MANAGEMENT OF COLLAR ROT AND WEB BLIGHT OF COWPEA WITH COMPOSTS AND COMPOST TEAS

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by
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DECLARATION

I, hereby declare that this thesis entitled ‘‘**Management of Collar Rot and Web Blight of Cowpea with Composts and Compost Teas’’** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associate ship, fellowship or other similar title of any university or society.

Vellayani
26/08/2014

Arathy Rajan
(2012-11-157)

CERTIFICATE

Certified that this thesis entitled "**Management of Collar Rot and Web Blight of Cowpea with Composts and Compost Teas"** is a record of research work done independently by Ms. Arathy Rajan (2012-11-157) under my guidance and supervision that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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26/08/2014

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LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Per cent
µm	Micro meter
@	At the rate of
°C	Degree Celsius
CD	Critical difference
cfu	Colony Forming Unit
cm	Centimeter
CRD	Completely Randomised Design
DI	Disease Incidence
et al.	And other co- workers
Fig.	Figure
g	Gram
h	Hour(s)
i.e.	That is
kg	Kilogram
L	Litre
m	Meter
max	Maximum
mm	Milli meter
mg	Milli gram
min	Minute(s)
ml	Millilitre
rpm	Rotation(s) per minute
sec	Second(s)
SE	Standard error
sp.	Species
viz.	Namely
v/ v	Volume/ Volume

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Introduction

INTRODUCTION

Cowpea (*Vigna unguiculata* subsp. *unguiculata* (L.) Verdcourt) is an important leguminous vegetable crop grown throughout the year in Kerala. It is raised as floor crop in coconut gardens; intercrop in tapioca and as pure crop in rice fallows during rabi and summer seasons. The tender pods are rich source of proteins, vitamins, minerals and dietary fibre. However the crop is very susceptible to pests and diseases which can cause considerable reduction in its yield. Collar rot and web blight is a major disease of the crop that can affect the crop in all its growth stages and cause serious yield loss.

Collar rot and web blight caused by *Rhizoctonia solani* Kuhn is an important soil-borne disease of cowpea (Lakshmanan *et al*., 1979). Under field condition, collar rot phase of the disease is more severe and widespread than the web blight phase.

R. solani is a ubiquitous soil inhabiting plant pathogen with great diversity and wide host range. It is often difficult to control this soil and root-inhabiting pathogen that survive saprophytically in soil organic matter and exist for long periods in the absence of a host plant in the form of sclerotia. The situation is worsened by the lack of resistant varieties. Though the disease can be managed with elaborate and repeated use of fungicides (Upamanyu *et al*., 2002), the detrimental effect on human and animal health and environmental pollution as well as the huge cost involved necessitate the disease to be managed by cheaper and eco- friendly methods.

Inadequacy of effective and economic crop protection strategies is one of the key factors limiting the expansion of organic farming in vegetables. Composts and manures are applied to improve soil fertility in organic farming systems. There is increasing evidence of the impact of the composts and compost extracts on promotion of beneficial microorganisms and suppression of pest and disease incidence and severity in agricultural and horticultural crops. The application of composts to soils has been shown to improve the beneficial soil

microflora and to cause suppression of some soil-borne diseases in field crops. Compost extracts or teas have also been shown to reduce the incidence and severity of foliar disease in some crops (Scheuerell and Mahafee, 2002; Weltzein, 1990). This programme is intended to assess the effect of composts and compost teas on the incidence and severity of collar rot and web blight in cowpea with the purpose of developing an eco-friendly strategy for managing the disease. The study was undertaken on the following lines:

- Symptomatology of collar rot and web blight.
- Isolation of *R. solani* causing collar rot and web blight of cowpea, characterization and pathogenicity testing.
- Identification of plant with antagonistic potential to *R. solani* for preparation of leaf compost.
- Standardization and preparation of compost tea.
- Isolation and enumeration of mycoflora associated with composts and compost teas.
- Screening the antagonistic potential of the saprophytic fungi and selection of the most effective antagonistic fungus.
- Preparation of antagonistic fungus enriched composts and compost teas.
- Testing the effect of various composts and compost teas and the antagonist enriched products for the management of collar rot and web blight and promotion of growth and yield of cowpea.
- Study of population dynamics of saprophytic fungi in soils amended with composts and compost teas and their antagonistic fungus enriched forms.

Review of literature

2. REVIEW OF LITERATURE

2.1. SYMPTOMATOLOGY

Lakshmanan *et al*. (1979) gave a detailed description of the symptoms of collar rot and web blight of cowpea caused by *R. solani*. Under field conditions, collar rot phase of the disease was more common than the web blight phase. Collar rot began as brownish black lesions at soil level near the collar region resulting in girdling at the basal portion of the stem. White cottony mycelial growth along with small sclerotia was noticed in the affected area. The leaves of the affected plants turned yellow and finally dropped off. The initial symptoms of web blight appeared on the leaves as small circular, light greyish-brown spots surrounded by irregular water soaked areas. These lesions then enlarged to oblong to irregular shapes. Under congenial conditions, the spots coalesced covering major portion of the leaf lamina or entire leaf with mycelial growth, leading to shedding of affected leaves.

Viswanathan and Viswambharan (1979) described collar rot and web blight symptoms of cowpea caused by *R. solani*. They recorded that the first visible symptom of the disease was the occurrence of water soaked lesions in the leaves accompanied by rotting of stem in collar region. With the advancement of the disease, enlargement of lesions along with white cottony mycelial web and numerous creamy white globular sclerotia appeared on the affected region. The final stages of infection represented yellowing of leaves with withering and drying off of the whole plant.

Nitzan *et al*. (2012) reported varying symptoms of web blight in cowpea as water soaked lesions and web like mycelia on theaffected leaves. Severe infection may result in necrosis of the leaves, petioles, pods and young stems along with defoliation.

Collar rot began as oval or spindle shaped brownish black lesions having length ranging from 0.2 to 8.0 cm and breadth ranging from 0.2 to 2.5 cm. Girdling of the basal portion of stem, poor root development, rotting of the tap root and lateral root are the other observable symptoms reported by Bhadrasree (2007).

Weber (1939) for the first time gave a detailed account of web blight of beans caused by *R. microsclerotia*. He observed that the pathogen was capable of producing two types of mycelia; superficial mycelia and sub epidermal mycelia. But *R. microsclerotia* causing web blight of beans produces small superficial sclerotia by means of which the fungus is disseminated in soil. Even though artificial inoculation of micro sclerotia on several other crop plants like tomato, carrot, beet root, brinjal, cucumber, water melon and lima beans also developed similar symptoms by artificial inoculation, the typical symptoms of web blight were observed only in members of the family Leguminosae. The symptoms of web blight on urdbean was observed on roots, stems, petioles and pods but the disease is the most destructive on foliage and caused seedling mortality during the second and third week of plant growth (Shailbala and Tripathi, 2007).

Lambe and Dunleavy (1961) observed that the symptoms of *Rhizoctonia* root rot and stem rot in soybean appears as rotting or decay of the root along with the development of large red oval lesions at the collar region. The above ground symptoms of diseased plants included chlorosis, wilting, drying and premature leaf drop. Sharma and Sohi (1980) found that *R. solani* caused pre-emergence and post-emergence mortality, collar rot, stem canker and pod rot symptoms in French bean. At the collar region of stem on rotting tissues, superficial frosty growth of pathogen was observed. High temperature and high humidity were congenial for disease development and spread whereas the development was limited by dry weather or low temperature. The incidence of disease is much more in areas where organic matter accumulates near the plant (Kadam *et al*., 2011).

Sasi (1978) reported the symptoms of *Rhizoctonia* damping off in cardamom as light brown discoloration at the collar region followed by decaying and collapse of the entire seedlings.Priyadarsini (2003) observed that the symptoms of amaranthus leaf blight caused by *R. solani* appeared as small irregular whitish cream spots on the foliage which enlarge under high humidity. Severely infected leaves showed shot-hole symptoms leading to defoliation.

2.2. ETIOLOGY

2.2.1.Pathogen

R. solani is a very common soil borne pathogen with a great diversity of host plants. Farr *et al*. (1989) listed over 500 hosts. It is known to cause a large number of distinct diseases on a wide variety of plants (Sinclair andBackman, 1989).*R. solani*was originally described by Kuhn on potato in 1858. It is a basidiomycete fungus that does not produce any conidia and produce only basidiospores (Agrios, 1997). In nature, *R. solani* reproduces asexually and exists primarily as vegetative mycelium and sclerotia (Sneh*et al*., 1991). The genus *Rhizoctonia* was described to accommodate the non-sporulating root pathogen *R. crororum* [*Helicobasidium purpureum*] (Shailbala and Tripathi, 2007).

The perfect stage of *R. solani* was reported to be *Thanatephorus cucumeris* (Frank) Donk (Talbot, 1970). He treated *T. cucumeris* as a collective species that included *T. praticola*, *Corticium microsclerotia*and *C. sasakii*. The sclerotial stage is common and is principally carried through soil. The teleomorph stage has been first reported from India by Saksena and Chaubey (1972).

R. solani was reported to cause aerial blight of several leguminous plants (Mundkur, 1935; Verma and Thapliyal, 1976). The pathogen was indicated to cause wide spread destruction of cowpea causing both collar rot and web blight of the crop in Nigeria (Williams, 1975). Menon (1979) reported the incidence and etiology of this disease in cowpea for the first time from Kerala. Lakshmanan *et al*. (1979) reported that *R. solani* was the incitant of collar rot and web blight of

cowpea that appeared in an endemic form in Kerala. *R. solani* affect potato plants from planting to harvest by inhibition in eyes germination, killing of underground sprouts, stem canker and stolon canker resulting in subsequent yield reduction (Banville, 1989). They observed that the mycelium of the fungus was initially creamy white which turned light brown at maturity. Fresh isolation from infected cowpea produced only micro sclerotia which were oval in shape, light to dark brown and flat.However, a comparative analysis of different *R. solani* isolates indicated that fast growing isolates having macro sclerotia were highly virulent compared to slow growers with micro sclerotia (Manoj*et al*., 2008).

Tiwari and Khare (2002) reported that *R. solani* produces both imperfect stage consisting of hyphae and sclerotia and perfect stage *ie.*,basidiospores in soil. Dwivedi and Saksena (1975) reported that *R. solani* produces basidiospore *ie.*, the perfect stage in soil and its importance in the survival of the pathogen.

2.2.2. Morphology

The isolates of *R. solani* have been divided into many groups according to various cultural, physiological or pathological criteria (Houston, 1945; Exner, 1953) which were inconsistent due to diversity in pathogenicity.

Parmeter and Whitney (1970) described the identifying characters of *R. solani*. According to them, the hyphae of the fungus could be distinguished by the brown colour,branching near the distal septum of hyphae, hyphal branches inclined to the direction of growth, constriction at the point of origin from the main hyphae, formation of septa near the point of origin of the branch, septal pore apparatus and multinucleate cells in young hypha. They also reported characters like the presence of monilioid cells, sclerotiawithout differentiated rind and medulla, hyphae greater than 5 µ in diameter, rapid growth rate and pathogenicity are usually associated with *R. solani*, with occasionally one or few of these characters might be lacking in individual isolates.

Dugger (1915) observed that in *R. solani*, young hyphal branches were inclined in the direction of growth and constricted at the point of union with the main hyphae. Palo (1926) noted that in certain cases the young branches arise at right angles to the main hyphae but they later bend towards the direction of growth of the main filaments. Butler and Bracker (1970) reported that theseptal pore in *R. solani* is 0.1- 0.2 µm in diameter.

The grouping of *R. solani* on the basis of hyphal anastomosis between different strains has gained much importance in the study of the pathogen. Flentje *et al*. (1970) reported that *R. solani* consists of a great number of isolates differing in various characters. Parmeter and Whitney (1970) observed that each anastomosis group has its general tendency in host range and pathogenicity. Lakshmanan *et al*. (1979) from Kerala reported the hyphal anastomosis between strains of *R. solani* from rice and cowpea. Vijayan and Nair (1985) studied the diversity and anastomosis pattern of the *R. solani* isolates of sheath blight of rice and assigned them to AG-I group. Sunder *et al*. (2003) collected 112 isolates of *R. solani* from soil, root and collar rot or foliage blight of various plant species from different locations in Haryana. Of these, 43% belonged to anastomosis group-1 (AG-1 lA, AG-1 IC), 37% to AG-4, 9% to AG-3 and 11% to AG-7. They also found that 20 selected isolates of different AGs showed considerable variability in their cultural and morphological characters, growth and virulence. Budakov*et al* .(2009) reported that the fungus consists of a heterogeneous population with 14 anastomosis groups (AG 1-13 and AG BI).

