MANAGEMENT OF COLLAR ROT OF COWPEA CAUSED BY *Rhizoctonia solani* Kuhn USING BIOFUMIGANTS

by

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(2015-11-022)

THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"Management of collar rot of cowpea caused by *Rhizoctonia solani* Kuhn using biofumigants"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

@	At the rate of
^{0}C	Degree Celsius
CD	Critical difference
cfu	Colony forming units
cm	Centimetre
CRD	Completely Randomised Design
DI	Disease Incidence
et al.	And other co-workers
Fig.	Figure
g	Gram
g L^{-1}	Gram Per litre
h	Hour (s)
ha	Hectare
ha^{-1}	Per hectare
i.e.	That is
KAU	Kerala Agricultural University
kg	Kilogram
kg ha^{-1}	Kilogram per hectare
km	Kilometre
L	Litre
L^{-1}	Per litre

mg	Milli gram
mL^{-1}	Per millilitre
mm	Milli metre
NS	Non –significant
No.	Number
%	Per cent
PDA	Potato Dextrose Agar
$Plant^{-1}$	Per Plant
rpm	Revolutions per minute
SE	Standard error
Sl.	Serial
sp. or spp.	Species (Singular and Plural)
viz.	Namely
v/v	Volume/ volume

Introduction

1. INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp), an important leguminous vegetable crop of Kerala, is cultivated in the uplands and in rice fallows. It can function as an emerging economy by trading the seeds and processed food products from cowpea and all the above ground parts except the pods provide a good source of animal fodder. The semi-erect bush cowpea spread over the ground as a mulch and provide a protective cover from soil erosion and also suppress weed growth. Soil fertility restoring habit make it an essential component of almost all cropping systems. The cultivation of the crop is affected by collar rot and web blight caused by *Rhizoctonia solani* Kuhn in all its growth stages which results in severe crop and yield loss.

Collar rot is an important soil borne disease causing concern to the cultivation of vegetable cowpea in Kerala (Lakshmanan *et al.*, 1979). Under congenial climatic conditions, collar rot symptom of the disease is more prevalent than web blight symptoms.

It is often difficult to control ubiquitous soil and root-inhabiting pathogens like *R. solani* that survive saprophytically in soil organic matter and exist for long periods in the absence of a host plant in the form of sclerotia, except with the elaborate and repeated use of fungicides (Upamanyu *et al.*, 2002). The detrimental effect of these chemicals as well as the huge cost involved, necessitate the disease to be managed by cheaper and environment friendly methods. The wide host range and high variability of the pathogen make it difficult to properly manage the disease through alternative means, though attempts through biological, cultural and host resistance as well as integrated packages involving different compatible strategies to manage the disease have been made by several workers. Lack of effective, economic crop protection strategies is one of the key factors limiting expansion of the organic farming in vegetables.

Biofumigation of soil is an emerging ecofriendly means of disinfecting soil. It refers to the suppression of soil-borne pests and pathogens by biocidal compounds

such as isothiocyanates (ITCs), terpenoids and other volatile compounds which are released through hydrolysis of incorporated plant tissues or seed meal extracts or oilcakes (Kirkegaard *et al.,* 1993).

This programme was intended to evaluate the efficacy of various plants/plant oils/oilcakes on the incidence and severity of collar rot of cowpea with the purpose of developing an ecofriendly strategy for managing the disease. The results, if promising, could be extended for management of other soil borne pathogens in cowpea as well as in other vegetable crops. The study was undertaken with the following objectives:

- Symptomatology of collar rot disease of cowpea.
- Isolation of the pathogen, its identification and pathogenicity testing.
- Testing the antifungal and biofumigant nature of plants, plant oils and oil cakes against *R. solani* under *in vitro* conditions.
- Evaluation of the biofumigant nature of selected plants, plant oils and oilcakes for suppression of *R. solani* under *in vitro* confined conditions.
- Testing the effect of selected plants, plant oils and oil cakes for the management of collar rot disease of cowpea under *in vivo* using pot culture experiment.
- Isolation and enumeration of microflora associated with biofumigation and testing their direct effect on pathogen suppression by *in vitro* dual culture technique.

Review of Literature

2. REVIEW OF LITERATURE

Cowpea is an important source of protein for the vegetarian population throughout the globe. In Kerala, cowpea (*Vigna unguiculata* (L.) Walp) is grown largely as a vegetable crop and is cultivated in an area of 3668 ha (FIB, 2017). Collar rot caused by the soilborne pathogen, *Rhizoctonia solani* Kuhn, is a major biotic stress affecting the economic yield of the crop which under congenial climatic conditions leads to severe crop losses.

2.1. IMPORTANCE AND YIELD LOSS

Collar rot is devastating soil borne disease, endemic to the cowpea growing tracts in Kerala. Lakshmanan *et al.* (1979) and Viswanathan and Viswambharan (1979) gave detailed account on incidence of this disease. The pathogen, *R. solani* has been reported to infect the plants at any stage of its growth. However, the infection in the younger stage was noticed to be more damaging to the crop. Sreeja (2014) while surveying the major diseases affecting the crop observed that the disease was noticed in major cowpea growing areas of Thiruvananthapuram district to the tune of 5-10 per cent. Vavilappalli (2012) recorded collar rot incidence on cowpea to the extent of 0 to 99.81 per cent and web blight disease index to the extent of 4.33 to 53.78 per cent. Vavilappalli *et al.* (2014) reported collar rot and web blight as the most important soilborne disease of Kerala especially during high humidity and temperature conditions. The highly virulent pathogen was attributed as a regular cause for crop loss and subsequent yield loss in cowpea.

In the global scenario also, the disease is of great significance in this crop. In Nigeria, severe crop loss was reported by collar rot and web blight disease of cowpea in a devastating form that caused wide spread destruction of the crop (Williams, 1975). Singh and Allen (1979) described that in Nigeria the incidence of collar rot disease in cowpea caused enormous loss of foliage and grain yield. According to

Galvez *et al.* (1989) a yield loss up to 90 per cent occurred in Central America due to web blight of beans.

Yield losses on account of *R. solani* infection in other pulse crops have also been reported. Sharma and Sohi (1980) described web blight disease of french bean caused by *R. solani* and observed that at different stages of crop growth, the green pod loss varied from 8.45 to 64.68 per cent.

Dubey and Mishra (1990) recorded yield loss of 10-60 per cent in horse gram (*Macrotyloma uniflorum*) due to incidence of web blight caused by *R. solani.* In horse gram, Dubey (1998) recorded the maximum disease incidence and intensity of the collar rot disease at a temperature of 26-28^{0}C and at a relative humidity (RH) of 90-100 per cent in the crop.

Gupta and Singh (2002) observed that in mung bean, *R. solani* induced foliar blight disease and premature defoliation at early stage of crop growth, and deformity of pods at the mature stage. They observed that the disease intensity varied from 6.66 to 75.35 per cent. Dubey (2003) reported that the pathogen caused considerable yield losses in mung bean and urd bean in India. Gupta *et al.* (2010) reported that infection by *R. solani,* causing web blight disease, reduced the grain yield of mung bean from 33.4 to 37.8 and test weight of seeds from 23.12 to 28.6 per cent. Singh *et al.* (2012) observed that in web blight disease of mung bean recorded maximum yield loss of 40.32 per cent and that severity and yield loss were positively correlated.

Sharma (1999) reported that in urd bean, the yield reduced by 30 per cent due to the incidence of foliar blight by *R. solani.* According to Santhosh and Tripathi (2013), disease severity changed in proportion to age of the crop in web blight disease of this crop. Though susceptibility to the pathogen was reported at all stages, 30-60 days old plants were found more susceptible.

Anwar *et al.* (1995) opined that the infection of *R. solani* caused severe disease in soybean and resulted in heavy yield loss. Supporting this, Fenille (2002) recorded yield loss of 31-60 per cent on soybean due to the incidence of foliar blight disease. On soybean, root rot disease by *R. solani* was found to reduce yield by 12-30 per cent (Bradley *et al.*, 2005).

R. solani infection in other crops also resulted in severe yield losses. Banville (1989) noticed that *R. solani* caused large scale destruction of potato crop by inhibition in tuber germination, killing below ground sprouts, causing stem canker, stolon canker and resulting in subsequent yield reduction. Crop losses due to sheath blight in rice and maize (Girija,1995; Rajput and Harlapur 2014) and leaf blight in amaranthus caused by *R. solani* (Nayar *et al.*, 1996; Gokulapalan *et al.*, 2000) have been recorded.

2.2. SYMPTOMATOLOGY

The symptoms of infection by *R. solani* are apparent in above ground and below ground parts of plants. Out of the two stages noticed due to *R. solani* infection in cowpea, collar rot stage noticed on lower parts of plant adjacent to soil, is more prominent and damaging as compared to the foliar blight stage, also known as web blight.

The first detailed description on symptoms of collar rot disease of cowpea from Kerala was given by Lakshmanan *et al.* (1979). According to him, collar rot symptom was noticed initially as brownish black water soaked lesions at the basal stem portion of the plant which later expanded and resulted in girdling of the basal part of the stem. On the infected leaves, initial symptoms were observed as small, circular, light greyish-brown irregular water soaked lesions. Gradually these spots coalesced and enlarged to form oblong to irregular lesions surrounded by dark brown margins.that further covered major portions of leaf lamina. Lakshmanan *et al.* (1979) also reported that under congenial conditions, superficial mycelial growth of the

pathogen along with small sclerotia was observed both at the infected collar and leaf lamina. The affected leaves completely turned yellow, leading to defoliation.

Viswanathan and Viswambharan (1979) gave a comprehensive account on collar rot symptoms of cowpea caused by *R. solani*. According to them, the first noticeable symptom was the appearance of water soaked lesions in the foliage, accompanied by decaying stem at collar region. Subsequently on the diseased areas, the enlarged lesions developed white cottony mycelial web followed by development of numerous globular creamy white sclerotia. The infection finally resulted in withering and drying of the entire plant.

Bhadrasree (2007) described the collar rot symptoms as the development of spindle or oval shaped brownish black lesions of length 0.2 to 0.8 cm and breadth 0.2 to 2.5 cm near the collar region of stem. Stem girdling at basal region was also noticed which was accompanied by yellowing and shedding of leaves. Under severe infection, the whole plant became withered as a result of decaying of the tap root as well as the lateral roots. On the basal part of the infected stem, white cottony mat was visible along with small sclerotia.

Rajan (2014) also observed that collar rot was prevalent at the seedling stage at the stem close to the soil level of cowpea and observed as brownish black lesions followed by stem girdling followed by defoliation. Poor root development and root rotting and appearance of white mycelial mat on the affected portions, often along with numerous, small sclerotia were also reported as symptoms of collar rot disease of cowpea.

Vavilapalli (2012) described the collar rot symptoms of cowpea as initial development of brownish black water soaked lesion at the collar region. Later stem girdling at the level nearer to soil which resulted in yellowing and drooping of leaves and finally rotting of the root. On the infected regions white mycelial growth of the pathogen studded with small mustard like sclerotia was observed.

R. solani infection has been reported in several pulses and leguminous crops. *Rhizoctonia* aerial blight of soybean was reported by Atkins and Lewis (1954) where initial light brown spots were noticed on leaves, petioles and young stems that later coalesced and resulted in defoliation along with rotting of the whole plant. Lambe and Dunleavy (1961) reported that Rhizoctonia stem rot and root rot disease in soybean began at collar region as large oval, reddish lesions in the lower part of stem as well as rotting of root. The other observable symptoms found associated with the disease in this crop were chlorosis, wilting, drying and premature leaf drop.

In French bean, *R. solani* resulted in pre-emergence and post emergence mortality, collar rot, stem canker and pod rot symptoms. White cottony mycelium along with sclerotial initials of the incitant was noticed on decayed stem portions at the collar region (Sharma and Sohi, 1980). High temperature coupled with high humidity favoured the development and progress of the infection in collar rot disease of groundnut, while persistence of dry climate always limited the spread of infection (Kadam *et al.*, 2011). Mishra (2011) documented the incidence of web blight disease on yard long bean, groundnut and soybean.

Rhizoctonia induced damping off of cardamom resulted initially light brown discoloration at the collar region and later the enlarged lesions accompanied by rotting and collapse of the entire seedling (Sasi, 1978). Farr *et al.* (1989) reported that in general, a variety of symptoms such as collar rot, pre- and post emergence damping-off, foot rot, root rot, sore shin, fruit rot, and foliar and web blights to be caused by this pathogen, *R. solani*. In amaranthus leaf blight, symptoms were noticed as small creamy white irregular spots on the foliage. Under congenial conditions, lesions enlarged and covered major portion of the leaf lamina, accompanied by shot–hole symptoms (Gireesh, 2016).

2.3. ETIOLOGY

2.3.1. Pathogen

Rhizoctonia solani Kuhn (teleomorph—*Thanatephorus cucumeris* (Fr.) Donk) is an economically important soilborne plant pathogen with broad host range infecting several agricultural and horticultural crops, including legumes, responsible for several diseases worldwide (Sneh *et al.*, 1966; Ogoshi, 1987 and Farr *et al.*, 1989).

R. solani was reported to cause a variety of symptoms depending upon the climatic conditions. In several leguminous plants, *R. solani* was the incitant of aerial blight of disease (Mundkur, 1935; Verma and Thapliyal, 1976). In general, *R. solani* reproduce asexually and survive by means of vegetative mycelium and sclerotia (Sneh *et al.*, 1966). Baker (1970) observed that in countries with tropical, temperate and sub polar climate, aerial diseases such as aerial blight, web blight, sheath blight, leaf blight and leaf spots, were common. Under humid tropical conditions, damping off, collar rot or root rots were observed. The perfect stage of the pathogen is a basidiomycete fungus that never formed conidia and produced only basidiospores (Agrios, 1997). The hyphae and sclerotia were produced by the imperfect stage, *R. solani*, whereas, basidiospores were formed by the perfect stage in soil (Tiwari and Khare, 2002).

The taxonomy and nomenclature of *T. cucumeris* (Frank) Donk has been discussed by Talbot (1965). He placed the genus *T. cucumeris* in the family Tulasnellaceae and order Tulasnellales. Earlier the perfect stage *T. cucumeris* was known under different names like *Hypschnus cucumeris, H. solani, Corticium solani, C. sasakii and T. particolus* (Kotila) Flintje. These synonyms which were earlier considered as distinct species had been included under *T. cucumeris* by Talbot (1970). The teleomorph or perfect stage of the *R. solani* has been described as *T.*

cucumeris (Frank) Donk (Talbot, 1970). First report on teliomorphic stage from India was given by Saksena and Chaubey (1972).

The *R. solani* incited collar rot of cowpea was reported first time in Kerala by Menon (1979) and later detailed by Lakshmanan *et al.* (1979). Manoj *et al.* (2008) conducted studies on morphological and virulence characterization of *R. solani* isolates causing sheath blight disease of rice. According to them, different isolates of *R. solani* produced two types of sclerotia; flat micro sclerotia by the slow growing isolates and round macro sclerotia by the fast growing isolates.

2.3.2. Morphology

Morphological and cultural characteristics often help to distinguish and delineate isolates of *R. solani.* The grouping of different *R. solani* isolates has been performed according to several cultural, physiological or pathological criteria (Exner, 1953; Vijayan, 1986; Ogoshi, 1987). On the basis of morphological characteristics, Kuiry *et al.* (2014) observed that *R. solani* isolates could be easily separated from *R. oryzae-sativae* isolates. The number of sclerotia, hyphal length, weight of sclerotia and mycelial growth rate are the important morphological markers for differentiation of *R. oryzae-sativae* from *R. solani* isolates.

Townsend and Willets (1954) opined that the sclerotia developed as a result of loosely woven hyphae without any definite pattern / arrangement in *R. solani*. Parmeter and Whiteny (1970) detailed those distinguishable characters of hyphae under microscopy such as, its brown colour, right angled branching, development of septa near the point of origin of hyphal branching, inclination of hyphal branches in the direction of growth, constriction nearer to branching, in young hypha presence of multinucleate cells and septal pore apparatus. They also noticed the production of barrel-shaped monilioid cells, sclerotia and hyphae having more than 5 micrometer in diameter and in general, the pathogen is highly virulent with rapid growth rate.

Dugger (1915) denoted the bending of young hyphal branches in the direction of growth of the main hyphae and hyphal constriction near to the origin of branching as characteristics of *Rhizoctonia.* Palo (1926) reported certain situations in which originally young hyphal branches were perpendicular to the main filaments, but later they were bent in the direction of growth of the main hyphae. According to Butler and Bracker (1970) the diameter of septal pore ranged from 0.1 to 0.2 µm. *R. solani* produced colourless young vegetative mycelium, which later turned to brown colour. The hyphae were divided into unique individual cells by septum having doughnut-shaped pore (Ogoshi, 1987). Rashmi *et al.* (2016) conducted detailed study on blight disease of cabbage and noted initial creamy white mycelial growth of *R. solani* and hard globular to irregular sclerotial bodies with a diameter of 1-4 mm on maturity. On the abaxial side of blighted leaves, white powdery mass of spores of the pathogen were observed. The size of basidia ranged from 7.3 to 11.1 µm X 4.4 to 6.8 µm and that of basidiospores in basidia was ranging from 4.1 – 7.9 X 3.5 – 5.9 µm.

According to Sadowski (1970) several strains of *R. solani* differed in cultural characters, ecological requirements and pathogenicity during field and laboratory experiments. Gokulapalan and Nair (1983) reported the morphological characteristics, anastomosis reaction and pathogenicity of four isolates of *R. solani.* On the basis of anastomosis, mycelial colour, growth and sclerotial size, *R. solani* isolates were categorised into four groups (Vijayan and Nair, 1985).

The concept of anastomosis has importance in the grouping of *R. solani* isolates. Different strains are characterized depending upon hyphal fusion between them. Flentje *et al.* (1970) described that the isolates of *R. solani* varied among themselves in several characters. Parmeter and Whitney (1970) reported that host range and pathogenicity varied among each anastomosis group. Hyphal anastomosis between the strains of *R. solani* attacking rice and cowpea was evident (Lakshmanan *et al.,* 1979). The anastomosis of *R. solani* isolate from cowpea with isolates from soybean, green gram, periwinkle and rice showed that those two were genetically

related (Das and Balakrishnan, 1983). In sheath blight of rice, the anastomosis pattern and diversity of *R. solani* was studied and included under AG-I group (Vijayan and Nair, 1985). Anastomosis group 1 (AG-1) of *R. solani* included the isolates which severely infect field crops such as rice, maize, and soybean causing diseases like sheath blight on rice, banded leaf sheath blight on maize, and aerial blight on soybean. AG-1 has been mentioned as a single species with a very wide host range (Pascual *et al.*, 2000). Harikrishnan and Yang, 2004 observed that 35.6 per cent of multinucleate *R. solani* isolates collected from different soybean fields belonged to AG-1group and 28 per cent grouped under AG-4. Profused sclerotia production was obtained with AG-1 at optimum temperature ranged between 25-30^{0}C. Shailbala and Tripathi (2007) noted the difference among AGs in morphology, physiology, host range, biochemical and molecular characteristics, and virulence. Considerable variability was observed between the 20 chosen isolates of different AGs in their morphological and cultural characters, growth and virulence (Sunder *et al.*, 2009).

2.3.3. Survival

Sclerotia and mycelia of *R. solani* from infected plant have been reported to colonize soil organic matter contributing to the inoculums reserves in the absence of the host plant. This enabled infection in the succeeding crops (Leach and Devey, 1938). Kotila (1947) suggested that basidiospores released in excess amount functioned as a prominent source of inoculum and dispersed the disease up to a distance of 30 meter. The airborne spores caused epidemics on upper plant part under congenial climatic conditions. Sclerotia were denoted as the common survival structures and were reported to be principally carried through soil. But basidial stage was rarely noticed in rice (Singh and Pavgi, 1969). Kaiser (1970) reported that 80 per cent of sclerotia were developed in the top 10 cm layer of the rhizosphere. Dwivedi and Saksena (1975) observed that *R. solani* developed the perfect stage with the formation of basidiospores in soil, which contributed to extended persistence and

survival of the fungus. *R. solani* could colonize saprophytically in treated soil substrates and this habit probably contributed to the failure of the disease control strategies (Dhingra *et al.*, 2004). In cowpea, the sclerotia produced on well developed lesions that eventually got detached and reached the soil contributed to the soilborne inoculum (Bhadrasree, 2007). The polyphagous fungus, *R. solani*, infecting more than 250 plant species also survived on other weeds that functioned as secondary hosts (Khandaker *et al.*, 2008). The ability of *R. solani* AG-1 IA, the causal agent of rice sheath blight, to survive in diseased rice straw and as sclerotia and mycelia was confirmed by Feng *et al.* 2016.

2.4. ECOFRIENDLY MANAGEMENT OF *R. solani*

The management of soil borne pathogens including *R. solani* is very difficult due to its long persisting and abundant inoculum levels in soil. The successful use of fungicides for the management of diseases caused by *R. solani* through soil drenching, seed treatment and foliar spray were reported by several workers. But this approach has been found to be costly and hazardous to human beings and animals. Continued use of fungicides also is reported to cause pollution of environment, development of fungicidal resistance and harmful effects on beneficial microflora. This situation has necessitated the need to reduce the dependence on chemical control means and to develop ecofriendly management strategies (Gurjar *et al.*, 2012).

Cultural, biological control methods and host resistance are considered as environment safe strategies. Soil disinfection through solarisation reduced the population of soilborne pathogens such as *R. solani* causing web blight in mung and urd beans and also improved soil fertility (Kaiser, 1970). According to Katan *et al.* (1976) soil solarization with white, transparent polyethylene sheets enhanced soil temperature by transmitting most of the solar radiation to heat up the soil. The efficacy varied with quality of the polyethylene material used for tarping the soil as well as the atmosphering temperature. Through soil solarization seedling and seed

rot disease of many crops caused by *R. solani* could be effectively managed. Gireesh (2016) reported significant reduction of leaf blight disease in amaranthus on soil solarization along with application of biocontrol agents (*Pseudomonas fluorescens*, *Trichoderma harzianum*), chemical activators (Acibenzolar-S-Methyl).

