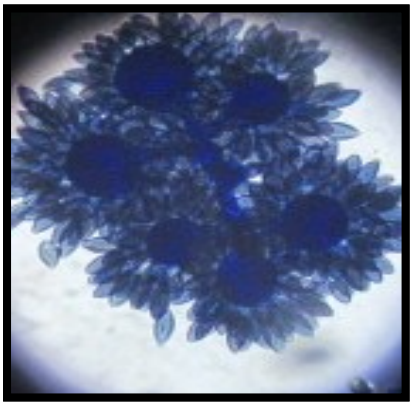
a. Mycelium (10 X)



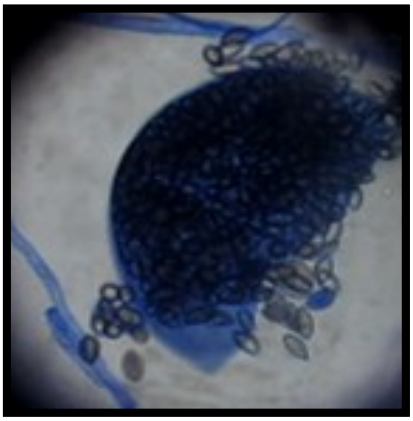
b. Sporangiophore with sporangium (10X)



c.Drooping sporangium (45 X)

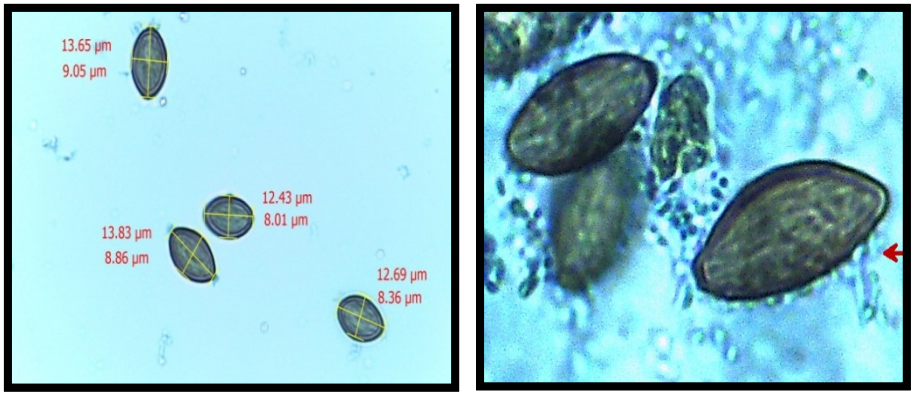


d. Monosporous sporangiola (45 X)

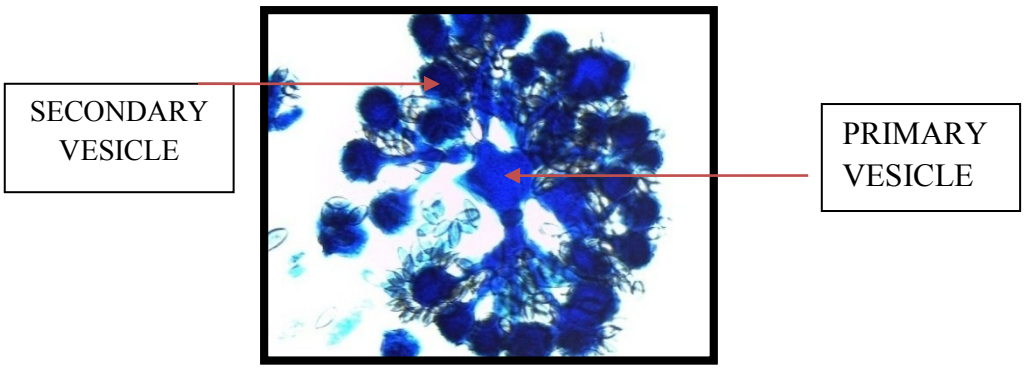


e. Dehisced sporangium (45X)

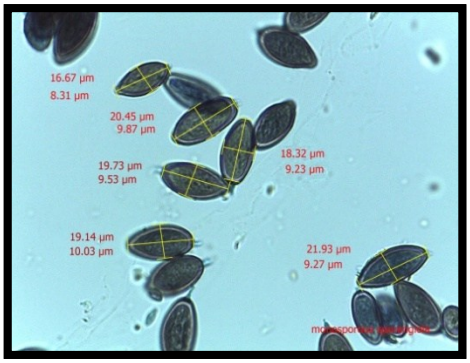
Plate 6 a-e: Morphological characteristics of *Choanephora cucurbitarum*



f. Sporangiospores(45X and 100X)



g. Primary and secondary vesicle(45X)



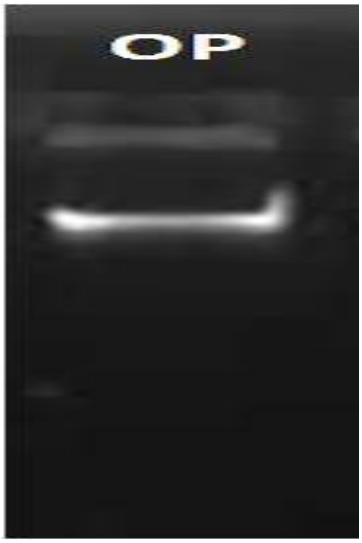
h. Monosporous sporangiola(45X)

Plate 6 f-h: Morphological characteristics of *Choanephora cucurbitarum* (continued)

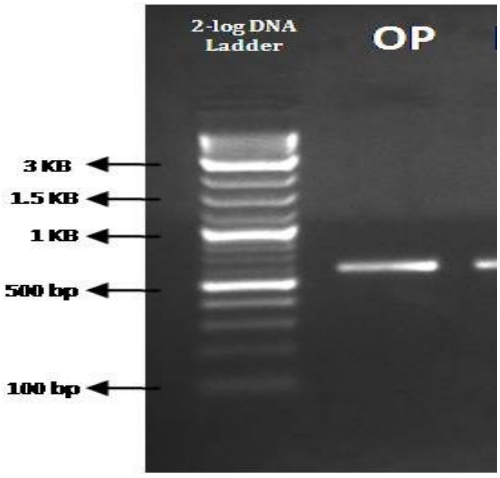
Table 8: Cultural and Morphological characters of virulent isolate of *C.cucurbitarum* obtained from cowpea pods

Isolate	Cultural characters		Morphological characters			
	Colony color					
	Upper view	Lower view	Colour of hyphae	Size of sporangia (µm xµm)	Size of monosporous sporangiola (µm xµm)	Size of sporangiospore (lxb) (µm xµm)
Virulent cowpea isolate	White	Light yellow	hyaline	90.7 – 110.0	12.0-20.0 x 6.0-14.0	16.0-22.0 x 8.23 -10.0
**Choanephora cucurbitarum*	White	yellow	hyaline	40.0- 130.0	8.0-16.0 x 9.12 – 20.0	7.0-14.0 x 9.0-26

*Cultural and morphological description of *Choanephora cucurbitarum* given by Kirk *et al.* (1984).



a. DNA



b. PCR products -Amplicon

Plate 7a-b: Molecular characterization of the pathogen obtained from cowpea pods (virulent isolate) using ITS sequencing

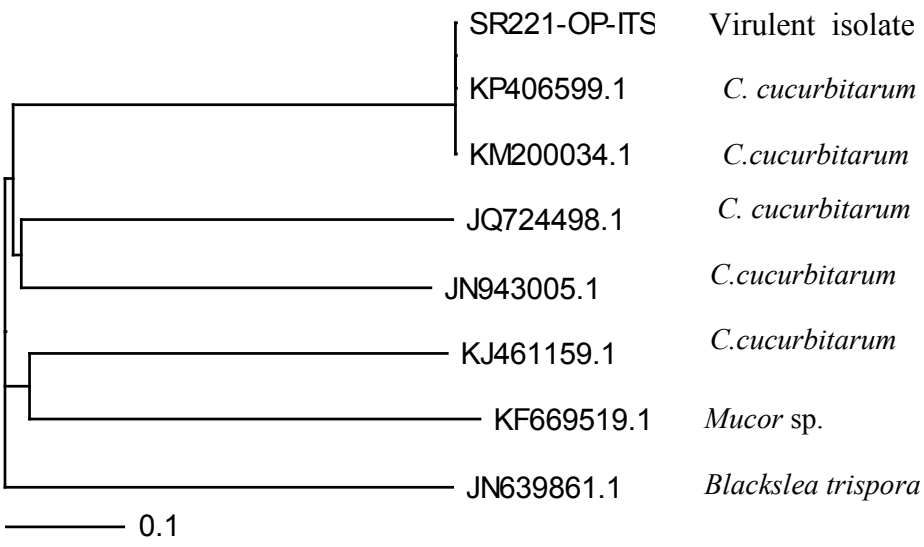


Figure 3: Phylogenetic tree generated from ITS – rDNA sequences of *Choanephora* sp. obtained from cowpea pods (virulent isolate)

Symptoms of natural incidence of Choanephora rot were observed on different crops such as bhindi (Plate 8a), bittergourd (Plate 8b), amaranthus (Plate 8c), chilli (Plate 8d) and brinjal (Plate 8e) and the pathogen, *C. cucurbitarum,* was isolated from these. The description of the symptom exhibited by various host is given in the Table (9). The isolates obtained from different host were serially numbered as C8 (bhindi), C9 (bittergourd), C10 (chilli), C11 (amaranthus) and C12 (brinjal). On bhindi, the disease was observed in immature fruits and leaves, in bittergourd on mature fruits, in chilli on both mature fruits and leaves, in amranthus on inflorescence and on brinjal on mature fruit. Water soaked lesions, wet rotting, white mycelial growth and development of sporangia were the commonly observed symptom on all the isolates, apart from this symptoms, necrosis was observed in all isolates except bittergourd and blighting were observed on amarathus inflorescence.

Natural incidence of the disease was not observed in any of the common weeds in the cowpea fields or in the adjoining area. So, artificial inoculation of the pathogen was given on the weeds growing in and around the cowpea field such as *Amarnthus viridis* L., *Cynodon dactylon* (L.) Pers, *Chromolaena odorata* (L.) King & H.E. Robins, *Boerhaavia diffusa* L. Nom. Cons and *Cyperus rotundus* L. The leaves of *B. diffusa* (Plate 8f) took up the infection while the other weeds did not did not show symptoms of disease (Table 10). In these weed, the initial symptoms appeared as water soaked lesions followed by wet rotting and necrosis. In advanced stages, from the necrotic region white mycelial growth appeared which on maturity produced sporangia which appeared as black pin head like structures.

4.4.1. Comparison of *Choanephora* Isolate from Different Hosts

The isolates C3, C8, C9, C10, C11 and C12 were grown in PDA medium for comparing the cultural, morphological and molecular characterization of the isolates. The isolates C3, C8, C9, C10, C11 and C12 grew rapidly on PDA medium and could cover the petri plates (9cm) within 24 h. All the isolates

a. Bhindi

b. Bittergourd

c.Amaranthus

d.Chilli

e. Brinjal

f.*Boerhaaviadiffusa*

Plate 8 a-f: Symptoms of infection of *C.cucurbitarum* on other vegetable hosts and weed plant

Table 9: Incidence of *Choanephora* induced rot in other vegetable crops

Host	Family	Parts affected	Lesion size (cm x cm)	Isolate No:	Nature of symptoms					
					W	R	N	WMG	S	B
Bhindi	Malvaceae	Immature fruits	3.0 x 1.2	C8	+	+	+	+	+	-
Bittergourd	Cucurbitaceae	Mature fruits	12 x 5.3	C9	+	+	-	+	+	-
Chilli	Solanaceae	Mature fruits	4 x 0.5	C10	+	+	+	+	+	-
Amaranthus	Amaranthaceae	Inflorescence	5 x 0.2	C11	+	+	-	+	+	-
Brinjal	Solanaceae	Fruit	6 x 3.2	C12	+	+	+	+	+	-

W: Water soaked lesion, R : Rotting of pods, N : Necrosis, WMG: White mycelial growth, S : Sporangial production, B : Blight

"+" symptoms present, " _" symptoms absent

Table 10: Response of common weeds of cowpea field to artificial inoculation of *C.cucurbitarum*

Name of the weeds	Family	Disease reaction
Amarnthus viridis L.	Amaranthaceae	-
Cynodon dactylon (L.) Pers	Poaceae	-
Chromolaena odorata (L.) King &H.E.Robins	Asteraceae	-
Boerhaavia diffusa L. Nom . Cons	Nyctaginaceae	+
Cyperus rotundus L.	Cyperaceae	-

+ Symptoms developed - Symptoms absent

appeared white in colour on the upper surface and yellowish in colour on the lower surface.

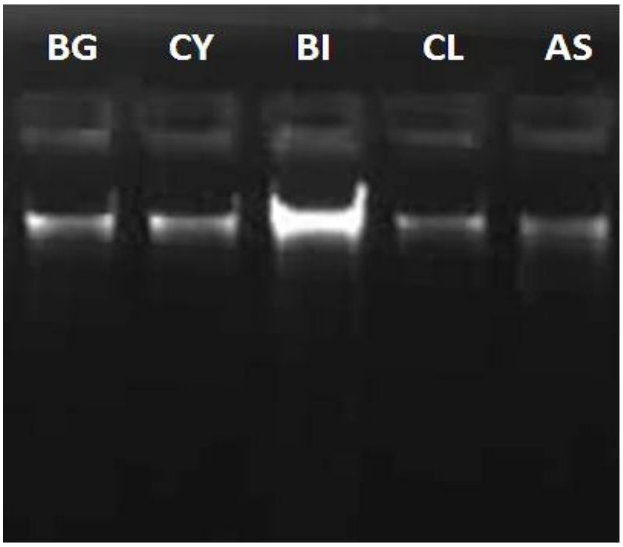
The morphological characters observed in C8, C10, C11, C13 and C14 were similar to that of C3. The mycelia of all the isolate were unbranched, hyaline and without any sepatations. The sporangiophores were non – collumellate. All the isolates produced two types asexual reproductive structures: drooping sporangia and monosporous sporangiola. The sporangia were sub-globose in shape and monosporous sporangiola were elliptic, fusiform or ovoid, striate. The sporangiophore (conidiophore) from which the monosporous sporangiola arose were long slender, branched at the apex with primary vesicle from which secondary vesicles were produced on the stalks which bears sporangiospores (conidia). The sporangiospores were elliptic, fusiform or ovoid in shape, light brown or dark brown in color. The size of sporangia, monosporous sporangiola and sporangiospores varied slightly within the isolates but were within the range described by Kwon and Jee (2005). The details of the morphological characters of the isolates are given in the Table 11.

The ITS - rDNA region of five isolates (Appendix III) of *Choanephora* sp. was sequenced for molecular characterization and identification of the pathogen. Amplification using the primers ITS 1 and ITS 4 resulted in amplicon of approximately 540 bp long (Plate 9a-b). Sequences of the isolates of *Choanephora* sp. obtained from different host were deposited in the GenBank and used for similar sequences in NCBI database using BLAST program. Alignment of the sequences of representative isolates collected for this study with other known sequences of *Choanephora* spp. obtained from the GenBank revealed that an identity of 83- 100% exist among the sequences.