2.3. IMPORTANCE AND YIELD LOSS

R. solani is a soil borne fungus able to survive in the soil for longer period of time even in the absence of host plant. Singh and Allen (1979) reported that web blight prevalent during the rainy season caused devastating loss of foliage yield loss of cowpea in Nigeria. Web blight caused by *R. solani* on horse gram (*Macrotylom auniflorum*) caused 10-60 per cent reduction in yield (Dubey and Mishra, 1990). Maximum disease severity occurred in early varieties followed by

medium and late duration varieties (Lakpale *et al*., 1997). Web blight disease causes 30 per cent yield loss in urdbean has been reported by Sharma (1999). *R. solani* isolates from urdbean was able to cause typical web blight symptoms on members of family Leguminosae (Sharma and Tripathi, 2001). Gupta and Singh (2002) reported that *R. solani* causing foliar blight of mungbean at early stage of crop growth caused premature defoliation and reduction in size of pods with a disease intensity ranging from 6.66 to 75.35 per cent. Root rot caused by *R. solani* on soy bean caused 12- 30% yield loss (Bradley *et al*., 2005). High rainfall coupled with 100 per cent soil moisture, relative humidity and soil temperature (23-25°C) favoured the development of the disease (Bhardwaj, 2005). Optimum temperature for sclerotia production is 18- 25°C (Harikrishnan and Yang, 2004).

R. solani is the most widely distributed and destructive pathogen that has a wide host range of over 450 genera of plants (Tripathi, 2000). Sharma and Sankaran (1991) reported widespread occurrence of web blight disease caused by *R. solani* in *Albizia falcatoria*, a fast growing leguminous tree. The pathogen survives by means of sclerotia during off season or as mycelium in *soil* or as thick walled brown hyphae in plant debris depending on temperature and moisture conditions (Prasad, 2010).

The loss in test weight of mung bean ranged from 23.12 to 28.6 per cent due to attack by *R. solani* (Gupta *et al*., 2010).Incidence of web blight disease on groundnut as well as on soybean and yard long bean during kharif season has been reported by Mishra (2011). Among different species of *Rhizoctonia*, Singh *et al.* (2012 a) reported that the loss in yield of mungbean due to web blight is directly related to the severity of the disease and a maximum yield loss of 40.32% was observed. The plant at all growth stages was found to be susceptible, however, disease severity varied with age. Young plants (30-60 days) were more prone to infection (Santhosh and Tripathi, 2013).

2.4. ECOFRIENDLY MANAGEMENT OF *R. solani*

Plant disease management in modern scenario has become more independent of chemical treatment due to its hazardous implications.Scientists involved in plant protection are focusing on alternate methods for disease management. Biological control has been reported to provide protection against many fungal diseases (Blakeman and Fokkeman, 1982). Several fungal antagonists like *Trichoderma* sp., has been found to be effective in checking diseases caused by *R. solani* (Elad*et al*., 1982).

2.4.1. Effect of compost in controlling pathogen

Most effective and cheapest method of alteration of soil environment is amendment of soil with decomposable organic matter especially composts. It is one of the methods of biological control of plant diseases. The decomposition of organic matter helps in alteration of physical, chemical and biological condition of the soil. The altered condition reduces the inoculum potential of soil inhabiting plant pathogens. They improve the soil structure and promote plant growth.

Composts made from a very wide range of plant residues have been tested for their potential in preventing or controlling root and soil borne diseases. The most predictable and successful pathogen suppression has been reported in container production systems (Hoitink and Fahy, 1986; Nelson and Craft, 1992). Weltzein (1991) studied extensively the effectiveness of using composts for disease control, particularly against fungal pathogens. The mode of action of composts leading to plant disease control and stimulation of microorganisms is complex and dependent on the nature of the amendments (Akhtar and Malik, 2000).

The application of composts to soils has been shown to alter the balance of soil microflora and to suppress some soil-borne diseases in field crops (Hoitink and Fahy, 1986; Hoitink *et al*., 1997). Soil borne pathogens like *R. solani* and *S. rolfsii* were suppressed in container media containing composted cattle manure

(Gorodecki and Hadar, 1990). Hoitink *et al.* (1991) and Huang and Huang (1993) reported that potting substrates containing compost suppress soil-borne diseases of floriculture and vegetable crops.

Diseases caused by *Pythium ultimum* and *R. solani* were considerably reduced in container media amended with composted organic household wastes (Schueler*et al*., 1989). Amendment of soil with compost made from residues of pearl millet, neem and weeds reduced resident populations of *Macrophomina phaseolina*, the incitant of dry root rot of legumes by 20 - 40% in comparison with unamended controls (Lodha and Burman, 2000). Application of cattle manure composts to soils significantly reduced the incidence of black scurf on organic potato tubers caused by *R. solani* (Tsror *et al*., 2001). Soils amended with composts provided 20-60 per cent reduction in *P. ultimum, R. solani, Phytophthora, F. oxysporum, V.dahliae* and *Sclerotinia* (Noble and Coventry, 2005).

Widmer *et al.* (1998) found that compost suppressed *P. nicotianae* on citrus plants. Lievens*et al.* (2001) showed that composts can induce systemic resistance to *Pythium* root-rot in cucumber when applied to a section of the root system using a split root system. Coventry *et al.* (2002) reported that compost prepared from onion waste reduced the number of plants infected by Allium white rot (*S. cepivorum*) by over 50% in the field. Composts and compost extracts derived from poultry manure had the potential to provide effective control of *Rhizoctonia* root rot and angular leaf spot in French bean (Joshi *et al.*, 2009).

Hoitink and Boehm (1999) suggested the possible mechanisms of disease suppression with compost to be successful competition for nutrients by beneficial micro-organisms, antibiotic production by beneficial micro-organisms, successful parasitism against pathogens by beneficial micro-organisms or activation of disease-resistance genes in plants by micro-organisms (induced systemic resistance). Suppressive effect of composts against diseases of several cereal and vegetable crop diseases, including clubroot has been reported by Tilston *et al.*

(2002). Hadar and Gorodecki (1991) reported that compost made from grape pomace contained highconcentration of sugars and low levels of cellulosic substances, tends to be colonized by *Aspergillus* and *Penicillium* sp. which suppressed *S. rolfsii*. Abbasi *et al*. (2002) reported that compost prepared from cannery waste suppressed anthracnose and bacterial spot disease in soil grown tomato crops. Gupta *et al.* (1986) reported that compost from spent mushroom reduced the number of sclerotia of *S. sclerotiorum* causing stalk rot of cauliflower.

Disease suppression effects of vermicompost on some soil-borne plant pathogens such as *Pythium* (damping-off), *Rhizoctonia* (root rot), *Verticillium* (wilt) has been reported by Chaoui *et al*. (2002). Manandhar and Yami (2006) studied the effect of vermicompost in controlling sheath blight disease of rice caused by *R. solani.* They also reported a significant increase in yield when compared with that of the control. Singh *et al.* (2012 b) found that use of vermicompost in *Coleus forskohlii* reduced the incidence of root rot disease caused by *F. chlamydosporum* and *R. solanacearum* by 73% and 82%, respectively.

Edwardand Fletcher (2000) reported that application of commercially produced vermicompost suppresses the attack of *Pythium* on cucumber, *Rhizoctonia* on radishes, *Verticillium* on strawberries and *Phomposis* on grapes in the field. They also concluded that the ability of pathogen suppression disappeared when vermicompost was sterilized. Nelson (2004) reported that vermicompost and liquid vermicompost extract suppressed *Pythium* damping-off in cucumber seedlings. He also concluded that seed colonizing microorganisms from vermicompost interfere with the pathogen's ability to chemical lysense the presence of a seed and hence aid in suppression.

Vermicomposts improve seed germination, enhance seedling growth and development and increase overall plant productivity (Atiyeh *et al*., 2000). Foliar sprays with aqueous vermicompost extracts on tomato provided reduction in late

blight disease (*P. infestans*) and yield was increased (Zaller, 2006). Sarma *et al*. (2010) reported the suppressive effect of leaf compost on *P. ultimum.*

2.4.2. Effect of compost teas in controlling pathogen

Compost tea has been increasingly used as an alternative plant disease control measure in organic agriculture. Al-Dahmani *et al.* (2003) found that compost teas produced using a range of materials and methods varied in their ability to control bacterial spot on tomato. They also found that foliar application of compost tea produced with composted cow manure significantly reduce the infection rate in many vegetable crops. Ingham and Alms (2003) found that aerated compost tea (ACT) is generally more effective than non- aerated compost tea (NCT) because they tend to have higher microbial populations and diversity. Dianez *et al.* (2006) reported that production of siderophores by the microbial species within the compost tea was responsible for disease suppression of soil-borne fungal pathogens.

Brinton *et al*. (1996) reported that principal active agents in compost tea mycoflora are fungi belonging to the genera *Penicillium* and *Trichoderma* that are involved in disease suppression. Compost extracts or teas have shown to reduce the incidence and severity of foliar disease in some crops (Scheuerell and Mahafee, 2002; Weltzein, 1990). Ma *et al*. (2001) reported that Fusarium wilt of pepper and cucumber was controlled by soil drenching non-aerated compost tea in greenhouse experiments. Compost teas have also been used to reduce the impact of plant pathogens by increasing the abundance and diversity of beneficial microbial species and by stimulating systemic resistance in plants (Al-Dahmani *et al.,* 2003; Dianez*et al.,* 2006). Scheuerell and Mahaffee (2004) reported that the development of *Pythium* damping-off of cucumber grown in soil-less media was significantly reduced by the application of aerated and non-aerated compost teas, with aerated compost teasfermented with kelp and humic acid nutrients displaying the most consistent disease suppression.

Aerated compost tea prepared from garden wastes was reported to suppress naturally occurring powdery mildew disease produced by the *Erysiphe polygoni* in tomato plants (Al-Dahmani *et al*., 2003). Al-Mughrabi and Khalil (2006) reported that aerated compost tea suppressed black scurf of potato caused by *R. solani* when used as a soil drench. Siddiqui *et al*. (2009) reported that non-sterilized actively aerated compost tea made from rice straw and empty fruit bunch of oil palm composts inhibited conidial germination of *Choanephora cucurbitarum,* the causal pathogen for wet rot of okra. They also reported that induced host resistance was stimulated in okra plants treated with compost teas during glass house trials.

Haruna *et al*. (2011) reported the efficacy of compost extracts on *Fusarium oxysporum. In vitro*efficacy of poultry manure based compost extract, cowdung based compost extract and neemleaf based compost extract was tested on *F. oxysporum f. sp. lycopersici,* the causal agentof tomato wilt disease and concluded that neem leaf based compost extract inhibited the radial mycelial growth by46.5 per cent.

Liping*et al*. (1999, 2001) reported effective control of Fusarium wilt of greenhouse grown cucumber (*F. oxysporum* f. sp. *cucumerinum*) and sweet pepper (*F. oxysporum* f. sp. *vasinfectum*) using drench applications of compost tea made from pig, horse, and cow manures. They found that compost tea had a mycolytic effect on chlamydospores and microsporeswhich suggested that disease suppression was achieved through the destruction of the propagules of the pathogen. Dianez*et al*. (2006, 2007) reported that nine fungi including *R. solani* and *P. aphanidermatum* were controlled *in vitro* using aerated compost tea made from grape marc compost.

Gangaiah *et al.* (2004) reported the suppression of *Septoria* leaf spot disease of tomato using aerated compost tea prepared from vermicompost. Use of microbial-enriched compost tea was found to inhibit the conidial germination of *Golovinomyces cichoracearum* causing powdery mildew in melon (Siddiqui *et al*.,

2012). They found that in vitro conidial germination was reduced by 85% upon treatment with microbial enrichedcompost tea, 96 hours after incubation.

Zhang *et al.* (1998) reported that compost teas significantly reduced the severity of bacterial speck caused by *Pseudomonas syringe* pv. *Maculicola* on *Arabidopsis* plant. A significant reduction in the incidence of early blight caused by *Alternaria solani* was observed with 14 day old compost extract prepared in 1:5 ratio of compost to water (Tsror and Bieche, 1999).

Application of vermicompost extract on tomato increased plant germination, growth and effectively suppressed a range of plant diseases. The beneficial response was due to plant growth regulators or hormones produced by the high microbial activity in vermicompost (Edward *et al.*, 2006). Compost teas suppressed disease by promoting the proliferation of beneficial microbes, which then acted as a biological control over pathogens (Dianez *et al*., 2007). Souleymane *et al.* (2010) reported that compost tea significantly inhibited mycelial growth of *Botrytis cinerea* by 57- 75 per cent and *P. infestans* by 100% when compared with control.

2.4.3. Effect of neem based preparations in controlling pathogen

Active components of neem leaves and its products are shown to have inhibitory effect on plant pathogens. Higher plants have been recognized to release volatile substances which reduce the population of pathogenic microorganisms in their surroundings. The use of neem cake and extract as a soil treatment measure have produced good result against various soil borne fungilike *P. aphanidermatum* and *R. solani* (Khan *et al*., 1974). Application of liquid medium by extracts of leaf, trunk bark, fruit pulp and oil of *A. indica* inhibited the growth of *F. oxysporum f.sp. ciceri, R. solani, S. rolfsii* and *S. sclerotiorum.* The inhibitory effect of a plant extract is owing to the presence of antifungal agents of various groups like antibiotics and phenolic compounds (Agrios, 1997).