The application of biocontrol agents has been considered as a sustainable strategy in integrated disease management in several crops. Several fungal and bacterial antagonists such as *Trichoderma* spp., *P. fluorescens* and *Bacillus subtilis* have been reported to be effective in suppressing the infections caused *R. solani* on economically important crops like rice, cotton and pea (Elad *et al.*, 1982). Three isolates of *Trichoderma* spp. were tested for their *in vitro* efficacy against *R. solani*, the causal agent of sheath blight of rice and the maximum suppression was noticed with TN3. In field condition also, the particular treatment was effective by reducing disease incidence and increasing grain yield (Prasad and Kumar, 2011). Sudhakar *et al.* (2013) screened the effect of different *P. fluorescens* strains on growth promotion, drought tolerance and yield enhancement of peanut cultivar "Narayani" under green house conditions. They reported the improved drought tolerance and higher biometric traits such as plant height, shelling percent and harvest index over control on treatment with *P. fluorescens*. Chandel and Sharma (2014) evaluated the antagonistic action of five species of *Trichoderma* and two bacterial antagonists, *B. subtilis* and *P. fluorescens* for *in vitro* suppression of *R. solani*, causing stem rot disease in carnation. All the antagonists showed inhibitory action against test pathogen, but the most virulent one was *T. viride* with an inhibition of 65.08 per cent followed by *T. harzianum* (63.77 per cent). Among the two bacterial antagonists, *B. subtilis* was found better than *P. fluorescens* with a percentage inhibition of 60.50. Sriraj *et al.* (2014) studied the *in vitro* effect of bioagents on *R. solani* and recorded more than 50 per cent suppression by using native isolates of *Bacillus, Pseudomonas* and *Trichoderma* species. Elzerjawi (2015) carried out studies on antagonistic effect

of *Trichoderma* species and noticed 82.21 per cent inhibition on mycelial growth of *R. solani* with *T. harzianum*.

2.5. SOIL BIOFUMIGATION

Soil biofumigation is an emerging ecofriendly strategy for reducing the soilborne plant pathogens such as *R. solani*. The research in this direction has gained momentum with the ban imposed on the use of methyl bromide and other chemical fumigants owing to the damage caused through ozone depletion and carcinogenic effects (Prasad *et al.*, 2016).

The term biofumigation was originally coined by J. A. Kirkegaard in 1993. Biofumigation is the process of growing, macerating / incorporating certain *Brassica* or related species into the soil leading to the release of the endogenous enzyme, myrosinase, that hydrolyzed glucosinolates (GSLs) to isothiocyanate compounds (ITCs) (Kirkegaard *et al.*, 1993). Sarwar *et al.* (1998) suggested that propenyl ITC and 2-phenyl–ethyl ITC, from *Brassica* sp. suppressed soilborne pathogens such as *R. solani, F. graminearum, and Gueaumanomyces graminis tritici.* Matthiessen and Kirkegaard (2006) reported that the volatile principle, ITCs in *Brassica* sp. resembled methyl isothiocyanate, the active component of several chemical fumigants, and therefore, they were identified to be suitable alternatives to chemical fumigants. Redovnikovic *et al.* (2008) detailed GSLs as secondary metabolites with sulphur and nitrogen groups. Plants of the order Capparales and specifically in the family Brassicaceae were reported to contain GSLs. Griffiths *et al.* (2011) noticed that in addition to disease management, the incorporation of biofumigants improved soil health, its water holding capacity and organic matter content and also controlled soil erosion.

2.5.1. Biofumigation with plant, plant oils and oil cakes

The biofumigant crops, in general contained both antifungal compounds and volatile principles. Plants from Cruciferae family (mustard, cabbage, radish,

cauliflower, etc.) released large amount of these toxic chemicals capable of suppressing diseases in the soil and were considered as ideal bio-fumigants. The type and concentration of glucosinolates have been found to vary between *Brassica* species as well as between cultivars of the same species (Kirkegaard and Sarwar, 1998).

Virtance and Matikkala (1959) suggested that the presence of antifungal substances *viz*., methyl and n-propyl compounds in filtered extracts of *Allium* spp. contributed to its effective use in the management of several soilborne pathogens. Datar (1999) explored the bioefficacy of garlic (*Allium sativum*) against sorghum root rot caused by *Macrophomina phaseolina.* Chandel and Sharma (2014) reported significant suppression of *R. solani* with the seed extracts of *Melia azedarach* and plant extract of *Adhatoda vasica.* The leaf extracts of *A. sativum*, *Eucalyptus globulens,* and *Zingiber officinale* were validated as the most effective in completely suppressing mycelial growth of *R. solani* (Kane *et al.,* 2002). Burgie (2005) studied the fungicidal effect of *Brassica* sp. on mycelial growth of *R. solani* causing damping off disease of vegetables. He found that the growing medium incorporated with horse radish (3g 100 cm^{-3}) caused complete inhibition of the pathogen. The medium amended with leaf extracts of savoy cabbage, red cabbage and fringed cabbage resulted in 85 per cent suppression of the pathogen. The inhibitory effect of aqueous extracts of seven plant species such as *Calotropis gigantea, Vinca rosea, Ocimum sanctum, A. indica, Eucalyptus citriodora, A. cepa* and *Z. officinale* were evaluated under *in vitro* on *R. solani* of green gram. The treatment with *Z. officinale* (10 per cent) recorded the highest suppression (86.11 per cent) on *R. solani* (Mishra *et al.*, 2005). Dubey (2006) noticed significant *in vitro* antifungal effect of *Pongamia glabra* (10 per cent) on *R. solani*, causing web blight disease of urd bean. Out of the eleven plant species, tested against *M. phaseolina* of green gram, the maximum suppression on mycelial growth of the pathogen was noted with onion bulb extract (10%) followed by acacia, ginger, neem, garlic and karanj extracts (Tandel *et al.*,

2010). Murugapriya *et al.* (2011) conducted *in vitro* experiments on antifungal activity of 30 plant species against the dry root rot pathogen, *M. phaseolina* ,in mung bean. The extracts of garlic creeper (*Adenocalymma alliaceum*) and zimmu (*Allium* sp.) induced complete inhibition on the mycelial growth of the pathogen in agar well diffusion method. Rajak (2014) showed that the phyto extract of neem (*A. indica*), at all the tested concentrations, gave excellent suppression of *R. solani*, the causal agent of root rot in mung bean, under *in vitro* conditions. They opined that the presence of azadiractin and monoterpenes in the neem extracts were responsible for the toxicity and effectiveness.

Thobunluepop (2009) conducted studies on fungicidal action of tea tree oil obtained from *Melaleuca alternifolia* that belonged to family Theaceae, on *R. solani* causing of sheath blight in rice. The fungistatic nature of essential oils such as tea tree oil and lemon grass oil was attributed to the presence of terpenoids and aromatic compounds (Gujar *et al.*, 2012). Essential oils are volatile heterogeneous mixtures of terpenes and phenolics and are formed as secondary metabolites in plants (Guesmi *et al.*, 2013). Muthukumar (2015) evaluated the antifungal activity of 16 plant oils on mycelial growth of *S. rolfsii.* Lemongrass oil caused complete suppression of the pathogen at a concentration of 0.002 per cent, whereas, similar effect was obtained with neem oil at a higher concentration (1.0 per cent) only.

Neem, sesamum and groundnut oil cake caused inhibition of *Thanatephorus cucumeris* causing web blight of mung and urd bean. Incorporation of groundnut cake (10 per cent) gave maximum inhibition of the pathogen (38.9 per cent) compared to til (30.8 per cent) and neem cake (Dubey and Patel, 2000). Jha *et al.* (2000) conducted experiment with oil cakes on root rot pathogen of okra and reported highest inhibition of mycelial growth of *M. phaseolina* with 5 per cent *Brassica juncea* cake. Bhadrasree (2007) evaluated the antifungal nature of extracts of different oil cakes on *in vitro* mycelial growth of *R. solani* and found 48.61 per cent suppression with coconut oil cake and 41.24 per cent suppression with groundnut oil

cake. However, she reported that incorporation of neem cake into the growing medium did not cause any suppression of the pathogen. Lenka and Pun (2014) assessed the effectiveness of oilcakes on inhibition of mycelial growth and sclerotial production of *R. solani* under laboratory conditions by poisoned food technique. They observed that mustard oilcake was the most effective followed by sunflower oilcake and neem oilcake. The treatment with mustard oilcake was the most promising in decreasing the sclerotia formation in *R. solani.* In the case of oil cake extracts, the antifungal activity were found to be dose dependent and effectively reduced the pathogen at higher concentration (Meena *et al.*, 2014).

The suppressive effect of biofumigants are also generally attributed to the presence of biocidal compounds, principally ITCs that possess both antifungal and biofumigant action. Gamliell and Stapleton (1993) detailed the inhibitory effect of volatiles released from cabbage residues on *Pythium ultimum* and *Sclerotium rolfsii.* Phenyl isothiocyanate, allylisothiocyanate, dimethyl sulphide, acetic acid and ethanol were the major biocidal components present in the cruciferous vegetables. The biocidal effect of volatiles released from *Brassica* leaf tissue on mycelial growth of *P. ultimum* was studied by Charron and Sams (1999). *B. juncea* (10g 500 mL^{-1} jar) resulted in complete suppression after 48 hours exposure to the pathogen. Booth *et al.* (2000) evaluated the *in vitro* biofumigant action of above ground and below ground materials of different *Brassica* sp. against *R. solani,* the incitant of black scurf disease of potatoes. The result validated the biofumigant effect of below ground root material from *B. oleraceae, B. campestris* and *B. juncea* (500mg petri $plate^{-1}$) in reducing the colony size of *R. solani.* Harvey *et al.* (2002) evaluated allyl isothiocyanate mediated suppression of *S. rolfsii* by exposing the pathogen to different concentrations of macerated tissues of Indian mustard and noted 50per cent and 90 per cent inhibition on mycelial growth at a concentration of 0.7 and 1.0 g L^{-1}, respectively. Naz (2006) screened the effect of 26 plant extracts for suppression of *R. solani* causing black scurf disease of potato and found that the extracts from

Brassica rapa (turnip), *B. napus* (canola) and *Allium cepa* (onion) showed 100per cent suppression in radial mycelial growth of the fungus.

The biofumigant action of thymol on *R. solanacearum* under *in vitro* has already been established by earlier workers (Momol *et al.,* 2000; Pradhang *et al.,* 2003). The essential oil extracted from mustard seeds (*Brassica rapa*) completely reduced the saprophytic growth of *R. solani* under *in vitro* conditions at a concentration of 50 µL. Higher mustard oil concentrations were required for field level application (Dhingra *et al.,* 2004). The fumigant effect of 39 essential oils on soilborne pathogens such as *R. solani* was tested and 68 per cent inhibition was obtained with *Origanum vulgare* oil alone. The major constituents were analysed with gas chromatography-mass spectrometry (Lee *et al.,* 2007). *In vitro* growth of *R. solanacearum* was significantly reduced on biofumigation with palmarosa oil and lemongrass oil (Alves *et al.,* 2014). Xu *et al.* (2017) investigated the biofumigant action of tea tree oil (TTO) against *Botrytis cinerea* under *in vitro* and *in vivo*. The TTO reduced total lipid content in the cell membrane by 50 per cent and also altered the mycelial morphology and cellular ultra structure of the pathogen.

Defatted seed meal produced after the processing of brassica seeds for oil has been reported as a convenient source of high glucosinolates (GSL) (Brown and Mazzola, 1997). Dubey (2002), in his experiment with soil application of five per cent oil cakes, neem cake showed best performance as it increased seed germination and grain yield of mung bean and decreased seedling mortality and disease intensity of web blight. The application of brassica seed meal altered both microbial and pathogenic communities present in soil. Fayzalla *et al.* (2009) explained the biofumigant effect of mustard seed meal in the control of soil-borne pathogens of soybean under laboratory situations. In petridishes the linear growth of *R. solani* was significantly suppressed (92.2 per cent) on exposure to volatiles from mustard seed meal (25mg petri plate^{-1}).

Dohroo and Gupta (1995) reported neem oil to possess inhibitory effect against sclerotia of *Rhizoctonia* sp. and claimed that 0.5 per cent neem oil effectively

controlled sheath blight disease caused by *R. solani*. Germination of sclerotia was negatively correlated with the concentration of Allyl ITC (AITC). Sclerotia, the hard resistant survival structure was found to require higher concentration of AITC than mycelia to suppress its germination. 50.0 and 90.0 per cent suppression on mycelial regeneration from sclerotia were noted with AITC at concentrations of 249.0 µmol L^{-1} and 528.0 µmol L^{-1}, respectively after 42 hours exposure (Harvey *et al.*, 2002). Biocidal effect of tea tree oil on sclerotial germination of *R. solani* was analysed *in vitro*. Maximum suppression (71.61 per cent) was recorded with tea tree oil at 2.0 per cent v/v concentration (Thoubunluepop *et al.*, 2009). An experiment was conducted in confined condition, with essential oil of mustard to evaluate its biofumigant effect on sclerotial viability of *S. rolfsii*. 100 per cent mortality of sclerotia was recorded on exposure to 100 µL mustard oil for one week (Dhingra *et al.*, 2013). Riad *et al.* (2014) evaluated the fungicidal effect of leaf extracts of *moringa oleifera* against *R. solani in vitro*. According to them the extract of moringa leaves at 30 and 40 per cent concentrations caused 41.2 per cent to 74.2 per cent and 61 to 90 per cent inhibition on sclerotial germination respectively. Warmington and Clarkson (2016) studied the effect of soil biofumigation with dried biofumigants in confined conditions on sclerotial germination of *Sclerotinia sclerotiorum*. The highest inhibition on sclerotial germination was obtained with *B. juncea* (6g 500 mL^{-1} jar).

The strategy of plastic mulching to retain the volatiles released from crucifer crop residue, in soil, is known to increase their effect on incidence of various diseases (Gamliel and Stapleton, 1993; Block *et al.*, 2000). Amending soil with plant tissue of *Brassica* spp. have been found to reduce the inoculum of several soilborne pathogen including *R. solani* and the diseases caused by them (Charron and Sams, 1999; Lodha and Sharma, 2002; Marwar and Lodha, 2002). Arnault *et al.* (2004) reported that plant extracts from garlic, onion, and leek of the plant family Alliaceae on decomposition emitted sulphur volatiles such as thiosulfinates and zwiebelanes which were subsequently converted into disulfides that possessed biocidal action against

several soilborne pathogens. The addition of plant residues also contributedconsiderable amount of organic matter that promoted soil health. In consonance with plastic tarping of soil, the residues incorporated caused anaerobic condition leading to enhancement of the suppressive effects on soilborne pathogens. Soilborne diseases could be suppressed by the incorporation of fresh plant material such as green manures, seed meals/ oil cakes /byproduct after oil extraction or dried plant material having biofumigant action (Kirkegaard and Matthiessen, 2004; Matthiessen and Kirkegaard, 2006). Chandel and Sharma (2014) evaluated the biofumigant effect of cabbage and cauliflower residues (5 per cent) on stem rot of carnation caused by *R. solani* under *in vivo* and recorded 22.28 per cent and 24.25 per cent disease incidence with cauliflower and cabbage, respectively.

The potting mixture containing inoculum of *R. solanacearum* was incorporated with palmarosa oil and lemongrass oil to evaluate the biofumigant action on pathogen. The assessment on bacterial population after an incubation period of one weekrevealed a drastic reduction of the pathogen (Paret *et al.*, 2010). Reitz *et al.*, 2008 conducted studies on the the sustainable management of tomato spotted wilt with essential oils such as lemongrass oil, and tea tree (*Melaleuca alternifolia*) oil integrated with kaolin. Both the essential oils significantly reduced the disease incidence, though, better results were obtained with the combination of tea tree oil with kaolin (Reitz *et al.*, 2008).

Van Os *et al.* (2004) studied biofumigation against *Pythium* root rot infection in flower bulb production and reported that soil amended with *B. juncea* seed meal resulted increase in bulb yield and drastic reduction of root rot. Incorporation of brassica seed meal into soil at 0.5 per cent weight of soil caused long term reduction in population of *Pythium* and subsequent infection in apple seedlings (Cohen *et al.*, 2007; Mazzola *et al.*, 2007). *B. juncea* seed meal was found to be superior to *B. napus* or *Sinapsis alba* seed meal for the control of root infection by *Pythium* spp. (Mazzola *et al.*, 2007). Differential capacity of brassica seed meal to suppress

soilborne pathogens and the diseases caused by them was discussed by Mazzola *et al.* (2009). Handiseni *et al.* (2012) found that tomato and pepper seedling emergence from soil amended with *B. juncea* seed meal was significantly higher indicating suppression of *P. ultimum* induced root rot. Incorporation of soybean seed meal amendment also significantly improved pathogen suppression and the seedling emergence. Wang *et al.* (2014) reported that the incorporation of rape seed meal (4g kg^{-1} soil) reduced the incidence of *Phytophthora* blight and improved the growth of pepper seedlings.

2.6. MICROFLORA INTERACTION AND SOIL BIOFUMIGATION

Agronomic and plant protection practices significantly influence the structure and function of soil microbial community. The suppression of soilborne pathogen as a result of incorporation biofumigant material may be mediated not only through the release of toxic metabolites but also through enhanced activity of saprophytic microflora. Galletti *et al.* (2008) carried out studies on the tolerance of *Trichoderma* spp. to biofumigation with seed meal of *Brassica carinata*. A fungistatic effect was noticed on antagonistic microflora on biofumigation with *Brassica* seed meal, but the effect was less compared to that on fungal pathogens. The integrated approach involving biofumigation and biocontrol agent application to soil was promising, even though some *Trichoderma* isolates were reduced by the concentration of allyl ITC. Omirou *et al.* (2011) detailed that the structure and function of soil microbial community was altered on biofumigation with broccoli residues. The released ITC rapidly reduced the proportion of fungi and gram negative bacteria in biofumigated soil. Wang *et al.* (2014) reported the least disease incidence of *Phytophthora* blight on biofumigation with rapeseed meal. Using the denatured gradient gel electrophoresis, they analysed the biofumigation effect on soil microflora and disease incidence was found negatively correlated with soil bacterial community and positively correlated with soil fungal community. The soil microbial structure also was altered by biofumigation. Hu *et al.* (2015) described the response of soil

microflora to various isothiocyanates during biofumigation. Soil fungal population was reduced by 85 per cent with allyl ITC ,whereas, the bacterial population was less influenced by ITC, though there was a short increase in the population of antagonistic group Firmicutes. The impact on soil miroflora varied with the type of biofumigant. Rokunuzzaman *et al.* (2016) studied the effect of soil disinfection with chemical and biological methods on bacterial communities. Mulching with mustard was reported to exert the least effect on the bacterial community structure when compared with the effect of fumigation with chloropicrin. Initially, phylum Firmicutes dominated and after two months, the population of both Bacteroidetes and Proteaobacteria were found to be elevated. Mustard (*B. juncea*) caused much less harm on soil bacterial community than chemical fumigation.

Materials and Methods

3. MATERIALS AND METHODS

The study on management of collar rot of cowpea caused by *Rhizoctonia solani* Kuhn using biofumigants was carried out at the Department of Plant Pathology, College of Agriculture, Vellayani during 2015-17. The materials and methods adopted for conducting the study are presented in this chapter.

3.1. SYMPTOMATOLOGY

The symptoms of collar rot disease of cowpea based on the descriptions detailed by Lakshmanan *et al.*, (1979) were observed in field conditions and recorded.

3.2. ISOLATION, IDENTIFICATION AND PROVING PATHOGENICITY OF PATHOGEN ASSOCIATED WITH COLLAR ROT DISEASE OF COWPEA

3.2.1. Isolation of the pathogen

Cowpea plants showing typical collar rot symptom were collected from the Instructional Farm, College of Agriculture, Vellayani during *Kharif* 2016. The infected portions were first washed in running water and cut into small bits, surface sterilized with 0.1% mercuric chloride ($HgCl_2$) solution for one min. followed by washings in two successive changes of sterile water. The bits were transferred into a sterile filter paper for absorption of existing water on its surface and then cultured on sterile petriplates containing Potato Dextrose Agar (PDA) medium (Appendix I) under laboratory conditions. These petriplates were sealed with parafilm and incubated at room temperature, 28±1°C, for 24- 48 h and observed for fungal growth. The fungal growth obtained on petri dishes from the bits were transferred to PDA slants (Aneja, 2003).

3.2.2. Purification of the isolates

The fungal cultures thus obtained were purified by transferring the growing tip of the fungal mycelium to PDA slants. Thus the typical colonies of four pathogen isolates obtained from collar region of cowpea plants at different

locations were purified by hyphal tip method (Parmeter *et al.*, 1969). The purified isolates were maintained on PDA slants for further research purpose.

3.2.3. Virulence rating

Virulence rating was performed to identify the most virulent one among the collected isolates based on rate of growth, morphological and cultural characteristics, lesion development on artificially inoculated cowpea seedlings.

3.2.3.1. Identification based on morphological and cultural characteristics

The morphological and cultural characteristics of the isolates of collar rot disease were studied and obtained by observing the mycelial growth of the pathogen isolates on PDA medium in petri plates. Mycelial discs of 5 mm diameter were taken from 7 day old culture of the pathogen isolates and placed at the centre of petriplate with PDA medium. Three replications were maintained. The inoculated plates were incubated at 28±1°C. The radial growth was measured periodically. From the culture plates, the rate of growth, colour and appearance of the mycelium, presence of monilioid cells and the number of sclerotia were recorded and compared.

3.2.3.2. Selection based on lesion development on artificial inoculation

Cowpea seedlings were raised in teacups and after fifteen days, seedlings were inoculated by placing 5 mm sized mycelial bits of isolates of pathogen at the collar region and proper humidity was maintained and observed for lesion development at collar region.

3.2.4. Pathogenicity testing

The pathogenicity testing was performed with the most virulent isolate on cowpea plants (variety, Bhagyalakshmy) to prove Koch's postulates. Cowpea seeds were sown in teacups and pathogen was artificially inoculated at collar region of fifteen days old seedlings by placing one sclerotium at the collar region. The inoculated plants were kept within polypropylene cover provided with

sufficient holes and moist cotton swabs to maintain humidity. Plants without inoculation served as control. Three replications were maintained. The plants were incubated at room temperature 28±1°C, and observed for development of symptoms. The pathogen was re-isolated and compared the morphological and cultural characteristics with that of the original culture.

3.2.5. Identification of the pathogen

The most virulent pathogen was further identified based on morphological, microscopic and cultural characteristics in the laboratory of the Department of Plant Pathology, College of Agriculture, Vellayani. Confirmation of the pathogen was done based on morphological and molecular characteristics at the Agharkar Research Institute, Pune.

3.2.5.1. Nuclear staining

Nuclear staining was performed to detect the nuclear status of the fungi. 0.5% trypan blue in lactophenol was prepared and used to stain the nucleus (Burpee *et al.*, 1978) (Appendix II). Two per cent water agar was prepared and sterilized. Sterilized petri plates were plated with two per cent agar and cellophane discs having 8 cm diameter were placed on those plates and inoculated with 1 cm diameter mycelial disc of the pathogen. After an incubation period of 5 days at 28±1°C, cellophane pieces of dimension 3 cm × 2 cm were cut and placed on drop of trypan blue stain mounted at the centre of a glass slide and the cellophane was kept in position with a cover slip, mounted over the slide. The prepared slide was gently warmed over a flame till stain started to boil. After cooling the slide was observed under the microscope.