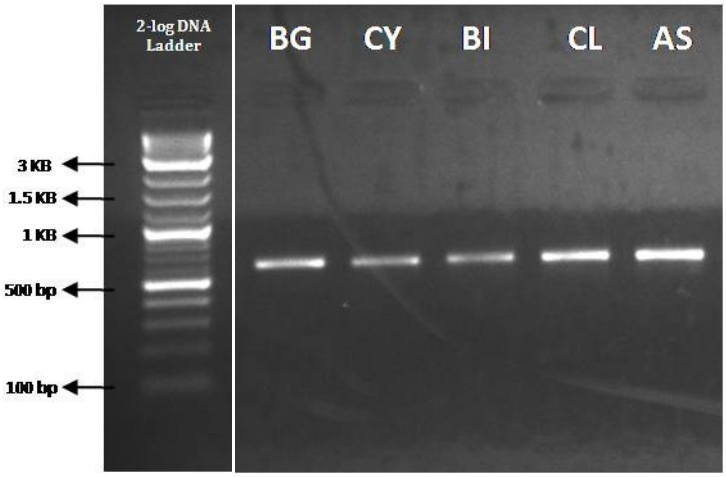
All the isolates obtained from different hosts belonged to same cluster as that of virulent isolate (KR261840) all these isolates showed 100% similarity to the isolates of *C. cucurbitarum* (KP406599.1 and KM200034.1) which was obtained from bhindi and *Phlox paniculata* reported from Korea (Figure 4).

Table 11: Morphological and cultural characteristics of *Choanephora* isolates obtained from different vegetable crops.

	Cultural characters (colony color)		Morphological characters			
	Upper view	Lower view	Colour of hyphae	Size of	Size of	Size of
Cowpea	White	Yellow	Hyaline	90.7	16.7 × 8.3	13.3 × 8.2
Bittergourd	White	Yellow	Hyaline	89.2	15.9× 8.5	13.2 × 8.0
Bhindi	White	Yellow	Hyaline	91.0	16.5 ×9.0	12.5 ×9.0
Chilli	White	Yellow	Hyaline	92.5	17.2 × 8.3	13.7 × 10.0
Amaranthus	White	Yellow	Hyaline	90.0	16.1× 8.2	13.0× 10.0
Brinjal	White	Yellow	Hyaline	91.2	15.7× 9.2	12.7× 9.2



a. DNA



b. PCR products

Plate 9 a-b: Molecular characterization of the pathogen obtained from different hosts using ITS sequencing

*BG: bittergourd, CY:chilli, BI: bhindi, CL: brinjal, AS:amranthus

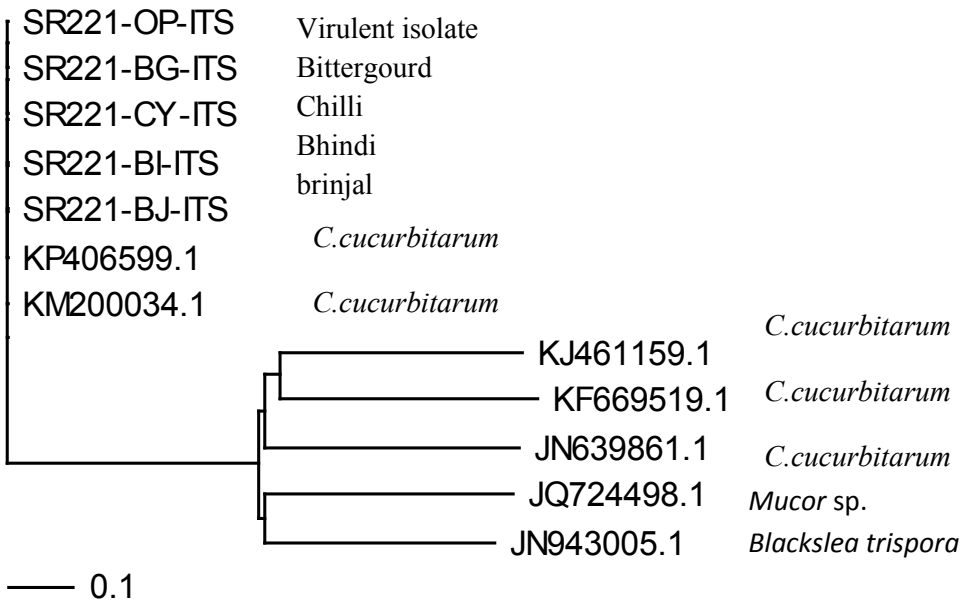


Figure 4: Phylogenetic tree generated from ITS – rDNA sequences of *Choanephora* spp. obtained from isolates obtained from different hosts

4.5. ISOLATION AND TESTING OF ANTAGONISTIC MICROORGANISMS

4.5.1. Isolation of the Antagonistic Micro flora from Healthy Cowpea Pods

The saprophytic micro flora was isolated from cowpea pods, rhizosphere soil and leaves from disease free cowpea plants among the Choanephora pod rot infected cowpea plants in the field. The details of the fungal and bacterial isolates are given in the Table 12.

4.5.2. *In vitro* evaluation of the antagonistic fungi and bacteria for suppression of the pathogen by dual culture technique

4.5.2.1. Antagonistic Fungi

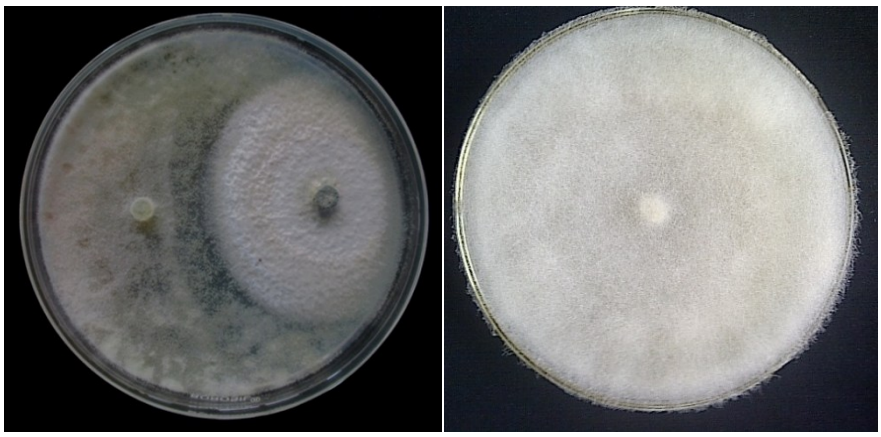
The three fungal genera: *Trichoderma* sp., *Aspergillus* sp. and *Penicillium* sp. obtained through isolation were evaluated for their antagonistic efficacy against the pathogen *C. cucurbitarum* under *in vitro* conditions. Among these, *Trichoderma* sp. was found to be most effective in controlling the pathogen giving an inhibition of 79.5% (Plate 10) and was significantly superior over the other two fungi while the antagonistic effect of *Aspergillus* sp. and *Penicillium* sp. was on par (Table 13). Hence, *Trichoderma* sp. was selected for evaluating the efficiency of disease suppression under *in vivo* conditions.

4.5.2.2. Anatgonistic Bacteria

The three bacterial isolates, *viz., Pseudomonas* sp., *Serratia* sp. and *Bacillus* sp. were evaluated for their efficacy in suppressing the growth of pathogen under *in vitro*. *Pseudomonas* sp. as found to be effective in controlling the pathogen and the percentage of inhibition was found to be 55 % (Plate 11) followed by *Serratia* sp. and *Bacillus* sp. were least effective against *C. cucurbitarum* (Table 14).

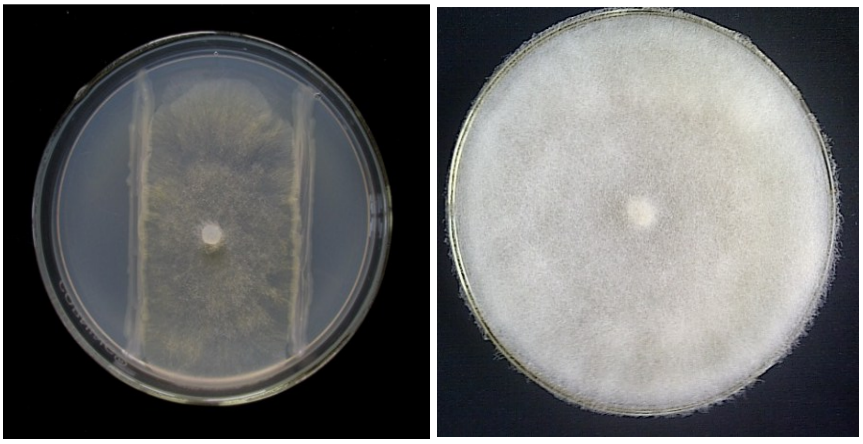
Table 12: Population of saprophytic micro flora obtained from phyllosphere, rhizosphere and fructosphere of cowpea obtained through serial dilution

Area from were isolate	Fungal population (cfu/g of soil or plant material)		Bacterial population (cfu/g of soil or plant material)	
Phyllosphere	*Aspergillus* sp.	9×10^4	*Pseudomonas* sp.	13×10^6
	Penicillium sp.	10×10^4	*Bacillus* sp.	10×10^6
Rhizosphere	*Trichoderma* sp.	19×10^4	*Pseudomonas* sp.	12×10^6
	Aspergillus sp.	8×10^4	*Serratia* sp.	22×10^6
	Penicillium sp.	8×10^4	*Bacillus* sp.	11×10^6
Fructosphere	*Trichoderma* sp.	7×10^4	*Pseudomonas* sp.	10×10^6
	Aspergillus sp.	8×10^4	*Bacillus* sp.	12×10^6



Trichoderma virens x *C. cucurbitarum* control (*C.cucurbitarum*)

Plate 10: Effect of *T. virens* on suppression of *C.cucurbitarum* under *in vitro* conditions



Pseudomonas sp. x *C.cucurbitarum* control (*C.cucurbitarum*)

Plate 11: Effect of *Pseudomonas* sp. on suppression of *C.cucurbitarum* under *in vitro* conditions

Table 13: Effect of saprophytic fungi against *C. cucurbitarum*

Treatments	Mycelial growth (cm)	Percentage inhibition *
Aspergillus sp.	8.83	0.56 (4.30)[b]
Penicillium sp.	8.60	2.82 (9.67)[b]
Trichoderma sp.	1.83	79.50 (63.15)[a]
Control	9	0.000 (0.0)[b]
CD (0.05)		(8.78)

*Mean of three replications

Values in the parenthesis are arc-transformed

Treatments with same alphabets in the superscript, do not differ significantly

Table 14: Effect of saprophytic bacteria on the mycelial growth of *C.cucurbitarum*

Treatments	Mycelial growth (cm)	Percentage disease suppression *
Pseudomonas sp.	4.00	55.00 (47.87)[a]
Bacillus sp.	8.26	4.07 (11.65)[b]
Serratia sp.	8.60	2.82 (9.67)[b]
Control	9.00	0.00(0.00)[c]
CD (0.05)		(8.22)

*Mean of three replications

Values in the parenthesis are arc-transformed

Treatments with same alphabets in the superscript, do not differ significantly

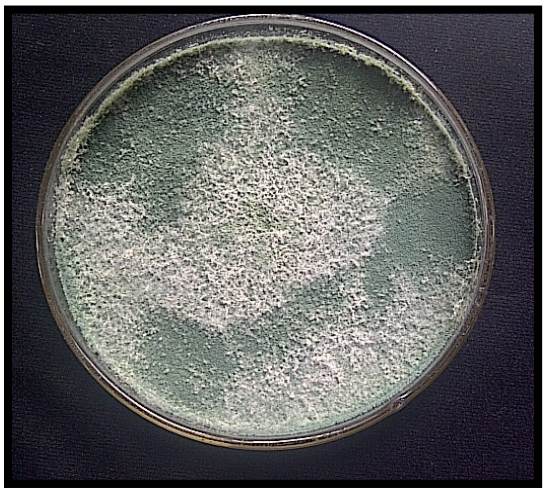
4.5.3. Identification of Fungus

The fungus was identified as *Trichoderma virens* based on the cultural (Plate 12a) morphological studies (Plate 12b) of the fungus. The fungus was fast growing, initially in the culture the colony appeared white in colour which later turns to greenish white mycelium. The conidiophores were sub- hyaline, measured 30-300 µm in length and 2.5 – 4.5 µm in diameter. The conidiophores were branched at right angles and each conidiophores terminated by a cluster of 3-6 closely appressed phialides. Conidia were ellipsoidal to ovoid 3.5 – 4.4 µm in size and dark green in color.

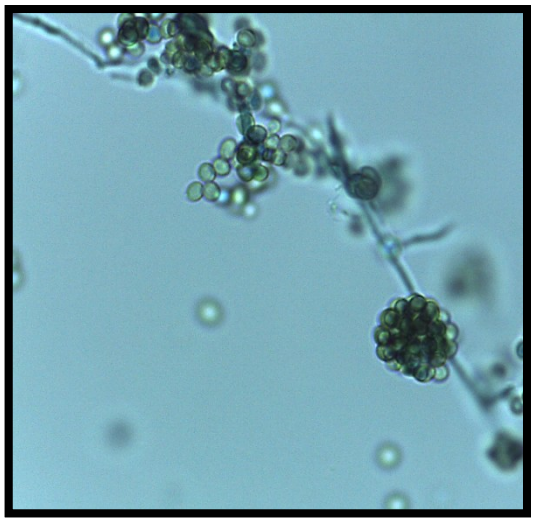
4.5.4. Identification of Bacteria Isolates

Three isolates of bacteria obtained were named as isolate 1, isolate 2 and isolate 3 (Plate 13a – 13c). The gram staining technique results revealed that isolate 1 was gram negative while isolate 2 and 3 were gram positive.

The isolates were then transferred to nutrient agar media and found that all the bacteria grow rapidly in nutrient agar. The bacterial isolate 3 appeared red in colour on nutrient agar and was identified as *Serratia* sp. When grown on King's B medium, the bacterial isolate 1 grew rapidly and the colonies were fluorescent when observed under UV Transilluminator GeNei^{TM} (Plate 13d). It was identified as *Pseudomonas fluorescens* based on the biochemical analysis carried out Cashew Export and Promotion Council of India (CEPC), Kollam. The colonies of bacterial isolate 2 showed an arborescent growth pattern on the nutrient agar medium, and smear prepared from 48 h old colonies revealed endospores when examined with oil immersion objective (100X) of compound microscope after endospore staining with malachite green. Hence, the isolate 2 was identified as *Bacillus* sp.