Sindhan*et al*. (1999) studied the efficacy of leaf extracts of neem against the mycelial growth of *R. solani* and concluded that the plant extract was inhibitory to *R. solani* even at 5% concentration. Sivakumar and Sharma (2000) reported that neem oil five per cent was highly effective in reducing mycelial growth and sclerotial germination of *R. solani* causing sheath blight of rice.Disease suppression by neem leaves was reported by Santhosh and Tripathi (2013) against *R. solani* causing web blight of urd bean.

Ehteshamul – Haque*et al.* (1998) reported use of neem seed cake as a soil amendment was significantly effective against *R. solani* and *F. solani.* Naz *et al*. (2006) reported the antifungal activity of *A. indica* against *R. solani* and reported that increase in the concentration of plant diffusates diminishes the radial mycelial growth of fungal pathogen *in vitro*. The composted leaves of neem showed 100% inhibition of *R. solani* and it was also reported that the sclerotia formation of *R. solani* was directly related with the degree of inhibition of its mycelial growth (Hossain and Bashar, 2011). Jaya and Dhananjay (2012) evaluated six plant extract for against *R. solani*and reported *A. indica* was significantly most effective with 90-98 % inhibition in radialmycelial growth of the pathogen.

Antifungal activity of *A. indica* has been reported to have inhibitory effects on *R. solani* (Sivakadacham, 1988; Sharma andJandaik, 1994). Use of neem leaves and seed extracts was found to suppress plant pathogenic fungi such as *R. solani* (Moslem and El-Kholie, 2009). Aye and Matsumoto (2011) observed that neem leaf extract inhibited the growth of *R. solani* by 87.5%, *R. oryzae* by 92.5% and *R. oryzae- sativae* by 80%. El-Kholie *et al*. (2012) studied the effect of ethanolicneem leaf extract on *R. solani* and reported complete inhibition percentage of the pathogen. Compost prepared from leaves and bark of *A. indica* effectively inhibited the growth of *R. solani* by 55 per cent (Swami and Alane, 2013).

Lakshmanan (1979) observed that the viability of sclerotia of *R. solani* decreased drastically in soil incorporated with fresh neem leaves. The decreased

viability may be due to their ability to increase the antagonistic micro-organisms in the soilor may be due to its fungitoxic principle involved. Ahmed *et al*. (2002) found that plant extracts of neemwere effective against *Bipolaris oryzae* causing brown spot disease of rice. Alkhail (2005) reported that aqueous extracts of *A. indica* is known to have strong antifungal activity against fungi *viz., F. oxysporum, B. cinerea*and *R. solani.*

2.4.4. Enrichment process

Process of enrichment has been reported to increase the disease suppressive activity of composts and compost teas. Enrichment with a known biocontrol agent such as *Trichoderma* sp. has been reported to have antagonistic activity towards many soil inhabiting pathogens. Several bacterial and fungal antagonists like *Trichoderma* sp., *Gliocladium* sp., *Bacillus subtilis* and fluorescent *Pseudomonas* sp. have been found to be effective in checking the disease caused by *R. solani* on crops *viz*. rice, pea and cotton (Elad *et al*., 1982). Antagonistic potentiality of *Trichoderma* sp. has been assessed against many soil borne plant pathogens (Bose *et al*., 2005; Pan, 2009). In recent years, direct application of antagonistic micro-organisms to control foliar and root infecting pathogens has gained momentum (Whipps, 1992).

2.4.4.1. Biocontrol Potential of Trichoderma sp.

Antagonistic fungi especially *Trichoderma*spp. against several phytopathogens has been reported by Rini and Sulochana (2006).*T. harzianum* inhibited growth of *R. solani* to 50.13 per cent and *T. virens* by 45.92 per cent *in vitro* (Upamanyu *et al*., 2002). Mallesh (2008) reported that among different biocontrol agents namely *T. virens, T. viride, T. harzianum, T. koningii* evaluated for their in vitro antagonism against *R. solani* causing root rot of sage, the bioagent *T. viride* provided maximum inhibition of 94.4 per cent. Bhat *et al.* (2009) obtained 65.8 and 65.1 per cent reduction in mycelial growth of *R. solani* when tested in dual culture with *T. harzianum* and *T. viride,* respectively.

Sharma and Tripathi (2001) reported that among the biocontrol agents tested against web blight, *T. virens* was found to be highly effective in comparison to under field condition. *T. virens* sprayed plots recorded enhanced grain yield and comparatively low disease severity in comparison to fungicide (antracol) sprayed plots. Meena *et al*. (2003) observed that volatile chemicals released by *T.harzianum* were effective in suppression of growth and sclerotial formation of *R. solani* f.sp. *sasakii*. Also, *T. harzianum* inhibited more than 80 per cent growth after 72 hours of incubation and 33.5 per cent inhibition of sclerotial formation after 10 days of incubation followed by *T. viride* which caused above 70 per cent inhibition of growth and 25.9 per cent inhibition of sclerotial formation by *R.solani* f.sp. *sasakii*. Rini and Sulochana (2007) reported inhibition of *R. solani* by *Trichoderma* sp. to 59 per cent within six days of inoculation.

T. viride was reported to exhibit higher degree of antagonism to *R. solani* under in vitro condition (Gokulapalan, 1981). The antagonistic effect of *Trichoderma* sp. may be due to faster mycelia growth than pathogenic fungi (Wei *et al*., 1999). Durman *et al*. (2000) reported that *Trichoderma* sp. had antagonistic ability against *R. solani.* Priyadarsini, (2003) found that the fungal antagonist *T. harzianum* was effective against *R. solani* causing foliar blight in amaranthus. She also reported an enhanced growth and increased level of total phenol in the plants treated with *T. harzianum.*

Soil application followed by foliar spray with talc based formulation of *T. longibrachiatum* was reported to be effective in lowering the intensity of foliar blight of amaranthus caused by *R. solani* (Smitha, 2000). Sprays of *Trichoderma* sp. at ten days interval against web blight of urdbean decreased the disease severity (Sharma and Tripathi, 2001). Khan and Sinha (2005) reported the efficacy of *T. harzianum* in the management of sheath blight of rice. Naeimi *et al.* (2010) found that the spore suspensions of *Trichoderma* isolate significantly reduced disease severity and disease incidence of rice sheath blight when compared with the control. Singh *et al*. (2014) reported that rhizospheric fungal

bioagent (*Trichoderma*) can enhance protection of sunflower against the *R. solani* through augmented elicitation of host defence responses.

Integration of *Trichoderma* with other biocontrol agents or other methods of disease management have also been attempted by several workers. Evaluation of the comparative performance of biocontrol agents and their integration for management of web blight (*R. solani* Kuhn) in French bean conducted by Gupta *et al*. (2003) revealed that all the bioagents tested significantly inhibited the mycelial growth as well as number of sclerotia of *R. solani* under *in vitro* conditions. The maximum mycelial growth inhibition (62.12 %) was observed by *Trichoderma harzianum*followed by *Trichoderma viride* (57.28 %), *Trichoderma virens* (51.38 %) and *Aspergillusniger* (46.72 %), respectively. The per cent reduction in the number of sclerotia was also maximum by *T. harzianum* (69.76 %). Dubey (2003) reported that seed treatment with slurry or water mixed spores of *T. viride* and *G. virens* had synergistic effect and afforded significant protection to germinating seeds of urd bean/ mung bean against *R. solani*. In the same study, seed treatment with *G. virens* / *T. viride* along with vitavax and *Rhizobium* gave maximum reduction in seed mortality and web blight intensity as compared to *T. viride* used alone. This treatment also afforded maximum seed germination, plant vigour, nodulation and yield in urd and mung bean.

2.5. USE OF FUNGICIDE

Sharma *et al.* (1975) observed that effective management of seedling blight of mungbean caused by *R. solani* was possible by seed treatment with systemic and non-systemic fungicides and lowest disease index was noted with benomyl treatment.Effectiveness of copper oxychloride against damping off of tomato and chilli in nursery beds caused by *Pythium* sp. was described by Vir and Hooda (1989). Use of copper oxychloride (Blitox 50 WP) against French bean web blight caused by *R. solani* was reported by Mathew and Gupta (1996).

Sasi (1978) reported the efficacy of Dithane M- 45 and Thiride in controlling *Rhizoctonia* damping off in cardamom. He also reported that, Bavistin

250 ppm and Thiride 1000 ppm caused complete inhibition of *R. solani*under *in vitro* condition. Effectiveness of copper oxychloride in controlling *Rhizoctonia* damping off in tomato was described by Satija & Hooda (1987). Mukhopadhyay *et al.* (1992) reported that seed treatment with antagonist followed by vitavax gave excellent control of chickpea and lentil wilt complex. Dubey (1998) observed the effectiveness of bavistin, contaf and indofil M- 45 in reducing the web blight severity of French bean.

Sharma and Thripathi (2001) observed that seed treatment along with two foliar sprays of tilt (0.1%) at 15 days interval was effective in reducing web blight disease severity to 30- 32% in urd bean during Kharif 1997-98, 98-99 crop season in terai region. Gaikwad and Nimbalkar (2003) reported use of copper oxychloride against *R. solani* as seed, seedling root dip and soil treatment of bell pepper which decreased root rot upto 90.91 per cent and increased the weight, number and yield of bell pepper. Senthil Kumar (2003) reported the use of copper oxychloride both under *in vitro* and *in vivo* conditions for the management of Fusarium wilt of vegetable cowpea. Singh *et al.* (2004) applied different concentrations of copper oxychloride as soil drench to betel vine for the management of collar rot disease caused by *S. rolfsii*. Raju and Naik (2006) reported that copper oxychloride at 0.3 per cent concentration exhibited 88.29 per cent suppression of *Aspergillus niger* and recorded the complete inhibition of radial growth of *Penicillium digitatum*. Kumar *et al*. (2014) reported that among the systemic fungicides, seed treatment with bavistin and three prophylactic sprays at 25 days interval was found to be superior in reducing web blight severity in urd bean. Maximum increase in grain yield (kg/ ha) 40.8 was recorded with bavistin followed by tilt.

Materials and Methods

3. MATERIALS AND METHODS

3.1. ISOLATION AND PROVING PATHOGENICITY OF PATHOGEN ASSOCIATED WITH COLLAR ROT AND WEB BLIGHT OF COWPEA

3.1.1. Symptomatology

The symptoms of collar rot and web blight were observed in field conditions. The symptoms were described and documented.

3.1.2. Isolation of the Pathogen

Cowpea plants showing typical collar rot and web blight symptoms caused by *Rhizoctonia solani* were collected from Crop Museum, College of Agriculture, Vellayani. The collar region and leaves of infected cowpea plants showing the disease symptoms were washed in running water and cut into small bits containing diseased portion along with some healthy tissue. The pieces were then surface sterilized in 0.1 per cent mercuric chloride solution for one min followed by three successive washings in sterile water and transferred into sterile petridishes containing potato dextrose agar (PDA) medium (Appendix I) under aseptic conditions. The petridishes were sealed with parafilm, incubated at room temperature for 24- 48 h and observed for fungal growth. The fungal growth emerging from the bits were transferred to PDA slants.

3.1.3. Purification of the Isolates

Typical colonies of *R. solani* were transferred to PDA slants. The three isolates obtained from collar region and leaves were purified by hyphal tip method (Parmeter *et al*., 1969) and pure cultures were maintained on PDA slants for further studies.

3.1.4. Identification of the Pathogen

The identification of *R. solani* was done by observing the morphological and cultural characteristics. The morphological and cultural characteristics were

studied by inoculating one cm diameter mycelial disc of the pathogen into sterile petridishes with PDA medium incubated at 28 ± 1°C. Radial growth was measured at 24 h intervals. The rate of growth, colour and appearance of the mycelium, presence of monilioid cells, nuclear status and the number of sclerotia formed were recorded.

3.1.5. Nuclear Staining

The nuclear status of the pathogen was determined by nuclear staining. A modified method of Burpee *et al.,* (1978) was followed for nuclear staining. The stain used was 0.5 per cent trypan blue in lactophenol (Appendix II).

Cellophane discs of eight cm diameter were placed on Petri plates plated with two per cent water agar. One cm diameter mycelial disc of the pathogen was inoculated at the centre of the cellophane discs. The plates were incubated at 28 ± 1°C for five days. Rectangular pieces of cellophane of dimension 3 cm × 2 cm was carefully cut from the petri plate. These pieces were carefully mounted on a drop of trypan blue stain placed at the centre of a glass slide and a cover slip was placed over it to hold the cellophane in position. The slide was warmed gently over a spirit lamp till the stain starts to boil. The slide was then allowed to cool and was observed under the microscope.