3.3 *IN VITRO* STUDIES

3.3.1. Evaluation of antifungal property of different plants, plant oils and oil cakes against *R. solani* under *in vitro* conditions

3.3.1.1. Plants

Fresh leaf tissues (100g) of ten different plants known to contain biocidal compounds [*viz.* cabbage (*Brassica oleracea* var *capitata* L.), cassava (*Manihot esculenta* Crantz), garlic creeper (*Mansoa alliacea* Lam.), lemon grass (*Cymbopogon flexuosus* [Nee ex Steudel] J.F. Watson), moringa (*Moringa oleifera* Lam.), mustard (*Brassica juncea* L.), neem (*Azadirachta indica* A. Juss.), papaya (*Carica papaya* L.), radish (*Raphanus sativus* L.), sweet potato (*Ipomoea batatas* (L.) Lam)] were collected, washed and drained to remove excess water. The extracts were prepared by the method of Paul and Sharma (2002). These plants were ground in 100 mL of sterile water. The product was filtered through muslin cloth and filtrate obtained was centrifuged at 6000 rpm for 25 min. The supernatant filtered through Whatman no. 42 filter paper. The final filtrate was collected in sterilised screw capped bottle. Aliquots of 2.5 mL of each of the concentrated leaf extract was diluted with 7.5 mL sterile water, and finally the diluted 10 mL volume of leaf extract preparation was added to equal volume of sterilized double strength PDA medium taken in conical flask and the amended medium was poured into petri plates.

3.3.1.2. Plant oils

Four oils *viz.*, neem oil (source: seeds of *Azadirachta indica* A. Juss.), lemongrass oil (source: leaves of *Cymbopogon flexuosus* [Nee ex Steudel] J.F. Watson), mahua oil (source: seeds of *Madhuca longifolia* (J. Konig)) and tea tree oil (source: leaves of *Melaleuca alternifolia* (Maiden & Betche)) each at 5 per cent concentration, was tested for their antifungal activity on *R. solani* under *in vitro* conditions. Each oil (5 mL) was filtered using sterilised bacterial proof filters, mixed with 0.1 mL emulsifier *viz.,* Polysorbate (Tween 80) and added into 250 ml conical flask containing 95 mL of sterilised PDA.

3.3.1.3. Oil cakes

Under *in vitro* conditions, studies were carried out on the fungicidal action of four oilcakes/seed meals such *viz.*, groundnut oil cake (source: *Arachis hypogaea* (Linn.), neem cake (source: *Azadirachta indica* A. Juss.), mahua cake (source: *Madhuca longifolia*) and mustard oil cake (source: *Brassica juncea* L.) over the pathogen *R. solani* at 10% concentration.

The extract was prepared based on the method given by Bhadrasree (2007). The oil cakes were dried at room temperature for 3-4 days, broken into small pieces, ground well with mortar and pestle and soaked in sterilised water in the ratio 1:2 (W/V). After 24 h, the hydrated cake was squeezed through four folds of sterilised muslin cloth and cake extract collected and centrifuged at 5000 rpm for 10 min. The supernatant was filtered through bacterial proof filters and decanted into a 250 mL conical flask.

The antifungal nature of different plants, plant oils and oil cakes over *R. solani* was tested by poisoned food technique (Nene and Thapliyal, 1993). Mycelial disc (5 mm diameter) was taken from 7 day old culture of *R. solani* and placed at the centre of petriplate. Three replications were maintained. The unamended medium poured into the sterile petriplate with pathogen at the centre served as the control. The inoculated plates were incubated at 28±1°C. Observation on colony diameter was recorded when the control plates were fully covered by *R. solani*. Percentage inhibition in colony growth was recorded as per Vincent (1947):

$$I = \frac{(C-T)}{C} X100$$ where, I= Percentage inhibition of mycelial growth

C= Growth of the pathogen in control plates (cm)

T= Growth of the pathogen in treatment plates (cm)

3.3.2. Evaluation of biofumigant nature of different plants, plant oils and oil cakes against *R. solani* under *in vitro* conditions

3.3.2.1. Preparation of plants, plant oils and oil cakes for biofumigation

The plants, plant oils and oil cakes used for the experiment were prepared as follows

3.3.2.1.1. Plants

Plant extracts were prepared as per the method suggested by Charron and Sams (1999). Those leaves already tested for their antifungal action (3.3.1.1.) on *R. solani* were macerated with mortar and pestle along with addition of sterile water in the ratio of 1:1 (w/v).

3.3.2.1.2. Plant oils

Plant oils, at 5% concentration (mentioned in 3.3.1.2.) were prepared by mixing 5 mL of plant oil with 0.1% emulsifier and diluting with 95 mL sterile water to a final volume of 100 mL.

3.3.2.1.3. Oil cakes

As mentioned in 3.3.1.3. the oil cake extracts were prepared and 10 mL of extract was taken and diluted to 100 mL by adding 90 mL sterile water. Thus 10% extract was prepared.

The biofumigant nature of plants, plant oils and oil cakes against *R. solani* under *in vitro* conditions was studied by Paired plate technique (Prasad *et al.*, 2016). The bases of two equal sized sterilized petri plates were taken. 2.5 g of the macerated plant tissue/ sterilised filter paper disc dipped in 5% plant oil/ 10% concentrate extract of oil cake was placed at the centre of the one kept at the bottom. The sterile molten PDA was poured into the other lid and this was inoculated with 5 mm mycelial disc cut from 7 day old *R. solani* culture grown on PDA and was inverted over the other plate containing the macerated plant tissue/ filter paper disc dipped in plant oil/ concentrate extract of oil cake. The adjoining

portions of two petri plates were sealed with parafilm. The paired plates with upper inverted plate carrying PDA and inoculated pathogen alone served as control. Percentage suppression of the pathogen over control was calculated using the formula,

$I = \frac{(C-T)}{C} \times 100$ where, I = Percentage Inhibition of mycelial growth

C = Growth of the pathogen in control plates (cm)

T= Growth of the pathogen in treatment plates (cm)

3.3.3. Mycelial regeneration from sclerotia

The effect of biofumigation on mycelial regeneration from sclerotia was tested in confined containers under *in vitro* with those plants described in 3.3.1.1. (50 g kg^{-1}soil), plant oils described in 3.3.1.2. (5% soil drench) and oil cakes described in 3.3.1.3. (10 g kg^{-1} soil). Plant oil (5%) was prepared as mentioned in 3.3.2.2. The methodology was given by Dhingra *et al*., (2013).

Garden soil was collected in polypropylene covers and sterilized at 1.02 kg cm^{-2} for 1 h (Plate 1a). Sclerotia were taken from seven day old cultures of *R. solani* and kept in sterilized muslin cloth bags at the rate three per bag under aseptic conditions (Plate 1b). The plastic container of capacity 1 L was selected and its half portion was filled with sterilized garden soil. Biofumigant such as macerated fresh plant tissue (50 g kg^{-1} soil)/ plant oil (5% soil drench)/ oil cake (10 g kg^{-1} soil) was incorporated into the soil filled in plastic container and over that after sprinkling sterile water another layer of sterilized garden soil was given. Four muslin cloth bags were added per container and plastic container was closed with its lid (Plate 1c). The effect of volatiles from biofumigants on mycelial re-generation from sclerotia was assessed by inoculating treated sclerotia on PDA at intervals of 24 h, one week, two weeks and one month. The confined container with inoculum and without biofumigant served as control. The percentage

a. Sterilised soil

b. Muslin cloth bag carrying sclerotia

c. Confined container

Plate 1 a-c. Biofumigation in confined condition

suppression of the pathogen over control was calculated using the formula described in 3.3.1.

3.4. *IN VIVO* STUDIES

3.4.1. Treatments

T1 : Cabbage (*Brassica oleracea* var *capitata* L.)

T2 : Cassava (*Manihot esculenta* Crantz)

T3 : Garlic creeper (*Mansoa alliacea* Lam.)

T4 : Mustard (*Brassica juncea* L.)

T5 : Lemongrass oil

T6 : Tea tree oil

T7 : Ground nut oil cake

T8 : Mustard oil cake

T9 : Inoculated control

T10: Un-inoculated control

T11: Chemical control check (Carbendazim 0.1%)

A pot culture experiment was conducted with cowpea, variety Bhagyalakshmy. The experimental design was CRD with 11 treatments in 5 replications. Fertilizers were applied timely as per the Package of Practices recommendations of Kerala Agricultural University (KAU, 2016).

3.4.2. Preparation of Inoculum and application of Inoculum and Biofumigants

3.4.2.1. Preparation of Pathogen Inoculum

The pathogen *R. solani* was mass multiplied in sand oats media (Lewis and Papavizas, 1984). On a cleaned surface, sand and oats were mixed at the ratio 9:1. This mixture was moistened to induce fungal growth. One litre conical flasks were filled with 700 g of the sand-oats mixture and sterilized at 1.02 kg/cm^2 for 1 h. Mycelial discs of (5 mm sized) the pathogen taken from the advancing edge of a seven day-old culture of *R. solani* isolate R1 were aseptically placed in those conical flasks containing sterilized mixture. The flasks were incubated at room temperature for 14 days.

3.4.2.2. Application of Inoculum

The mass multiplied inoculums of *R. solani* isolate R1 was mixed with garden soil in the ratio 1:2 and 250 g of this mixture was added into potting mixture two weeks before sowing cowpea seeds. Thus the soil was artificially inoculated.

3.4.2.3. Application of Biofumigants

The promising biofumigants under *in vitro,* were selected for further pot culture studies (Chandel and Sharma, 2014). In pots (10 kg capacity), after application of inoculum another layer of garden soil was added and over that the selected biofumigants such as macerated plant tissue (50 g kg^{-1} of soil)/ plant oil (soil drench, 5%) /oil cake (10 g kg^{-1} of soil) were incorporated separately. An appropriate quantity of water was added to maintain soil moisture content to 50% of water holding capacity. Amended soil was immediately covered with double layered transparent plastic film to minimize loss of fumigant gases (Plate 2). Biofumigation was continued for a period two weeks. Thereafter, the plastic film was removed, the soil was gently mixed thorougly and left open for 3 days to

Plate 2. Biofumigation under *in vivo* conditions

ensure the release of any residues of isothiocyanates (Wang *et al.*, 2014). Cowpea seeds were sown in those biofumigated pots.

3.4.2.4. Application of Fungicide

Carbendazim (0.1 per cent) solution was prepared and the soil inoculated as mentioned under 3.4.2.2. was drenched at the rate of 1L/pot with the fungicidal solution before sowing cowpea seeds.

3.5. MICROFLORA FROM BIOFUMIGATED SOIL

3.5.1. Population dynamics of saprophytic fungi

The population of saprophytic fungi in biofumigated soil was enumerated at different intervals ie., before biofumigation, immediately after biofumigation, one week after sowing the seeds and two weeks after sowing the seeds using serial dilution and plating technique (Wakesman,1922) and expressed as cfu g^{-1}.

One gram of ground soil sample was transferred into a 250 mL conical flask containing 100 mL sterile water. The sample was mixed thoroughly and shaken for 20 min in a mechanical shaker. It represented a dilution of 10^{-2}. One mL of that suspension was pipetted out to 99 mL sterile water under laboratory conditions. Again the flasks were kept on a shaker for 15 min. From this 10^{-4} dilution one mL of suspension was pipetted out to sterile Martin's Rose Bengal Agar medium (Appendix I) and gently swirled for a while for thorough mixing of sample with media. The petriplates were incubated at room temperature and the development of fungal colonies was observed.

3.5.1.1. Mass screening of saprophytic fungi

Cross culture method was adopted to test the antagonism of fungi isolated from dilution plates on *R. solani* (Henis *et al.*, 1979). 5 mm diameter mycelial discs of four saprophytic fungi were placed in a petri dish at a distance of three cm from the centre and near to the periphery at equidistant points. 5 mm diameter

mycelial disc of actively growing pathogen was introduced at the centre. Three replications were maintained. The dishes were incubated at 28±1°C and visually examined for inhibition of mycelial growth of the pathogen.

3.5.1.2. In vitro screening of saprophytic fungi for suppression of R. solani

Using predominant fungi from biofumigated soil dual culture was performed to assess its inhibition and per cent inhibition on *R. solani* (Skidmore and Dickinson, 1976).

The saprophytic fungus obtained from serial dilution technique were grown in seperate petri dishes. Mycelial discs of 5 mm size were cut from the periphery of actively growing fungal cultures grown on PDA, one from the pathogen and the other from the saprophytic fungi. The discs were placed 3 cm apart diametrically opposite and 1 cm away from the periphery, on a PDA plate. Both cultures were allowed to grow. The mycelial disc of pathogen alone served as the control. When the pathogen attained full growth in control plate, the mycelial growth was recorded and the percentage inhibition was calculated using the formula described in 3.3.1.

3.5.2. Population dynamics of saprophytic bacteria

The suspension at a dilution of 10^{-4} was prepared as described in 3.4.3.1. One mL was pipetted out to 99 mL sterile water collected in 250 mL conical flask. It was shaken for another 15 min. From the 10^{-6} dilution, one mL was transferred to petriplates containing sterile soil extract agar medium and mixed thoroughly (Appendix I). The petriplates were incubated at room temperature. The population was estimated as cfu g^{-1} of soil before biofumigation, immediately after biofumigation, one week and two weeks intervals thereafter sowing the seeds.

3.5.2.1. Mass screening of saprophytic bacteria

Using the methodology described in 3.5.1.1. mass screening of saprophytic bacteria was done. Twenty four hours old bacterial cultures were used for

inoculation. The bacterial isolates were spotted at the rate of four isolates per petri dish containing PDA at four equidistant points near the periphery at a distance of three cm the centre. Two days later, the actively growing test pathogen was introduced at the centre of the petri dish.

3.5.2.2. In vitro screening of saprophytic bacteria for suppression of R.solani

The predominant bacterial isolates selected after initial mass screening for antagonism to *R. solani* were tested by dual culture technique (Utkhede and Rahe, 1983). The bacterial cultures were singly streaked at a spacing of three cm from 5 mm diameter mycelial disc of the pathogen placed on PDA medium in sterilized petri dishes. The remaining procedure as described in 3.5.1.2.

3.5.3. Observations

During the course of experiment, on cowpea plant the following observations were made.

3.5.3.1. Disease Incidence (%)

The incidence of collar rot disease on cowpea plants were recorded on the next day of sowing the seeds at an interval of five days till the time of uprooting the crop. Collar rot incidence was calculated using the formula,

$$\text{Disease incidence (\%)} = \frac{\text{Number of plants affected}}{\text{Total number of plants}} \times 100$$

(Mayee and Datar, 1986)

3.5.3.2. Disease Intensity

Disease intensity was rated 14 days after inoculation. Disease scoring was completed using modified 0-4 disease scale developed by Haware and Nene (1980) (Plate 3).

Grade	Description
0	No visible symptom
1	1-25% - Water soaked lesion at collar region and rotting
2	26-50% - Lesion progression and rotting
3	51-75 % - Horizontal and vertical spread and rotting
4	76-100 % - Advanced stage of rotting, yellowing of foliage and collapse of plant

Percentage disease index was calculated using the formula:

$$PDI = \frac{\text{Sum of grades of each plant}}{\text{Number of plants assessed}} \times \frac{100}{\text{Maximum grade}}$$

McKinney (1923)

3.5.3.3. Shoot length (cm)

Shoot length from ground level to the growing tip of each plant was measured after uprooting the plant.

3.5.3.4. Root length (cm)

Root length of each plant was measured after uprooting.

3.5.3.5. Fresh weight of plants (g)

Fresh weight of plants was recorded immediately after uprooting the plant.

3.5.3.6. Dry weight of plants (g)

Dry weight of plants was recorded after drying the samples to a constant weight in an oven at 60°C.

3.5.3.7. Yield (g per plant)

Plate 3. 0-4 scale for assessment of collar rot intensity

Number of pods per plant and pod weight was recorded.

*3.5.3.8. **Nodulation character***

Number of root nodules per plant was recorded.

3.6. STATISTICAL ANALYSIS

The data obtained from the studies conducted under laboratory and field conditions were subjected to analysis of variance (ANOVA) after appropriate transformations wherever needed.

Results

4. RESULTS

Experiments were conducted both under *in vitro* and *in vivo* conditions to study the symptomatology, morphological and cultural characteristics of *R. solani* and to evaluate the effect of biofumigants such as plants, plant oils and oil cakes for ecofriendly management of collar rot in cowpea.

4.1. SYMPTOMATOLOGY

The infected plants were collected during June - July from different cowpea fields of College of Agriculture, Vellayani. The studies carried out on the symptomatology of collar rot disease in cowpea revealed that at the seedling stage, water soaked lesions appeared at collar region (Plate 4a). This further expanded horizontally across the stem and developed into deep seated brownish black lesions (Plate 4b). Later the lesions progressed vertically upwards and also further in horizontal direction resulting in rotting of the major parts of basal portion of the stem (Plate 4c). The infected plants became stunted, followed by complete yellowing and shedding of foliage and resulted in drying up of the entire plant. In severe stages, roots became decayed and white cottony mycelial growth was visible along with small white sclerotial pin heads that eventually turned into brown mustard like sclerotia (Plate 4d).

In the mature plants, at the flowering stage, web blight symptoms were observed on the lower leaves. Initially, small, irregular, dull green water soaked lesions appeared on the leaves (Plate 5a) that turned to straw colour delimited by dark brown margins (Plate 5b). Under congenial humid climatic conditions, these lesions advanced rapidly to cover major portion of leaf lamina and finally resulted in complete yellowing or drying of the affected leaves (Plate 5c). The infected leaves were found webbed together by the mycelium of the pathogen (Plate 5d) and at later stages of infection small sclerotial pin heads were noticed on the affected leaves (Plate 5e).

a. Water soaked lesion at collar region

b. Brownish deep seated lesion with horizontal spread

c. Vertical spread

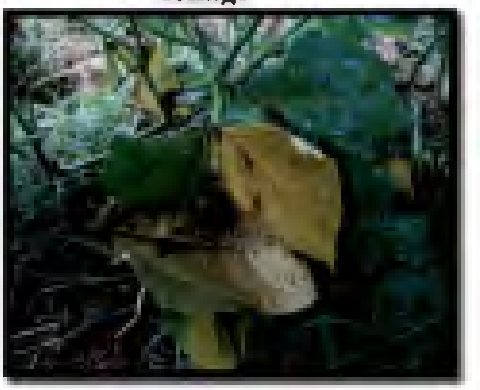
d. Cottony mycelial growth with formation of sclerotia

Plate 4 a-d. Cowpea plants showing symptoms of collar rot

a. Water soaked lesion

b. Necrotic lesion

c. Complete yellowing of foliage



d. Webbed leaves

e. Sclerotia formation

Plate 5 a-e. Cowpea plants showing symptoms of web blight

4.2. PATHOGEN

4.2.1. Isolation of Pathogen

The incitant of collar rot disease of cowpea was isolated from the collar rot affected cowpea plants collected from four different locations in the Instructional Farm, College of Agriculture, Vellayani during *Kharif* 2016. Four isolates designated as R1, R2, R3 and R4 were obtained and were maintained on PDA medium under aseptic conditions (Plate 6).

4.2.2. Virulence rating

For virulence rating, the isolates were compared and screened based on various attributes like growth rate, morphological and cultural characteristics on PDA medium as well as lesion development on artificial inoculation of healthy cowpea plants.

4.2.2.1. Morphological and cultural characteristics of collar rot isolates

The four collar rot isolates, R1, R2, R3 and R4 exhibited differences in their morphological and cultural characteristics. The isolates differed primarily in their growth rate and pattern, mycelial and sclerotial characteristics (Table 1,2). The maximum average growth rate was obtained with the isolate R1 (3.07 cm/day). This was followed by the isolate R2 with an average growth rate of 2.73 cm/day. The average growth rate of the isolate R3 was 2.5 cm/day and that of R4 was 2.13 cm/day. The R1 isolate took 3 days to cover the entire area (63. 59 cm^2) on PDA on petri plate. But the isolates R2 and R3 attained full growth on 4^{th} day after inoculation on PDA. However, the isolate R4 took 5 days to attain full growth in nine cm diameter (63. 59 cm^2) of petri plate.

The isolates differed with regard to mycelial colour. R1 produced off-white coloured mycelium, while R2 and R4 formed white coloured mycelium. The

Table 1. Mycelial characteristics of collar rot isolates on PDA medium

Isolates	Average growth/ day* (cm)	No. of days taken to cover petridish	Mycelial colour	Colour of medium on the under side	Mycelial texture
R1	3.07[a]	3	Off - white	Light brown	Appressed
R2	2.73[b]	4	White	Light brown	Appressed
R3	2.50[b]	4	Light brown	Yellowish brown	Appressed
R4	2.13[c]	5	White	White	Fluffy
SE m (±)	0.16	-	-	-	-
CD (0.05)	0.29	-	-	-	-

*Mean of three replications

Table 2. Sclerotial characteristics of collar rot isolates of cowpea on PDA medium

Isolates	Sclerotial production	Days needed to form sclerotial initials	No. of sclerotia / petridish (63.59 cm^2) in 12 days	Averge size of sclerotia (mm^2) (mean of 100 sclerotia)*
R1	+	5	146	2.84
R2	+	7	124	2.41
R3	+	6	109	1.35
R4	-	-	-	-

+/- : Presence / absence of sclerotia

R1 (Vellayani isolate1)

R2 (Vellayani isolate 2)

R3 (Vellayani isolate 3)

R4 (Vellayani isolate 4)

Plate 6. Isolates of collar rot pathogen on PDA medium after 8 days of inoculation

mycelial colour of R3 was light brown. The colour of medium on the underside of petri plate varied among the collar rot isolates from white to yellowish brown. The mycelia appeared appressed in all the isolates except R4 which showed a fluffy mycelial texture (Table 1).

The collar rot pathogen isolates varied in their sclerotial characteristics. Sclerotial production was noticed in all the collar rot isolates except R4, which had fluffy mycelial texture. Collar rot isolate R1 produced sclerotia earlier to all the other isolates. With this isolate, the sclerotial initials appeared on the 5^{th} day of inoculation on PDA medium and 146 sclerotia with an average size of 2.84 mm^2 were formed per petri plate within 14 days. The isolate R2 produced sclerotial initials on 7^{th} day of inoculation and 124 sclerotia were produced per petri plate, with an average size of 2.41 mm^2. The isolate R3 produced sclerotial initials on 6^{th} day of inoculation and formed 109 sclerotia per petri plate with an average size of 1.35 mm^2 (Table 2).