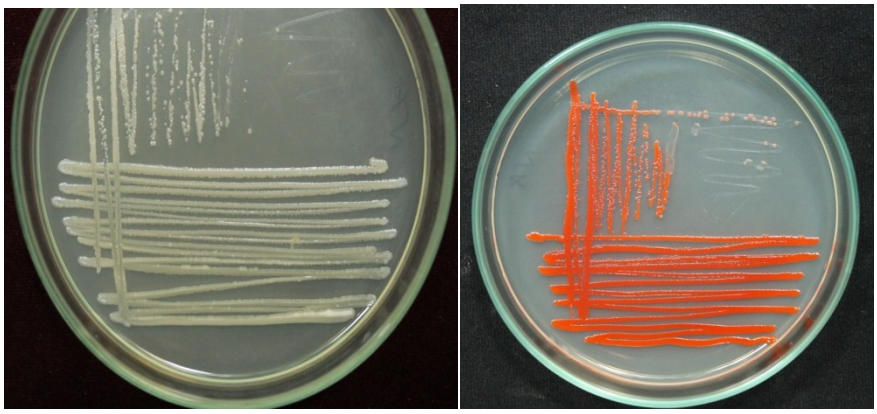


a.Mycelial growth and sporulation

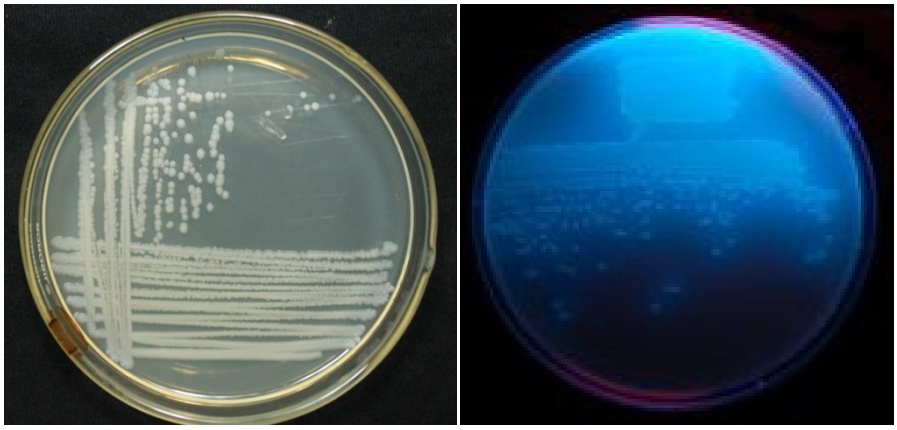


b. Phialides and gleoid mass

Plate 12a-b: Cultural and morphological characters of *Trichoderma virens*



a. *Pseudomonas* sp. b. *Serratia* sp.



c.*Bacillus* sp. d. Fluorescent pseudomonads viewed under UV light

Plate 13a-d: Colony characteristics of bacterial antagonists obtained through serial dilution

4.6. *IN VITRO* EVALUATION OF ORGANIC PREPARATIONS AGAINST *C. cucurbitarum*

Organic preparations such as panchagavya, jeevamruth, fish amino acid, vermiwash and compost tea were evaluated for its efficiency to suppress the disease caused by *C. cucurbitarum.* The treatments were tested at field concentration (5%) and at lower (2.5%), and higher (10%) concentration with autoclaving, with filtration and without filtration. The results of effect of various treatments following different method was described in Table 15 (Plate 14a-e).

The effect of organic preparations when used by different methods such as autoclaved, non- autoclaved and without filtration had varied effect on the growth of pathogen. Under *in vitro* conditions, effect of vermiwash was significantly superior than any other treatments and inhibited the growth of the *C.cucurbitarum* by 100% when used autoclaved and without filtration. Also, the effect of jeevamruth without filtration and panchagavya after filtered was on par with vermiwash.

In general autoclaving, reduced the suppressive effect of the organic preparations but panchagavya which was autoclaved inhibited the growth of *C. cucurbitarum* by 94.41% and was statistically superior while the other treatments which was autoclaved did not had any effect in suppression of *C. cucurbitarum* and was on par with the control.

The effect of filtration had varied effect on the growth of *C. cucurbitarum.* Vermiwash at three concentrations yielded 100% suppression of the *C. cucurbitarum* and was on par with effect of panchagavya at 10% while the other treatments were least effective.

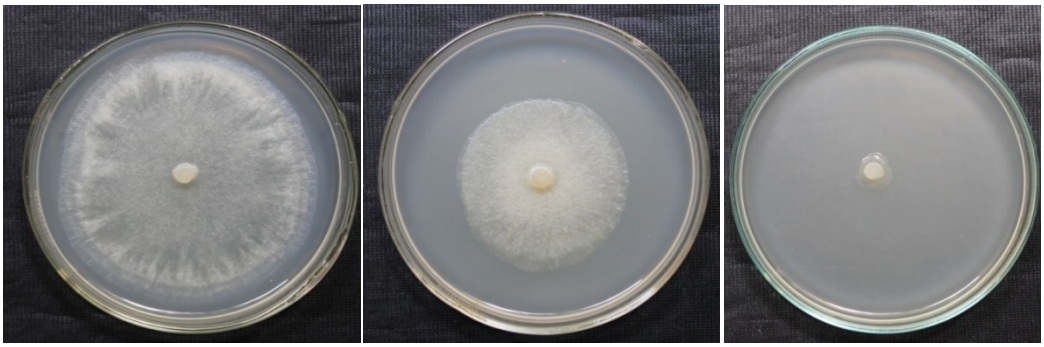
Non – filtered organic preparations generally caused less suppression of the pathogen, however vermiwash at 5% and 10% inhibited the growth of *C. cucurbitarum* completely and was on par with the effect of jeevamruth and panchagavya when used at 10% while the other treatments were less effective.

Table 15: Effect of organic preparations on mycelial suppression of *C. cucurbitarum*, the incitant of pod rot of cowpea under *in vitro* conditions

Treatments	Concentration of organic preparations								
	2.5% *			5% *			10% *		
	Method of preparation								
	A	WF	F	A	WF	F	A	WF	F
	Percentage mycelial suppression of *C.cucurbitarum*								
Panchagavya	14.86 (22.67)[a]	4.22 (11.58)[b]	0.00 (0.00)[b]	61.12 (42.86)[a]	39.95 (39.20)[b]	8.92 (17.38)[c]	94.41 (76.33)[a]	100.00 (90.00)[a]	100.00 (90.00)[a]
Fish amino acid	0.00 (0.00)[b]	0.68 (4.73)[bc]	0.00 (0.00)[b]	0.00 (0.00)[b]	21.04 (27.30)[c]	0.00 (0.00)[d]	0.00 (0.00)[c]	42.55 (40.72)[b]	4.55 (12.32)[c]
Jeevamruth	0.00 (0.00)[b]	0.00 (0.00)[c]	34.75 (36.11)[a]	0.00 (0.00)[b]	1.28 (6.48)[d]	58.60 (49.95)[b]	8.69 (17.15)[b]	8.40 (16.86)[d]	100.00 (90.00)[a]
Vermiwash	0.00 (0.00)[b]	100 (90)[a]	13.96 (21.94)[a]	0.00 (0.00)[b]	100.00 (90)[a]	100.00 (90.00)[a]	0.00 (0.00)[c]	100.00 (90.00)[a]	100.00 (90.00)[a]
Compost tea	0.00 (0.00)[b]	0.00 (0.00)[c]	0.00 (0.00)[b]	0.00 (0.00)[b]	0.37 (3.48)[d]	0.00 (0.00)[d]	0.00 (0.00)[c]	30.67 (33.62)[c]	0.00 (0.00)[d]
Control	0.00 (0.00)[b]	0.00 (0.00)[c]	0.00 (0.00)[b]	0.00 (0.00)[b]	0.00 (0.00)[d]	0.00 (0.00)[d]	0.00 (0.00)[c]	0.00 (0.00)[d]	0.00 (0.00)[d]
CD(0.05)	(2.81)	(7.86)	(13.43)	(1.102)	(7.62)	(10.90)	(11.31)	(2.09)	(7.59)

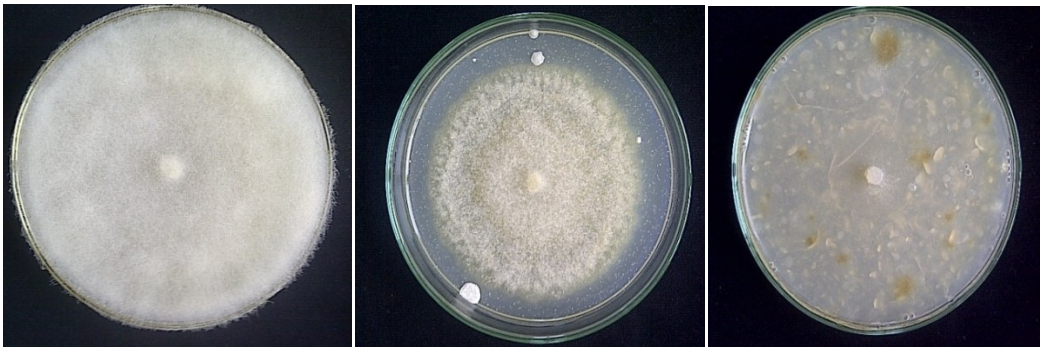
A : Autoclaved WF : Filtered , F: Fresh (Non – filtered and non – autoclaved)

Mean of three replications , Values in the parenthesis are arc- transform, treatments with same alphabets in the superscript, do not differ significantly

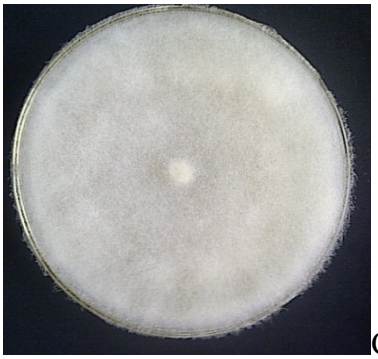


Panchagavya autoclaved @ 2.5%, 5%, 10%

Panchagavya filtered @ 2.5%, 5%, 10%



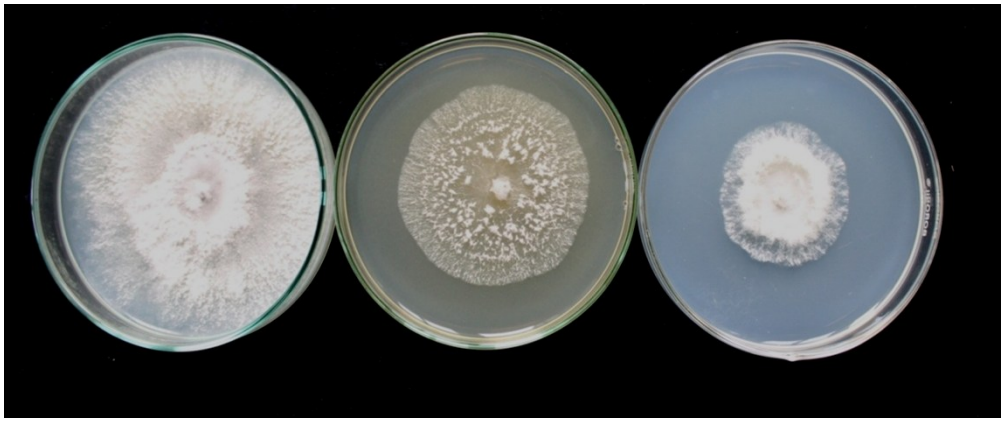
Panchagavya without filtration @ 2.5%, 5%, 10%



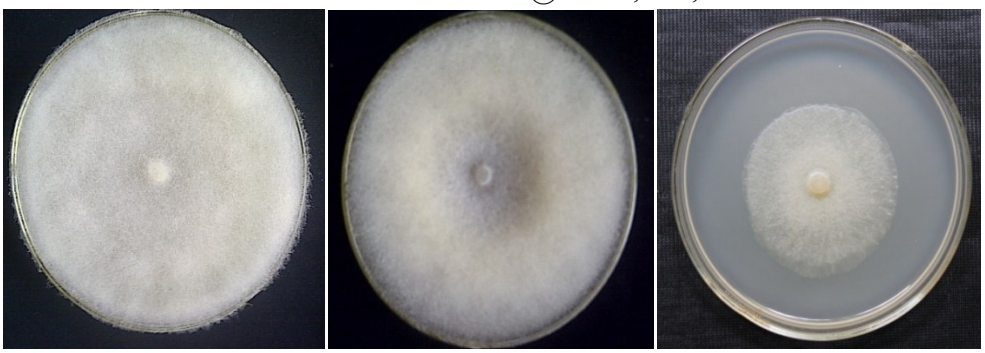
Control

Plate 14a: Effect of panchagavya on the *in vitro* suppression of *C. cucurbitarum*

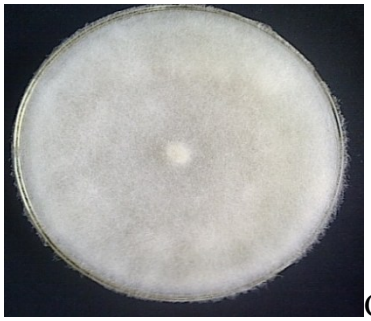
Fish amino acid autoclaved @ 2.5%, 5%, 10%



Fish amino acid filtered @ 2.5%, 5%, 10%

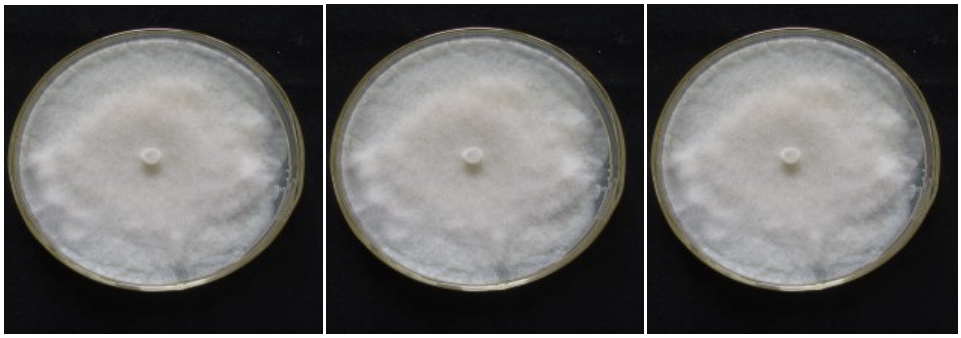


Fish amino acid without filtration @ 2.5%, 5%, 10%

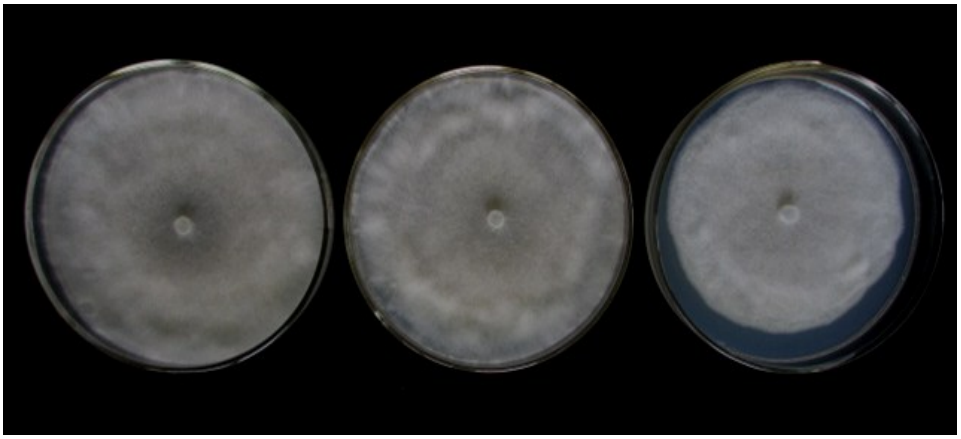


Control

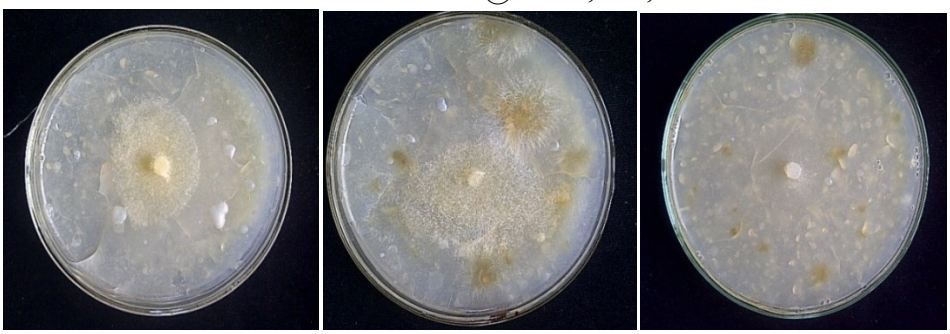
Plate 14b: Effect of fish amino acid on the *in vitro* suppression of *C. cucurbitarum*