3.1.6. Pathogenicity

Pathogenicity of the isolates was proved on cowpea plants following Koch's postulates. Cowpea seedlings of the variety Bhagyalakshmy released from Kerala Agricultural University were raised in cups. Ten to fifteen days old seedlings were artificially inoculated on aerial part (leaves) as well as at the collar region. The pathogen was inoculated on the leaves by placing sclerotia on the leaf surface. Collar region of the plant was inoculated by placing sclerotia at the collar region after giving injury by pin pricking. A thin layer of moist cotton was placed over the inoculated area on the leaf and the stem. The inoculated plants were covered with a polypropylene cover having sufficient holes to maintain humidity. Control plants without inoculation were also maintained. The plants were incubated at room temperature (28 ± 1°C) and observed for symptom

development. Re-isolation of the pathogen was done from the artificially inoculated plants and compared with that of the original culture.

3.1.7. Cross Infectivity Study

Cross infectivity of isolates causing collar rot and web blight in cowpea were studied by inoculating the isolate of collar rot on leaf and vice versa.

Sclerotia of the isolate causing collar rot were inoculated on the leaves after giving pin pricks. A piece of thin layer of moist cotton was then placed over the leaves to provide moisture. To ensure humidity the plant was covered with a polypropylene cover having sufficient holes. The number of days taken for the development of lesion was recorded. Similarly, cross infectivity of web blight isolate was also studied by inoculating the isolate at the collar region of the plant. The development of symptom, if any, was recorded in both the cases.

3.2. PREPARATION OF COMPOSTS AND COMPOST TEAS

3.2.1. *In vitro* Evaluation of Leaf Extracts Against *R. solani*

Leaf extracts of five plants selected based on their antifungal property against *R. solani* as evidenced from earlier reports were tested (Table- 1, Plate- 1) were tested for their efficacy in inhibiting the mycelial growth of the pathogen under *in vitro* conditions.

Table 1. List of plants with antifungal property against *R. solani*

No	Common Name	Scientific Name	Reference
1	Neem	*Azadirachta indica*	Khan *et al*., (1974)
2	Henna	*Lawsonia inermis*	Shivpuri *et al.,* (1997) Kuruchev *et al.,* (1997)
3	Tulsi	*Ocimum sanctum*	Kishore *et al.,* (2001)
4	Clerodendron	*Clerodendron infortunatum*	Verma *et al.,* (2008)
5	Pongamia	*Pongamia pinnata*	Kishore *et al.,* (2001)

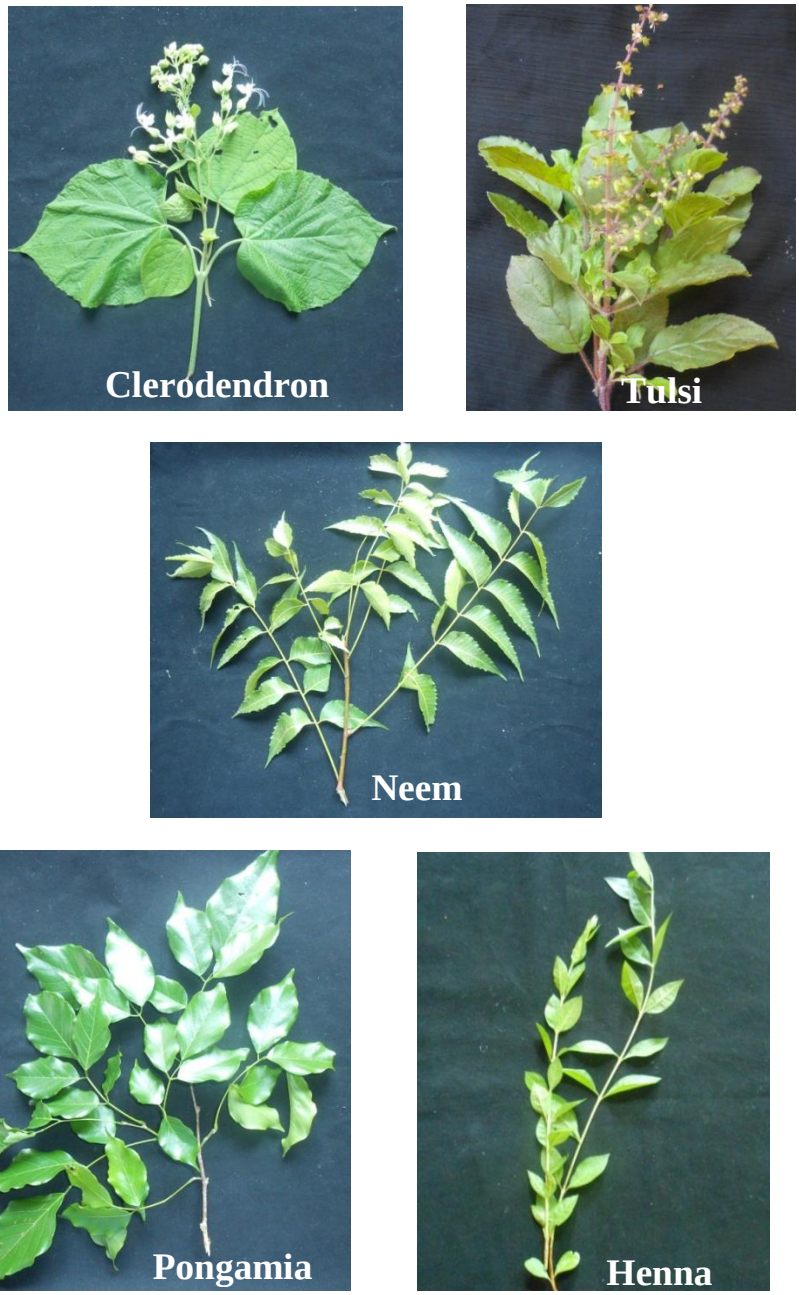


Plate 1. Plants with antifungal property against *Rhizoctonia solani*

The plant extract was prepared following the method given by Paul and Sharma (2002). 100 g fresh healthy leaves were collected and washed and were ground in 100 ml of sterile water. The product was filtered through muslin cloth and the filtrate thus obtained was centrifuged @ 6000 rpm for 25 min. The supernatant obtained was poured into a funnel containing Whatman no. 42 filter paper. The filtrate was fed into membrane filter unit for sterilization. The final filtrate was collected in aseptic condition in a sterilised screw capped bottle. The supernatant thus obtained was designated as concentrated leaf extract.

Five ml of the extract obtained was added to double strength PDA medium. The extracts were tested against *R. solani* following poisoned food technique (Nene and Thapliyal, 1993). Five mm disc of actively growing culture of *R. solani* was inoculated in the centre of the petriplates and incubated at room temperature. Three replications were maintained for each treatment. The diameter of the colony was measured and recorded.

The per cent inhibition in the mycelial growth of R. *solani* was calculated using the formula,

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

where, I = Inhibition of mycelial growth

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

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

(Vincent, 1927)

Based on the extent of inhibition, the most effective plant was identified for the preparation of leaf compost.

3.2.1.1. *Preparation of Leaf Compost*

On the basis of the results obtained in the experiment mentioned under 3.2.1.1 neem leaf was selected to prepare leaf compost as follows.

A plastic container with broad base was selected to prepare leaf compost. The collected leaves were shredded into pieces. Four parts of ground leaves were thoroughly mixed with one part fresh cow dung (Plate- 2a). Adequate moisture

a. Preparation of neem leaf compost

b. Mature compost

Plate 2 a-b. Preparation of neem leaf compost

was provided by sprinkling water over it. To ensure humidity, it was covered with moist newspaper. The heap was turned at every fifteen days so as to provide aeration. The composting process was completed within 60 days (Plate- 2b).

3.2.2. Preparation of Aerated Compost Tea

Bucket-Bubbler Technique proposed by Ingham (2005) was modified for the preparation of aerated compost tea.

A 10 L of water was taken in a 20 L bucket with an air bubbler which was then attached to an aquarium type aeration pump (Plate- 3a). Air was blown into it from the air bubbler. 2 kg compost was then taken in a muslin cloth, tied and then suspended into the water from a glass rod. The aerator provided continuous flow of air and created enough turbulence to mix the brew (Plate- 3b). To harvest the brew, the aerator was turned off for half an hour to allow the solids to settle down at the bottom of the bucket (Plate- 3c).

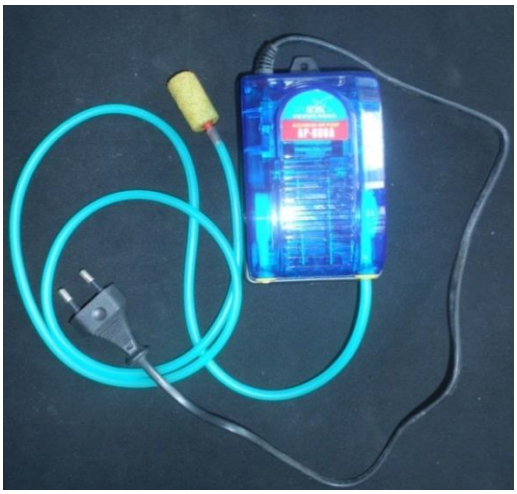
3.2.2.1. *Standardization of Compost Tea Brewing Period*

Compost extract, watery fermented compost extract and steepages are approximate synonyms defined as a 1:5 to 1:10 (v:v) dilutions of compost to water. In order to standardize the ratio and brewing period of compost tea, 1:5 and 1:10 dilution of compost tea was prepared and brewed for three days. Samples were drawn at 12 h interval and the microbial population was estimated by serial dilution and plate counting method (Wakesman, 1992). The dilution and the time interval at which maximum microbial population was attained were regarded as the optimum dilution and brewing period for the preparation of compost tea.

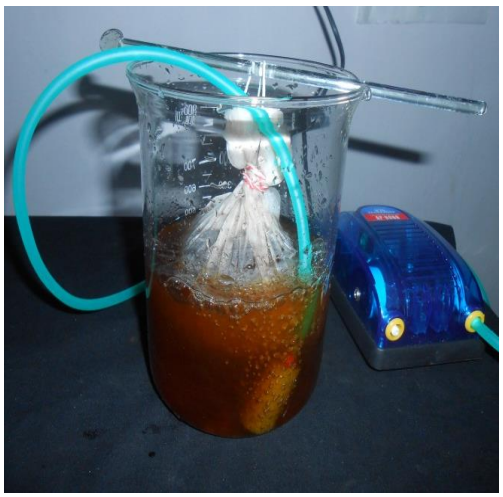
3.3. SCREENING THE ANTAGONISTIC POTENTIAL OF SAPROPHYTIC FUNGI

3.3.1. Isolation and Enumeration of Mycoflora Associated with Composts and Compost Teas

Isolation and enumeration of mycoflora associated with composts and compost teas were done by serial dilution and plate counting technique (Wakesman, 1922).



a. Aquarium type aeration pump



b. Aeration of the brew

c. Compost tea after preparation

Plate 3 a-c. Preparation of aerated compost tea by modified bucket bubbler method

One g sample of thoroughly mixed composts (vermicompost, coirpith compost, vermiwash, leaf composts) were transferred aseptically into 250 ml conical flasks containing 100 ml of sterile water and shaken for 20 min in a mechanical shaker. One ml of the suspension was pipetted out to 99 ml of sterile water in a 250 ml conical flask under aseptic condition. The flasks were again shaken for 15 min in a mechanical shaker. One ml of suspension was pipetted out to sterile petri dishes, melted and cooled Martin's Rose Bengal Agar medium (Appendix I) was poured at the rate of 20 ml per dish and gently rotated for thorough mixing. The petri dishes were incubated at room temperature. Population of saprophytic fungi was enumerated and colony count of composts and compost teas was recorded as cfu/ g and cfu/ ml respectively. Each colony was then transferred separately into PDA slants and stored at 4 °C for further studies.

3.3.2. Mass Screening of Saprophytic Fungi

The fungi isolated from dilution plates were tested for their antagonism to *R. solani* by cross culture method (Henis *et al*., 1979). Five mm diameter mycelial disc of antagonistic fungi and pathogen were used for the study. Four discs of different antagonistic fungi were placed in a petridish at a distance of three cm from the centre and near to the periphery at equidistant points. Actively growing test pathogen was introduced at the centre of the dish. Five replications were maintained. The dishes were incubated at 28 ±1 °C and visually examined for inhibition of growth of the pathogen.

3.3.3. *In vitro* Screening of Saprophytic Fungi for Suppression of *R. solani*

The fungus that exhibited inhibition on the mycelial growth of *R. solani* was further tested for its per cent inhibition on the pathogen by dual culture method (Skidmore and Dickinson, 1976). The most efficient one was selected for enriching composts and compost teas.

The saprophytic fungi obtained from serial dilution technique were grown in separate petri dishes. Mycelial discs of five mm diameter 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 four cm apart at diametrically opposite points one cm away from the periphery on a PDA plate. Both the cultures were allowed to grow. The mycelial disc of pathogen alone placed at the centre of the petri dish served as the control. When the pathogen attained full growth in the control plate, the mycelial growth was recorded and the percentage inhibition was calculated.