4.2.2.2. Development of lesions

Lesion development at the collar region of two weeks old cowpea seedlings on artificial inoculation of all the pathogen isolates was observed three days after inoculation which was significantly different. Among all the isolates tested, the largest lesion (an average size of 2.65 cm^2) was developed on inoculation with the isolate R1 (Plate 7). This was followed by R2, R3 and R4 with values 2.03 cm^2, 0.65 cm^2 and 0.33 cm^2, respectively (Table 3).

4.2.2.3. Selection of the virulent isolate

Based on the virulence rating, growth rate, morphological and cultural characteristics the isolate R1 was identified as the most virulent and was selected for further studies.

Table 3. Lesion development by artificial inoculation by collar rot isolates on cowpea seedlings
Var. Bhagyalakshmy

Isolates	Days taken for lesion initiation	Lesion size (3 DAI*) (cm^2)
R1	1	2.65^a
R2	2	2.03^b
R3	2	0.65^c
R4	3	0.33^d
SE m (±)	-	0.12
CD (0.05)	-	1.30

* Days after inoculation

Plate 7. Lesion development on artificial inoculation on cowpea seedlings with the collar rot isolates

4.2.3. Pathogenicity Testing

Pathogenicity testing was conducted with the selected collar rot pathogen isolate, R1 and Koch's postulates were proved. Ten to fifteen days old cowpea seedlings were inoculated artificially with R1 isolate and collar rot symptoms were obtained on 3 days after inoculation. On re-isolation, the pathogen yielded pure culture identical in all respects to the original culture of the pathogen earlier isolated from infected cowpea plants collected from the field (Plate 8).

4.2.4. Identification of the pathogen

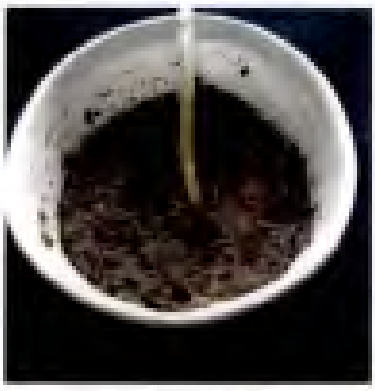
The pathogen was tentatively identified based on morphological, microscopic and cultural characteristics in the laboratory, Department of Plant Pathology, College of Agriculture, Vellayani. Confirmation of identification at Type Culture Collection was done based on morphological and molecular characteristics at Agharkar Research Institute, Pune.

4.2.4.1. Microscopic characteristics

The mycelial growth of the pathogen was examined under the microscope and following characteristics were observed (Plate 9):

- Right angled hyphal branching (Plate 9a).
- Formation of septum in the branch near the point of origin of hyphal branching (Plate 9b).
- Constriction of hyphal branches at the point of origin from the main hyphae and inclination of hyphae to the direction of growth (Plate 9c).
- Average mycelial diameter ranging from 6- 10.5 µm (Plate 9a).
- Production of monilioid cells (Plate 9e).
- Presence of multinucleate hyphae (Plate 9d).
- Hyphal anastamosis (Plate 9f).

a. R1 isolate



b. Symptom development on inoculation on cowpea at collar region

c. Re-isolated culture

Plate 8 a-c. Pathogenicity testing with the virulent isolate

a. Right angled branching

b. Septum at the origin of branching

c. Constriction at the base of branching

d. Multinucleate hyphae

e. Monilioid cells

f. Hyphal anastomosis

Plate 9 a-f. Microscopic characteristics of R1 isolate

4.2.4.2. Cultural characteristics

The isolate R1 produced off -white rapidly growing appressed mycelia which was light brown on the underside (Plate 10).

The above microscopic and cultural characteristics were similar to those of *Rhizoctonia solani* by Parmeter and Whitney (1969), hence the isolate, R1 was tentatively identified as *R. solani.*

4.2.4.3. Confirmation of the pathogen

The identity was confirmed based on morphological characteristics as *Rhizoctonia solani* Kuhn at the National Fungal Culture Collection of India, Agharkar Research Institute, Pune. This was further confirmed using molecular identification by ITS sequencing using universal primers ITS-4 and ITS-5 which showed 100 per cent similarity with *Thanatephorus cucumeris* (Frank) Donk, the perfect stage of *R. solani* (f. *Ceratobasidiaceae*). The accession number assigned was: EF429212.

4.3. *IN VITRO* STUDIES

4.3.1. Evaluation of antifungal property of different plants, plant oils and oil cakes against *R. solani* under *in vitro* conditions

4.3.1.1. Plants

Using poisoned food technique (Nene and Thaplial, 1993), the antifungal action of ten different plants [*viz.* cabbage (*Brassica oleracea* var *capitata* L.), cassava (*Manihot esculenta* Crantz), garlic creeper (*Mansoa alliacea* Lam.), lemon grass (*Cymbopogon flexuosus* [Nee ex Steudel] J.F. Watson), moringa (*Moringa oleifera* Lam.), mustard (*Brassica juncea* L.), neem (*Azadirachta indica* A. Juss.), papaya (*Carica papaya* L.), radish (*Raphanus sativus* L.) and sweet potato (*Ipomoea batatas* (L.) Lam.)] on the mycelial growth of *R. solani* was evaluated. Among the leaf extracts tested, 100 per cent mycelial inhibition of the collar rot pathogen was

a. Off white mycelia on upper side

b. Light brown colour on underside

Plate 10 a-b. Cultural characteristics of R1 isolate

obtained on PDA incorporated with leaf extracts of cabbage and garlic creeper. The incorporation of leaf extract of sweet potato showed 37.44 per cent inhibition of *R. solani*, whereas, with radish leaf extract mycelial inhibition of 30.00 per cent was noticed. These two treatments were statistically on par. The leaf extract of neem and mustard also caused statistically significant inhibition on mycelial growth of the pathogen as compared to control. The per cent inhibition was found to be 16.67 and 5.22, respectively (Table 4, Plate 11).

4.3.1.2. Plant oils

The plant oils *viz*., neem oil, lemongrass oil, mahua oil and tea tree oil were evaluated for their antifungal action on the collar rot pathogen. Among the four plant oils tested, the highest inhibition (100 per cent) was obtained on incorporation of lemongrass oil and tea tree oil into PDA medium. The addition of mahua oil caused 40.74 per cent suppression on mycelial growth of the pathogen and this was followed by neem oil with 16.29 per cent suppression of the pathogen as compared to control (Table 5, Plate 12).

4.3.1.3. Oil cakes

Four oil cakes *viz*., groundnut, neem, mahua and mustard were tested for their inhibitory effect on mycelial growth of *R. solani*. Mustard oil cake showed the maximum pathogen suppression (100 per cent) which was significantly high. This was followed by groundnut oil cake and neem cake which gave 79.62 per cent and 76.29 per cent inhibition, respectively (Table 6, Plate 13).

Table 4. Effect of antifungal nature of *R.solani* under *in vitro* conditions

Plants	Mycelial growth* (cm)	% Inhibition
P1 (Cabbage)	0.00	100.00[a] (89.04)
P2 (Cassava)	9.00	0.00[e] (0.96)
P3 (Garlic creeper)	0.00	100.00[a] (89.04)
P4 (Lemongrss)	9.00	0.00[e] (0.96)
P5 (Moringa)	9.00	0.00[e] (0.96)
P6 (Mustard)	8.53	5.22[d] (13.07)
P7 (Neem)	7.50	16.67[c] (24.09)
P8 (Papaya)	9.00	0.00[e] (0.96)
P9 (Radish)	6.30	30.00[b] (32.97)
P10 (Sweet potato)	5.63	37.44[b] (39.80)
Control	9.00	-
SE m (±)	-	0.24
CD (0.05)	-	0.73

(Figures given in the paranthesis are transformed values).
*Mean of three replications.
Means followed by a common letter(s) are not significantly different by one- way ANOVA at P = 0.05)

Table 5. Effect of the antifungal nature of plant oils against *R. solani* under *in vitro* conditions

Plant oils	Mycelial growth* (cm)	% Inhibition
O1 (Neem oil)	7.53	16.29^{c} (23.70)
O2 (Lemongrass oil)	0.00	100.00^{a} (89.04)
O3 (Mahua oil)	5.33	40.74^{b} (39.66)
O4 (Tea tree oil)	0.00	100.00^{a} (89.04)
Control	9.00	-
SE m (±)	-	0.76
CD (0.05)	-	3.02

Table 6. . Effect of antifungal nature of oil cakes against *R.solani* under *in vitro* conditions

Oil cakes	Mycelial growth*(cm)	% Inhibtion
OC1 (Groundnut oilcake)	1.83	79.62^{b} (63.18)
OC2 (Neem cake)	2.13	76.29^{c} (60.88)
OC3 (Mahua cake)	9.00	0.00^{d} (0.96)
OC4 (Mustard oilcake)	0.00	100.00^{a} (89.04)
Control	9.00	-
SE m (±)	-	0.39
CD (0.05)	-	1.56

*Mean of three replications.

Means followed by a common letter(s) are not significantly different by one- way ANOVA at P = 0.05)

(Figures given in the parenthesis are transformed values).

Plate 11. Effect of antifungal nature of plants against *R. solani* under *in vitro* conditions

Plate 12. Effect of antifungal nature of plant oils against *R. solani* under *in vitro* conditions

Plate 13. Evaluation of antifungal nature of oil cakes against *R. solani* under *in vitro* conditions

4.3.2. Evaluation of biofumigant nature of different plants, plant oils and oil cakes against *R. solani* under *in vitro* conditions

4.3.2.1. Preparation of plants, plant oils and oil cakes for biofumigation

Plants, plant oils and oil cakes were prepared for biofumigation

4.3.2.1.1. Plants

The *in vitro* biofumigant effect of the plants tested against *R. solani* showed significant differences among the treatments. The maximum suppression (100 per cent) was noticed with cabbage, cassava, garlic creeper and mustard which were statistically on par with the control. This was followed by radish, moringa, neem and lemon grass which gave suppression of 87.80 per cent, 83.30 per cent, 77.88 per cent and 76.67 per cent, respectively. Here, the treatment with radish, moringa, neem and lemongrass were significantly different compared to the control. However, under *in vitro* conditions, the biofumigant activity of neem and lemongrass were statistically on par. The suppression of pathogen in treatments with sweet potato and papaya recorded lower values of 52.20 per cent and 27.78 per cent, respectively (Table 7, Plate 14).

4.3.2.1.2. Plant oils

Of the four plant oils evaluated for their effectiveness as a biofumigant on mycelial growth of *R. solani*, the maximum suppression (100 per cent) was obtained with both lemongrass oil and tea tree oil. But neem oil and mahua oil did not have any inhibitory biofumigant effect on the collar rot pathogen (Table 8, Plate 15).

4.3.2.1.3. Oil cakes

The biofumigant effect was found maximum (100 per cent) with mustard oil cake only. The suppression of pathogen in treatments with groundnut and neem cake

Table 7. Biofumigant effect of plants on *R. solani* under *in vitro* conditions

Plants	Mycelial growth* (cm)	% Inhibition
P1 (Cabbage)	0.00	100.00[a] (89.04)
P2 (Cassava)	0.00	100.00[a] (89.04)
P3 (Garlic creeper)	0.00	100.00[a] (89.04)
P4 (Lemongrass)	2.10	76.67[d] (61.59)
P5 (Moringa)	1.50	83.30[c] (66.18)
P6 (Mustard)	0.00	100.00[a] (89.04)
P7 (Neem)	2.00	77.80[d] (61.89)
P8 (Papaya)	6.50	27.78[f] (31.73)
P9 (Radish)	1.10	87.80[b] (69.56)
P10 (Sweet potato)	4.30	52.20[e] (46.28)
Control	9.00	-
SE m (±)	-	1.30
CD (0.05)	-	2.14

(Figures given in the paranthesis are transformed values).
*Mean of three replications.
Means followed by a common letter(s) are not significantly different by one- way ANOVA at P = 0.05)

Table 8. Biofumigant effect of plant oils against *R.solani* under *in vitro* conditions

Plant oils	Mycelial growth* (cm)	% Inhibition
O1 (Neem oil)	9.00	0.00[b] (0.96)
O2 (Lemongrass oil)	0.00	100.00[a] (89.04)
O3 (Mahua oil)	9.00	0.00[b] (0.959)
O4 (Tea tree oil)	0.00	100.00[a] (89.04)
Control	9.00	-
SE m (±)	-	1.12
CD(0.05)	-	1.20

Table 9. Biofumigant effect of oil cakes against *R.solani* under *in vitro* conditions

Oil cakes	Mycelial growth* (cm)	% Inhibition
OC1 (Groundnut cake)	8.50	5.56[b] (13.57)
OC2 (Neem cake)	8.73	3.00[c] (9.56)
OC3 (Mahua cake)	9.00	0.00[d] (0.96)
OC4 (Mustard oilcake)	0.00	100.00[a] (89.04)
Control	9.00	-
SE m (±)	-	0.82
CD(0.05)	-	3.28

*Mean of three replications.
Means followed by a common letter(s) are not significantly different by one- way ANOVA at P = 0.05)
Figures in the parenthesis are transformed

Plate 14. Biofumigant effect of plants against *R. solani* under *in vitro* conditions

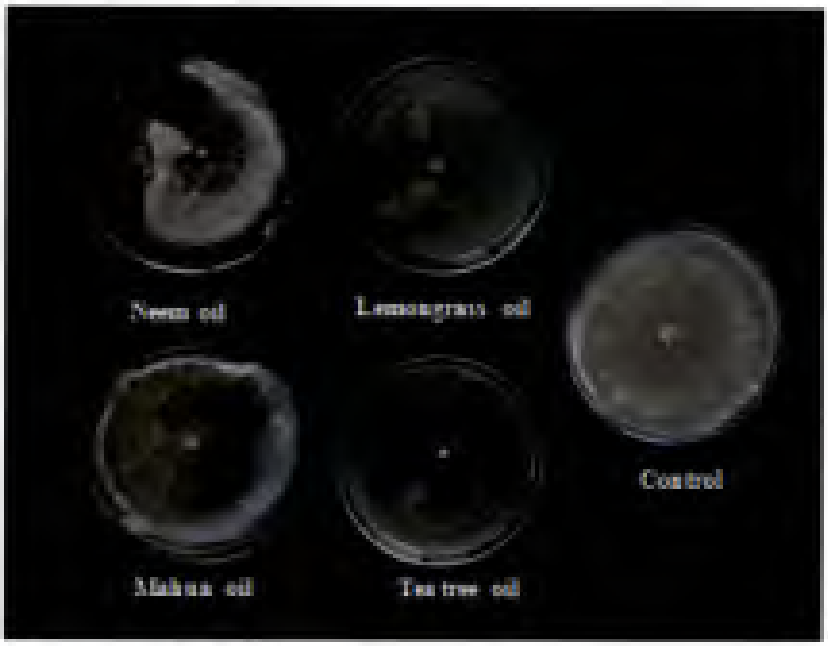


Plate 15. Biofumigant effect of plant oils against *R. solani* under *in vitro* conditions

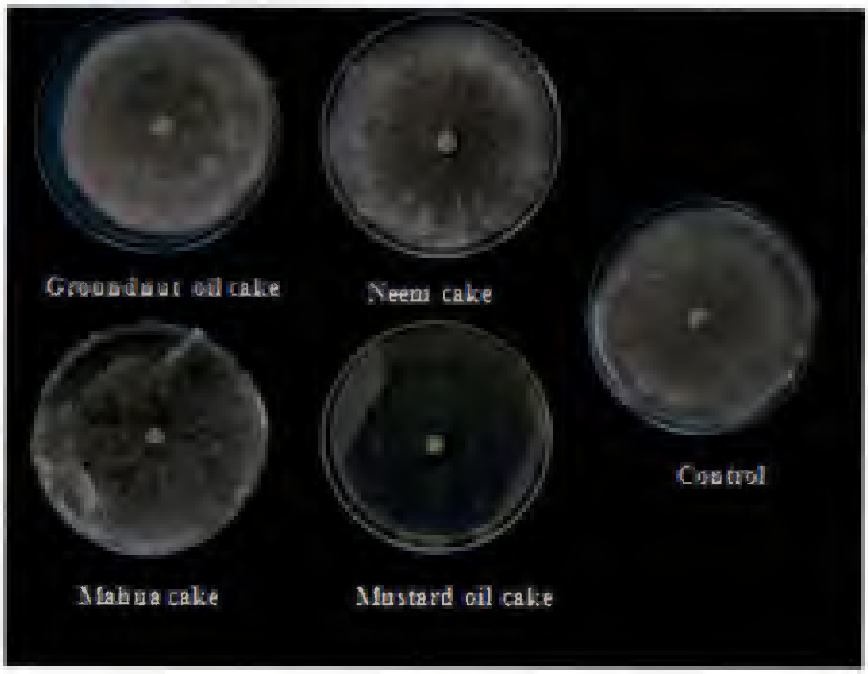


Plate 16. Biofumigant effect of oil cakes against *R. solani* under *in vitro* conditions

recorded lower values of 5.56 per cent and 3.00 per cent, respectively (Table 9, Plate 16).

4.3.3. Effect of biofumigants on mycelial regeneration from sclerotia of *R. solani*

Biofumigant effect of plants (50 g kg^{-1} soil), plant oils (5% soil drench), oil cakes (10 g kg^{-1} soil) on mycelial regeneration from sclerotia was assessed at intervals of 24 h, one week, two weeks and one month by inoculating the sclerotia exposed to biofumigants in confined container on PDA medium. The results indicated that biofumigation with plants, plant oils and oil cakes, in general, suppressed mycelial regeneration from sclerotia. The extent of suppression was found to increase with period of incubation of the biofumigants.

4.3.3.1. Plants

On biofumigation with cabbage, garlic creeper and mustard mycelial regeneration from sclerotia was completely suppressed after 24 h itself which was statistically significant over control. This was followed by the effect of lemongrass and radish causing a suppression of 44.1 per cent and 36.33 per cent, respectively, which were statistically on par. After an interval of one week, cabbage, garlic creeper and mustard showed 100 per cent suppression on re-generation from sclerotia. But this was followed by neem and cassava with a suppression of 75.56 per cent and 61.48 per cent, respectively, which varied significantly. After two weeks interval, complete suppression on mycelial regeneration from sclerotia was obtained with cabbage, cassava, garlic creeper and mustard. This was followed by sweet potato, papaya and neem with a suppression of 84.81 per cent, 80.37 per cent, 80.00 per cent respectively. After one month interval also, the plant extract of cabbage, cassava, garlic creeper and mustard resulted in 100 per cent suppression of the pathogen. These treatments were statistically on par between themselves, but they differed

significantly compared to control. This was followed by sweet potato and moringa with a suppression on mycelial growth of the pathogen by 81.85 per cent and 81.11 per cent, respectively. These were statistically on par with each other (Table 10, Plate 17-20).

4.3.3.2. Plant oils

Effect of soil biofumigation with selected plant oils under *in vitro* conditions in confined containers on mycelial regeneration from sclerotia of *R. solani* was assessed. After 24 h, 100 per cent suppression was obtained with lemongrass oil and tea tree oil. This was followed by neem oil with a suppression of 26.67 per cent. After one week of biofumigation with lemongrass oil and tea tree oil, there was complete suppression of the collar rot pathogen. No significant difference was noticed among the remaining two plant oils. The per cent inhibition was found to be 44.89 and 44.44 for neem and mahua oil, respectively. After two weeks also, 100 per cent suppression on mycelial re-generation from sclerotia was noticed with lemongrass and tea tree oil. The inhibition with neem oil was 54.44 per cent and this was followed by mahua oil with a suppression of 44.44 per cent. These two treatments were significantly different compared to the control. After one month interval, the biofumigation with neem oil, lemongrass oil and tea tree oil caused complete suppression on mycelial regeneration from sclerotia. The percentage inhibition with mahua oil was 61.56 which was significantly superior to control (Table 11, Plate 21-24).