Jeevamruth autoclaved @ 2.5%, 5%, 10%



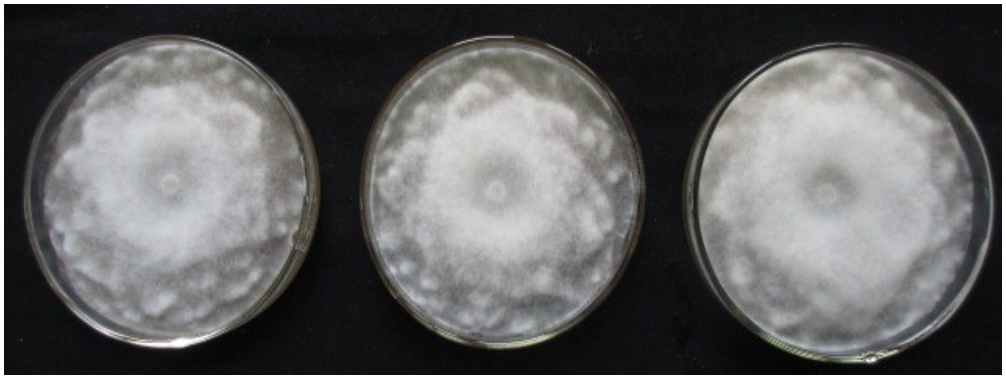
Jeevamruth filtered @ 2.5%, 5%, 10%



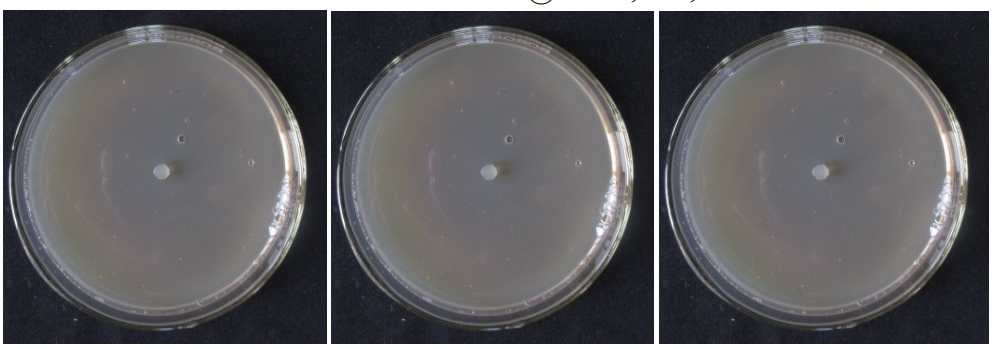
Jeevamruth without filtration @ 2.5%, 5%, 10%

Control

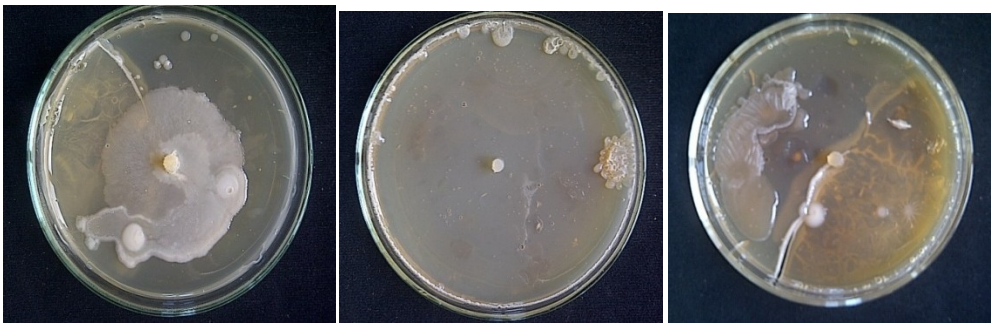
Table 14c: Effect of jeevamruth on the *in vitro* suppression of *C. cucurbitarum*



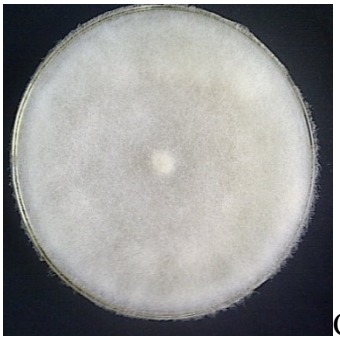
Vermiwash autoclaved @ 2.5%, 5%, 10%



Vermiwash filtered 2.5%, 5%,10%

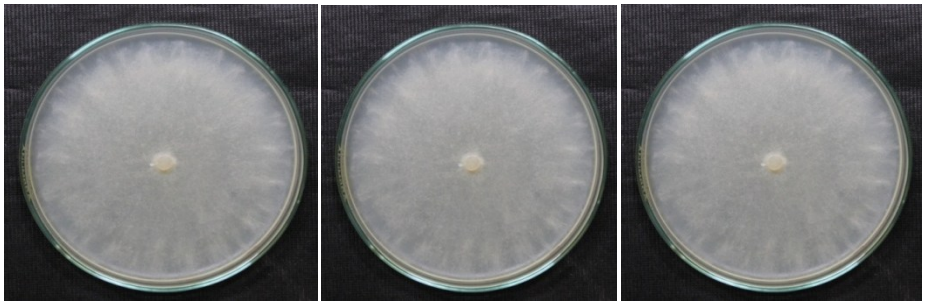


Vermiwash without filtration 2.5%, 5%, 10%

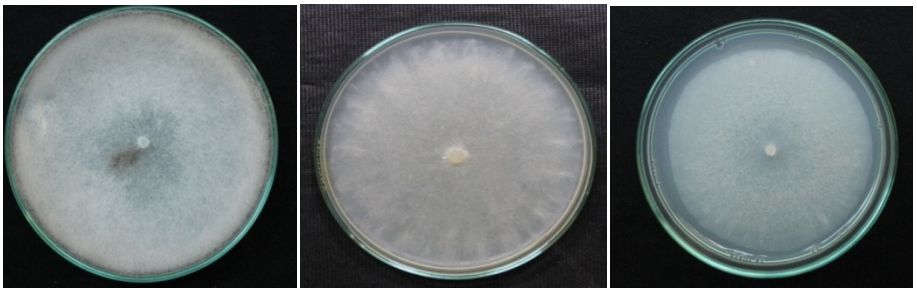


Control

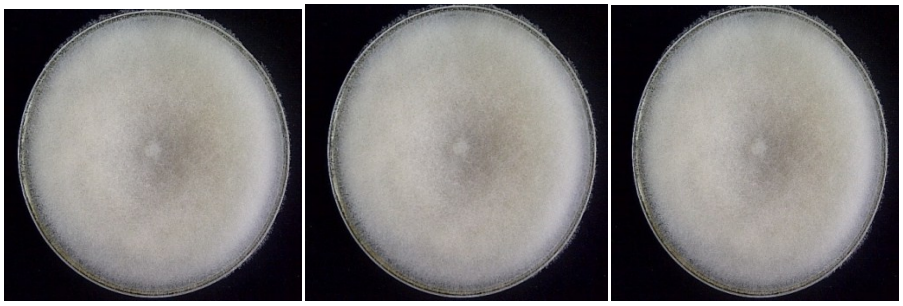
Table 14d: Effect of vermiwash on the *in vitro* suppression of *C. cucurbitarum*



Compost tea autoclaved @ 2.5%, 5%, 10%



Compost tea filtered @2.5%, 5%, 10%



Compost tea not filtered @ 2.5%, 5%, 10%

 Control

Table 14e: Effect of compost tea on the *in vitro* suppression of *C. cucurbitarum*

4.7. *IN VITRO* EVALUATION OF CHEMICALS IN SUPPRESSION OF *C.cucurbitarum*

The results (Table 16, Plate 15a- 15g) of the *in vitro* evaluation of nine fungicides by poisoned food technique revealed that six chemicals *viz*., mancozeb, copper oxy chloride, captan + hexaconazole, carboxin, carbendazim + mancozeb and propiconazole gave 100% inhibition to the growth of the pathogen under *in vitro* conditions at recommended dose and was significantly different from the effect of other chemicals. However, copper hydroxide gave 98.30% inhibition of *C. cucurbitarum* and was on par with the above treatments. Azoxystrobin and carbendazim gave 21.01% and 2.10% inhibition of the pathogen respectively and were less effective than other chemicals used for study under *in vitro* conditions.

4.8. *IN VITRO* SUPPRESSION OF POD ROT ON EXCISED PODS USING INDIGENOUS ORGANIC PREPARATIONS AND SELECTED ANTAGONISTS

The selected antagonist and the effective dose of organic preparations selected using poisoned food technique were sprayed on excised pods (Table 17, Plate 16a- 16g). Out of the treatments, application of *P. fluorescens* was found to be effective giving 100% inhibition and was significantly superior to other treatments followed by *Trichoderma virens* inhibited by 63.30%, fish amino acid (10%) by 60.40 %, vermiwash (2.5%) by 55.53% and panchagavya (10%) by 45.30%. The effect of jeevamruth (10%) and compost tea (10%) was statistically inferior to other treatments but was better than the control.

4.9. *IN VIVO* SUPPRESSION OF Choanephora POD ROT OF COWPEA USING THE SELECTED ANTAGONIST, ORGANIC PREPARATIONS AND CHEMICALS

The effect of selected antagonists, organic preparations and chemicals on the suppression of the Choanephora pod rot of cowpea was studied by pot culture experiment with 13 treatments (Plate 17). The treatments were applied as foliar

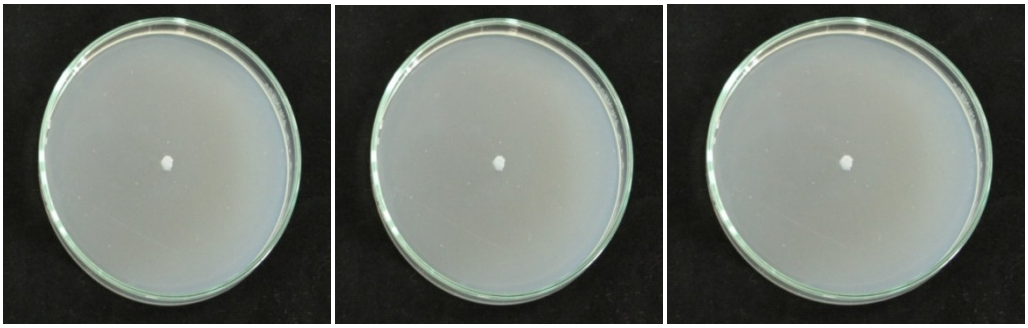
Table 16: Efficacy of fungicides on the *in vitro* suppression of *C. cucurbitarum*

Chemical name	Trade name	Lower dose*	Recommended dose*	Higher dose*
Mancozeb	Indofil M-45	100.00	100.00	100.00
Copper oxy chloride	Fyter	100.00	100.00	100.00
Captan+ hexaconazole	Taqat	70.74	100.00	100.00
Azoxystrobin	Amistar	12.90	21.01	24.00
Carbendazim	Bavistin	0.00	2.10	59.24
Carboxin	Vitavax	100.00 (90.00)[a]	100.00	100.00
Carbendazim+ mancozeb	Cosuit	92.62	100.00	100.00
Propiconazole	Tilt	100.00	100.00	100.00
Copper hydroxide	Kocide	90.86	98.30	99.61
Control		0.00	0.00	0.00
CD(0.05)		(2.09)	(4.58)	(2.93)

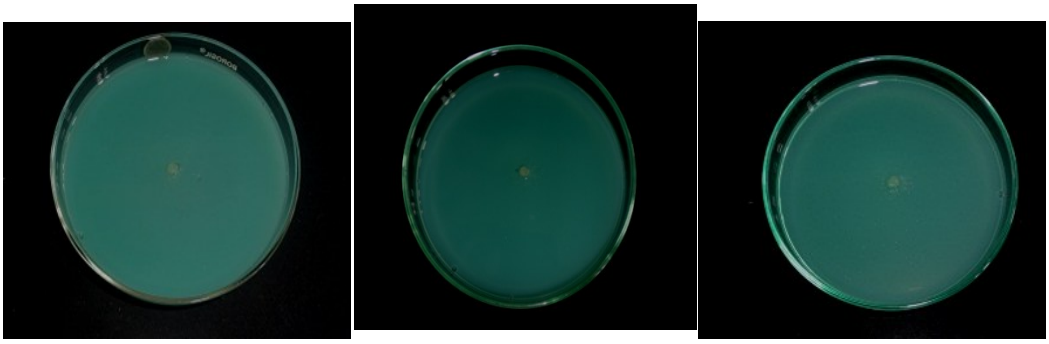
*Mean of three replications ,Values in the parenthesis are arc-transformed