The per cent inhibition of mycelial growth of *R. solani* was calculated using the formula,

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

where, I = Inhibition of mycelial growth

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

T = Growth of the pathogen in dual culture plate (cm)

(Vincent, 1927)

Based on the extent of inhibition, the most efficient antagonistic fungus was selected for the process of enrichment.

3.3.4. Identification of the Antagonistic Fungi

The most efficient antagonistic fungus obtained by dual culture method was identified by slide culturing technique (Riddel, 1974) and microscopic observations.

Sterile water agar medium (Appendix I) was poured in petridishes to a thickness of two mm. After solidification, 6 mm square pieces were cut using a sterile needle. One square disc was placed at the centre of a sterile slide and each of the four sides of the agar block was inoculated with mycelial bits of the pathogen. A cover slip was placed on the top of the inoculated agar disc and the slides were placed in moist petridish chambers consisting of wet filter paper in the bottom in which two glass rods kept as support for the slides. The dish with the slide was then incubated at room temperature for two to three days. After this, the cover slip was lifted off gently and mounted on another slide using lactophenol stain (Appendix I). The agar block was removed from the culture slide and

another mount was prepared on it without disturbing the fungal growth on it. The slides were examined under low and medium power objectives of a compound microscope and micro- morphological characters of phialides, conidia and conidiophores were observed.

3. 4. *IN VIVO* STUDIES

3.4.1. Treatments

T_1: Soil incorporation of vermicompost + *R. solani*

T_2: Soil incorporation of enriched vermicompost + *R. solani*

T_3: Spraying and drenching with vermiwash + *R. solani*

T_4: Spraying and drenching with enriched vermiwash + *R. solani*

T_5: Soil incorporation of coirpith compost + *R. solani*

T_6: Soil incorporation of enriched coirpith compost + *R. solani*

T_7: Spraying and drenching with coirpith compost tea+ *R. solani*

T_8: Spraying and drenching with enriched coirpith compost tea+ *R. solani*

T_9: Soil incorporation of leaf compost + *R. solani*

T_{10}: Soil incorporation of enriched leaf compost + *R. solani*

T_{11}: Spraying and drenching with leaf compost tea+ *R. solani*

T_{12}: Soil incorporation of enriched leaf compost tea + *R. solani*

T_{13}: *R. solani* alone (inoculated control)

T_{14}: Un-inoculated control

T_{15}: Chemical control (COC 0.2 %)

A pot culture experiment in C.R.D. involving the above mentioned treatments in three replications was conducted using the bush type cowpea variety Bhagyalakshmy. Timely application of fertilizers was given as per package of practice recommendations by KAU (2011).

3.4.2. Preparation of Inoculum, Composts and Compost Teas

3.4.2.1. Preparation of Pathogen Inoculum

R. solani was mass multiplied in sand oats mixture (Lewis and Papavizas, 1984) prepared by mixing oats with sand in the ratio 1:9. The mixture was then moistened with water, to promote fungal growth. The mixture was taken in a conical flask and sterilized at 1.02 kg/cm^2 for one h. Actively growing mycelial disc of the pathogen was aseptically transferred into the flasks and incubated at room temperature for 14 days (Plate- 4). This was then used for soil inoculation.

Seven days old mycelial growth of *R. solani* grown in petri dishes was used for foliar inoculation.

3.4.2.2. Preparation of Inoculum of Trichoderma for Enrichment Process

The most effective antagonist, *Trichoderma sp*. obtained by serial dilution method was grown in PDA plate. A five mm mycelial disc of actively growing Trichoderma culture was then transferred into conical flasks containing Potato Dextrose Broth (PDB) (Appendix I). It was then incubated at room temperature (28 ± 1 °C). When mycelium of *Trichoderma* has covered the entire surface of the broth, the mycelial mats were harvested, macerated in a blender and used for the enrichment process (Plate- 5).

3.4.2.3. Preparation of Enriched Composts

Leaf compost was enriched by adding *Trichoderma* at the last phase of composting process. After adding the inoculum the compost was mixed well so as to ensure equal distribution of the inoculum throughout the compost. Water was sprinkled over it and covered with a moist newspaper to provide humidity and to favour the fungal growth. The process of enrichment was completed within a period 20 days when white mycelial growth of *Trichoderma* was observed in the compost. Frequent mixing and moistening was done throughout the process.

Vermicompost, vermiwash and coirpith compost used for the study was obtained from Cropping Systems Research Centre (C.S.R.C), Karamana. The composts were enriched by adding *Trichoderma* after the process of composting.

Plate 4. Inoculum of *R. solani* of multiplied in sand- oats media

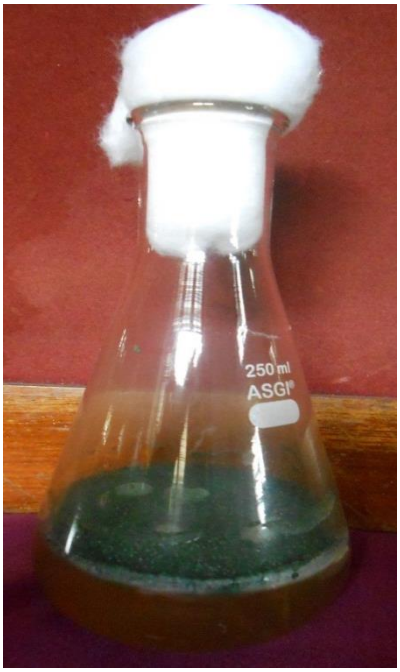


Plate 5. PDB with growth of *T. virens*

It was then moistened and covered with newspaper. Turning and moistening of compost was done at weekly intervals. The curing of compost was continued till a brown friable powdery material was generated.

3.4.2.4. Preparation of Enriched Compost Teas

The enriched compost tea was prepared from enriched compost by following the same procedure as described above.

3.4.2.5. Preparation of Enriched Vermiwash

The enriched vermiwash was obtained by adding *Trichoderma* into vermicompost 48 h prior to the extraction of vermiwash.

3.4.3.1. Application of Inoculum

For application at the collar region, 250 g of inoculum was mixed with soil in the ratio 1:2 was applied at the collar region of the cowpea seedlings after a month of planting. Three seedlings were maintained per pot.

For foliar application, the mycelial suspension of *R. solani* was prepared by harvesting the mycelial mats from fully grown culture of *R. solani* raised on PDA plates. Mycelial mats were suspended in sterile distilled water (SDW) and homogenized in a blender for one minute and strained through double layered muslin cloth and diluted with SDW in such a manner to contain 15-20 mycelial bits per microscopic field (200 X). It was then sprayed on to the leaves by a hand sprayer 30 days after sowing.

3.4.3.2. Application of Compost Teas

The compost teas prepared as given under bucket bubbler technique was applied as foliar spray (50ml/ plant) and as soil drenches (1L/ pot). The application was repeated at fortnightly intervals.

3.4.3.3. Application of Vermiwash

Vermiwash and enriched vermiwash prepared were diluted to five times by adding water. It was then applied as foliar spray (50ml/ plant) and soil drench (1L/ pot). The application was repeated at fortnightly intervals.

3.4.3.4. Application of Composts

Composts and enriched compost was incorporated into soil (100g/ plant). The application was repeated at fortnightly intervals.

3.4.3.5. Application of Fungicide

Copper oxychloride (0.2 per cent) prepared was thoroughly mixed and used for foliar spray and soil drenching in cowpea plants (1L/ pot). The application was repeated at fortnightly intervals.

3.4.4. Population Dynamics of Saprophytic Fungi in Soil

The population dynamics of saprophytic fungi in the soil at monthly interval was estimated by serial dilution and plating technique (Wakesman, 1922).

One g of thoroughly mixed soil sample was transferred aseptically into a 250 ml conical flask containing 100 ml of sterile water and shaken for 20 min in a mechanical shaker. One ml of the suspension was pipetted out to 99 ml of sterile water in a 250 ml conical flask under aseptic condition. The flasks were again shaken for 15 min in a mechanical shaker. One ml of suspension was pipetted out to sterile petri plates containing melted and cooled Martin's Rose Bengal Agar medium (Appendix I) at the rate of 20 ml per dish and gently rotated for thorough mixing of the sample. The petri plates were incubated at room temperature. Population of saprophytic fungi was enumerated as cfu/ g. The population of saprophytic fungi was estimated for a period of four months.

3.4.5. Observations

The following observations of cowpea plants were recorded during the course of the experiment.

3.4.5.1. Disease Incidence (%)

Observation on collar rot incidence was taken from the next day of inoculum application till the time of uprooting the crop. Observations were taken at an interval of five days. Disease 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)

3.4.5.2. Disease Intensity

Pathogenicity was rated 14 days after inoculation. Scoring of the disease was done using 0-5 disease scale developed by (Mathew and Gupta, 1996) (Plate-6).

Grade	Description
0	Healthy
1	1-20 per cent of leaf area infected
2	21- 40 per cent of leaf area infected
3	41-60 per cent of leaf area infected
4	61-80 per cent of leaf area infected
5	81- 100 per cent of leaf area infected

Percentage disease index was calculated using the formula:

$$\text{PDI} = \frac{\text{Sum of grades of each leaf}}{\text{Number of leaves assessed}} \times \frac{100}{\text{Maximum grade used}}$$

McKinney (1923)

3.4.5.3. Shoot length (cm)

Length of shoot from ground level to the growing tip of each plant was measured after uprooting the plant.

3.4.5.4. Root length (cm)

Length of roots of each plant was measured after uprooting.

Plate 6. 0-5 score for assessment of web blight intensity

3.4.5.5. Fresh weight of plants (g)

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

3.4.5.6. Dry weight of plants (g)

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

3.4.5.7. Yield (g/ plant)

Number of pods per plant and pod weight was recorded.

3.4.5.8. Nodulation character

Number of root nodules per plant was recorded.

3.5. 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 under *in vitro* and *in vivo* conditions to study the symptomatology, morphological and cultural characters of *R. solani*, to standardize and prepare compost tea for exploring the possibility of its use in eco-friendly management of collar rot and web blight in cowpea.

4.1. SYMPTOMATOLOGY

Symptomatology of collar rot and web blight was studied by collecting infected plants from the Crop Museum, College of Agriculture, Vellayani. Collar rot was noticed at the seedling stage at the stem near the soil level whereas the web blight appeared at the later stages of crop growth on the foliage. The collar rot symptom appeared at the seedling stage as brownish black lesions at the soil level near the collar region resulting in girdling at the basal portion of the stem (Plate- 7a). The lesion coalesced and enlarged and deep- seated to cover the entire lower portion of the stem (Plate- 7b). The affected plant became stunted followed by yellowing and shedding of leaves and ultimately drying up off the entire plant. In severe cases, poor root development and root rotting were observed. White cottony mycelial growth along with small brown mustard or irregular shaped sclerotia could be seen in the affected area (Plate- 7c).

The web blight symptoms were noticed in mature plants at flowering stage. The initial symptoms of web blight appeared on the leaves as small circular, light greyish-brown spots surrounded by irregular water soaked areas (Plate- 8a). These lesions then enlarged to form oblong to irregular shapes (Plate- 8b). Under congenial conditions, the spots coalesced covering major portion of the leaf lamina with mycelial growth, leading to shedding of affected leaves (Plate- 8c). With the advancement of the disease, lesions enlarged along with white cottony mycelial web and numerous globular sclerotia appearing on the affected region (Plate- 8d). In the final stage of infection, the leaves turned yellow and then necrotic and were shed in large numbers. The affected leaves were webbed together by the mycelium of the pathogen (Plate- 8e).

a. Brownish black lesion at collar region

b. Deep seated lesion

c. Formation of sclerotia on the affected stem

Plate 7a-c. Cowpea plants showing symptoms of collar rot

a. Initial water soaking

b. Advancement of lesions

c. Shedding of leaves

d. Mycelium and sclerotia on leaves

e. Webbed leaves

Plate 8 a-e. Cowpea leaves showing symptoms of web blight

4.2. PATHOGEN

4.2.1. Isolation of Pathogen

The pathogen causing collar rot and web blight of cowpea was isolated from the diseased plants collected from the Crop Museum, College of Agriculture, Vellayani. Three isolates were obtained (Plate- 9). The mycelial growth of the collar rot isolate was off-white in colour which produced sclerotia profusely. Out of the two web blight isolates obtained, W1 produced tan coloured mycelia with no sclerotial production and the web blight isolate, W2 produced off-white mycelia similar to that of the collar rot isolate. The sclerotia of the two isolates were large and dark brown in colour. The fungal cultures were maintained on PDA slants by periodic sub-culturing.

4.2.2. Identification of Pathogen

The isolates examined showed the following characteristics (Plate- 10):

- Off- white to pale rapidly growing mycelium with average dia varying from 5- 11.2 µm with branching near the distal septum of hyphal cells (Plate- 10a).
- Hyphal branches inclined to the direction of growth and constricted at the point of origin from the main hyphae (Plate- 10b).
- Production of monilioid cells, often called barrel-shaped cells or chlamydospores, in chains or in aggregates (Plate- 10c).
- Formation of septum in the branch near the point of origin (Plate- 10d).
- Presence of multinucleate hyphae (Plate- 10e).