4.3.3.3. Oil cakes

The performance of four oil cakes in suppressing mycelial regeneration from sclerotia was tested at four different time intervals. After 24 h, the treatments showed significant variation in the extent of suppression. The inhibition was maximum (100

Table 10. Effect of soil biofumigation with selected plants under *in vitro* in confined containers on mycelial regeneration from sclerotia of *R. solani*
(% suppression of mycelial regeneration from sclerotia)

Plants	After 24 h	After one week	After two weeks	After one month
P1	100.00[a] (89.04)	100.00[a] (89.04)	100.00[a] (89.04)	100.00[a] (89.04)
P2	28.56[b] (36.47)	61.48[c] (51.66)	100.00[a] (89.04)	100.00[a] (89.04)
P3	100.00[a] (89.04)	100.00[a] (89.04)	100.00[a] (89.04)	100.00[a] (89.04)
P4	44.11[b] (30.685)	44.11[e] (41.60)	77.40[de] (61.64)	76.67[d] (61.13)
P5	0.00[d] (0.96)	53.70[d] (47.12)	77.04[e] (61.37)	81.11[b] (64.25)
P6	100.00[a] (89.04)	100.00[a] (89.04)	100.00[a] (89.04)	100.00[a] (89.04)
P7	12.78[c] (12.95)	75.56[b] (60.39)	80.00[cd] (63.47)	77.04[d] (61.40)
P8	16.33[c] (15.44)	55.56[d] (46.71)	80.37[c] (63.74)	79.63[bc] (63.18)
P9	36.33[b] (36.72)	42.59[e] (40.73)	77.41[de] (61.62)	77.22[cd] (61.62)
P10	0.00[d] (0.96)	44.44[e] (40.730)	84.80[b] (67.08)	81.85[b] (64.79)
C	0.00[d] (0.96)	0.00[d] (0.96)	0.00[d] (0.96)	0.00[d] (0.96)
SE m ((±)	2.26	0.67	0.57	0.45
CD (0.05)	3.02	2.44	2.07	1.64

Means followed by a common letter(s) are not significantly different by one- way ANOVA at P = 0.05)
Figures in the parenthesis are transformed values
P1(cabbage), P2 (cassava), P3 (garlic creeper), P4(lemon grass), P5 (moringa), P6 (mustard), P7 (neem), P8 (papaya), P9 (radish), P10 (sweet potato), C (Control)

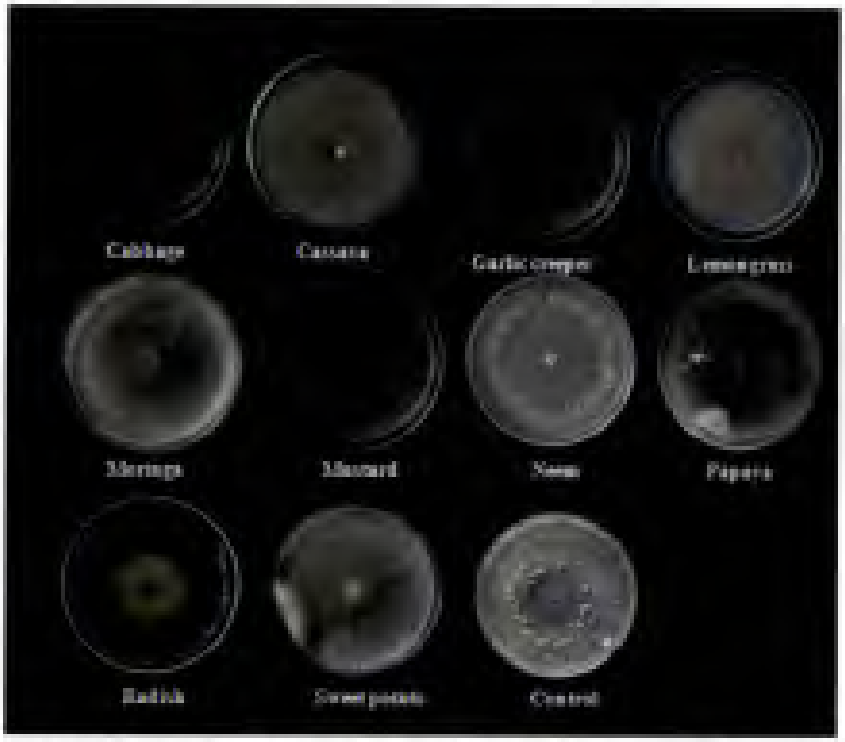


Plate 17. Effect of soil biofumigation with selected plants in confned containers on mycelial regeneration from sclerotia of *R. solani* (after 24h)

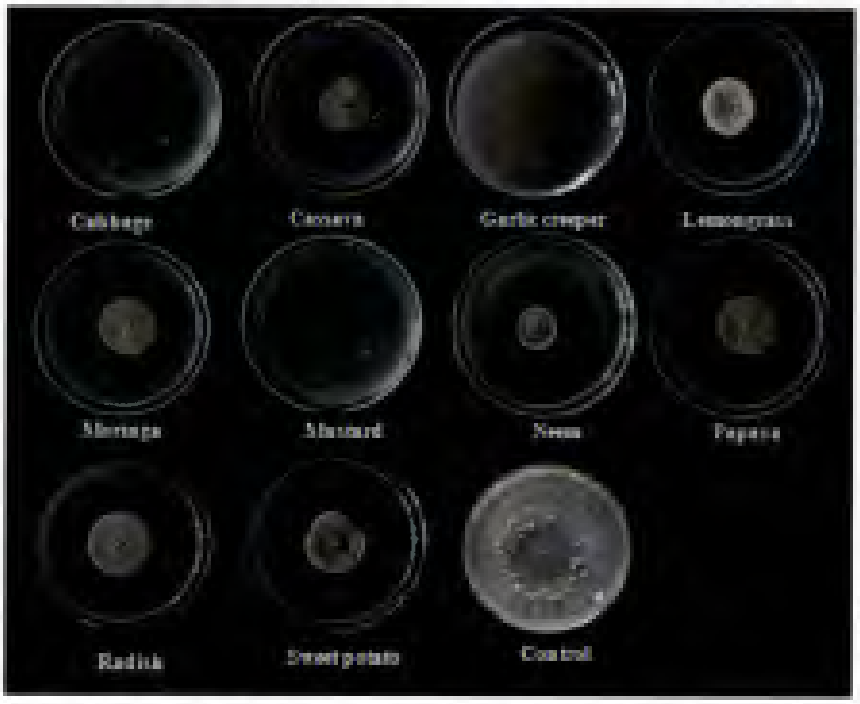


Plate 18. Effect of soil biofumigation with selected plants in confined containers on mycelial regeneration from sclerotia of *R. solani* (after one week)

Plate 19. Effect of soil biofumigation with selected plants in confined containers on mycelial regeneration from sclerotia of *R. solani* (after two weeks)

Plate 20. Effect of soil biofumigation with selected plants in confined containers on mycelial regeneration from sclerotia of *R. solani* (after one month)

Table 11. Effect of soil biofumigation with selected plant oils under *in vitro* in confined containers on mycelial regeneration from sclerotia of *R. solani*

	% Suppression of mycelial regeneration from sclerotia			
Plant oils	After 24 h	After one week	After two weeks	After one month
O1 (Neem oil)	26.67^{b} (31.09)	44.89^{b} (42.03)	54.44^{b} (47.55)	100.00^{a} (89.04)
O2 (Lemon grass oil)	100.00^{a} (89.04)	100.00^{a} (89.04)	100.00^{a} (89.04)	100.00^{a} (89.04)
O3 (Mahua oil)	10.00^{c} (19.45)	44.44 ^{b} (41.75)	44.44^{c} (41.81)	61.56^{b} (51.63)
O4 (Tea tree oil)	100.00^{a} (89.04)	100.00^{a} (89.04)	100.00^{a} (89.04)	100.00^{a} (89.04)
Control	0.00^{d} (0.96)	0.00^{d} (0.96)	0.00^{d} (0.96)	0.00^{d} (0.96)
SE m (±)	0.29	0.15	0.16	0.08
CD (0.05)	1.16	1.08	0.62	0.35

Means followed by a common letter(s) are not significantly different by one- way ANOVA at P = 0.05)
Figures in the parenthesis are transformed values

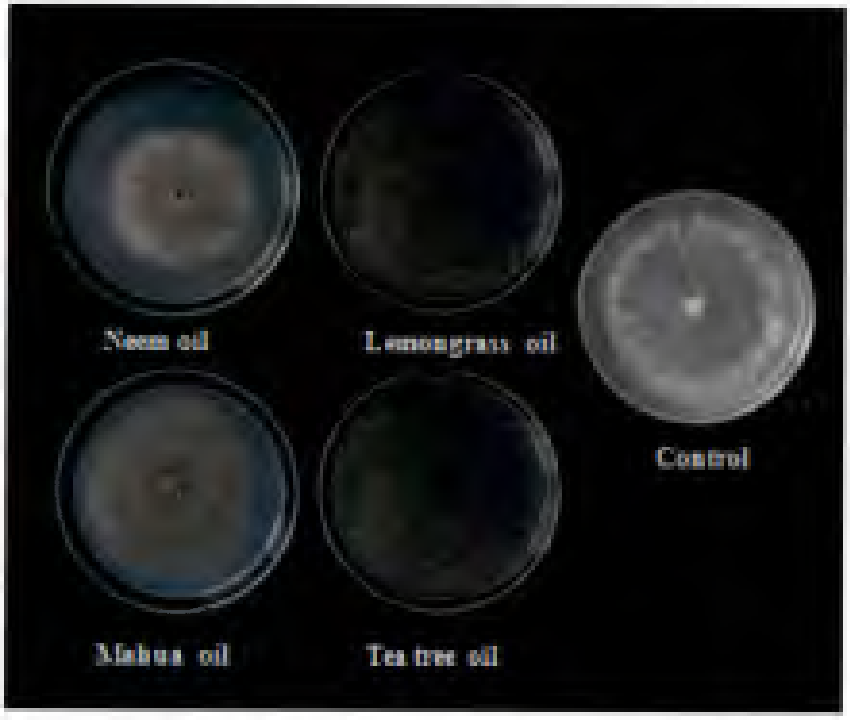


Plate 21. Effect of soil biofumigation with selected plant oils in confined containers on mycelial regeneration from sclerotia of *R. solani* (after 24 h)

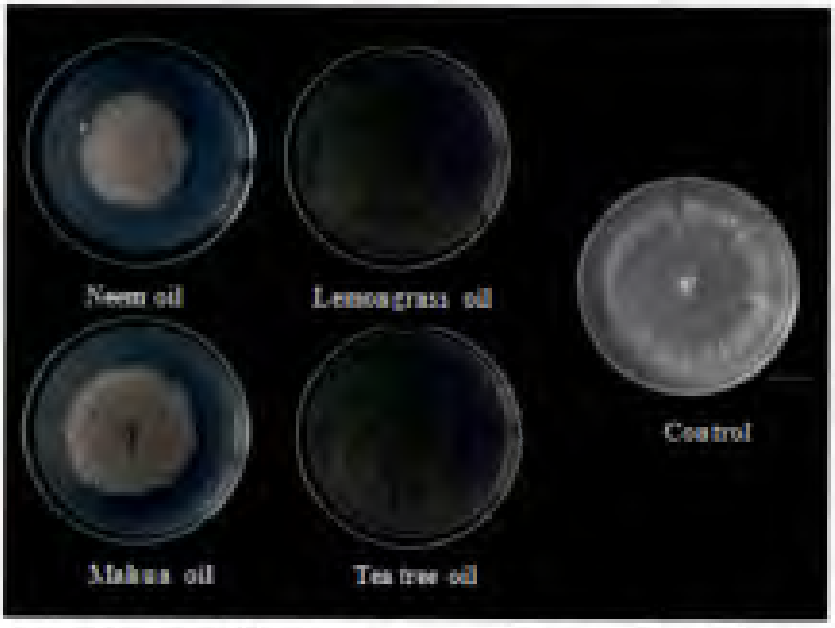


Plate 22. Effect of soil biofumigation with selected plant oils in confined containers on mycelial regeneration from sclerotia of *R. solani* (after one week)

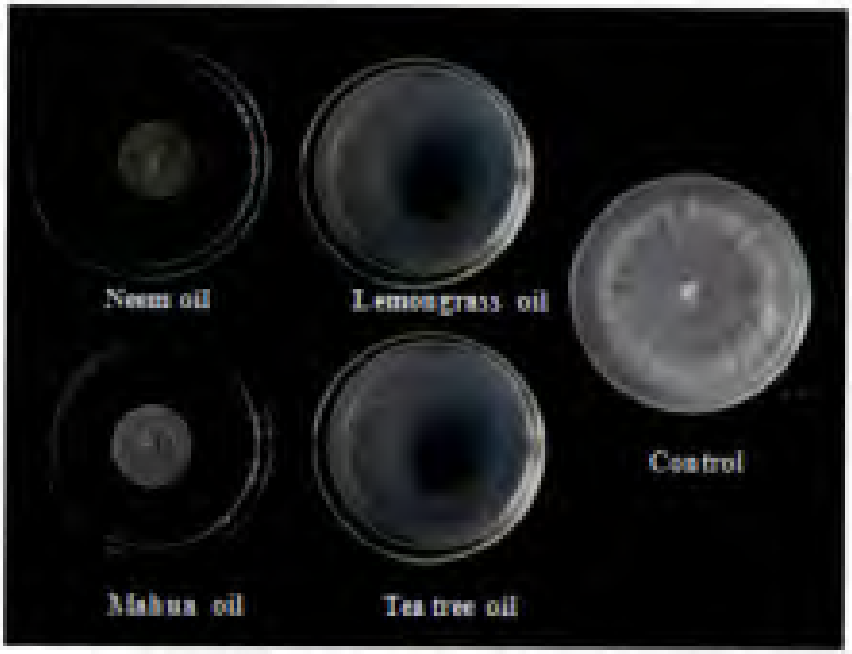


Plate 23. Effect of soil biofumigation with selected plant oils in confined containers on mycelial regeneration from sclerotia of *R. solani* (after two weeks)

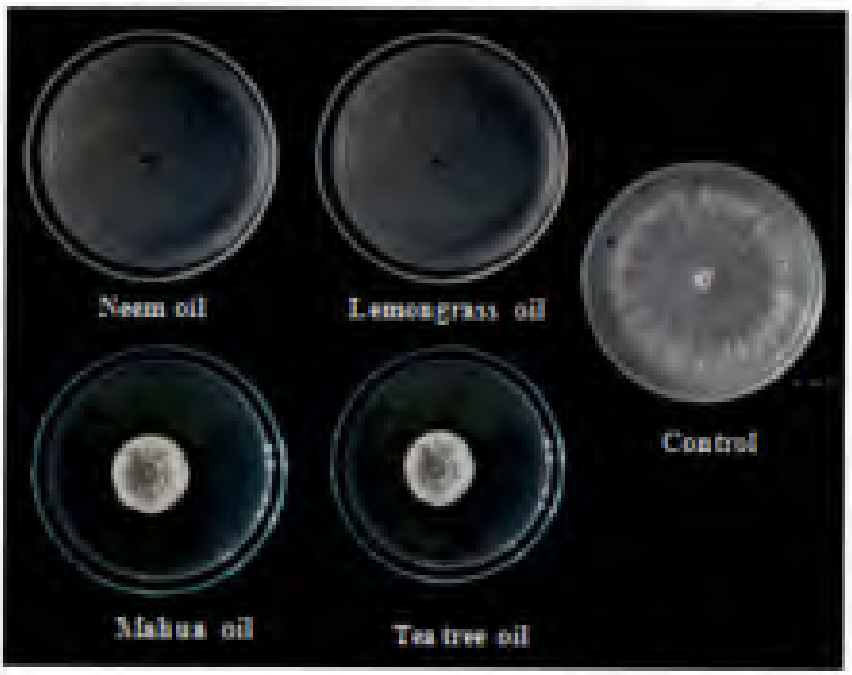


Plate 24. Effect of soil biofumigation with selected plant oils in confined containers on mycelial regeneration from sclerotia of *R. solani* (after one month)

per cent) with mustard oil cake. This was followed by groundnut oil cake with 35.56 percentage inhibition. After 24 h, neem and mahua cake did not have any inhibitory effect on mycelial regeneration from sclerotia. After one week interval, complete suppression was obtained with mustard oil cake, and this was followed by groundnut oil cake with 35.18 per cent suppression of collar rot pathogen. After two weeks interval, inhibition with mustard oil cake was maximum and this was followed by groundnut, neem and mahua cake with an inhibition of 69.44 per cent, 64.89 per cent and 54.44 per cent, respectively. The biofumigant effect of groundnut cake and neem cake were statistically on par. After one month interval, both mustard and groundnut cake gave 100 per cent suppression on mycelial re-generation from sclerotia. This was followed by neem and mahua cake with a value of 86.44 per cent and 73.33 per cent, respectively which were statistically significant (Table 12, Plate 25-28).

4.4. ASSESSMENT OF THE EFFECT OF BIOFUMIGATION FOR MANAGEMENT OF COLLAR ROT OF COWPEA

An experiment was conducted under pot culture conditions in C.R.D. to evaluate the efficacy of selected biofumigants involving 11 treatments and 5 replications. The potting mixture was artificially inoculated with the most virulent isolate of *R. solani*, R1. The treatments included the selected plants (50 g kg^{-1} soil), plant oils (5% soil drench) and oil cakes (10 g kg^{-1} soil). Cowpea seeds of variety, Bhagyalakshmy were sown in pots two weeks after biofumigation. The effect of biofumgation on collar rot incidence was compared with that of inoculated control, un-inoculated control and chemical control (0.1 per cent carbendazim).

The per cent disease incidence and intensity of collar rot were calculated based on standard procedures (Table 13). In collar rot disease disease incidence and intensity maximum suppression (100 per cent) was noticed on plants grown in soil biofumigated with mustard plant (50 g kg^{-1} soil) and mustard oil cake (10 g kg^{-1} soil)

Table 12. Effect of soil biofumigation with oilcakes under *in vitro* in confined containers on mycelial regeneration from sclerotia of *R. solani*

	% Suppression of mycelial re-generation from sclerotia			
Oil cakes	After 24 h	After one week	After two weeks	After one month
OC1 (Groundnut oil cake)	35.56[b] (36.59)	35.18[b] (36.59)	69.44[b] (59.03)	100.00[a] (89.04)
OC2 (Neem cake)	0.00[c] (0.96)	5.56 [c] (13.59)	64.89[b] (53.63)	86.44[b] (68.44)
OC3 (Mahua cake)	0.00[c] (0.96)	0.00[d] (0.96)	54.44[c] (47.55)	73.33[c] (58.91)
OC4 (Mustard oil cake)	100.00[a] (89.04)	100.00[a] (89.04)	100.00[a] (89.04)	100.00[a] (89.04)
Control	0.00[d] (0.96)	0.00[d] (0.96)	0.00[d] (0.96)	0.00[d] (0.96)
SE m (±)	1.66	0.67	0.57	0.45
CD(0.05)	2.26	4.96	5.78	1.14

Means followed by a common letter(s) are not significantly different by one- way ANOVA at P = 0.05)
Figures in the parenthesis are transformed values

Plate 25. Effect of soil biofumigation with selected oil cakes in confined containers on mycelial regeneration from sclerotia of *R. solani* (after 24 h)

Plate 26. Effect of soil biofumigation with selected oil cakes in confined containers on mycelial regeneration from sclerotia of *R. solani* (after one week)

Plate 27. Effect of soil biofumigation with selected oil cakes in confined containers on mycelial regeneration from sclerotia of *R. solani* (after two weeks)

Plate 28. Effect of soil biofumigation with selected oil cakes in confined containers on mycelial regeneration from sclerotia of *R. solani* (after one month)

Table 13. Effect of biofumigation on incidence and intensity of collar rot disease

Treatments	Disease Incidence	% disease Suppression	Disease Intensity	% disease suppression
T1	27.78^{bc} (31.55)	72.22	22.22^{b} (31.49)	77.78
T2	44.44^{bc} (41.75)	55.56	36.89^{b} (37.40)	63.11
T3	38.89^{bc} (38.50)	61.11	29.63^{b} (32.86)	70.37
T4	0.00^{f} (0.28)	100.00	0.00^{d} (0.28)	100.00
T5	50.00^{b} (45.00)	50.00	46.29^{b} (42.88)	53.71
T6	22.22^{cd} (27.81)	77.78	38.89^{b} (46.10)	61.11
T7	11.11^{de} (16.16)	88.89	11.11^{c} (16.16)	88.89
T8	0.00^{f} (0.28)	100.00	0.00^{d} (0.28)	100.00
T9	94.44^{a} (81.78)	5.56	90.27^{a} (78.91)	9.73
T10	5.56^{ef} (8.22)	94.44	5.56^{cd} (8.22)	94.44
T11	0.00^{f} (0.28)	100.00	0.00^{d} (0.28)	100.00
SE m (±)	1.41	-	0.61	-
CD (0.05)	14.40	-	15.32	-

Means followed by a common letter(s) are not significantly different by one- way ANOVA at P = 0.05)
Figures in the parenthesis are transformed values

T1 - Cabbage (*Brassica oleracea* var *capitata*), T2 - Cassava (*Manihot esculenta* Crantz), T3 - Garlic creeper (*Mansoa alliacea* Lam.), T4 - Mustard(*Brassica juncea* L.), T5 - Lemongrass oil, T6 - Tea tree oil, T7 - Ground nut oil cake, T8- Mustard oil cake, T9 - Inoculated control, T10- Un-inoculated control, T11- Chemical control check (Carbendazim 0.1%)

(Plate 29 a-b). This result was on statistically par with chemical control and un-inoculated control.

4.4.1. Plants

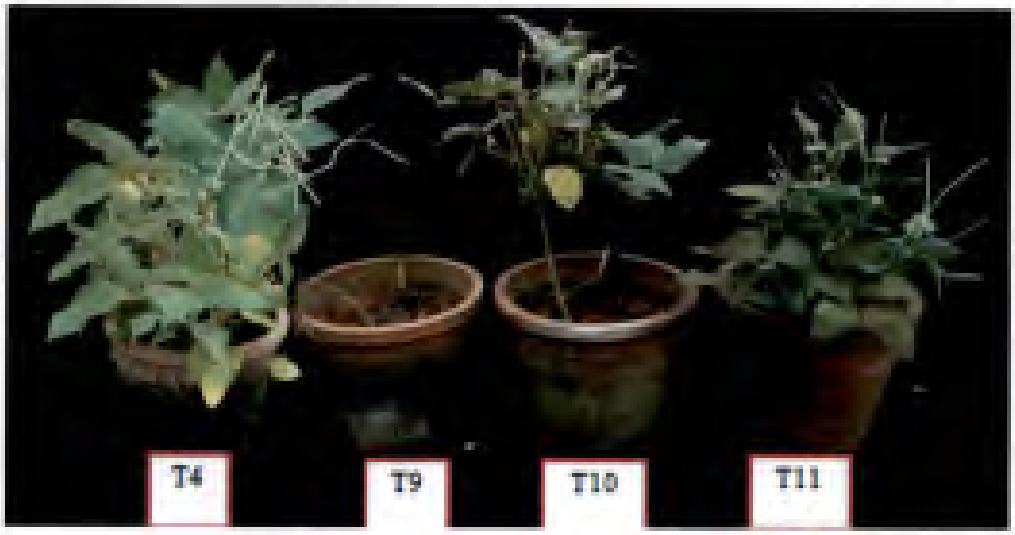
In the case of disease incidence on biofumigation with plants, the percentage disease suppression was found to be maximum on plants grown in pots biofumigated with mustard plant (T4). This result was statistically on par with chemical control and un-inoculated control. This result was followed by biofumigation with cabbage (T1) with a percentage disease suppression of 72.22 %. T1 was statistically on par with, T3 (garlic creeper) and T2 (cassava) with a percentage disease suppression of 61.11 % and 55.56 %, respectively.

In the case of disease intensity, complete collar rot suppression was obtained on plants grown in pots biofumigated with mustard plant (T4). This result was significantly different from biofumigation with other selected plants, such as cabbage, garlic creeper and cassava which were statistically on par between themselves, with a percentage disease suppression of 77.78 %, 70.3 % and 63.11 %, respectively.

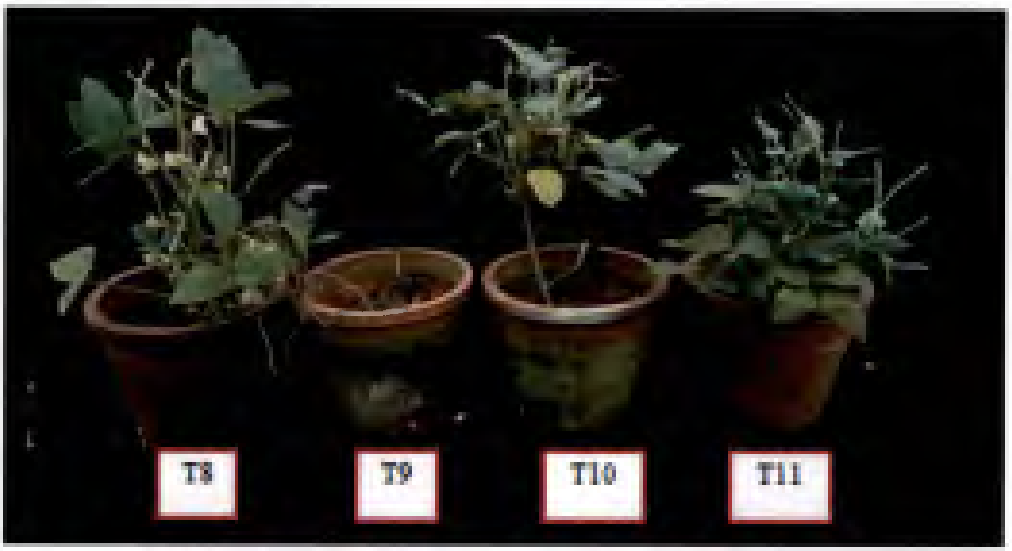
4.4.2. Plant oils

The least disease incidence was noticed, on plants grown in pots biofumigated with tea tree oil, T6 (22.22%) which was significantly different compared to inoculated control (T9), un-inoculated control (T10) and chemical control (T11) with a disease incidence of 94.44 %, 5.56 % and zero per cent. This was followed by biofumigation with lemongrass oil (T5) with a disease incidence of 50.00%.