** Concentrations are given in the order low dose, recommended dose and high dose.

a. Mancozeb @ 0.1, 0.2, 0.3g/100ml



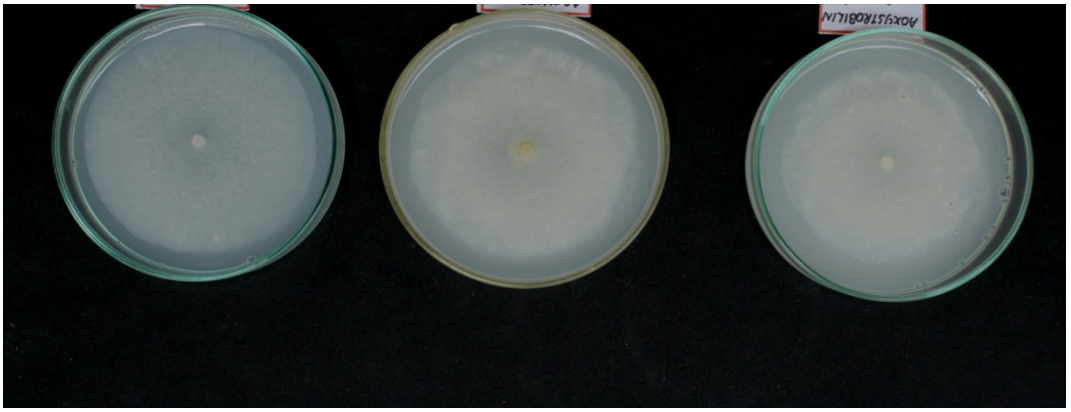
b. Propiconazole @0.05, 0.1, 0.15 m/100ml



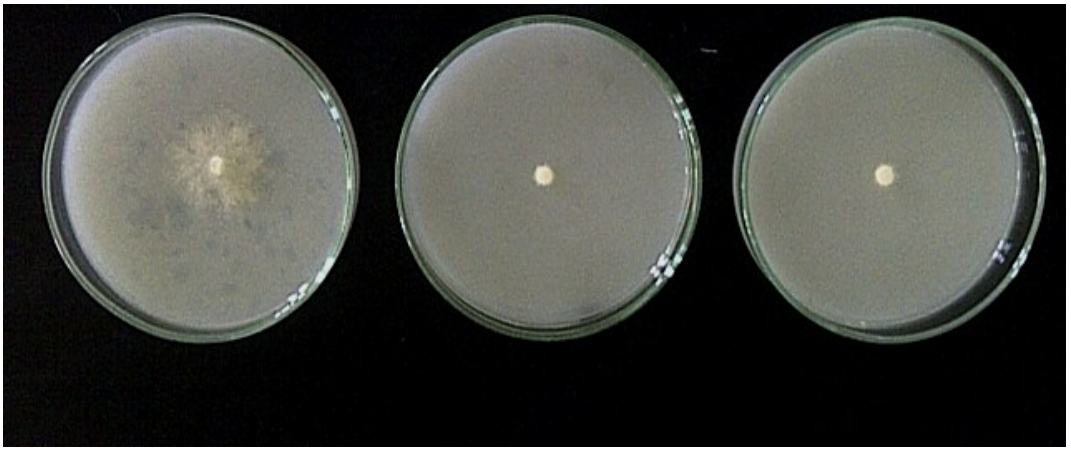
c. Copper hydroxide @ 0.1, 0.2, 0.3 g/100ml

Plate 15a-c: Effect of fungicides on the *in vitro* suppression of *C. cucurbitarum*

d. Copper oxy chloride @ 0.1, 0.2, 0.3 g/100ml

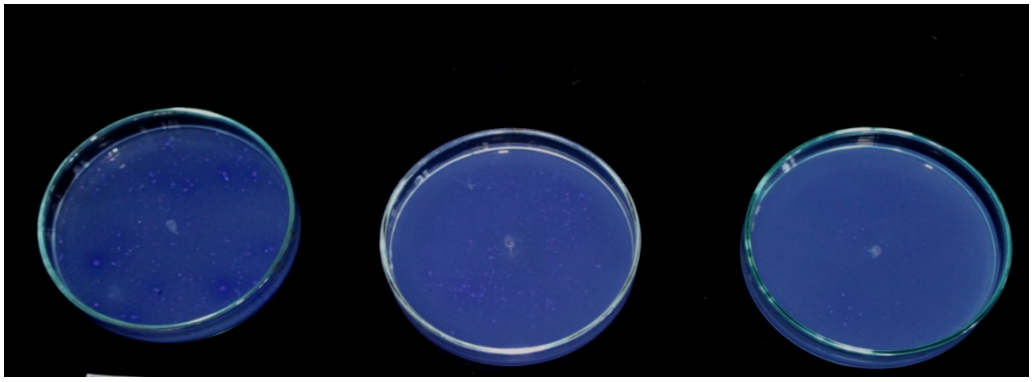


e. Azoxystrobin @ 0.05, 0.1, 0.15 ml/100ml



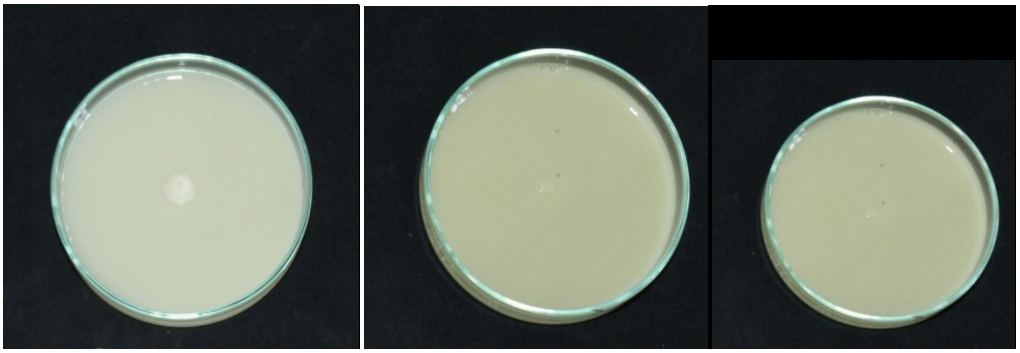
f. Taqat @ 0.15, 0.2, 0.25 g/100ml

Plate 15 d-f: Effect of fungicides on the *in vitro* suppression of *C. cucurbitarum* (continued)



g. Vitavax @0.2, 0.25, 0.30 g/100ml

h. Carbendazim @ 0.05, 0.10, 0.15 g/100ml



i. Cosuit @ 0.15, 0.2, 0.25 g/100ml

Control

Plate 15 g-i: Effect of fungicides on the *in vitro* suppression

of *C. cucurbitarum* (continued)

Table 17: Effect of selected dose of organic preparations and selected antagonists for suppression of rot caused by *C.cucurbitarum* on excised pods

Treatments	Lesion size (cm)	*Percentage disease suppression
Pseudomonas fluorescens (10^6cfu/ml)	0.00	100.00 (90.00)[a]
Trichoderma virens (10^6cfu/ml)	5.50	63.32 (52.72)[b]
Panchagavya (10%)	8.20	45.29 (42.30)[d]
Jeevamruth (10%)	9.80	34.62 (36.05)[e]
Vermiwash (2.5%)	6.66	55.54 (48.18)[c]
Fish amino acid (10%)	5.93	60.40 (51.00)[b]
Compost tea (10%)	10.73	28.37 (32.18)[f]
Control	20.00	0.000 (0.00)[g]
CD(0.05)		**(2.11)**

*Mean of three replications

Values in the parenthesis are arc-transformed

Treatments with same alphabets in the superscript , do not differ significantly

a.Compost tea (10%)

b. *Trichoderma virens* (10^6 cfu/ml)

c.panchagavya (10%)

d. *Pseudomonas* sp. (10^6 cfu/ml)

Plate 16 a-d: Effect of organic preparations and selected antagonist on *in vitro* suppression of rot on excised pods

e.Jeevamruth (10%)

f. Vermiwash (2.5%)

g. Fish amino acid (10%)

h. Control

Plate 16 e-h: Effect of organic preparations and selected antagonist on *in vitro* suppression of rot on excised pods

Plate 17: An overview of the pot culture study on suppression of pod rot of cowpea caused by *C. cucurbitarum*

spray on the plant followed by challenge inoculation of the pathogen. The observations were taken at weekly intervals for three weeks. The percentage disease index and percentage of suppression of disease were calculated based on the standard procedures.

The results (Table 18) revealed that application of all the treatments either organic preparations, biocontrol agents or chemicals were effective in suppressing the pathogen when compared to the uninoculated control. Lowest disease index was recorded in plants applied with chemicals. Among the chemicals used, the lowest disease index was 0.27 recorded by copper hydroxide (Kocide) with a percentage of disease suppression of 99.72 (Plate 18) which was followed by mancozeb and propiconazole with a disease index of 0.56 and 2.16 and the percentage of disease suppression was 99.44 and 97.83 respectively. This was statistically on par with the superior treatment copper hydroxide.

Accordingly the application of organic preparations and biocontrol agents gave good control of pathogen and was superior to the control but lower than the chemical treatment. Among the organic preparations and biocontrol agents the lowest disease index recorded was 12.63 by fish amino acid (10%) which was significantly different other treatments with the percentage of suppression of 87.33% (Plate 19) followed by the selected fungal antagonist *T. virens* with a disease index of 24.64 and the percentage of suppression was 75.27%. This treatment was on par with the disease index of selected bacterial antagonist *P.fluorescens* with 27.67, panchagavya (10%) with 27.97 and compost tea (10%) with 28.97, the percentage of disease suppression was 72.31, 71.95 and 74.87 respectively. The application of jeevamruth (10%) and the KAU released talc based *Trichoderma* formulation recorded the disease index of 46.36 and 37.63. The application of the KAU released talc based *Pseudomonas* formulations were least effective with a disease index of 60.62 but was better than the inoculated control.

Table 18: Effect of selected antagonists, indigenous organic preparations and chemicals on suppression of pod rot caused by *C.cucurbitarum* under *in vivo* conditions

Treatments	*Disease index			*Percentage disease suppression		
	Week after inoculation					
	1ST Week	2ND	3RD Week	1ST Week	2ND	3RD
Trichoderma virens (10^6cfu/ml)	0.39 (3.59)[c]	22.15 (28.08)[d]	24.64 (29.76)[d]	99.08	75.59	75.27
Pseudomonas fluorescens(10^6cfu/ml)	0.00 (0.00)[c]	19.84 (26.45)[d]	27.61 (31.70)[d]	100.00	78.14	72.31
Compost tea (10%)	11.69 (20.00)[b]	22.80 (28.52)[d]	28.97 (32.57)[d]	72.64	74.87	70.95
Panchagavya (10%)	0.00 (0.00)[c]	19.84 (26.45)[d]	27.97 (31.92)[d]	100.00	78.14	71.95
Fish amino acid (10%)	0.00 (0.00)[c]	11.20 (19.56)[e]	12.63 (20.82)[e]	100.00	87.65	87.33
Jeevamurth (10%)	0.00 (0.00)[c]	32.74 (34.90)[c]	46.36 (42.92)[c]	100.00	63.93	53.50
Copper hydroxide (0.2%)	0.00 (0.00)[c]	0.16 (4.30)[g]	0.27 (3.03)[f]	100.00	99.38	99.72
Mancozeb (0.3%)	0.00 (0.00)[c]	0.25 (2.89)[g]	0.56 (4.30)[f]	100.00	99.72	99.44
Propiconazole (0.1%)	0.00 (0.00)[c]	1.38 (12.08)[f]	2.16 (8.46)[f]	100.00	95.17	97.83
KAU released *Trichoderma*	0.00 (0.00)[c]	34.65 (36.06)[c]	37.63 (37.83)[c]	100.00	61.76	62.26
KAU released *Pseudomonas* (2%)	2.37 (8.85)[b]	50.87 (45.50)[b]	60.62 (51.13)[b]	94.45	43.95	39.20
Untreated control	0.00 (0.00)[c]	0.56 (4.30)[g]	1.63 (7.34)[f]			
Inoculated check	42.74 (40.83)[a]	90.76 (72.30)[a]	99.72 (86.96)[a]			
CD(0.05)	**16.13**	**6.41**	**6.41**			

*mean of three replications, Values in the parenthesis are arc transformed

Copper hydroxide treated plant | inoculated control | untreated control

Plate 18: Effect of copper hydroxide (0.2%) on suppression of pod rot of cowpea caused by *C.cucurbitarum*

Fish amino acid treated plants | inoculated control | untreated control

Plate 19: Effect of fish amino acid (10%) on suppression on pod rot of cowpea caused by *C.cucurbitarum*

In general, in all the treatments there was a progressive increase in development of disease over lapse of time. However, the rate of increase in disease index was negligible in case of copper hydroxide as well as with propiconazole, mancozeb and fish amino acid and *T.virens*.

On comparison of the plant height obtained from each treatment, it was found that the maximum plant height was obtained from the treatment with copper hydroxide (34.66 cm) which was on par with all other treatments such as treatment with selected antagonist fungus (31.33 cm), selected antagonist bacteria (30.0 cm), organic preparations such as compost tea (32.0 cm), panchagavya (33.3 cm), fish amino acid (32.8 cm), jeevamruth (31.33 cm), chemicals and KAU released talc based *Trichoderma* and *Pseudomonas* formulations (Table 19).

The maximum root length was observed in treatment with propiconazole (27.83 cm) which was on par with the treatment by selected antagonistic fungi (27.67 cm) while the treatments with selected bacterial antagonist, panchagavya, copper hydroxide, KAU released talc based *Trichoderma* and *Pseudomonas* formulations were on par followed by jeevamruth, compost tea and mancozeb treatments.

Comparison of the fresh root weight and dry weight, the maximum fresh root weight were obtained in the treatment with selected bacterial antagonist (17.10 g) and the maximum dry root weight were obtained in the treatment with KAU talc based *Pseudomonas* formulation and was found to be on par with other treatments.

Statistically significant values for shoot fresh weight was obtained in the plants which were treated with propiconazole (66.67 cm) and that of dry weight was noticed in plants treated with selected bacterial antagonist (4.47 cm) while all other treatments were on par with each other both in fresh and dry shoot weight.

Table 19: Effect of selected treatments on growth and nodulation of cowpea

Sl no:	*Plant height	*Root length	*Root fresh	*Root dry	*Shoot	*Shoot dry	*No: of pods	*No: of
1.	31.33	27.67^{a}	8.17^{cde}	1.33	46.67	4.37	24.67^{bcd}	22.00
2.	30	22.87^{abc}	17.10^{a}	1.17	58.93	4.47	19.33^{abcd}	22.33
3.	32	18.33^{c}	10.00^{bcd}	0.77	58.08	2.70	24.00^{cd}	22.67
4.	33.33	22.67^{abc}	11.60^{bc}	1.17	41.03	2.60	28.00^{a}	28.00
5.	32.8	19.167^{c}	6.77^{cde}	0.78	51.90	3.27	24.67^{bcd}	26.67
6.	31.33	20.33^{bc}	6.37^{de}	1.02	46.67	2.53	17.33^{bcd}	23.00
7.	34.66	21.87^{abc}	10.00^{bcd}	0.40	46.90	2.27	23.33^{d}	19.67
8.	32	19.30^{c}	8.67^{bcde}	1.23	46.67	2.67	27.00^{ab}	18.33
9.	37	27.83^{a}	13.47^{ab}	1.4	66.67	3.80	27.33^{bcd}	39.33
10.	31.66	22.60^{abc}	9.93^{bcde}	0.87	47.53	2.17	18.00^{a}	23.00
11.	33.33	21.267^{abc}	8.40^{bcde}	2.00	42.87	2.70	12.67^{abc}	19.33
12.	31.66	26.67^{ab}	11.37^{bcd}	1.77	39.77	1.73	24.67^{bcd}	28.67
13.	27.66	17.57^{c}	4.83^{e}	0.30	22.33	1.03	12.67^{d}	20.67
CD (0.05)	NS	6.59	5.16	NS	NS	NS	2.90	NS

*Mean of three replications, Values in the parenthesis are arc-transform

Treatments with same alphabets in the superscript, do not differ significantly

Maximum number of pods was obtained from the plants treated with compost tea and was on par with the treatment with KAU talc based *Pseudomonas* formulations. The maximum number of nodules was obtained in plants which were treated with propiconazole (38) and was on par with all the other treatments.