All the above characteristics conformed to those of *Rhizoctonia solani*, as described by Parmeter and Whitney (1969). Hence, the pathogen was identified to be *Rhizoctonia solani* Kuhn.

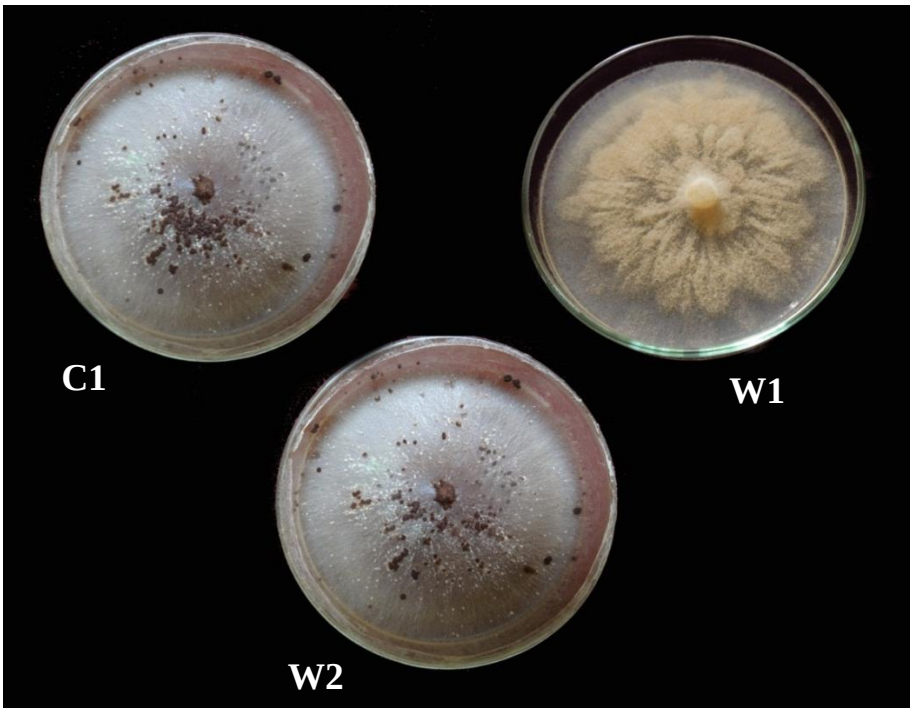
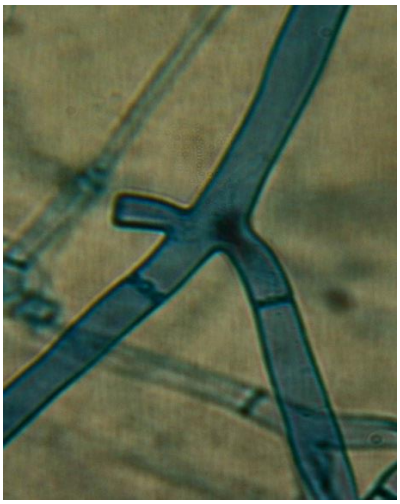


Plate 9. Isolates of *R. solani* on PDA medium

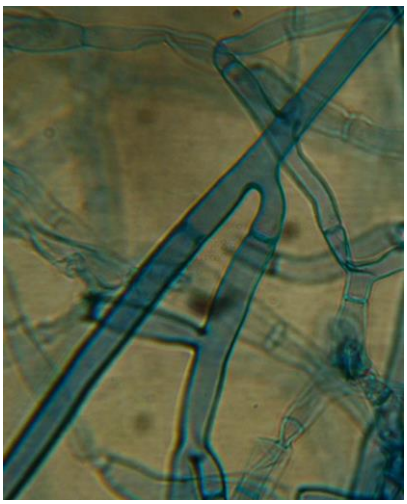
C1 - Collar rot isolate -1

W1- Web blight isolate -1

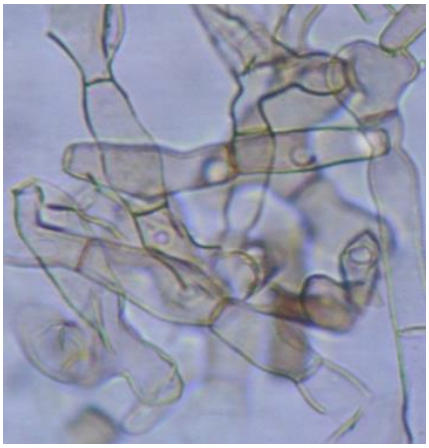
W2- Web blight isolate -2



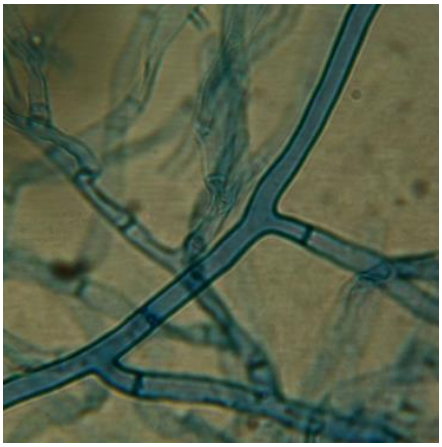
a. Branching near the distal septum



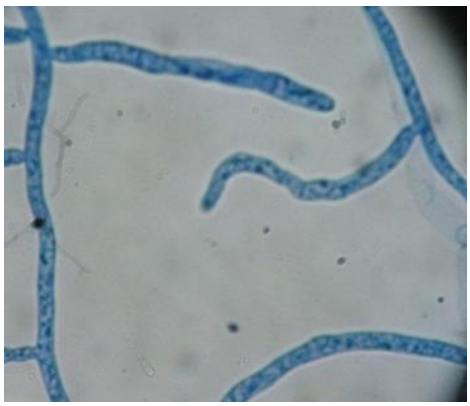
b. Constriction in hyphae



c. Monilioid cells



d. Septum near the point of origin



e. Multinucleate hyphae

Plate 10 a-e. Hyphal characteristics of *R. solani*

4.2.3. Pathogenicity Test

The pathogenicity of the isolates of *R. solani* was confirmed by proving Koch's postulates. Artificial inoculation of the pathogen at the collar region of the cowpea seedlings produced collar rot symptom within three to four days of inoculation (Plate- 11a) and artificial inoculation of the pathogen on the leaf lamina of the cowpea seedlings produced typical web blight symptom within 48 h of inoculation (Plate- 11b). Re-isolation of the pathogen from the artificially inoculated plants yielded *R. solani* identical to the original culture. The web blight isolate W1 did not produce any symptom when inoculated artificially.

4.2.4. Comparison of Growth of Collar Rot and Web Blight Pathogens

Collar rot isolates and web blight isolate exhibited difference in the average growth rate. The average growth rate of the collar rot isolate C1 was found to be 2.23 cm/day. The two web blight isolates differed significantly in their average growth rate. The average growth rate of the isolate W1 was 1.76 cm/day and that of W2 was 2.19 cm/day (Table- 2).

4.2.5. Morphological and Cultural Characters of the Isolates

The *R. solani* isolates used in this study showed difference in morphological and cultural characters (Tables- 3, 4). The isolates differed in their growth pattern, mycelial characters and sclerotial characters. The collar rot isolate C1 and the web blight isolate W2 took three days to attain full growth in the nine cm dia of the petri dish. The web blight isolate W1 took more days to attain its full growth *i.e.*, five days to cover nine cm of the petridish.

The colour of mycelium on PDA medium varied among the isolates (Table-2). The isolate that did not produce any sclerotia was tan coloured with fluffy mycelium whereas, the sclerotial isolates produced off- white mycelium that was regularly appressed to the surface. The isolate that produced tan coloured mycelium presented a floral pattern of mycelial growth in the petridish. The colour of the medium on the underside of the petridish also showed variation

a. Pathogenicity testing of collar rot isolate

b. Pathogenicity testing of web blight isolate

Plate 11 a-b. Pathogenicity testing of collar rot and web blight isolates of *R. solani*

Table 2. Mycelial characteristics of *R. solani* isolates of collar rot and web blight of cowpea

Isolates	*Average growth rate (cm/day)	No:of days taken to cover 9cm petridish	Mycelial Colour	Mycelial Texture	Colour of medium on the underside
Collar rot (C1)	2.23	3	Off - white	Appressed	Brown
Web blight (W1)	1.76	5	Tan	Fluffy	Chocolate brown
Web blight (W2)	2.19	3	Off- white	Appressed	Brown
SE	5.81	_			
CD (0.05)	0.2013	_			

*Mean of three replications

Table 3. Sclerotial characteristics of *R. solani* isolates of collar rot and web blight of cowpea

Isolates	Sclerotial production	No: of days taken for the formation of sclerotial initials	Sclerotial intensity	No:of sclerotial initials
C1	+	5	High	134
W1	_	_	_	_
W2	+	7	High	107

between isolates. Sclerotial isolate, W2 was light brown and that of non- sclerotial isolate, W1 was chocolate brown in colour.

The isolates differed in their sclerotial characters. Sclerotial initials were produced only in the isolates that produced off- white mycelia (C1 and W2). The isolate W1 with tan coloured mycelia did not produce any sclerotial bodies. Even though the isolates C1 and W2 produced sclerotia, the number of days taken for the formation of sclerotia varied. The isolate C1 produced sclerotia faster than the other. Isolate W2 took seven days for the production of sclerotial initials, whereas, isolate C1 produced sclerotia after five days of its growth (Table- 3).

4.2.6. Selection of Virulent Isolates

Based on the pathogenicity, virulence and growth rate, the isolate C1 and W2 was selected for further studies.

4.2.7. Cross Infectivity Study

This study revealed the cross infective nature of the two pathogenic isolates. The collar rot isolate (C1) and the web blight isolate (W2) were cross infective *i.e,* the collar rot isolate (C1) produced web blight symptom when inoculated on the leaf lamina and the web blight isolate (W2) produced collar rot symptom when inoculated at the collar region of the cowpea seedlings. Even though the isolates exhibited cross infectivity, the number of days taken for the development of symptom varied. Isolate C1 produced both collar rot symptom and web blight symptom two days after inoculation. The isolate W2 produced web blight symptom two days after inoculation whereas it produced collar rot symptom three days after inoculation (Table- 4).

4.3. PREPARATION OF COMPOSTS AND COMPOST TEAS

4.3.1. Preparation of Leaf Compost

The leaves of the plants with antifungal properties were evaluated and neem emerged as the most effective in pathogen suppression based on which

Table 4. Cross infectivity study of *R. solani* isolates of collar rot and web blight of cowpea

Isolates	Leaf lamina	No. of days taken for lesion development	Collar region	No. of days taken for lesion development
C1	+	2	+	2
W2	+	2	+	3

+ = Pathogenic

neem leaf compost was prepared. The results of each part of the experiment are presented below.

4.3.1.1. In vitro Evaluation of Leaf Extracts Against R. solani Isolates

Leaf extracts of five plants *viz*., Neem (*Azadirachta indica*), Henna (*Lawsonia inermis*), Tulsi (*Ocimum sanctum*), Clerodendron (*Clerodendron infortunatum*) and Pongamia (*Pongamia pinnata*) selected on the basis of their antifungal property against *R. solani* were tested for the efficacy in suppressing the two isolates of *R. solani* by poisoned food technique (Nene and Thapliyal, 1993) (Plate- 12).

A comparison on the effect of leaf extracts on collar rot and web blight isolates of *R. solani* revealed that neem leaf extract is highly effective in causing *in vitro* suppression of both the isolates.

4.3.1.1. a. In vitro Evaluation of Leaf Extracts Against Collar Rot Isolate of R. solani

All the leaf extracts tested showed significant suppression of the pathogen. Among the different plant extracts tested against the collar rot isolate of *R. solani*, the highest inhibition per cent was obtained by incorporation of neem leaf extract in PDA medium. The percentage inhibition was found to be 60.7. The henna leaf extract caused an inhibition of 55.90 per cent which was statistically on par with neem leaf extract. The leaf extracts of clerodendron, tulsi and pongamia also resulted in suppression of growth of the pathogen as compared to that of control. The per cent inhibition was found to be 39.06, 34.73 and 38.70 respectively (Table- 5, Plate- 12a) which were statistically on par.

4.3.1.1.b. In vitro Evaluation of Leaf Extracts Against Web Blight Isolate of R. solani

All the tested leaf extracts significantly suppressed the mycelial growth of web blight isolate of *R. solani.* Among them, the maximum suppression was caused by neem leaf extract (77.88 per cent) followed by henna (54.46 per cent).