The least disease intensity, was obtained on plants, grown in pots biofumigated with tea tree oil (T6) with a recorded value of 38.89 % and this was followed by lemongrass oil with a disease intensity of 46.29 % and these two treatments were statistically on par.



a. Effect of biofumigation with mustard plant



b. Effect of biofumigation with mustard oil cake

Plate 29 a-b Biofumigation under *in vivo* with mustard plant and mustard oil cake

4.4.3. Oil cakes

In the case of disease incidence, percentage disease suppression was found to be maximum on plants grown in pots biofumigated with mustard oil cake (T8). The result was statistically on par with chemical control (T11) and un-inoculated control (T10) followed by biofumigation with groundnut oil cake, T7 (88.89 %).

The least disease intensity was recorded on plants, grown in pots biofumigated with mustard oil cake (T8). There was a total suppression disease in that treatment. This observation was on par with chemical control (T11) and un-inoculated control (T10). Treatment, T7 in which biofumigation was done with groundnut oil cake, an inhibition of 88.89 % was observed.

4.5. POPULATION DYNAMICS OF MICROFLORA IN BIOFUMIGATED SOIL

The population of saprophytic fungi and bacteria were enumerated from biofumigated soil at different time intervals ie., before biofumigation, immediately after biofumigation (24 h), one week after sowing cowpea and two weeks after sowing cowpea.

4.5.1. Population dynamics of saprophytic fungi

The population of saprophytic fungi exhibited a decrease in the biofumigated treatments and an increasing trend after raising the crop (Table 14). Though there was slight differences in the population of saprophytic fungi in the soil from different pots before the start of biofumigation all the values were statistically on par. In general, the population of saprophytic fungi in biofumigated soil was higher with respect to inoculated control.

The fungal population showed slight variations before biofumigation, however, the population variation was statistically non significant. Immediately after biofumigation, the population declined and the least value was recorded from soil

Table 14. Population dynamics of saprophytic fungi in biofumigated soil at different time intervals (cfu/g $\times 10^4$)

Treatments	Before biofumigation	After biofumigation	One week after sowing	Two weeks after sowing
T1	21.00[a]	12.00[ef]	32.33[e]	65.33[c]
T2	19.33[a]	14.00[d]	44.67[b]	78.00[b]
T3	21.33[a]	16.00[b]	40.00[c]	46.33[f]
T4	19.67[a]	10.50[g]	56.00[a]	91.67[a]
T5	19.67b[a]	12.20[e]	23.67[f]	33.33[h]
T6	22.00[a]	11.50[ef]	24.67[f]	46.00[f]
T7	19.67[a]	15.00[c]	25.67[f]	29.00[i]
T8	20.00[a]	11.33[f]	36.00[d]	64.00[c]
T9	21.00[a]	16.00[b]	30.00[e]	38.00[g]
T10	22.00[a]	22.00[a]	41.67[c]	55.00[d]
T11	20.67[a]	15.00[c]	31.67[e]	52.67[e]
SE m (±)	-	0.22	0.76	0.81
CD(0.05)	NS	0.79	2.74	2.32

Means followed by a common letter(s) are not significantly different by one-way ANOVA at P = 0.05)

T1 - Cabbage (*Brassica oleracea* var *capitata*), T2 - Cassava (*Manihot esculenta* Crantz), T3 - Garlic creeper (*Mansoa alliacea* Lam.), T4 - Mustard(*Brassica juncea* L.), T5 - Lemongrass oil, T6 - Tea tree oil, T7 - Ground nut oil cake, T8- Mustard oil cake, T9 - Inoculated control, T10- Un-inoculated control, T11- Chemical control check (Carbendazim 0.1%)

treated with mustard plant, T4 (10.50× 10^4 cfu g^{-1}) followed by mustard oil cake, T8 (11.33 × 10^4 cfu g^{-1}). The above values were, however, significantly different from each other. The treatment T8 was statistically on par with T6 (tea tree oil) that recorded 11.50 × 10^4 cfu g^{-1} population of fungi.

After one week interval also, the treatment with mustard plant (T4) recorded higher fungal population of 56 × 10^4 cfu g^{-1}, which was significantly superior to inoculated control (T7) with a population of 30 × 10^4 cfu g^{-1}. This was followed by T2 (cassava), T10 (sweet potato) and T3 (garlic creeper) with recorded values of 44.67× 10^4 cfu g^{-1}, 41.6 × 10^4 cfu g^{-1} and 40 × 10^4 cfu g^{-1} respectively. The treatment T10 was statistically on par with T3 (garlic creeper). The treatment with mustard oil cake (T8) recorded a population of 36 × 10^4 cfu g^{-1}.

The highest fungal population of 91.67 × 10^4 cfu g^{-1} was recorded from soil biofumigated with mustard plant (T4) after two weeks of raising the crop. Two weeks after raising the crop, the second highest fungal population (78.00× 10^4 cfu g^{-1}) was recorded from pots biofumigated with cassava (T2). This was followed by cabbage (T1) which was on par with mustard oil cake (T8) with a fungal population of 65.33 × 10^4 cfu g^{-1}. One week after raising the crop the second highest fungal population of 44.67 × 10^4 cfu g^{-1} was recorded from soil biofumigated with cassava (T2).

4.5.1.1. Effect of selected saprophytic fungi on collar rot pathogen

Among the saprophytic fungi obtained from biofumigated soil, the most frequent flora were selected and assessed for their ability to inhibit *R. solani* by dual culture technique. The percentage suppression of mycelial growth of *R. solani* was recorded under *in vitro* conditions (Table 15, Plate 30a).

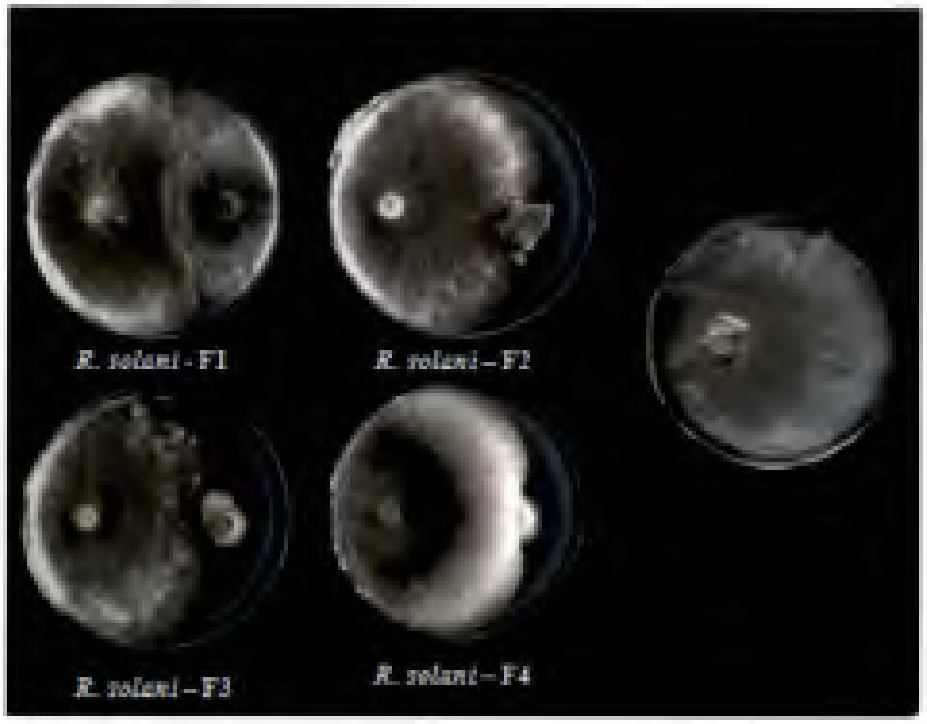
Four fungi were isolated and pure cultures were maintained. Dual culturing each fungus with the pathogen on PDA medium revealed that there was statistical

Table 15. Effect of predominant saprophytic fungi obtained from biofumigated soil on *R.solani* under *in vitro* conditions

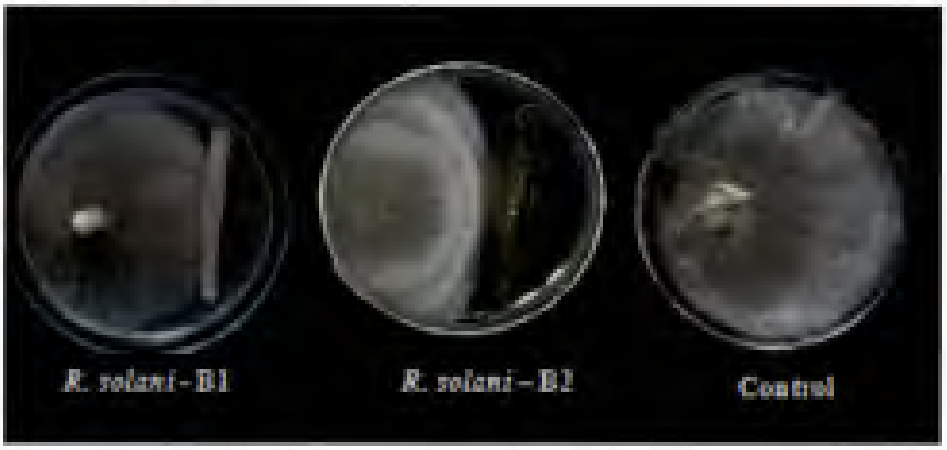
Fungi	Mycelial growth* (cm)	Percentage inhibition
F1	4.73	47.44[a]
F2	5.6	37.78[b]
F3	6.5	27.78[c]
F4	6.3	30.00[d]
Control	9.00	-
SE m (±)	-	0.23
CD (0.05)	-	0.88

* Mean of three replications

Means followed by a common letter(s) are not significantly different by one-way ANOVA at P = 0.05)



a. Effect of saprophytic fungi from biofumigated soil on *R. solani* under *in vitro* conditions



b. Effect of saprophytic bacteria from biofumigated soil on *R. solani* under *in vitro* conditions

Plate 30 a-b. Effect of saprophytic microflora from biofumigated soil on *R. solani* under *in vitro* conditions

difference in the extent of inhibition effected by the saprophytic fungi obtained from the soils of biofumigated treatments. The highest suppression (47.44 %) was noticed with isolate, F1. An inhibition of 37.78 per cent was recorded with the isolate, F2 and this was followed by fungal isolates, F4 and F3 with a suppression of 30 per cent and 27.78 per cent, respectively.

4.5.2. Population dynamics of saprophytic bacteria

The population of saprophytic bacteria declined immediately after biofumigation and, thereafter, showed an increasing trend. Before biofumigation bacterial population was unchanged and statistically non significant. But immediately after biofumigation a general decline was noticed and the least population (8.17×10^6 cfu g^{-1}) was recorded from pots treated with mustard plant (T4) followed by mustard oil cake, T8 (8.70×10^6 cfu g^{-1}). The highest bacterial population of 18×10^6 cfu g^{-1} was recorded from T11, chemical control check and this was followed by T7, un-inoculated control with a population of 17.33×10^6 cfu g^{-1} (Table 16).

After one week of raising cowpea, the highest bacterial population was recorded from soil biofumigated with mustard oil cake with 54.67×10^6 cfu g^{-1} and this was followed by mustard plant (T4) and chemical control check (T11) with a population of 51.67×10^6 cfu g^{-1} and 51×10^6 cfu g^{-1}, respectively. These three treatments T8, T4 and T11 were on par. A bacterial population of 45.67×10^6 cfu g^{-1} was recorded from T10 (un-inoculated control) and this bacterial count was on par with T7 (groundnut oil cake) and T6 (tea tree oil) with a bacterial population of 45×10^6 cfu g^{-1} and 40.67×10^6 cfu g^{-1}, respectively

The highest population of saprophytic bacteria was recorded two weeks after raising the crop, from the soil biofumigated with mustard oil cake (T8) with a bacterial population of 56.33×10^6 cfu g^{-1}. This result was on par with bacterial population (55.33×10^6 cfu g^{-1}) enumerated from soil biofumigated with mustard

Table 16. Population dynamics of saprophytic bacteria in biofumigated soil at different time intervals (cfu/g $\times 10^6$)

Treatments	Before biofumigation	After biofumigation	One week after sowing	Two weeks after sowing
T1	17.00^{a}	9.73^{f}	32.00^{de}	40.67^{de}
T2	17.78^{a}	10.63^{e}	29.33^{de}	33.00^{fg}
T3	18.43^{a}	11.00^{e}	27.67^{e}	32.00^{g}
T4	17.00^{a}	8.17^{g}	51.67^{a}	55.33^{a}
T5	17.00^{a}	12.13^{d}	33.67^{d}	37.67^{ef}
T6	18.43^{a}	9.47^{f}	40.67^{c}	44.67^{cd}
T7	18.00^{a}	13.30^{c}	45.00^{bc}	50.00^{b}
T8	17.00^{a}	8.70^{g}	54.67^{a}	56.33^{a}
T9	16.00^{a}	14.78^{b}	19.00^{f}	21.00^{h}
T10	18.00^{a}	18.00^{a}	45.67^{b}	49.33^{bc}
T11	18.33^{a}	17.33^{a}	51.00^{a}	54.00^{ab}
SE m (±)	-	0.20	0.80	1.36
CD (0.05)	NS	0.73	4.80	4.89

Means followed by a common letter(s) are not significantly different by one- way ANOVA at P = 0.05)

T1 - Cabbage (*Brassica oleracea* var *capitata*), T2 - Cassava (*Manihot esculenta* Crantz), T3 - Garlic creeper (*Mansoa alliacea* Lam.), T4 - Mustard(*Brassica juncea* L.), T5 - Lemongrass oil, T6 - Tea tree oil, T7 - Ground nut oil cake, T8- Mustard oil cake, T9 - Inoculated control, T10- Un-inoculated control, T11- Chemical control check (Carbendazim 0.1%)

plant (T4). There was no significant difference between treatments such as chemical control check (T11), groundnut oil cake (T7), un-inoculated control (T10) and tea tree oil (T6).

4.5.2.1. Effect of selected saprophytic bacteria on collar rot pathogen

Two predominant colony types were noticed among the saprophytic bacteria obtained from biofumigated soil, and were designated as B1 and B2. These two isolates were assessed for their inhibition to *R. solani* by dual culture technique. The percentage suppression on mycelial growth of *R.solani* was recorded under *in vitro* conditions (Table 17, Plate 30b).

The bacterial isolate, B2 caused maximum suppression of mycelial growth of *R. solani* (44.44 per cent). The isolate, B1 resulted in 37.41 per cent suppression of the collar rot pathogen. There was statistically significant difference between the inhibition exerted by these two isolates on collar rot pathogen.

4.6. BIOMETRIC OBSERVATIONS

Biometric observations like shoot length (cm), root length (cm), fresh weight-shoot (g), dry weight - shoot (g), fresh weight-root (g), dry weight-root (g), number of root nodules/ plant, number of pods/ plant and yield /plant (g) were recorded. The results on biometric observations revealed significant difference among all the parameters in the various treatments. In general, the maximum value for most of the biometric parameters were obtained in the plants grown on soils biofumigated with mustard plant and mustard oil cake (Table 18) (Plate 31).

4.6.1. Shoot length

The cowpea plants grown on soil biofumigated with macerated mustard plant (T4) recorded maximum shoot length of 37.62 cm followed by plants grown on soil biofumigated with mustard oil cake (34.94 cm). All the treatments were significantly

Table 17. Effect of predominant bacteria obtained from biofumigated soil on *R.solani* under *in vitro* conditions

Bacteria	Mycelial growth* (cm)	Percentage inhibition
B1	5.63	37.41[b]
B2	5.00	44.44[a]
Control	9.00	-
SE m (±)	-	0.021
CD (0.05)	-	0.031

* Mean of three replications

Means followed by a common letter(s) are not significantly different by one- way ANOVA at P = 0.05)

Table 18. Effect of different treatments on growth, nodulation and yield characteristics of cowpea

Treatments	Shoot length (cm)	Root length (cm)	Fresh weight-shoot (g)	Dry weight - shoot (g)	Fresh weight - root (g)	Dry weight - root (g)	No. of root nodules	No. of pods/ plant	Yield/ plant (g)
T1	32.30^{abc}	7.60^{bc}	43.20^{def}	6.17^{de}	11.05^{c}	6.17^{de}	5.40^{bc}	26.80^{cd}	133.60^{cd}
T2	33.90^{abc}	7.68^{abc}	42.50^{ef}	5.77^{ef}	11.17^{c}	5.77^{ef}	5.40^{bc}	28.80^{cd}	124.20^{d}
T3	30.08^{bc}	8.14^{abc}	37.90^{f}	4.84^{f}	7.76^{d}	4.84^{f}	3.40^{c}	28.80^{cd}	144.00^{cd}
T4	37.62^{a}	8.82^{abc}	77.00^{a}	9.79^{a}	12.39^{bc}	9.79^{a}	4.00^{c}	35.60^{b}	185.40^{b}
T5	27.96^{c}	7.00^{c}	45.26^{def}	5.70^{ef}	10.80^{c}	5.70^{ef}	3.40^{c}	23.80^{d}	119.00^{d}
T6	32.10^{abc}	8.30^{abc}	51.03^{cd}	7.08^{cd}	14.08^{ab}	7.08^{cd}	2.00^{c}	23.80^{cd}	136.00^{cd}
T7	31.84^{abc}	7.40^{bc}	42.88^{ef}	6.12^{de}	10.66^{c}	6.12^{de}	9.00^{b}	27.60^{cd}	138.00^{cd}
T8	34.94^{ab}	9.58^{a}	64.82^{b}	9.26^{ab}	15.04^{a}	9.26^{ab}	2.00^{c}	41.80^{a}	209.00^{a}
T9	10.80^{d}	3.60^{d}	14.49^{g}	2.07^{g}	3.50^{e}	2.07^{g}	4.00^{c}	2.00^{e}	10.00^{e}
T10	33.78^{abc}	9.04^{ab}	56.37^{c}	8.09^{bc}	15.68^{a}	8.09^{bc}	22.8^{a}	28.60^{cd}	143.00^{cd}
T11	32.48^{abc}	9.18^{ab}	49.19^{cde}	7.61^{c}	14.14^{ab}	7.61^{c}	22.6^{a}	31.00^{bc}	155.00^{bc}
SE m (±)	1.33	0.43	1.78	0.26	0.44	0.26	0.86	1.18	5.56
CD (0.05)	6.01	1.98	8.04	1.17	2.01	1.17	3.86	5.34	25.05

Figures followed by a common letter(s) are not significantly differed by one –way ANOVA at P= 0.05.

Plate 31. General view of pot culture experiment

different from inoculated control (10.80 cm). However, they were on par with each other.

4.6.2. Root length

Maximum root length (9.58 cm) was recorded for the plants grown on soil biofumigated with mustard oil cake (T8) followed by chemical control check (9.18 cm) and un-inoculated control (9.04 cm). All the other treatments were also significantly superior to inoculated control. But they were statistically on par with each other. The plants from soil biofumigated with mustard plant exhibited root length of 8.82 cm. The root length was more for those plants from pots biofumigated with mustard oil cake and chemical control check compared to the un-inoculated control.

4.6.3. Fresh weight of shoot

The highest fresh weight of shoot (77.0 g) was recorded in plants grown on soil biofumigated with mustard plant (T4) and this was followed by treatment with mustard oil cake (T8) with a fresh weight of 64.82 g. These two treatments were significantly different compared to all other treatments and showed a significant increase in fresh weight compared to that of inoculated control (14.49g). Except T4 and T8, all other treatments were statistically on par compared to the inoculated control. Fresh weight recorded from un-inoculated pot (T10) and chemical control check (T11) were 56.37 g and 49.19 g, respectively.

4.6.4. Dry weight of shoot

All the treatments showed increase in shoot weight compared to inoculated control. Maximum shoot weight of 9.79 g was recorded from plants grown on soil biofumigated with mustard plant (T4) followed by mustard oil cake (T8) with a shoot weight of 9.26 g. All the treatments were significantly different compared to that of

inoculated control (2.07 g) but on par with un-inoculated (8.09 g) and chemical control check (7.61 g).

4.6.5. Fresh weight of root

All the treatments showed increase in fresh weight of root compared to that of inoculated control (3.50 g). The highest fresh weight of root was recorded from un-inoculated control (15.68 g), which was on par with plants treated with mustard oil cake (15.04 g), chemical control check (14.14 g) and tea tree oil (14.08 g). These differed significantly when compared with inoculated control.

4.6.6. Dry weight of root

The highest dry weight of root was obtained in plants treated with chemical control check (4.46 g) and this result was on par with un-inoculated control (4.43 g) and treatment with mustard oil cake (4.36 g). All the treatments showed increase in dry weight of root compared to that of the inoculated control and significantly differed from the inoculated control (0.88 g).

4.6.7. Number of root nodules /plant

Maximum number of root nodules of 22.80 was recorded from un-inoculated control. This was on par with chemical control check (22.60). The un-inoculated control and chemical control check were significantly different from all other treatments. All the treatments showed a decrease in number of root nodules as compared to the un-inoculated control.

4.6.8. Number of pods/plant

The results revealed that number of pods was maximum in plants grown on soil biofumigated with mustard oil cake (41.80) which was significantly different from inoculated control (2.0). This was followed by mustard plant 35.60 pods/plant..

All the treatments showed an increase in pod number when compared to inoculated control.

4.6.9. Yield/plant

The highest pod yield was recorded from plants grown on soil biofumigated with mustard oil cake (209.0 g) followed by the treatment with mustard plant (185.4 g). The pod weight from plants grown on soil biofumigated with mustard oil cake was significantly higher than chemical control check (155.0 g), un-inoculated control (143.0 g) and inoculated control (10.0 g). All the treatments were significantly different from inoculated control and produced higher pod yield than inoculated control.