Discussion

5. DISCUSSION

Cowpea (*Vigna unguiculata* L. *Walp.*) is one of the popular and remunerative nutrient rich leguminous crops grown in Kerala. It is well adapted to cultivation in open fields and under protected cultivation. Several diseases such as wilt, root rot, and collar rot, foliar leaf diseases hamper the cultivation of the crop. Some of these diseases lead to total collapse of the plant, whereas, others cause significant loss of foliage thereby resulting in reduction of pod yield. Recently, a wet rot of pods has been noticed widely affecting the pod yield of the crop in different cowpea growing areas of Kerala. The causal organism of pod rot of cowpea was identified as *Choanephora cucurbitarum* (Berk. &Rav.) Thaxter (Bashir *et al.,*1985). High temperature and humidity aggravate the situation and result in severe yield loss (Hussein and Ziedan, 2013). Since the disease is noticed on the edible pods, biocontrol agents and ecofriendly methods are the best option to control the disease. The growing demand for organic produce also highlights the need for sustainable ecofriendly disease management strategies. With this in view, a study entitled "Choanephora pod rot of cowpea and its ecofriendly management" was conducted in the Department of Plant Pathology, College of Agriculture, Vellayani, Thiruvananthapuram during the period 2013-2015.

Choanephora pod rot incited by *C. cucurbitarum* is an emerging and serious issue faced by the cowpea growers in Kerala State. Wilson and Jose (1965) reported the incidence and etiology of this disease in cowpea (*Vigna sinensis*) in Kerala for the first time. In the present study, it was observed that the pod rot on cowpea appeared as water soaked lesions on pods, which subsequently developed into a wet rot affecting both young and mature pods. Diseased pods produced luxuriant white growth of the casual organism which on its tips bore black pin headed structures called sporangia. In advanced stages, complete rotting of the pods was observed. Similar symptoms were described by Singh and Allen (1979) in lamb's tail pod rot of cowpea caused by *C. cucurbitarum.*

High humidity and temperature are the two important pre disposing factors for the development of fungal disease. In the present study, it was observed that pod rot was severe when the temperature was 25°C - 30°C and the humidity was high, which was in accordance with the observations of Abel - Mortal *et al.* (2010). They reported that floral rot of Egyptian henbane incited by *C. cucurbitarum* occurred when humidity was high and the temperature was between 20°C - 30°C.

At the pod formation stage cowpea is attacked by a number of pests. Apart from the direct damage caused by feeding, the incidence of the pests also pre disposes the pod to diseases. Cutherbert and Ferry (1975) observed that the *Choanephora* sp. caused the rotting of cowpea pods through feeding damage and oviposition punctures made by the insect, cowpea cucurlio (*Chalcoderis aevious*). During the course of present study of field symptoms of pod rot, it was observed that the disease was severe in pods infested by the pod borer (*Maruca testulasis*) which was in tune with the observations by Irvine (1957) and Karel and Matary (1983).

C. cucurbitarum was found to cause infection mainly on pods of cowpea, even though the other parts of the plant such as leaves, stem, peduncle and flowers were also attacked to varying extent. In the present observations, the symptoms on the leaves could be seen as water soaked lesions which under dry conditions became necrotic and developed sporangiophores bearing pin-head like sporangia containing sporangiospores. On the stem, peduncle and flowers, the initial symptoms appeared as small water soaked lesions which later coalesced causing wet rot of the affected parts. In the advanced stages, white mycelial growth developed from the affected portion which at the tip bore black pin headed structures or sporangia containing spores inside. Similar symptoms of water soaking and fruiting body development on the affected tissues were noticed by Jose and Wilson (1965) and Turkensteen (1979).

Seven isolates of the pod rot pathogen obtained from the pods, leaves, peduncle, stem and flowers were sequentially numbered from C1 to C7. Pathogenicity studies were carried out with the isolates obtained from the cowpea pods after pure culture, following Koch's postulates. All the isolates produced symptoms of water soaking and wet rotting within 24 h of inoculation on healthy cowpea pods. Johnson *et al.* (2014) observed the development of symptoms of soft rot and decay in cowpea pods after five days of artificial inoculation. However, Yasmin and Siddique (1988) observed that in maize variety 'Shaheen' when inoculated with *C. cucurbitarum*, the development of water soaking and sporangial production appeared only one week after inoculation. The isolates obtained from cowpea pods showed variability in time taken for symptom expression as well as for the onset of sporulation. The isolate C3, isolated from cowpea pods, which took the least time (72 h) for complete rotting of the pods was selected as the most virulent isolate.

The virulent isolate C3 grew rapidly on PDA medium and covered the entire petri plate within 24 h. Initially, the colony appeared white which on maturity produced black pin heads. On the basis of microscopic examination, the morphological features of the isolate were found identical to that of *C. cucurbitarum* and observed to produce two types of asexual fruiting bodies *i.e.,* drooping multisporous sporangia and slender branched monosporous sporangiola. The monosporous sporangiola were 12-20 ×6-14 µm in size and the sporangia were sub-globose which measured 90.75-110 µm in size. The sporangiospores were ovoid, light brown and measured 16-22 × 8.23 -10 µm. These observations were in tune with the descriptions of *C. cucurbitarum*, the incitant of floral rot of Egyptian henbane by Abel- Mortal *et al.* (2010) and Kirk (1984) in cowpea. The isolates were further confirmed as *Choanephora cucurbitarum* (Berk and Ravenel) Thaxt. by the Fungal Identification Service, Mycology and Plant Pathology Group, Agharkar Research Institute, Pune, India (Accession no: NFCCI-3461).

The identity of the species of the pathogen was further confirmed through ITS - rDNA sequence analyses. The internal transcribed spacer region of rDNA is the most used target sequence in the molecular detection of fungi and is most employed marker used to infer taxonomy in lower fungi (Bruns, 2001). The ITS - rDNA sequencing of the virulent isolate of *C. cucurbitarum* showed maximum similarity (100%) with that of *C. cucurbitarum* (KP726891.1 and KP726889.1) reported from Korea on bhindi and *Phlox paniculata*, respectively. The sequence analysis supported the morphological identification of the isolate. The identity of the isolate was further confirmed by depositing the sequences at GenBank of NCBI and obtained an accession number KR261840. The morphological and genetical characterization of the pathogen causing rot of ice plant (*Mesembryanthemum crystallinum*) was studied by Kagiwada *et al.* (2010) and confirmed as *C. cucurbitarum.*

C. cucurbitarum is a facultative saprobe belonging to subdivision Zygomycotina with a wide host range. Choanephora pod rot of cowpea is an emerging threat to the cultivation of the cowpea. Similar symptoms are also widespread in several other vegetable crops grown in the State. The attempt to study the host range of the pathogen revealed that bhindi, bittergourd, amaranthus, chilli and brinjal were affected. Symptoms of rotting were visible in different plant parts. This agrees with the findings of Kacharek *et al.* (2003) who reported the incidence of *Choanephora* induced rots in other vegetable families such as Cucurbitaceae, Solanaceae and several other crops including cereals and millets (Yasmin and Siddique, 1988). Johnson *et al.* (2014) observed that *C. cucurbitarum* was pathogenic to *Abelmoschus esculentus, Amaranthus* sp.*, Cucumis sativus* and *Vigna unguiculata.* The morphological and genetic studies of *C. cucurbitarum* isolates from different vegetable crops showed conformity with that of virulent isolate causing pod rot of cowpea.

Exploration of antagonistic saprophytic microorganisms from different plant parts and their exploitation for management of plant pathogens is a relevant approach in view of the greater awareness of pollution free environment. An

attempt was made to isolate and screen antagonistic bacteria and fungi from the rhizosphere, phyllosphere and fructosphere for suppression of *C. cucurbitarum. Trichoderma virens* and *Pseudomonas fluorescens,* well known and proven candidates for biocontrol agent emerged as the effective antagonists under *in vitro* conditions for suppressing the mycelial growth and sporulation of the test pathogen. *T. virens* is a soil saprophyte known to suppress diverse soil borne plant pathogens (Kausar, 2014). Similarly, the Abou-Aly *et al.* (2015) observed that *P. fluorescens* isolated from the rhizosphere of the plants could be used as a biocontrol agent against some soil borne fungi and the mechanism of action include competition, parasitism, antibiosis and induction of host resistance.

Indigenous organic preparations like panchagavya, vermiwash, compost tea, fish amino acid and jeevamruth have also shown promise in management of diseases (Chadha *et al.,* 2012). Incorporation of this preparation into the media has found to suppress the mycelial growth and sporulation of several fungal pathogen. In the present study, effectiveness of organic preparations such as panchagavya, jeevamruth, fish amino acid, vermiwash and compost tea were tested at different concentration by poisoned food technique at three different concentrations 2.5%, 5% and 10%. Amendment of media was also done with autoclaved and filtered organic preparations. Fresh vermiwash (5% and 10%), Jeevamruth (10%) and Panchagavya (10%) when incorporated to media without sterilization or filtration gave complete inhibition of the pathogen. The suppression could be attributable to the presence and activity of antagonistic microorganisms present in the organic preparations. Vermiwash after bacteria proof filtration at 2.5%, 5% and 10% concentration significantly suppressed *C.cucurbitarum* and its effect was on par with the effect of panchagavya at 10%. Panchagavya (10%) after autoclaving gave 94.4% suppression of the pathogen. Patil (2009) observed that application of vermiwash and panchagavya, gave the best results, among the indigenous technology knowledge tested, in reducing the sorghum rust severity and increasing the grain yield.

Fungicides form an integral part of management of diseases of crop plants. When incorporated into media at varying concentrations, fungicides have shown inhibition of pathogens. An attempt was made with nine available fungicides to evaluate its efficiency in inhibition of the mycelial growth and sporulation of *C. cucurbitarum* under *in vitro* conditions. The study revealed that mancozeb, copper oxy chloride, propiconazole, carboxin, captan+ hexaconazole and carbendazim + mancozeb gave 100% inhibition to the growth of the pathogen at recommended dose and was significantly superior to the effect of other chemicals. Chahal and Grover (1974) and Jana *et al.* (2011) obtained similar results when mancozeb and copper oxy chloride were tested against *C. cucurbitarum.* The effect of propiconazole was contradictory to the results obtained by Northover and Zhou (2002), who observed only 20-60% control of *Rhizopus stolonifer*, the zygomycetous pathogen causing soft rot disease of peaches. The relevance of the combination fungicides containing carbendazim and mancozeb in controlling several fungal pathogens have been highlighted by several workers like Yadav and Anandi (2013) in controlling the growth of *Fusarium oxysporum* f.sp. *ciceri,* pathogen causing fusarial wilt of cowpea, Kumar and Solanki (2014) in managing blossom blight and anthracnose of mango. Copper hydroxide also gave 98.30% inhibition of *C. cucurbitarum* and was on par with the above treatments. Carbendazim and azoxystrobin were less effective in controlling the pathogen under *in vitro* conditions which was in accordance with the observations by Jana *et al.* (2011) and Sreeja (2014).

Observations on the application of effective dose of selected antagonists and organic preparations on excised pods revealed that among the selected antagonist *P.fluorescens* suppressed the disease completely. Among the organic preparations, fish amino acid suppressed the disease to 60.46% and was significantly superior to the other organic treatments which were in tune with the observations made by Anees (2014). While working on the management of Pythium stem rot disease of cowpea, he observed that plants sprayed with fish amino acid (5%) recorded the lowest disease incidence of 9.5%.

It was envisaged to develop management strategy for bringing down the severity of pod rot by ecologically safe, environmentally viable and adoptable technology. In order to achieve this, the selected antagonists, effective fungicides mancozeb (0.3%), copper hydroxide (0.2%) and propiconazole (0.1%) and organic preparations such as panchagavya (10%), fish amino acid (10%), jeevamruth (10%), compost tea (10%) and vermiwash (2.5%) were applied prophylactically at the pod formation stage followed by the challenge inoculation of the pathogen. The plants sprayed with fungicides showed least disease index. Among the fungicides evaluated, copper hydroxide (0.2%) showed maximum suppression of the disease (Figure 5). Similar reports were given by Musmade *et al.* (2013) in retarding growth of *Fusarium moniliforme* causing stalk rot of maize when applied at 0.25%. The application of mancozeb and propiconazole also caused statistically significant reduction in disease. Similar results were obtained by Gondal *et al.* (2012) and Gohel (2012) in controlling *Alternaria solani* causing early blight of tomato and *Alternaria alternata* causing leaf spot and fruit rot disease of chilli.

The application of biocontrol agents also caused reduction in disease index. Foliar spraying of *T. virens* suppressed the disease to 75.27% (Figure 6). This was adjudged the best among the biocontrol agents tested. *Trichoderma* spp. had received considerable attention as potential biocontrol agents against a wide range of soil borne plant pathogenic fungi (Chet, 1987). The ability of *Trichoderma* spp. to control plant diseases was attributed mainly to their direct effects on pathogens, competition and parasitism and to produce antibiotics (Chet *et al*., 1998; Harman & Kubicek, 1998; Howell, 1998) as well as through induction of systemic resistance (Harman, 2006).