Table 5. Effect of different leaf extracts on the mycelial growth of collar rot isolate of *R. solani*

Leaf extracts	*Mycelial growth (cm)	Percentage inhibition
Neem	3.53	60.70 [a] (7.85)
Clerodendron	5.33	39.06 [b] (6.32)
Tulsi	5.86	34.73 [b] (5.97)
Pongamia	5.5	38.70 [b] (6.30)
Henna	3.96	55.90 [a] (7.54)
Control	9.00	_
SE m (±)	_	0.22
CD (0.05)	_	0.69

(Figures given in the parenthesis 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.

The suppression caused by neem leaf extract and henna leaf extract was significantly different from all other treatments. No significant difference was noticed among the remaining three plant extracts. The per cent inhibition was found to be 35.90, 29.16 and 38.05 for clerodendron, tulsi and pongamia leaf extracts respectively (Table- 6, Plate- 12b).

4.3.1.1.c. Preparation of Neem Leaf Compost

The shredded neem leaves took 60 days for complete degradation to form bleck coloured fully matured compost.

4.3.2. Preparation of Aerated Compost Tea

Aerated compost tea was prepared by modifying the bucket bubbler technique proposed by Ingham (2005).

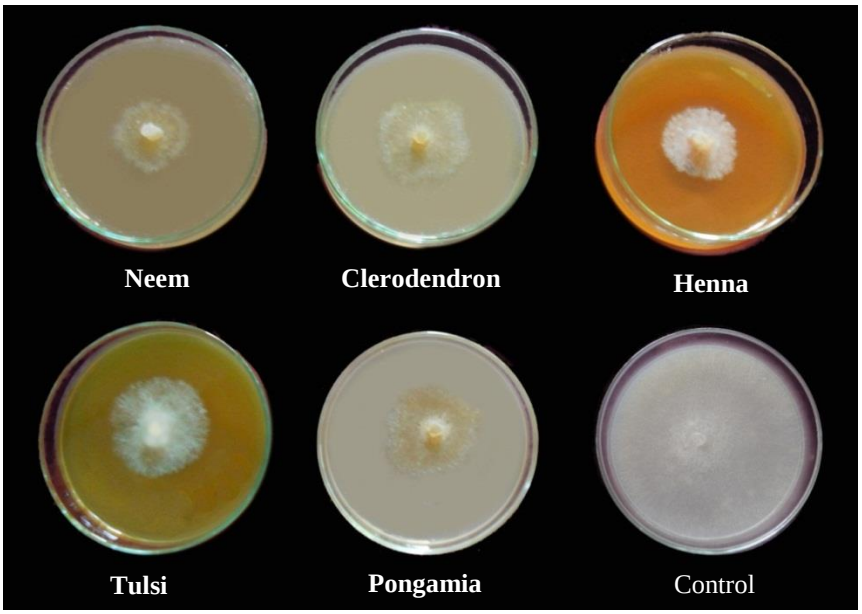
4.3.2.1. Standardisation of Brewing Period of Compost Tea

The brewing time and dilution best suited for the preparation of compost tea was standardized based on the population of saprophytic fungi present in it. Compost tea was prepared in 1:5 and 1:10 dilution and aerated by an aerator. Samples were drawn at regular intervals and the microflora present in compost tea was assessed by serial dilution and plate counting method. The dilution at which maximum microflora was obtained and the time at which highest colony count of mycoflora was obtained were considered ideal for the preparation of compost tea. The population of saprophytic fungi in 1:5 dilution before brewing was 3.65×10^4 cfu/ ml of compost tea. Thereafter, a slow increase in population of fungi was observed. But with the increase in brewing time there was a rapid increase in the fungal population (Fig.1). This is evident from the population of 72.52×10^4 cfu/ ml of compost tea at 24 h of brewing. However, with further increase in brewing time, the population of fungi decreased drastically to 1.96×10^4 cfu/ ml and then to 0.29×10^4 cfu/ ml of compost tea after 72 h of brewing (Table-7). Similarly, at 1:10 dilution, the population of fungi before brewing was 3.19×10^4 cfu/ ml of compost tea which subsequently increased to 25.88×10^4 cfu/ ml of compost tea after 24 h of brewing and finally reduced to zero after 72 h of brewing (Plate- 13).

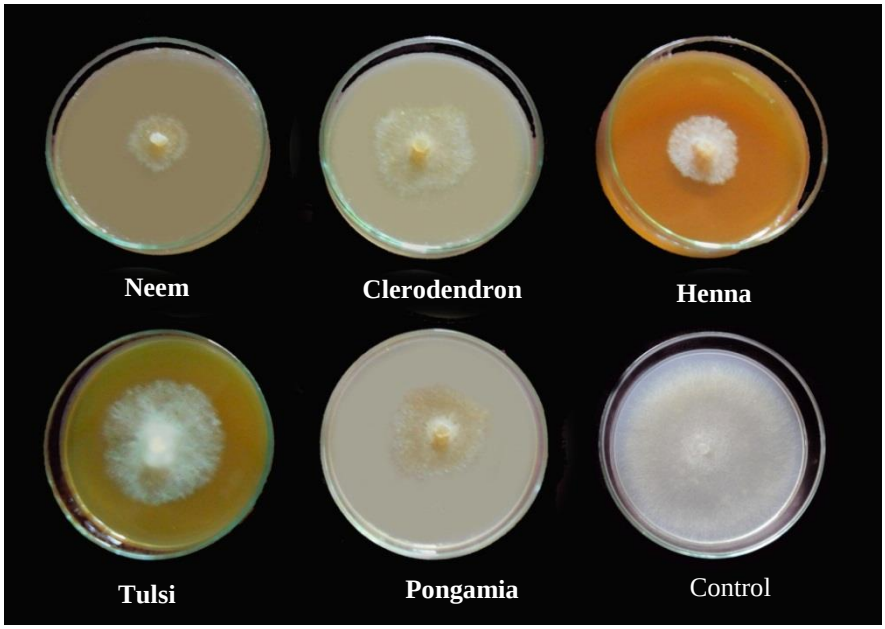
Table 6. Effect of different leaf extracts on the mycelial growth of web blight isolate of *R. solani*

Leaf extracts	* Mycelial growth (cm)	Percentage inhibition
Neem	2.00	77.88 [a] (61.92)
Clerodendron	5.76	35.90 [c] (36.79)
Tulsi	6.36	29.16 [c] (32.67)
Pongamia	5.56	38.05 [c] (38.07)
Henna	4.10	54.46 [b] (47.54)
Control	9.00	_
SE m (±)	_	1.80
CD (0.05)	_	5.70

(Figures given in the parenthesis 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.



a. ***In vitro*** **evaluation of leaf extracts for suppression of collar rot isolate**



b. ***In vitro*** **evaluation of leaf extracts for suppression of web blight isolate**

Plate 12 a-b. ***In vitro*** **evaluation of leaf extracts (5%) for suppression of *R. solani* isolates**

Table 7. Population of fungi (cfu/ ml) of the compost tea at different dilution and time intervals

Sample	Time	Ratio	*Population count ($\times 10^4$) (cfu./ ml)
I	Day 1 6am	1:5	3.65^{c} (2.15)
II		1:10	3.19^{c} (2.04)
III	Day 1 6pm	1:5	67.42^{a} (8.27)
IV		1:10	25.88^{b} (5.18)
V	Day 2 6am	1:5	72.52^{a} (8.57)
VI		1:10	20.30^{b} (4.61)
VII	Day 2 6pm	1:5	4.84^{c} (2.41)
VIII		1:10	0.54^{c} (1.24)
IX	Day 3 6am	1:5	1.96^{c} (1.72)
X		1:10	0.29^{c} (1.13)
XI	Day 3 6pm	1:5	1.16^{c} (1.47)
XII		1:10	0^{c} (1.00)
SE m (±)	_	_	0.702
CD (0.05)	_	_	2.05

(Figures given in the parenthesis are transformed values).
*Mean of three replications.

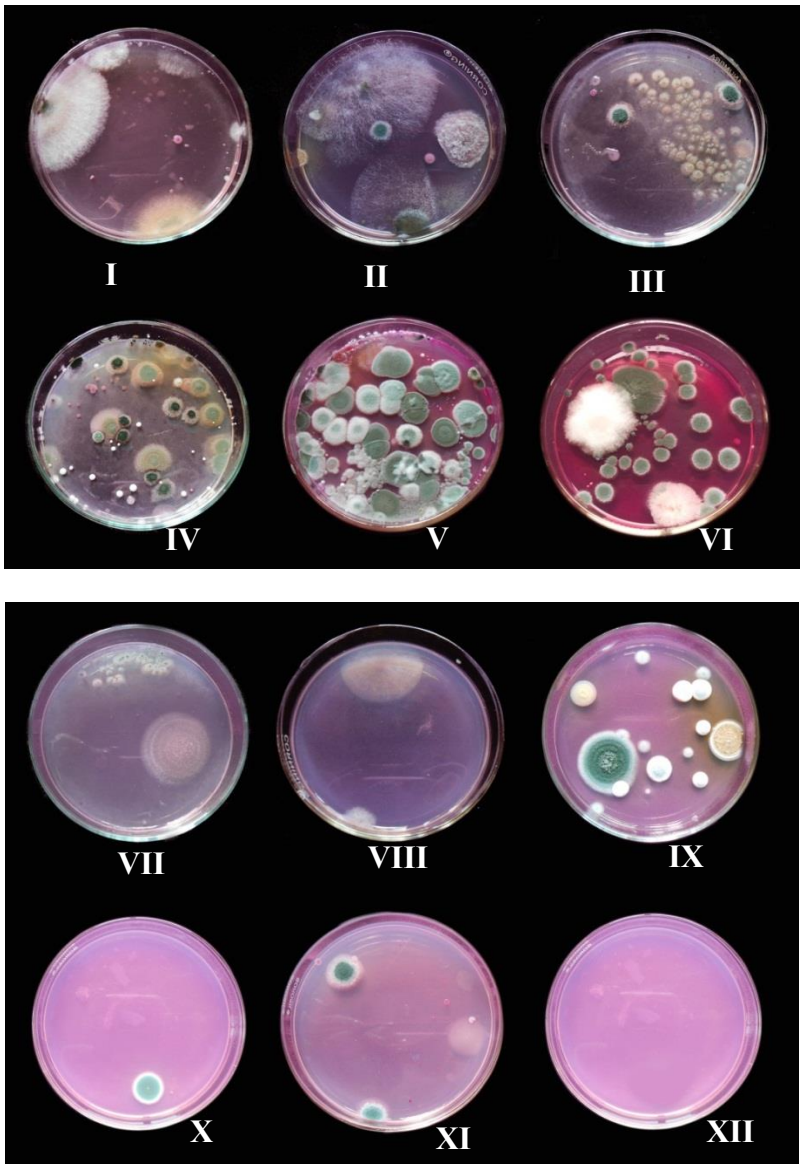


Plate 13. Saprophytic fungi in leaf compost tea at different dilution and brewing time

I-Day 1, 6 am; II-Day 1, 6 pm; III-Day 2, 6 am; IV- Day 2, 6 pm; V-Day 3, 6 am; VI-Day 3, 6 pm; VII-Day 4, 6 am; VIII-Day 4, 6 pm; IX-Day 5, 6 am; X-Day 5, 6 pm; XI-Day 6, 6 am; XII-Day 6, 6 pm.

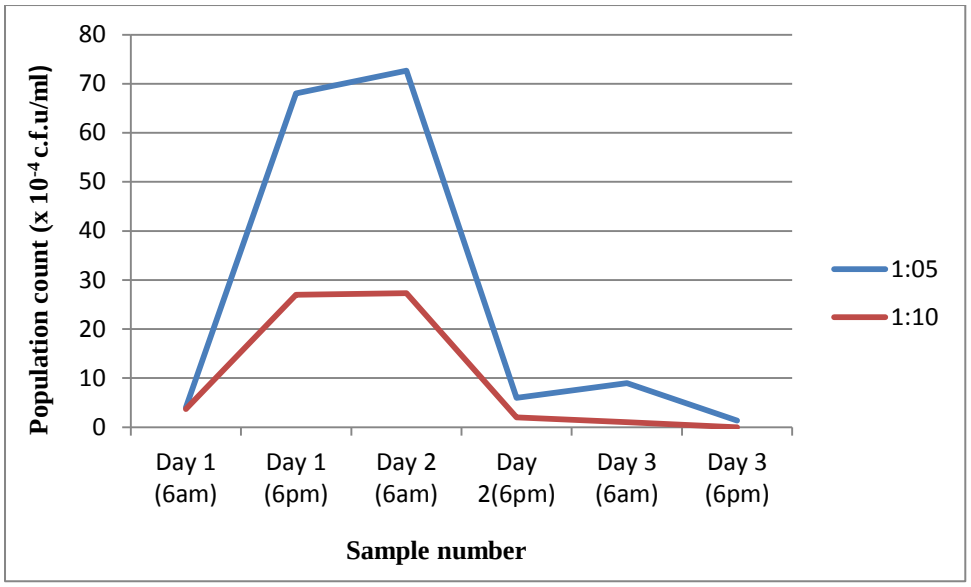


Fig.1. Effect of dilution and brewing time on the population of saprophytic fungi in leaf compost tea

The effect of dilution on fungal population could also be studied from this experiment. The population of the saprophytic flora was higher in 1:5 dilution when compared to 1:10 dilution at all stages of brewing. The initial population in 1:5 dilution was 3.65×10^4 cfu/ ml, whereas in 1:10 dilution it was 3.16×10^4 cfu/ ml. With the progress of brewing, the difference in population of mycroflora in dilution became significant. After 12 h of brewing 72.52×10^4 cfu/ ml was the population of fungi in compost tea diluted five times which is 2.8 times more when diluted ten times. After 24 h there was a decline in the population of saprophytic fungi in both the dilutions.