Discussion

5. DISCUSSION

Cowpea (*Vigna unguiculata* (L.) Walp) is remunerative crop in Kerala grown for its immature pods used as vegetable. Cowpea is susceptible to an array of pathogens which can result in substantial loss in yield. Among the fungal diseases collar rot caused by *Rhizoctonia solani* is a major concern. Considering the soil borne nature, survival mechanisms and wide host range, the management of the disease is difficult, though possible through several cultural, biological or chemical means (Sindhan *et al.*, 1999; Rodrigues *et al.,* 2001; Kumar *et al.*, 2014; Patel *et al.*, 2014; KAU, 2016). However, as with all endemic soilborne pathogens, attempts to reduce the soil inoculum reserves through crop rotation, soil solarisation or soil fumigation have been found to offer better results. The former two methods are time consuming, whereas, the latter involves the use of hazardous chemicals. As an ecofriendly alternative to the latter, biofumigation using plants or products derived from them could be tested for their usefulness for soilborne pathogen eradication. With this objective, the study entitled "Management of collar rot of cowpea caused by *Rhizoctonia solani* Kuhn using biofumigants" was taken up in the Department of Plant Pathology, College of Agriculture, Vellayani, Thiruvananthapuram during the period 2015-2017.

In the present study, symptoms of collar rot disease were documented under natural field conditions. The disease was observed as the appearance of brownish black water soaked lesion at the basal portion of cowpea plant which later spread in both vertical and horizontal direction and became deep seated. Later at the infected portions mycelial growth of the pathogen along with formation of mustard like sclerotia were also observed. Finally, at the severe stage of disease, the affected plants showed stem girdling and complete root rotting. The collar rot symptoms were prominent at the seedling stage as well as prior to flowering, and web blight phase was predominantly noticed in mature plants. Occasionally, on the aerial parts of cowpea, *R. solani* caused initially water soaked lesion that later became necrotic with sclerotial production and the mycelial strands of pathogen were seen to web infected

leaves together. In the final stages, complete yellowing and death of the plant were noticed. Menon *et al.* (1979) reported two phases of the disease: a) collar rot seen at the collar region and rotting of stem. b) web blight seen on foliage which causes blighting and webbing of leaves. These observations are in tune with those detailed for the same disease in cowpea by Lakshmanan *et al.* (1979). Vavilapalli *et al.* (2014), also observed that root rot/ collar rot was most severe at seedling stage on the stem near the soil level and web blight phase was severe at the vegetative stage. Lambe and Dunleavy (1961) described that in soybean the symptoms of *Rhizoctonia* root rot were seen as rotting of the root along with development of large reddish oval lesions at the collar region. In web blight phase, varying symptoms ranging from water soaked lesion to cobwebby mycelia on the affected leaves have been reported by several researchers (Viswanathan and Viswambharan, 1979; Shailbala and Tripathi, 2007; Nitzan *et al.*, 2012). Pascual *et al.* (2000) observed that the primary source of inoculum for infection by *R. solani* causing hypocotyls rot and foliar blight in soybean (*Glycine max*) was either sclerotia or hyphae in soil organic debris.

In the current study, the incitant of collar rot disease was isolated and pure cultured. Four different isolates were maintained, characterized and screened based on mycelial and sclerotial characteristics and lesion development on artificial inoculation on cowpea seedlings. The R1 was identified as the most virulent isolate based on its fast rate of growth (3.07 cm/day), profuse sclerotia production (146/63.59 cm^2 area of petri plate) and large sized lesion on inoculation (2.65 cm^2) compared to other isolates of collar rot pathogen. Shajahan *et al.* (1997) reported that the cultural characteristics and sclerotial production were related to virulence. Girija (1995) and Ranjit (2000) reported a hypovirulent culture as the one that produced tan coloured mycelia with floccose nature and without sclerotia. This was in conformity with the findings of Castanho and Butler (1978) who were the first to report hypovirulence in *R.solani*. Using the most virulent isolate, R1 Koch's postulates were proved. On microscopic examination, this isolate showed characters such as right angled hyphal branching, formation of septum at the origin of hyphal branching,

constriction at the point of branching from the main hyphae, production of monilioid cells in chains, multinucleate hyphae and had mycelial diameter that varied from 6.0-10.5 µm. It also exhibited cultural characteristics like rapid growth on PDA medium, appressed growth and production of mycelium off- white on upper side and light brown on the underside of petri plate. These observations were in conformity to the descriptions of *R. solani* given by Parmeter and Whitney (1970). Initially the hyphal branches were right angled to the main hyphae, but gradually they were bent and seen growing towards the direction of main hyphae (Palo, 1926). The identity was further confirmed using molecular identification by ITS sequencing as *R. solani* Kuhn (Accession no. EF429212) and showed 100 per cent similarity with *Thanatephorus cucumeris* (Frank) Donk, the perfect stage of *R.solani.* This is in tandem with the earlier report on the teleomorphic stage of the pathogen (Talbot, 1970; Saksena and Chaubey, 1972).

Plants synthesize secondary metabolites and some of them as well as their derivatives have antimicrobial activity such as alkaloids, flavonoids, isoflavonoids, tannins, coumarins, glucosides, terpenes and phenolic compounds. An attempt was made to evaluate the *in vitro* antifungal and biofumigant nature of ten different plants, four plant oils and four oil cakes on suppression of mycelial growth of *R. solani.* Among these plant extracts, total inhibition of the pathogen was obtained on PDA incorporated with leaf extracts of cabbage and garlic creeper. The extracts of leaves of sweet potato (37.44 per cent) and radish (30.00 per cent) also gave significant suppression. The inhibitory effect of extracts of different Brassicaceae plants such as savoy cabbage, red cabbage, horse radish and fringed cabbage on *R. solani* has already been established by Burgie (2005) who noted 100 per cent suppression with horse radish leaves and 85 per cent suppression with the remaining *Brassica* spp. Murugapriya *et al.* (2011) observed that in mung bean the extracts of garlic creeper (20%) induced complete suppression of *M. phaseolina.* Chandel and Sharma (2014) revealed that leaf extracts of *Melia azedarach* and *Adhatoda vasica* showed maximum inhibition on mycelial growth of *R. solani* to the extent of 44.38 to

44.25%. Islam (2008) reported that the addition of sweet potato plant extract to the growing medium suppressed the growth of food poisoning bacteria and highlighted the scope for further exploitation of anti-microbial properties of the crop. The percentage inhibition of linear growth of *R. solani,* as a result of incorporation of neem leaf extract, was very less in comparison to the extracts of garlic and madar (Sonakar *et al.*, 2014).

Under *in vitro* evaluation of the biofumigant nature of plants, complete suppression of the pathogen was obtained on treatment with cabbage, cassava, garlic creeper and mustard. This was followed by radish, moringa, neem and lemon grass which gave suppression of 87.80%, 83.30%, 77.80% and 76.67%, respectively. Kirkegaard *et al.* (1996) investigated the effect of volatile compounds released from the root, shoot and seed meal tissues of canola (*Brassica napus*) and Indian mustard (*Brassica juncea*) on the mycelial growth of five soilborne pathogens of cereals including *R. solani.* The root and shoot tissues of *Brassica* spp. taken at flowering stage were found to be more suppressive than those collected at maturity. The mustard tissues were generally more suppressive than canola. On the contrary, Mokhtar *et al.* (2014) observed that cabbage leaf extract exerted only 11.67% reduction on the radial growth of *R. solani,* the incitant of root rot of green gram. Charron and Sams (1999) reported that among the *Brassica* spp. the Indian mustard, *B. juncea* (10g/500 mL jar) complete suppression after 48 h exposure to the pathogen. Gamliell and Stapleton (1993) detailed the inhibitory effect of volatiles released from cabbage residues on *Pythium ultimum* and *Sclerotium rolfsii.* The effectiveness of cassava as biofumigant could be attributed to the production of HCN from the cyanogenic glycosides contained in the leaves (Mc Mahon *et al.*, 1995). The phytoconstituents from moringa such as carbamates and thiocarbamates have been shown to inhibit human pathogenic bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Das *et al.*, 1957).

Mahadevan and Sridhar (1982) highlighted the use of plant oils in the management of *R. solani* incited diseases. Plant derived essential oils are volatile

aromatic concentrated hydrophobic oily liquids which are reported to contain non-phytotoxic compounds effective against several microorganisms including fungal pathogens (Chuang *et al.*, 2007). The antimicrobial nature of plant oils is generally due to their content of terpenes, which are phenolic in nature (Tabassum and Vidyasagar, 2013). In the present study, under *in vitro* evaluation for the antifungal nature of plant oils, lemongrass oil and tea tree oil were found to be the most effective with 100 per cent suppression of mycelial growth of the pathogen. Mahua oil and neem oil afforded suppression of 40.74 and 16.29 per cent. Dhingra *et al.*, 2004 reported that mustard oil incorporated into the medium (50 $\mu L\ L^{-1}$) completely suppressed the mycelial growth of *R. solani* under *in vitro* conditions . The results obtained in our study was in conformity with the observation of Handique and Singh (1990), who tested the antifungal activity of essential oil extracted from a mutant strain, Lm-81 of lemongrass (*Cymbopogon flexuosus*) for antifungal activity against three soilborne pathogens including *R. solani* and found 60% decrease in growth of *R. solani* at 100 ppm and total suppression of growth at 1000 ppm. Amini *et al.* (2016) while studying the antifungal effect of plant based essential oil of *Cymbopogon citratus* and *Ocimum basilicum* found that *C. citratus* was more toxic than essential oil from *O. basilicum.* Thobunluepop *et al.* (2009) observed 100% suppression of *R. solani* with incorporation of tea tree oil (2% v/v).

Apart from antifungal action, when used as a biofumigant lemongrass oil and tea tree oil (5%) also gave 100% suppression. Earlier workers also highlighted the biofumigant effect of essential oil on suppression of plant pathogens, suppression of *Ralstonia solanacearum* with palmarosa oil and lemongrass oil (Alves *et al.*, 2014), thymol (Momol *et al.*, 2000; Pradhanang *et al.*, 2003).

The oil cakes are the by-products of oil production from the respective oil yielding crop, which is obtained after extraction of oil. The seed meal/oil cakes very often inherit high amount of biochemical constituents, including fungitoxic biomolecules, originally present in the respective oil yielding seeds. Brown and Morra (2005) reported high concentration of glucosinolates in canola seed meal. Out

of the four oil cakes tested for their inhibitory effect on mycelial growth of *R. solani*, mustard oil cake induced the maximum suppression (100 per cent). This was followed by groundnut cake and neem cake with a suppression of 79.62 and 76.29 per cent, respectively. Lenka and Pun (2014) investigated the *in vitro* antifungal action of oil cakes on *R. solani* and obtained promising results with mustard oilcake. However, they observed only mere suppression of the pathogen by treatment with neem cake. In a management study on the web bight disease of urd and mung bean caused by *R. solani*, Dubey and Patel (2000) observed maximum suppression of the pathogen with groundnut oil cake (10%).

In the present study, it was observed that among the oil cakes, only mustard oil cake gave effective biofumigant action on pathogen. Fayzalla *et al.* (2009) explained the biofumigant effect of mustard seed meal in the management of soil-borne pathogens such as *R. solani, Macrophomina phaseolina* and *Sclerotium rolfsii* causing damping off, root rot and wilt diseases of soybean under laboratory conditions and recorded 92.2% suppression *of R. solani* at a concentration of 25 mg petridish^{-1}.

R. solani survives in soil as sclerotia which are compressed mycelia that enable it to persist in soil for long periods in the absence of host plant (Upamanyu *et al.*, 2002). The primary spread of infection starts from sclerotia. In order to assess the effect of biofumigation on survival of sclerotia in the soil, *in vitro* soil biofumigation study was carried out under confined conditions in sealed containers with plants, plant oils and oil cakes. Similar experiments were performed by Warmington and Clarkson (2016) with six biofumigant crops to identify the potent biofumigant that can suppress sclerotial germination in *Sclerotinia sclerotiorum*. In the present study, in general, biofumigation with plants, plant oils and oil cakes exerted suppression of mycelial regeneration from sclerotia. The extent of suppression was found to increase with increase in period of incubation with biofumigants.

Among the plants, treatment with cabbage, garlic creeper and mustard were found to be very effective and caused 100 per cent suppression on mycelial regeneration from sclerotia 24 h after exposure to the treatment. Dubey *et al.* (2009) found inhibitory effect of neem extract on survival of sclerotia of *M. phaseolina* after 2 and 4 days of incubation. This was attributed to the presence of several bioactive compounds such as sulphur, neem, alkaloids, resins, glycosides, ammonia and fattyacids and ammonia evolved during the decomposition of neem oil and seed cake. Neem contains a variety of chemical constituents such as nimolicinol, isolimolicinolide, azadirachtin, azadirachtol, nimlinone, nimbocinol, nimbocinone, nimocin etc. (Tewari, 1992). Dubey and Kumar (2004) reported the antifungal effect of azadirachtin as effective as the fungicides: carbendazim and mancozeb. Biofumigation with plants belonging to Brassicaceae such as *B. juncae* and *Raphanus sativus* drastically reduced the viability from sclerotia of *S. sclerotiorum* (Warmington and Clarkson, 2016) and from microsclerotia of *Verticillium dahliae* (Neubauer *et al.*, 2014). After a period of two weeks of biofumigation with cassava 100 per cent suppression on sclerotial growth was noticed. Biofumigation with leaves of other plants such as sweet potato, moringa, papaya, neem and lemongrass, which are widely grown in the state afforded suppression of 81.85, 81.11, 79.63, 77.04 and 76.67 per cent, respectively one month after exposure to the treatment. Though the suppression was significantly less as compared to the best treatments in this study, the availability and affordability make them promising candidates for further exploitation for biofumigation. Biofumigation with lemongrass oil and tea tree oil resulted in 100 per cent suppression of sclerotial growth 24 h after exposure to treatments. After a period of one month, neem oil also caused complete suppression of mycelial regeneration from sclerotia. Dohroo and Gupta (1995) reported that neem oil (0.5%) successfully inhibited sclerotial production and germination in *R. solani,* the incitant of sheath blight disease of rice. On exposure to essential oil of mustard the sclerotia of both *S. rolfsii* and *S. sclerotiorum* became non-viable (Dhingra *et al.*, 2013).

The incorporation of soil amendments such as oil cakes in addition to improving soil health by supplying organic matter, also cause suppressive effect on soilborne fungal pathogens on account of release of volatile compounds, non-availability of nitrogen, CO_2 accumulation and increase in saprophytic microflora (Stover, 1987). In the present study, biofumigation with mustard oil cake gave 100 per cent suppression of *R. solani* 24 h after exposure to oil cake. After one month exposure, groundnut cake, neem cake and mahua cake exerted a suppression of 100.00, 86.49 and 3.33 per cent, respectively. Reduced sclerotia production in *R. solani* on biofumigation with mustard oil cake was already reported by several workers (Fayzalla *et al.*, 2009; Lenka and Pun, 2014).

The effect of various selected biofumigants such as plants, plant oils, oil cakes on collar rot disease of cowpea was studied by pot culture experiment using susceptible cowpea variety, Bhagyalakshmy. The selected plants (cabbage, cassava, garlic creeper and mustard) at the rate of 50 g kg^{-1} soil, plant oils (lemongrass oil and tea tree oil) at the rate of 5% (soil drench) and oil cakes (mustard and groundnut oil cake) at the rate of 10 g kg^{-1} soil were incorporated into the potting mixture two weeks before sowing the seeds. The incidence and intensity of collar rot disease in biofumigated pots were compared with that of inoculated control, un-inoculated control and chemical control check. Similar experiments were conducted by several workers to manage soil-borne pathogens in other crops (Momol *et al.*, 2000; Dhingra *et al.*, 2004; Wang *et al.*, 2014).

In collar rot disease incidence and intensity, maximum suppression (100 per cent) was noticed on plants grown in soil biofumigated with mustard plant (50 g kg^{-1} soil) and mustard oil cake (10 g kg^{-1} soil). This result was statistically on par with chemical control and un-inoculated control. The incorporation of plant tissues of *Brassica* spp. have been found to reduce the inoculum of several soilborne pathogens, including *R. solani* (Charron and Sams, 1999). Larkin and Griffin (2006) investigated the effect of biofumigation with *Brassica* sp. on *R. solani* and reported significant reduction of pathogen population on treatment with *B. juncea, B. napus,*

and *R. sativus* under greenhouse conditions. Van os and Lazzeri (2006) reported that in soil infected with *R. solani* the efficacy of seed meal was equal to the fungicidal treatment using azoxystrobin. Previous studies also revealed the superiority of mustard seed meal in suppressing *Pythium* incited damping off in lily and the results were comparable to the application of soil fungicide, Rhizolex. An assay was carried out on biofumigant action of mustard seed meal on *R. solani* infecting soybean and obtained complete suppression of disease along with enhanced plant growth (Fayzalla *et al.*, 2009).

The biofumigation with groundnut oil cake was found to cause significant reduction on incidence and intensity of collar rot disease (88.89%) (Fig.1). Biofumigation with tea tree oil, cabbage, cassava, garlic creeper and lemongrass oil also recorded good suppression of collar rot disease in the pot culture studies. The results are in agreement with previously reported works on exploitation of biofumigation for management of soilborne pathogens. Arnault *et al.* (2014) observed that the residues of *Allium* spp., contain dimethyl disulfide and dipropyl disulfide, gave significant biofumigant activity on soil borne pathogens as well growth promotion in crop. Sharma and Negi (2013) conducted a pot culture experiment for the management of collar rot disease in apple and observed that mulching with mustard and cabbage gave a disease suppression of 80.20 and 62.30 per cent, respectively. Successful suppression of pathogen population and management of disease through biofumigation with lemongrass oil have been recorded earlier for bacterial wilt diseases caused by *R. solanacearum* (Paret *et al.*, 2010; Alves *et al.*, 2014). The antifungal action of different biofumigants over the pathogen tested could be attributed to GSLs which when subjected to enzymatic hydrolysis released ITCs, oxazolidinethiones, ionic thiocyanate and organic cyanides through different pathways (Matthiessen and Kirkegaard, 2006).

Application of biofumigants benefit the plant not only through crop protection, but also directly contribute to growth and yield of the plant. In the present study, biofumigation enhanced the yield of cowpea (Fig.3), fresh and dry weight of

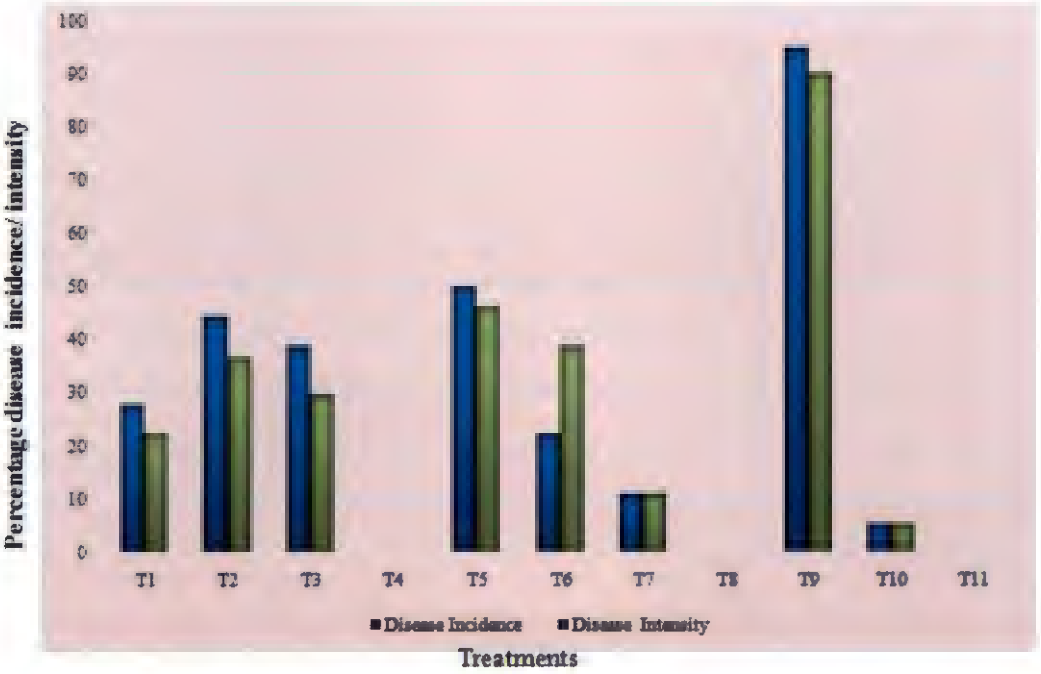


Fig.1. Effect of biofumigation on incidence and intensity of collar rot disease

T1 - Cabbage (*Brassica oleracea* var *capitata*), T2 - Cassava (*Manihot esculenta* Crantz), T3 - Garlic creeper (*Mansoa alliacea* Lam.), T4 – Mustard (*Brassica juncea* L.), T5 - Lemongrass oil, T6 - Tea tree oil, T7 - Ground nut oil cake, T8- Mustard oil cake, T9 - Inoculated control, T10- Un-inoculated control, T11- Chemical control check (Carbendazim 0.1%)

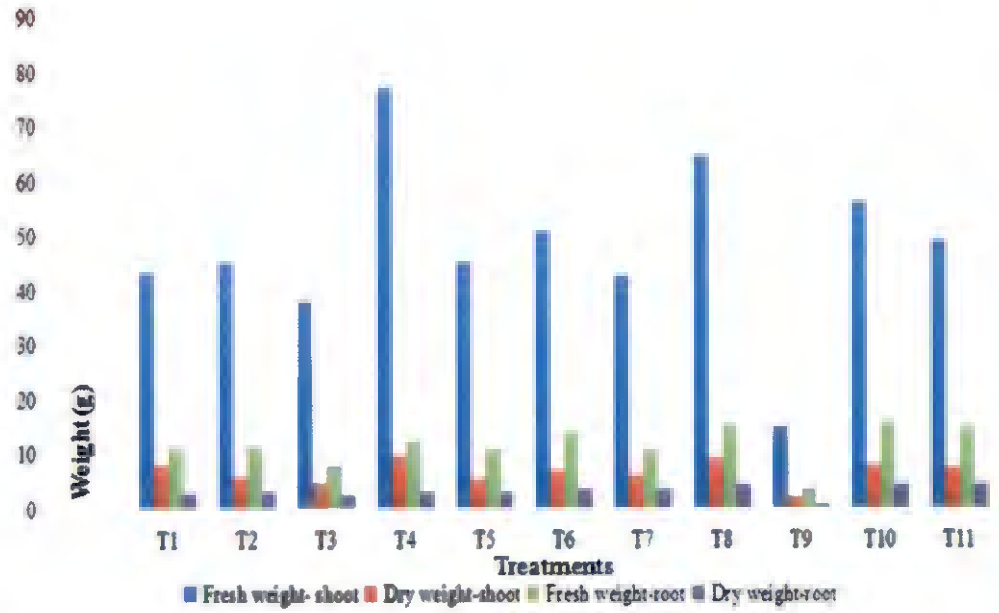


Fig.2. Effect of different treatments on growth related attributes of cowpea

T1 - Cabbage (*Brassica oleracea* var *capitata*), T2 - Cassava (*Manihot esculenta* Crantz), T3 - Garlic creeper (*Mansoa alliacea* Lam.), T4 - Mustard(*Brassica juncea* L.), T5 - Lemongrass oil, T6 - Tea tree oil, T7 - Ground nut oil cake, T8- Mustard oil cake, T9 - Inoculated control, T10- Un-inoculated control, T11- Chemical control check (Carbendazim 0.1%)

shoot (Fig.2) by 45.00, 11.36 and 21.68 per cent. Fayzalla *et al.* (2009) recorded the highest shoot weight of soybean plants (37.50 g plant^{-1}) from mustard seed meal incorporated pots challenge inoculated with *R. solani* in comparison with those in unamended control treatment. Application of defatted seed meal and liquid formulation from *B. nigra* enhanced the shoot dry weight of melons (Galletti *et al.*, 2015).