Use of organic liquid preparations is an age old practice in India intended to provide cheap and effective options to the farmers in the country. The organic preparations applied on cowpea plants at the pod bearing stage showed effective suppression of pod rot. Among these preparations, fish amino acid caused the lowest disease index of 12.63 which indicated suppression of 87.33% of pod rot

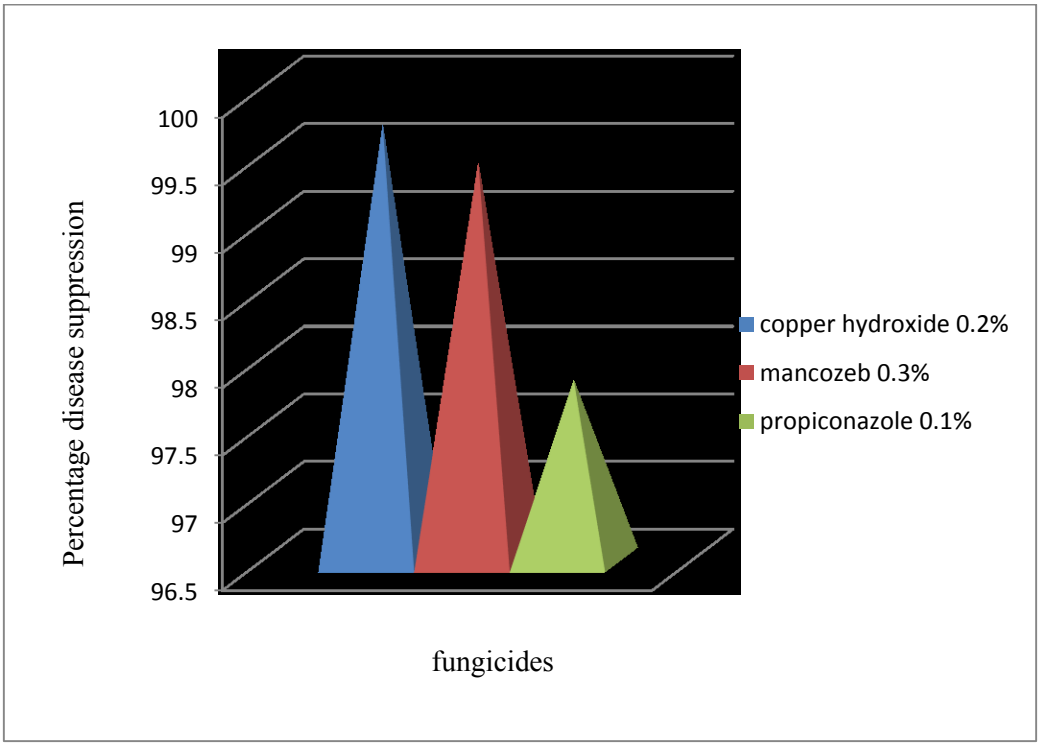


Figure 5: Effect of fungicides (at recommended dose) on suppression of pod rot of cowpea caused by *C.cucurbitarum*

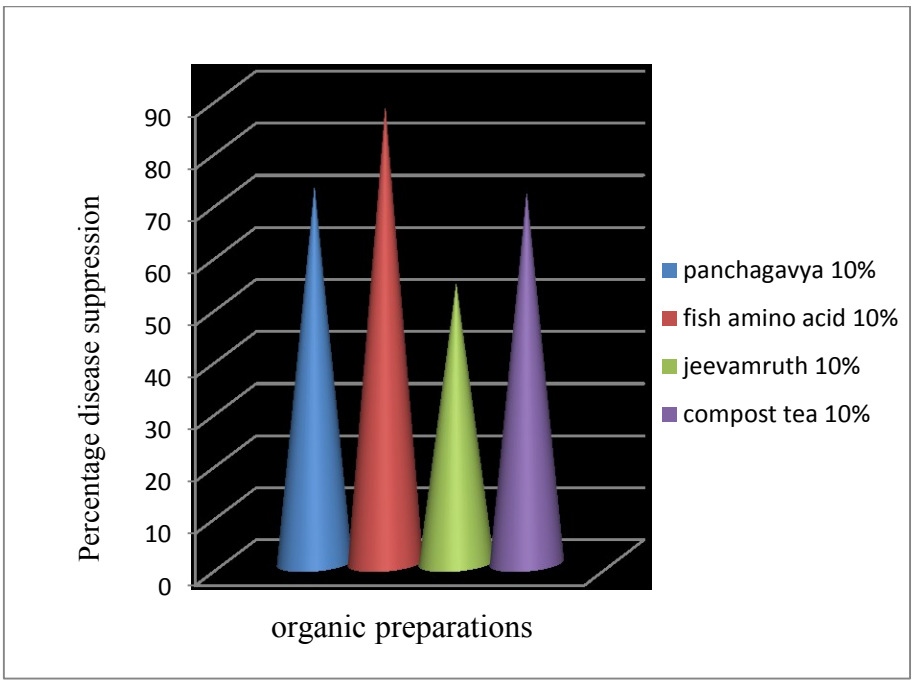


Figure 6: Effect of foliar application of organic preparations at 10% on suppression of Choanephora pod rot of cowpea

(Figure 7). Liquid ferments from animal products or fish that contained basic constituents such as amino acids, sugars, fatty acids macro and micronutrients in available form providing nourishment to the plants which in turn flourished with excellent growth, flowering, fruiting and resistance to several diseases (Nene, 2012). Panchagavya (10%), compost tea (10%) and jeevamruth (10%) suppressed the disease by 71.9, 74.87 and 53.53 per cent respectively. The observations were in accordance with the observations by Chadha *et al.* (2012), who concluded that the application of panchagavya, compost tea and jeevamruth were effective in enchancing the productivity of crop as well as in suppressing the growth of various pathogens. Foliar spray of 20 per cent jeevamruth was found very effective in managing damping off disease in papaya var. Red Lady and plant survival was recorded up to 88.33 per cent in comparison to 36.67 per cent in untreated seedlings (Rai *et al.*, 2014).

The maximum root length was observed on plants sprayed with propiconazole and root fresh weight on plants sprayed with *P. fluorescens*. Maximum number of pods was observed on plants sprayed with panchagavya. Panchagavya, jeevamruth, *etc.* are prepared from cow products (dung, urine, milk, ghee and curd) parts contains N fixers, P solublizers, biocontrol agents and are well known to protect plants from diseases, and also provide growth stimulants and other beneficial substances to the plants (Nene, 2012).

Choanephora pod rot sets in during the fleshy vegetable stage as well as during the later periods when pods are maintained for seed purpose. The management of rot can be approached from two different angles. The cowpea pods during the period of harvest for vegetable purpose, in order to manage the diseases at acceptable levels, prophylactic spraying of fish amino acid (10%) at the podding stage appear to be a viable choice. This also provides a solution for managing the disease in organically grown vegetable cowpea.

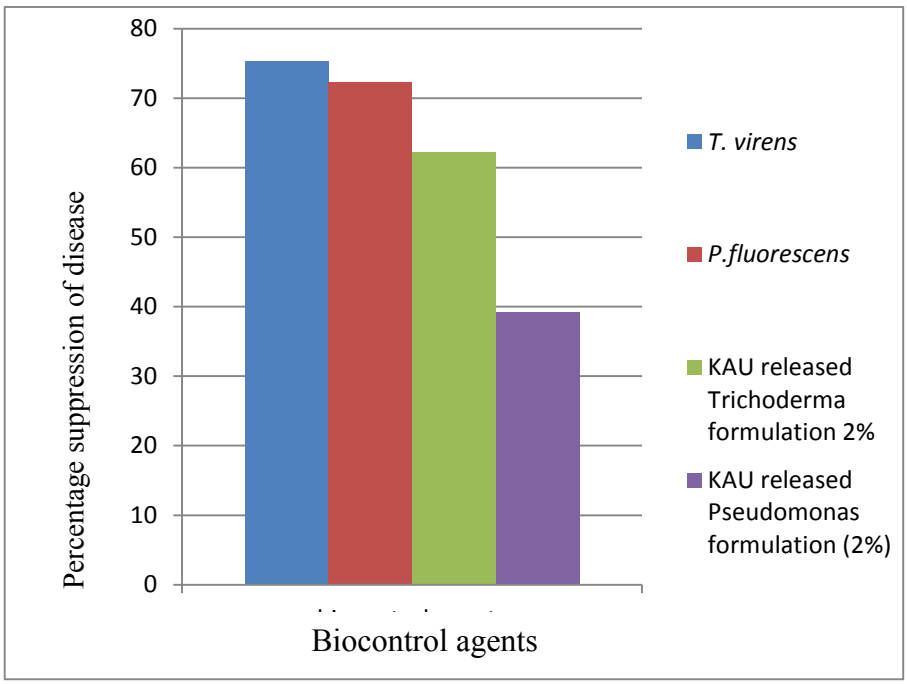


Figure 7: Effect of biocontrol agents on disease suppression of pod rot of cowpea caused by *C. cucurbitarum*

Prophylactic spraying fungicides like copper hydroxide (0.2%), mancozeb (0.3%) and propiconazole (0.1%) offer immense scope to bring down the pod rot to significantly lower levels. This aims at protecting the interest of the farmers by assured seed yield in terms of quality and quantity. Fungicides like copper hydroxide that are active against a wide spectrum of plant pathogens, but not against biocontrol agents, afford an opportunity for the integration of chemical fungicides with biological agents. Valarmathi *et al*. (2013) opined that apart from effective management of bacterial and fungal diseases by copper hydroxide (Kocide 3000), the compatibility with bacterial and fungal biocontrol agents enhances its scope in the agro ecosystem with the minimal residual effect.

Summary

6. SUMMARY

Pod rot of cowpea caused by *Choanephora cucurbitarum* (Berk. &Ravenel) Thaxt. is an emerging disease affecting the cultivation of the crop in Kerala. The present study entitled "Choanephora pod rot of cowpea and its ecofriendly management" was undertaken at College of Agriculture, Vellayani during 2013-2015 with an objective to study the associated symptoms, pathogen and host range and to locate and integrate suitable cultural, biological and chemical methods to manage the disease in a sustainable way.

The onset and development of Choanephora pod rot of cowpea under field conditions revealed that the disease occurred throughout the year. The disease initiated as small water soaked lesions which further coalesced together forming the wet rotting of the pods. White mycelial growth was visible on the affected parts from which during later stages of infection, black pin headed sporangia arose in large numbers giving a lamb's tail like appearance. The symptom expression on different parts of cowpea plant such as pods, pedicel and flowers were also studied.

Seven isolates of *Choanephora* sp. were isolated from the diseased pods, stem, leaves and peduncle of cowpea collected from College of Agriculture, Vellayani and coded C1 – C4. Potato dextrose agar medium was selected as the best media for the growth and sporulation of the pathogen.

Pathogenicity of the isolates obtained from pods (C1- C4) was proven by following Koch's postulates and the most virulent isolate was selected based on the time taken by each isolate to cause complete rotting of the pods after artificial inoculation on fresh pods. The time taken for complete rotting of pods varied among the isolates. C3 isolate took the least time (72 h) for complete rotting of the pods, and was thus identified as the most virulent isolate and was used for the further studies.

The cultural and morphological characteristics of the fungus showed conformity with those described for *C. cucurbitarum* by Krik (1984). Hence, this was tentatively identified as *Choanephora cucurbitarum.* It was further confirmed as *Choanephora cucurbitarum* (Berk. &Ravenel) Thaxt. by the Fungal Identification Service, Mycology and Plant Pathology Group, Agharkar Research Institute, Pune, India (Accession no: NFCCI-3461). The molecular characterization of the pathogen (C3 isolate) through ITS sequencing of rDNA revealed that the pathogen showed 100% similarity with the *Choanephora cucurbitarum* isolates from Korea reported to cause Choanephora blight on *Phlox paniculata* and bhindi.

Apart from cowpea, Choanephora induced rots were also observed on other vegetable crops such as bhindi, cucurbits, chilli, amaranthus and brinjal. The morphological, cultural and molecular characterization of the pathogen isolated from different hosts showed identity with the virulent isolate C3. Natural incidence of the disease was not observed on any of the weeds in and around the cowpea fields, and on artificial inoculation of the pathogen on common weed flora, only *Boerhaavia diffusa* L. Noms. Cons took up infection.

Two saprophytic bacteria isolated from the rhizosphere and fructosphere and one fungus isolated from the fructosphere were found to be efficient in suppressing of the mycelial growth of the pathogen *in vitro*. The most effective fungal and bacterial antagonists were selected. The bacterium was identified as *Pseudomonas fluorescens* based on cultural and biochemical characters. The selected antagonistic fungus was identified as *Trichoderma virens* based on cultural and morphological characters. *P. fluorescens* gave 55% and *T. virens* gave 79.5% inhibition of mycelial growth of the pathogen on potato dextrose agar medium.

Organic preparations like panchagavya, jeevamruth, compost tea and fish amino acid were prepared and their effectiveness in suppression of mycelial growth of *C. cucurbitarum* on PDA under *in vitro* conditions was checked at

different concentrations. Fresh, autoclaved or filtered through bacteria proof filters organic preparations were incorporated into melted PDA at ambient temperature. In general, autoclaving reduced the suppressive effect of the organic preparations, however panchagavya inhibited the growth of *C. cucurbitarum* significantly (94.41%). The effect of filtration had varied effect on the growth of *C. cucurbitarum*. Vermiwash at three different concentrations and panchagavya at 10% completely inhibited the pathogen. Non-filtered organic preparations generally caused less suppression of the pathogen, but vermiwash at 5% and 10%, jeevamruth and panchagavya when used at 10% inhibited the growth of *C. cucurbitarum* completely under *in vitro* conditions.

Bioassay of fungicides such as mancozeb, copper oxy chloride, captan + hexaconazole, carboxin, carbendazim + mancozeb, propiconazole, copper hydroxide, azoxystrobin and carbendazim for suppression of *C. cucurbitarum* showed that all the fungicides, except copper hydroxide, azoxystrobin and carbendazim at recommended doses, completely inhibited the growth of the pathogen while copper hydroxide (0.2%), carbendazim (0.1%) and azoxystrobin (0.1%) inhibited the growth upto 98.30%, 54.24% and 24.00%, respectively under *in vitro* conditions. The mycelial inhibition resulting from copper hydroxide incorporation in the medium was statistically on par with the superior treatments.

Application of effective dose of organic preparations on excised pods revealed that panchagavya (10%) caused maximum suppression of pod rot (60.64%). Among the selected antagonistic microorganisms isolated from rhizosphere, phyllosphere and fructosphere, *P. fluorescens* when applied on excised pods followed by the challenge inoculation of the *C. cucurbitarum* gave 100% suppression of the disease.

A pot culture experiment was conducted to evaluate the efficiency of organic preparations, biocontrol agents and fungicides for management of pod rot of cowpea, revealed that all the treatment were effective in suppression of disease. Fungicide sprayed plants showed the least disease index. Among the fungicides,

application of copper hydroxide showed 99.72% maximum suppression of the disease. The fungicides mancozeb and propiconazole also showed 99.38% and 97.83% suppression of disease, respectively. Among the biocontrol agents, the selected biocontrol agent *T. virens* suppressed the disease to 75.27%. Fish amino acid suppressed the disease by 87.33% and was considered as the best treatment among the organic preparations tested.

Thus this study clearly establishes the involvement of *Choanephora cucurbitarum* (Berk. & Ravenel) Thaxt. with the pod rot of cowpea and depicted the stages of infection as well as the morpho-molecular features of the pathogen. The *in vitro* studies highlight the effectiveness of *P. fluorescens* and *T. virens* and help to fix the effective dose of organic preparations and fungicides for suppression of pathogen. The pot culture studies indicate that fungicides like copper hydroxide (0.2%), mancozeb (0.3%) and propiconazole (0.1%), organic preparations such as fish amino acid (10%) or biocontrol agents like *T. virens* (10^6cfu/ml) could be exploited to manage the disease by prophylactic application, at the pod formation stage.

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Appendix

APPENDIX-I

COMPOSITION OF MEDIA USED

1. Potato Dextrose Agar

Peeled and sliced potatoes	- 200 g
Dextrose ($C_6H_{12}O_6$)	- 20 g
Agar-agar	- 20 g
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 to the mixture. The volume was made upto 1000 ml with distilled water and medium was sterilized at 15 psi and 121 ºC for 15 min.

2. Potato Sucrose Agar

Peeled and sliced potatoes	- 200 g
Sucrose	- 20 g
Agar-agar	- 20 g
Distilled water	- 1000 ml

3. Czapek-Dox Agar

$NaNO_3$	- 2 g
K_2HPO_4	- 1 g
$Mg(SO_4).7H_2O$	- 0.5 g
KCl	- 0.5 g
$FeSO_4$	- 0.1 g
Sucrose	- 30g
Agar	- 20 g

Distilled water - 1000 ml

4. Martin's Rose Bengal agar

Dextrose - 10 g

Peptone - 5 g

KH_2PO_4 - 1 g

$MgSO_4$. $7H_2O$ - 0.5 g

Rose Bengal - 33 mg/l

Agar - 20 g

Distilled water - 1000 ml

5. **Oat Meal Agar**

Oats - 30 g

Agar - 20 g

Distilled water - 1000 ml

6. **Carrot Agar**

Carrot - 20 g

Agar - 20 g

Distilled Water - 1000 ml

7. **Corn Meal Agar**

Corn flakes - 60 g

Agar - 20 g

Distilled Water -1000 ml

8. Malt extract agar

Malt extract - 20 g

Agar - 20 g

Distilled water - 1000 ml

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 in 3ml glycerol.

2. Crystal violet

One volume saturated alcohol solution of crystal violet in four volumes of one per cent aqueous ammonium oxalate.

3. Gram's iodine

Iodine crystals	- 1.0 g
Potassium iodide	- 2.0 g
Distilled water	- 300 ml

4. Safranin

Ten ml saturated solution of safranin in 100 ml distilled water.

5. Malachite green

Malachite green	- 5.0 g
Distilled water	- 100 ml

APPENDIX III

ITS - SEQUENCE OF *Choanephora spp.* OBTAINED FROM VARIOUS HOSTS

Virulent isolate obtained from cowpea

AAGGATCATTAAATAAATAAGAAGTATTTCGGGCTTGTCCTGATCTACTATCTATTTT
ACTGTGAACTGTTTTATTTCATGGCGTTTGAGGGATGTTCTTGTGCTATATGGGTAGGC
ATGAGGAATGTTAACCGAGCTATGGTCAAGCTTAGGCTTGGTACCCTGTTTATATACT
TTCAATTGATCAGATTATAAAATGTAACATAGGTAGTAATATCTATAAAACAACTTTT
AACAATGGATCTCTTGGCTTTTGCATCGATGAAGAACGTAGCAAATTGCGATAACTAG
TGTGAATTGCAAATTCAGTGAATCATCGAGTCTTTGAACGCATCTTGCGCTCATTGGT
ATTCCAGTGAGCACGCCTGTTTCAGTATCAAAAACAACCCTCATTCAAAATTTTTTTTT
GAATGGTCATGAAGGAAGCTAGCAATGGCGACCTTTTAAATTGAGTAAGGCCTGAAT
CTGTTCATCTAGCCTGAACTTTTTTTTAATATAAAGGAAAGCTCTTGCGACTTGGACTT
TGTTGGGGCCTCCCAAATAAAACTCTTTCATCTTGATCTGAAATCAGGTGGGACTACC
CGCTGAACTTAA

Bittergourd

AAGGATCATTAAATAAATAAGAAGTATTTCGGGCTTGTCCTGATCTACTATCTATTTT
ACTGTGAACTGTTTTATTTCATGGCGTTTGAGGGATGTTCTTGTGCTATATGGGTAGGC
ATGAGGAATGTTAACCGAGCTATGGTCAAGCTTAGGCTTGGTACCCTGTTTATATACT
TTCAATTGATCAGATTATAAAATGTAACATAGGTAGTAATATCTATAAAACAACTTTT
AACAATGGATCTCTTGGCTTTTGCATCGATGAAGAACGTAGCAAATTGCGATAACTAG
TGTGAATTGCAAATTCAGTGAATCATCGAGTCTTTGAACGCATCTTGCGCTCATTGGT
ATTCCAGTGAGCACGCCTGTTTCAGTATCAAAAACAACCCTCATTCAAAATTTTTTTTT
GAATGGTCATGAAGGAAGCTAGCAATGGCGACCTTTTAAATTGAGTAAGGCCTGAAT
CTGTTCATCTAGCCTGAACTTTTTTTTAATATAAAGGAAAGCTCTTGCGACTTGGACTT
TGTTGGGGCCTCCCAAATAAAACTCTTTCATCTTGATCTGAAATCAGGTGGGACTACC
CGCTGAACTTAA

Chilli

AAGGATCATTAAATAAATAAGAAGTATTTCGGGCTTGTCCTGATCTACTATCTATTTT
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GAATGGTCATGAAGGAAGCTAGCAATGGCGACCTTTTAAATTGAGTAAGGCCTGAAT
CTGTTCATCTAGCCTGAACTTTTTTTTAATATAAAGGAAAGCTCTTGCGACTTGGACTT
TGTTGGGGCCTCCCAAATAAAACTCTTTCATCTTGATCTGAAATCAGGTGGGACTACC
CGCTGAACTTAA

Bhindi

AAGGATCATTAAATAAATAAGAAGTATTTCGGGCTTGTCCTGATCTACTATCTATTTT
ACTGTGAACTGTTTTATTTCATGGCGTTTGAGGGATGTTCTTGTGCTATATGGGTAGGC
ATGAGGAATGTTAACCGAGCTATGGTCAAGCTTAGGCTTGGTACCCTGTTTATATACT
TTCAATTGATCAGATTATAAAATGTAACATAGGTAGTAATATCTATAAAACAACTTTT
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CTGTTCATCTAGCCTGAACTTTTTTTTAATATAAAGGAAAGCTCTTGCGACTTGGACTT
TGTTGGGGCCTCCCAAATAAAACTCTTTCATCTTGATCTGAAATCAGGTGGGACTACC
CGCTGAACTTAA

Brinjal

AAGGATCATTAAATAAATAAGAAGTATTTCGGGCTTGTCCTGATCTACTATCTATTTT
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TTCAATTGATCAGATTATAAAATGTAACATAGGTAGTAATATCTATAAAACAACTTTT
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GAATGGTCATGAAGGAAGCTAGCAATGGCGACCTTTTAAATTGAGTAAGGCCTGAAT
CTGTTCATCTAGCCTGAACTTTTTTTTAATATAAAGGAAAGCTCTTGCGACTTGGACTT
TGTTGGGGCCTCCCAAATAAAACTCTTTCATCTTGATCTGAAATCAGGTGGGACTACC
CGCTGAACTTAA

Amarathus

AAGGATCATTAAATAAATAAGAAGTATTTCGGGCTTGTCCTGATCTACTATCTATTTT
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TTCAATTGATCAGATTATAAAATGTAACATAGGTAGTAATATCTATAAAACAACTTTT
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ATTCCAGTGAGCACGCCTGTTTCAGTATCAAAAACAACCCTCATTCAAAATTTTTTTTT
GAATGGTCATGAAGGAAGCTAGCAATGGCGACCTTTTAAATTGAGTAAGGCCTGAAT
CTGTTCATCTAGCCTGAACTTTTTTTTAATATAAAGGAAAGCTCTTGCGACTTGGACTT
TGTTGGGGCCTCCCAAATAAAACTCTTTCATCTTGATCTGAAATCAGGTGGGACTACC
CGCTGAACTTAA

Abstract

Choanephora Pod Rot of Cowpea and its Ecofriendly Management

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ABSTRACT OF THE THESIS
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ABSTRACT

The study entitled "Choanephora pod rot of cowpea and its ecofriendly management" was undertaken in the Department of Plant Pathology, College of Agriculture, Vellayani during 2013-2015 with the objective to study the symtomatology, etiology and to develop an ecofriendly management strategy for Choanephora pod rot of cowpea.

The pathogen causing the pod rot of cowpea was isolated from the diseased pods, leaves, peduncle, stem and flowers. Pathogenicity was proven following Koch's postulates, virulence rating was done and the C3 isolate obtained from cowpea pods were found to be the most virulent isolate. Based on cultural and morphological characters, the pathogen was identified as the *Choanephora cucurbitarum* (Berk. &Ravenel) Thaxt., which was further confirmed by ITS sequencing.

The study revealed that the *C. cucurbitarum* had wide host range encompassing other vegetable crops such as bhindi, cucurbits, chilli, amaranthus and brinjal. Natural incidence of the disease was not observed on any weeds in and around the cowpea fields. However, on artificial inoculation of the pathogen, the spreading hogweed (*Boerhaavia diffusa* L. Noms. Cons) took up infection.

The dual culture technique to study the antagonism of the saprophytic mycoflora isolated from the rhizosphere and fructosphere through serial dilution and plating indicated that *Trichoderma virens* and *Pseudomonas fluorescens* exhibited 79.50% and 55% percentage inhibition of the pathogen.

The effectiveness of organic preparations such as panchagavya, jeevamruth, fish amino acid, vermiwash and compost tea was tested at different concentrations by poisoned food technique. Amendment of media was also done with autoclaved and filtered organic preparations. Vermiwash at 2.5%, 5% and 10% filtered as well as incorporated to media as such, significantly suppressed *C.cucurbitarum.* Jeevamruth (10%) and Panchagavya (10%) gave complete

inhibition of the pathogen. Panchagavya (10%) incorporated after filtration and autoclaving also gave 100% and 94.4% suppression of the pathogen respectively.

The application of effective dose of organic preparations on excised pods showed that panchagavya (10%) caused maximum suppression of pod rot (60.64%). Among the biocontrol agents, the selected bacterial antagonist *i.e., P. fluorescens* showed complete suppression of the disease when applied on the excised pods.

A pot culture study was conducted to evaluate the efficiency of organic preparations, biocontrol agents and fungicides. The maximum disease suppression of 99.72% was observed in plants sprayed with copper hydroxide. The organic preparation, fish amino acid suppressed the disease to 87.33%. Application of effective biocontrol agent *T. virens* yielded 75.27% suppression of pod rot.

The ecofriendly management of the disease can be achieved by application of fish amino acid (10%) or *T. virens* (10^6cfu/ml) or fungicides such as copper hydroxide (0.2%), mancozeb (0.3%) and propiconazole (0.1%) during the pod formation stage.

സംഗ്രഹം

വെള്ളായണി കാർഷിക കോളേജിലെ സസ്യരോഗ വിഭാഗത്തിൽ, 2013 മുതൽ 2015 വരെ, വള്ളിപ്പയറിലെ കായ്ചീയൽ രോഗത്തിന്റെ ലക്ഷണങ്ങളും, കാരണങ്ങളും, അവയെ നിയന്ത്രിക്കാൻ മാർഗ്ഗങ്ങൾ അവലംബിക്കുക എന്ന ലക്ഷ്യത്തോടുകൂടി കൊയിനിഫോറ പോട് റൊട്ട് ഓഫ് കൗപീ ആൻഡ് ഇറ്റ്സ് ഇക്കോഫ്രണ്ട്ലി മാനേജ്മെന്റ് എന്ന് വിഷയത്തിൽ പഠനം നടത്തി.

വള്ളിപയർ കായ്ചീയൽ രോഗം ഉണ്ടാക്കുന്ന കുമിളിനെ രോഗബാധയേറ്റ കായ്, ഇല, പൂവ് തുടങ്ങിയ ഭാഗങ്ങളിൽ നിന്നും വേർതിരിച്ച് എടുത്തു. കോച്ച് പോസ്റ്റുലേറ്റ് വഴി കുമിളിന്റെ രോഗം വരുത്തുവാനുള്ള കഴിവിനെ തെളിയിക്കുകയും, സി 3 എന്ന ഐസൊലേറ്റ് കടുത്ത രോഗബാധ ഉണ്ടാക്കുന്നതായും കണ്ടെത്തി. കൃത്രിമ മാധ്യമങ്ങളിൽ വളർത്തി എടുത്ത രോഗഹേതുവായ കുമിളിന്റെ വളർച്ചാപരമായും, രൂപപരമായുമുള്ള സ്വഭാവങ്ങളുടെ അടിസ്ഥാനത്തിൽ ഈ കുമിൾ കൊയിനിഫോറ കുക്കർബിറ്റാറം ആണ് എന്ന് കണ്ടെത്തി. ജനിതക പഠനത്തിലൂടെ ഈ കുമിൾ കൊയിനിഫോറ കുക്കർബിറ്റാറം ആണ് എന്ന് സ്ഥിരീകരിച്ചു.

പയറു കൂടാതെ മറ്റു പച്ചക്കറി ഇനങ്ങളായ വെണ്ട, വെള്ളരി, മുളക്, ചീര, വഴുതന മുതലായവയിലും ഈ കുമിൾ രോഗം ഉണ്ടാക്കുന്നതായി ഈ പഠനത്തിലൂടെ കണ്ടെത്താൻ കഴിഞ്ഞു. പാടത്തിനു ചുറ്റുമുള്ള കളകളിൽ സ്വാഭാവികമായ രോഗലക്ഷണം കണ്ടെത്തിയില്ല.

ഈ കുമിളിന്റെ വളർച്ചയെ നിയന്ത്രിക്കുന്ന കുമിളുകൾ ചെടിയുടെ വേരിനോടുള്ള മണ്ണിൽ നിന്നും, കായിൽ നിന്നും വേർതിരിച്ച് എടുത്തു. ഈ കുമിളുകളോടൊപ്പം രോഗഹേതുവായ കുമിളിനെയും വളർത്തിയതിൽ ട്രൊക്കോഡർമ്മയും, സ്യൂഡോമൊണാസ് ഫ്ളൂറസെൻസും യഥാക്രമം 79.59-തും, 55 ശതമാനം രോഗഹേതുവായ കുമിളിനെ നിയന്ത്രിക്കുന്നതായി കണ്ടെത്തി.

ജൈവ കൂട്ടുകളായ പഞ്ചഗവ്യം, ജീവാമൃതം, മത്തി ശർക്കര മിശ്രിതം, വെർമിവാഷ്, കമ്പോസ്റ്റ് എന്നിവ ഉപയോഗിച്ച് രോഗത്തെ നിയന്ത്രിക്കാൻ നടത്തിയ പരീക്ഷണങ്ങളിൽ പഞ്ചഗവ്യം (10%), വെർമിവാഷ് (2.5%, 5%, 10%), ജീവാമൃത് (10%) എന്നിവ കൊയിനിഫോറയുടെ വളർച്ചയെ നിയന്ത്രിക്കുന്നതായി കണ്ടെത്തി.

ചെടികളിൽ നടത്തിയ പരീക്ഷണങ്ങളിൽ കോപ്പർ ഹൈഡ്രോക്സൈഡ് (0.2%), മത്തി ശർക്കര മിശ്രിതം,(10%) ട്രെക്കോഡർമ്മ എന്നിവ രോഗത്തെ യഥാക്രമം 99.72%, 85.33%, 75.27% നിയന്ത്രിക്കുന്നതായി ഈ പഠനത്തിൽ കണ്ടെത്തി.