The population dynamics of saprophytic fungi and bacteria from biofumigated soils were assessed. In general, soil microflora exhibited a decrease in the biofumigated treatments and an increasing trend after raising the crop. Immediately after biofumigation least population (10.50×10^4 cfu g^{-1}) of saprophytic fungi and bacteria (8.17×10^6 cfu g^{-1}) were recorded from soil biofumigated with mustard plant. However, Rokunuzzman *et al.* (2016) observed that bacterial composition of soil treated with wheat bran and mustard were almost similar to the untreated control. They attributed this to the preponderance of facultative anaerobic bacteria that could survive even after soil reduction with the C sources. In the present study, two weeks after sowing cowpea, the highest saprophytic fungal population of 91.67×10^4 cfu g^{-1} was recorded from soil biofumigated with mustard plant (Fig.4) and that of bacterial population of 56.33×10^6 cfu g^{-1} from soil biofumigated with mustard oil cake (Fig.5). Though there was difference in values for the population of saprophytic fungi in the soil from the different pots, before incorporation of biofumigants, all the recorded values were statistically on par. There was a temporary suppression of the population of soil microflora following biofumigation. However, the population of saprophytic microflora regained immediately after raising the crop. The studies on effect of soil disinfection with chemical and biological methods on bacterial communities by Rokunuzzaman *et al.* (2016) showed that compared to chemical fumigants, biofumigation with mustard green manures caused much less damage on soil bacterial community. The biofumigation with seed meals of *B. juncea* induced more than 60% reduction of fungal population but also showed noticeable enrichment of bacterial antagonists such as *Bacillus, Pseudomonas, Streptomyces* spp. (Hollister

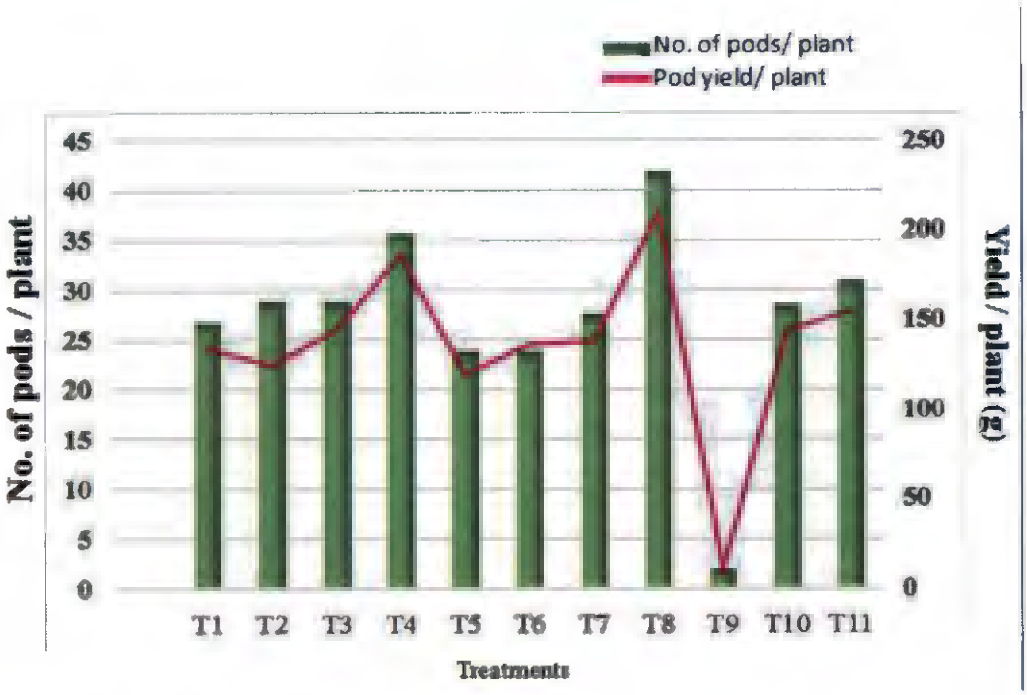


Fig.3. Effect of different treatments on yield attributes of cowpea

T1 - Cabbage (*Brassica oleracea* var *capitata*), T2 - Cassava (*Manihot esculenta* Crantz), T3 - Garlic creeper (*Mansoa alliacea* Lam.), T4 – Mustard (*Brassica juncea* L.), T5 - Lemongrass oil, T6 - Tea tree oil, T7 - Ground nut oil cake, T8- Mustard oil cake, T9 - Inoculated control, T10- Un-inoculated control, T11- Chemical control check (Carbendazim 0.1%)

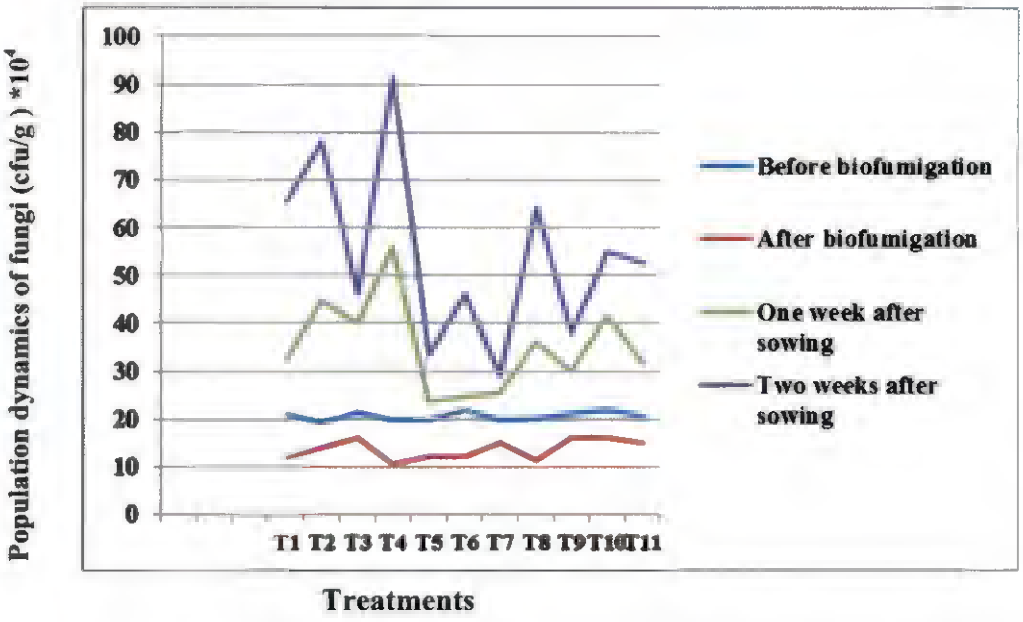


Fig.4. Population dynamics of saprophytic fungi in biofumigated soil at different time intervals (cfu g^{-1})

T1 - Cabbage (*Brassica oleracea* var *capitata*), T2 - Cassava (*Manihot esculenta* Crantz), T3 - Garlic creeper (*Mansoa alliacea* Lam.), T4 – Mustard (*Brassica juncea* L.), T5 - Lemongrass oil, T6 - Tea tree oil, T7 - Ground nut oil cake, T8- Mustard oil cake, T9 - Inoculated control, T10- Un-inoculated control, T11- Chemical control check (Carbendazim 0.1%)

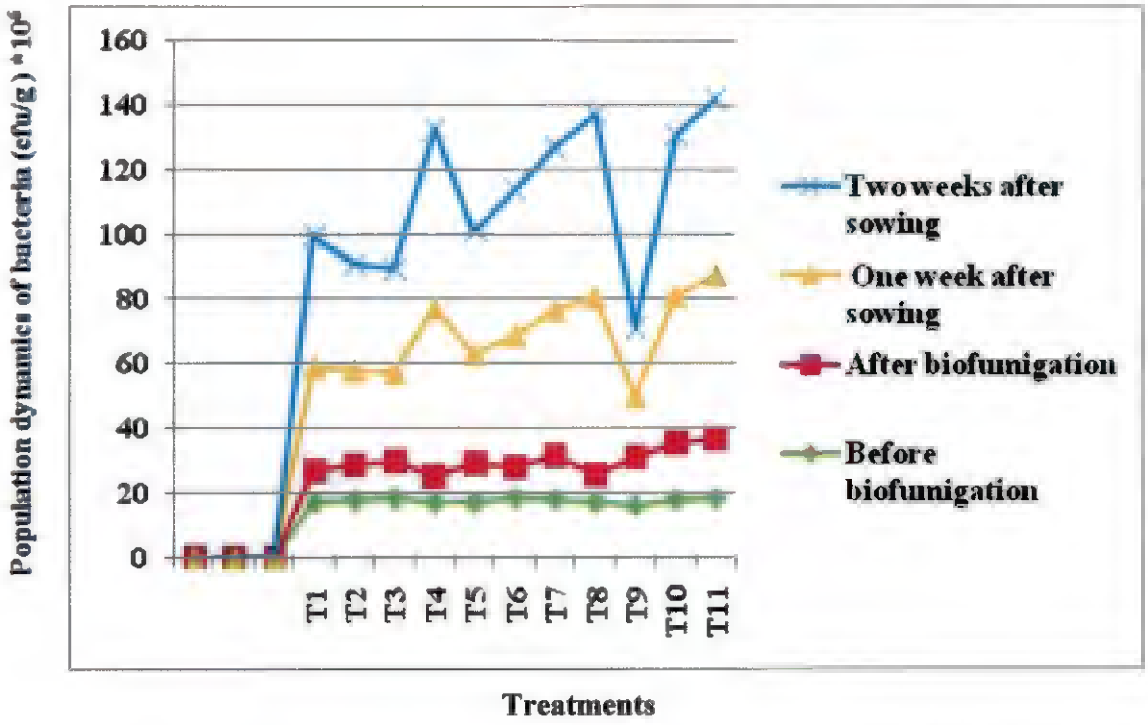


Fig.5. Population dynamics of saprophytic bacteria in biofumigated soil at different time intervals (cfu g^{-1})

T1 - Cabbage (*Brassica oleracea* var *capitata*), T2 - Cassava (*Manihot esculenta* Crantz), T3 - Garlic creeper (*Mansoa alliacea* Lam.), T4 – Mustard (*Brassica juncea* L.), T5 - Lemongrass oil, T6 - Tea tree oil, T7 - Ground nut oil cake, T8- Mustard oilcake, T9 - Inoculated control, T10- Un-inoculated control, T11- Chemical control check (Carbendazim 0.1%)

et al., 2012). They mentioned a dramatic decrease of fungal communities after incorporation of allyl isothiocyanates. Omirou *et al.* (2011) indicated that bifumigation induced change in the structure and function of microbial community and it did not have direct toxicity on microbial relationships in the soil.

The effect of biofumigation was not only due to volatiles released in the soil but also due to saprophytic microflora present in the soil. This was supported by *in vitro* studies conducted using predominant fungi and bacteria. Among the predominant saprophytic fungi obtained from biofumigated soil, the fungal isolate F1 exhibited 47.44 per cent suppression and among the two bacterial isolates, the isolate B2 caused 44.44 per cent inhibition of the pathogen. Mass multiplication and addition of microflora with biocontrol potential along with biofumigants could enhance the result of biofumigation. Soil incorporation of *Brassica* based biofumigants along with effective *Trichoderma* strains has been reported to have enhanced suppression of soilborne plant pathogens owing to mycoparasitism, induction and secretion of cell wall degrading enzymes, antibiotic production, competition with plant pathogen and induction of systemic resistance in plants. Galletti *et al.* (2008) reported the enhanced effects through combination of *Trichoderma* with biofumigation.

Soil-borne diseases are one of the major factors contributing to low yields of organic cultivation of major vegetable crops. Vegetable cowpea in Kerala is vulnerable to an array of such diseases that reduce yield by damaging whole plants or the valued products .The onset and spread of diseases like collar rot becomes a concern when the diseases assume an epidemic form causing enormous crop losses. The *in vitro* studies, biofumigation in confined conditions and the pot culture experiment taken up in the present research programme, highlight the suppressive effect of brassicaceous plants and mustard oilcake in inducing suppression of the pathogen/disease. However, incorporation of various locally grown plants/ their products also afforded appreciable suppression. There is scope for further research to boost up the effects obtained from these cheaper agrowastes or byproducts, through integration with proven biocontrol agents and successful techniques like soil

solarisation. To deal with the diverse mechanisms of survival, dissemination and infection displayed by soilborne pathogens such as *R. solani* causing collar rot of cowpea, multipronged action has to be developed. Biofumigation, in consonance with soil solarisation and effective biocontrol agents, could provide an integrated package for providing a successful ecofriendly integrated management strategy for diseases incited by pathogens like *R.solani*, especially in organic cultivation.

Summary

6. SUMMARY

The present study was conducted in the Department of Plant Pathology, College of Agriculture, Vellayani during 2015-2017with the objective to evaluate biofumigant nature of plants, plant oils and oil cakes against *Rhizoctonia solani* and to develop an ecofriendly management strategy for collar rot of cowpea using biofumigation. The salient findings of the study are summarised below.

1. Pathogen causing collar rot disease of cowpea was isolated from infected cowpea plants collected from the Instructional Farm, College of Agriculture, Vellayani. Four collar isolates R1 –R4 were obtained and pure cultures were maintained.

2. Based on the comparison of cultural and morphological characters and lesion development on inoculation with four collar rot isolates, R1 having fast rate of growth (3.07cm/day), profuse sclerotia production (146/63.59 cm^2), large sized lesion on inoculation (2.65 cm^2) was identified as the most virulent isolate.

3. Pathogenicity testing was done with R1, the most virulent isolate and Koch's postulates were proved.

4. The R1 isolate was confirmed at National Fungal Culture Collection of India, Agharkar Research Institute, Pune as *Rhizoctonia solani* Kuhn (Accession no. EF429212) based on morphological characteristics. On molecular identification 100 per cent similarity was observed with *Thanatephorus cucumeris* (Frank) Donk, the perfect stage of *R. solani.*

5. Among the different leaf extracts evaluated for their *in vitro* antifungal action on mycelial growth of *R. solani*, cabbage and garlic creeper caused complete suppression of the pathogen. The biofumigant action was maximum (100%) with the leaf extracts of cabbage, cassava, garlic creeper and mustard.

6. Four plant oils tested for antifungal and biofumigant action under *in vitro* conditions, the lemongrass oil and tea tree oil caused the highest inhibition.

7. Among the four oil cakes evaluated under *in vitro*, 100 per cent mycelial suppression of the pathogen was shown by mustard oil cake both as antifungal and as biofumigant.

8. The biofumigant action of ten different plants, four plant oils and four oil cakes with biocidal principles was tested by subjecting the sclerotia of *R. solani* to biofumigant action under confined conditions in sealed containers revealed that the extent of suppression was proportional to the period of incubation with biofumigants. After 24 h itself, the mycelial regeneration from sclerotia was completely suppressed on biofumigation with cabbage, garlic creeper, mustard, lemongrass oil, tea tree oil and mustard oil cake. One month after biofumigation, 100 per cent suppression of mycelial regeneration from sclerotia was obtained on biofumigation with cabbage, cassava, garlic creeper, mustard, lemongrass oil, tea tree oil, neem oil, mustard oil cake and groundnut oil cake.

9. The incidence and intensity of collar rot disease were found to be the least in soil biofumigated with mustard plant and mustard oil cake and the particular treatments were on par with both chemical control check and un-inoculated control.

10. However, the highest pod yield was recorded from plants grown in soil biofumigated with mustard oil cake followed by those grown in soil biofumigated with mustard plant, which were significantly superior to the chemical control check and un-inoculated control. This revealed the benefits of biofumigation through growth promotion in addition to soilborne disease suppression.

11. The population of soil microflora exhibited a decrease in the biofumigated treatments and an increasing trend after raising the crop. The saprophytic

fungi and bacteria were found to be maximum *viz.*, 91.67 × 10^4 cfu g^{-1} and 56.33 × 10^6 cfu g^{-1}, respectively in samples taken two weeks after planting cowpea from soil biofumigated with mustard plant. The least population of microflora was recorded in soil biofumigated with mustard plant immediately after biofumigation.

12. Among the predominant saprophytic fungi obtained from the biofumigated soil, the isolate F1 exhibited 47.44% and among the two bacterial isolates, the isolate B2 caused 44.44% inhibition of the pathogen.
13. Biofumigation of the soil through incorporation of either mustard plant (50 g kg^{-1} soil) or tea tree oil (5% soil drench) or mustard/ groundnut oil cake (10 g kg^{-1} soil) for two weeks can effectively manage *R.solani* incited collar rot disease of cowpea.

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Appendices

APPENDIX-I

COMPOSITION OF DIFFERENT MEDIA

Potato Dextrose Agar

Potato	-200.0 g
Dextrose	-20.0 g
Agar-agar	-20.0 g
Distilled water	-1000 ml

Martin's Rose Bengal Agar

Dextrose	- 10.0 g
Peptone	- 5.0 g
Potassium dihydrogen phosphate	- 1.0 g
Magnesium sulphate	- 0.5 g
Rose bengal	- 33.0 mg
Streptomycin solution (1%)	- 3.0 mL
Agar-agar	- 15.0 g
Distilled water	- 1000 ml

Soil Extract Agar

Glucose	- 1.0 g
Dipotassium phosphate	- 0.5 g
Soil extract	- 100.0 mL
Agar	- 15.0 g
Distilled water	- 1000 ml

Soil extract was prepared by steaming 1 kg garden soil in 1L water in an autoclave for 30 min at 1.05 kg cm^{-2}.

Nutrient agar

Beef extract	- 3.0 g
Peptone	- 5.0 g
Sodium chloride	- 5.0 g
Agar	- 15.0 g
Distilled water	- 1000 ml

APPENDIX-II

COMPOSITION OF DIFFERENT STAINS

Lactophenol - Cotton blue

Anhydrous lactophenol	- 67.0 mL
Distilled water	- 20.0 mL
Cotton blue	- 0.10 g

Anhydrous lactophenol was prepared by dissolving 20g phenol in 16 mL lactic acid and 31 mL glycerol.

Management of collar rot of cowpea caused by *Rhizoctonia solani* Kuhn using biofumigants

APARNA K.P.

(2015-11-022)

Abstract of the thesis

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ABSTRACT

The study entitled "Management of collar rot of cowpea caused by *Rhizoctonia solani* Kuhn using biofumigants" was undertaken in the Department of Plant Pathology, College of Agriculture, Vellayani during 2015-2017 with the objective to evaluate biofumigant nature of plants, plant oils and oil cakes against *Rhizoctonia solani* and to develop an ecofriendly management strategy for collar rot of cowpea using biofumigation.

The pathogen causing collar rot disease of cowpea was isolated from the infected cowpea plants collected from the Instructional Farm, College of Agriculture,Vellayani. Pathogenicity was confirmed, virulence rating was done and the collar rot isolate R1 was found to be the most virulent one. Based on morphological and cultural characteristics, the pathogen was identified as the *Rhizoctonia solani* Kuhn (Accession no. EF429212). This was further confirmed using molecular identification by ITS sequencing and showed 100 per cent similarity with *Thanatephorus cucumeris*, the perfect stage of *R. solani.*

The antifungal and biofumigant action of ten different plants, four plant oils and four oil cakes/ seed meals on mycelial growth of *R. solani* was evaluated under *in vitro conditions* in petridishes. Among these, incorporation of the leaf extracts of cabbage / garlic creeper, lemongrass oil / tea tree oil and mustard oil cake extract in PDA medium caused 100 per cent suppression of the pathogen. The *in vitro* biofumigant activity of cabbage/ cassava/ garlic creeper / mustard, lemon grass oil / tea tree oil and the mustard oil cake was found to be statistically superior to all other treatments, and resulted in 100 per cent suppression of mycelial growth.

The biofumigant action of ten different plants, four plant oils and four oil cakes with biocidal principles was tested by subjecting the sclerotia of *R. solani* to biofumigant action under confined conditions in sealed containers. Biofumigation with plants, plant oils and oil cakes, in general, exerted suppression of mycelial regeneration from sclerotia. The extent of suppression was found to increase with increase in period of incubation with the biofumigants. Cabbage,

cassava, garlic creeper and mustard were found to completely suppress the regeneration of mycelial growth from the treated sclerotia. Biofumigation with lemongrass oil / tea tree / mustard oil cake resulted in 100 per cent suppression of regeneration of mycelium. Accordingly, plants such as cabbage, cassava, garlic creeper and mustard, plant oils such as lemongrass oil and tea tree oil, oil cakes such as mustard and groundnut oil cake were selected for further *in vivo* studies.

An experiment was conducted under pot culture conditions to evaluate the efficacy of selected biofumigants involving 11 treatments and 5 replications in C.R.D. The treatments included the selected plants (50 g kg^{-1} soil), plant oils (5% soil drench) and oil cakes (10 g kg^{-1} soil) for suppression of collar rot of cowpea. The inoculated untreated control showed severe collar rot incidence (94.44%) compared to the treated pots. The maximum disease suppression (100%) was observed in cowpea plants grown on soil biofumigated with mustard plant /mustard oil cake which was on par with both chemical control check (0.1% carbendazim) and un-inoculated control. But the highest pod yield was recorded from plants grown on soil biofumigated with mustard oil cake (209.0 g) followed by treatment with mustard plant (185.4 g), which were significantly superior to the chemical control check (155.0 g) and un-inoculated control (143.0 g). These results revealed the enhanced effects of biofumigation for both disease suppression and growth enhancement.

The population of soil microflora exhibited a decrease in the biofumigated treatments and an increasing trend after raising the crop. Among the predominant saprophytic fungi obtained from biofumigated soil, the isolate F1 exhibited 47.44 % and among the two bacterial isolates, the isolate B2 caused 44.44 % inhibition of the pathogen.

The experiments revealed the scope of biofumigation for the management of *R. solani* under *in vitro* as well as *in vivo*. The collar rot caused by soil-borne pathogen, *R. solani* could be successfully managed by incorporation of either mustard plant (50 g kg^{-1} soil) or tea tree oil (5% soil drench) or mustard /groundnut oilcake (10 g kg^{-1} soil) two weeks before raising cowpea.