4.4.1. Isolation and Enumeration of Mycoflora Associated with Composts and Compost Teas

Saprophytic fungi were isolated from compost and compost tea by serial dilution and plating on Rose Bengal Agar (RBA) medium. The predominant genera of saprophytic fungi obtained from the different compost and compost tea are listed in Table- 8.

The populations of mycoflora present in compost and compost teas were assessed and recorded as cfu/ g and cfu/ ml respectively. The result revealed that the population of saprophytic fungi was found to be higher in compost teas when compared with that of their respective solid composts. A total of 99.33×10^4 fungal colonies were identified from one ml of neem leaf compost tea. The predominant fungal genera were *Aspergillus* sp., *Trichoderma* sp., *Fusarium* sp. and *Penicillium* sp. This showed maximum diversity in fungal flora compared to other composts or compost teas. The genera that were common to all the composts and compost teas examined were *Aspergillus* sp. and *Penicillium* sp. The population of fungi in neem leaf compost tea (99.33×10^4 cfu/ ml) was on par with neem leaf compost (81.66×10^4 cfu/ g). There was no significant difference in the population count of fungi in vermicompost, coirpith compost, vermiwash and coirpith compost tea. These were in the order of 29.66×10^4, 30.66×10^4, 41.66×10^4 and 33.66×10^4 cfu/ g of the sample respectively (Table- 8, Plate- 14).

Table 8. Population of saprophytic fungi in composts and compost teas

Compost/ Compost tea	*Population count (10^4) (cfu/ g or cfu/ ml)	Predominant fungal genera
Vermicompost	29.66 [b]	*Fusarium* sp. *Penicillium* sp. *Trichoderma* sp.
Coirpith compost	30.66 [b]	*Aspergillus* sp. *Penicillium* sp.
Neem leaf compost	81.66 [a]	*Aspergillus* sp. *Fusarium* sp.
Vermiwash	41.66 [b]	*Aspergillus* sp. *Trichoderma* sp. *Penicillium* sp. *Fusarium* sp.
Coirpith compost tea	33.66 [b]	*Aspergillus* sp. *Fusarium* sp. *Penicillium* sp.
Neem-leaf compost tea	99.33 [a]	*Aspergillus* sp. *Trichoderma* sp. *Fusarium* sp. *Penicillium* sp.
SE m (±)	7.96	
CD (0.05)	24.56	

*Mean of three replications.

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

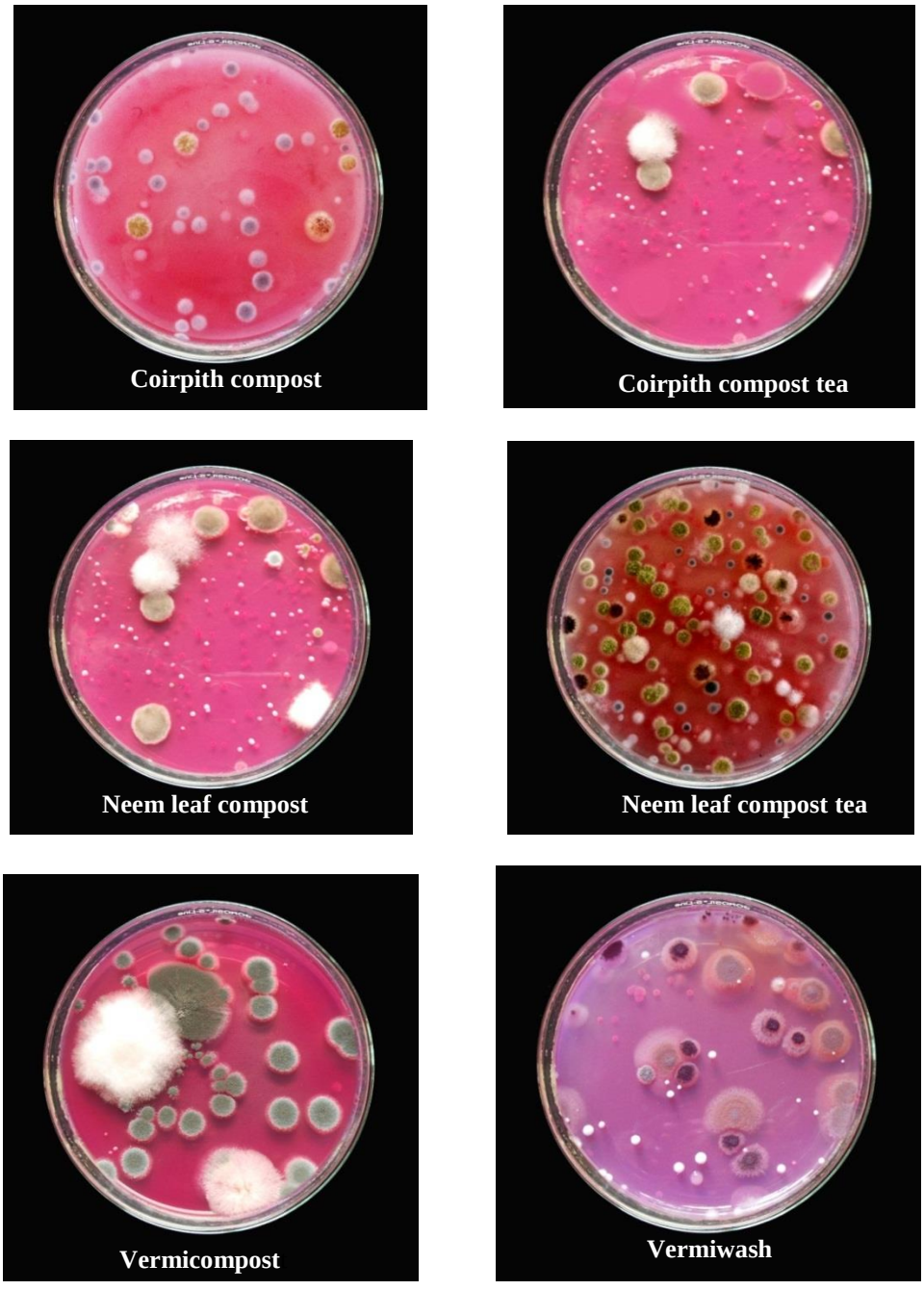


Plate 14. Saprophytic fungi in different composts and compost teas

4.5. SELECTION OF ANTAGONISTIC FUNGI FOR ENRICHMENT

The saprophytic fungi obtained from compost and compost teas were assessed for their inhibition on *R. solani* by dual culture technique. The fungus which showed the maximum suppression of the pathogen was selected for enrichment of composts and compost teas.

4.5.1. *In vitro* Inhibition of Collar Rot Isolate of *R. solani* by Saprophytic Fungi

Among the four saprophytic fungi tested, *Trichoderma* sp. caused significant suppression of *R. solani.* Percentage inhibition of 44.73 was obtained with *Trichoderma* sp. which was significantly different from all the other three fungi namely, *Aspergillus* sp., *Penicillium* sp., and *Fusarium* sp. The other three fungi exhibited varying levels of suppression. *Aspergillus* sp. caused inhibition to the extent of 25.07 per cent which was on par with that of *Penicillium* sp. (22.39 per cent) and *Fusarium* sp. (22.18 per cent) (Table- 9, Plate- 15).

4.5.2. *In vitro* Inhibition of Web Blight Isolate of *R. solani* by Saprophytic Fungi

A comparison of inhibition of four different saprophytic fungi on web blight isolate of *R. solani* revealed that *Trichoderma* sp. was highly effective in causing *in vitro* suppression of web blight isolate. The highest inhibition of 44.42 per cent was obtained with that of *Trichoderma* sp. which was significantly different from that of all the other three fungi. On the contrary, *Aspergillus* sp. and *Penicillium* sp. exerted inhibition of 28.85 per cent and 31.81 per cent respectively which was statistically on par with each other (Table- 10, Plate- 16)

4.5.3. Selection of Antagonist for Enrichment of Composts and Compost Teas

Trichoderma sp. showed maximum suppression for the mycelial growth with respect to both the isolates under *in vitro* condition. Hence it was selected for enriching composts and compost teas.

Table 9. Effect of saprophytic fungi on the mycelial growth of collar rot isolate of *R. solani*

Saprophytic fungi	* Mycelial growth (cm)	Percentage inhibition
Aspergillus sp.	6.73	25.07 [b] (5.10)
Penicillium sp.	6.93	22.39 [b] (4.83)
Fusarium sp.	7.00	22.18 [b] (4.81)
Trichoderma sp.	5.10	44.73 [a] (6.76)
Control	9.00	_
SE m(±)	_	0.312
CD (0.05)	_	1.020

(Figures given in the parenthesis 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.

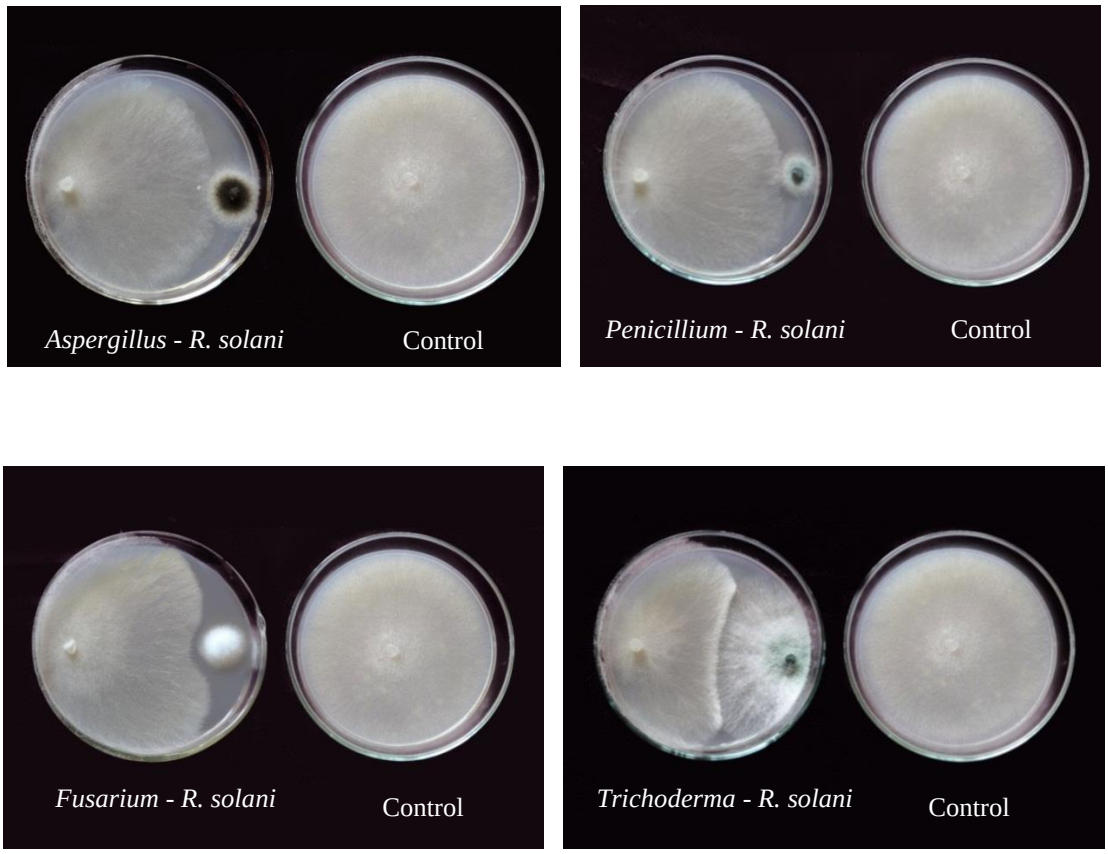


Plate 15. Suppression of collar rot isolate of *R. solani* by saprophytic fungi under *in vitro* condition

Table 10. Effect of saprophytic fungi on the mycelial growth of web blight isolate of *R. solani*

Saprophytic fungi	* Mycelial growth (cm)	Percentage inhibition
Aspergillus sp.	6.40	28.85 [b] (5.46)
Penicillium sp.	6.13	31.81 [b] (5.72)
Fusarium sp.	7.03	21.82 [c] (4.77)
Trichoderma sp.	5.00	44.42 [a] (6.73)
Control	9.00	_
SE m(±)	_	0.112
CD (0.05)	_	0.366

(Figures given in the parenthesis 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.

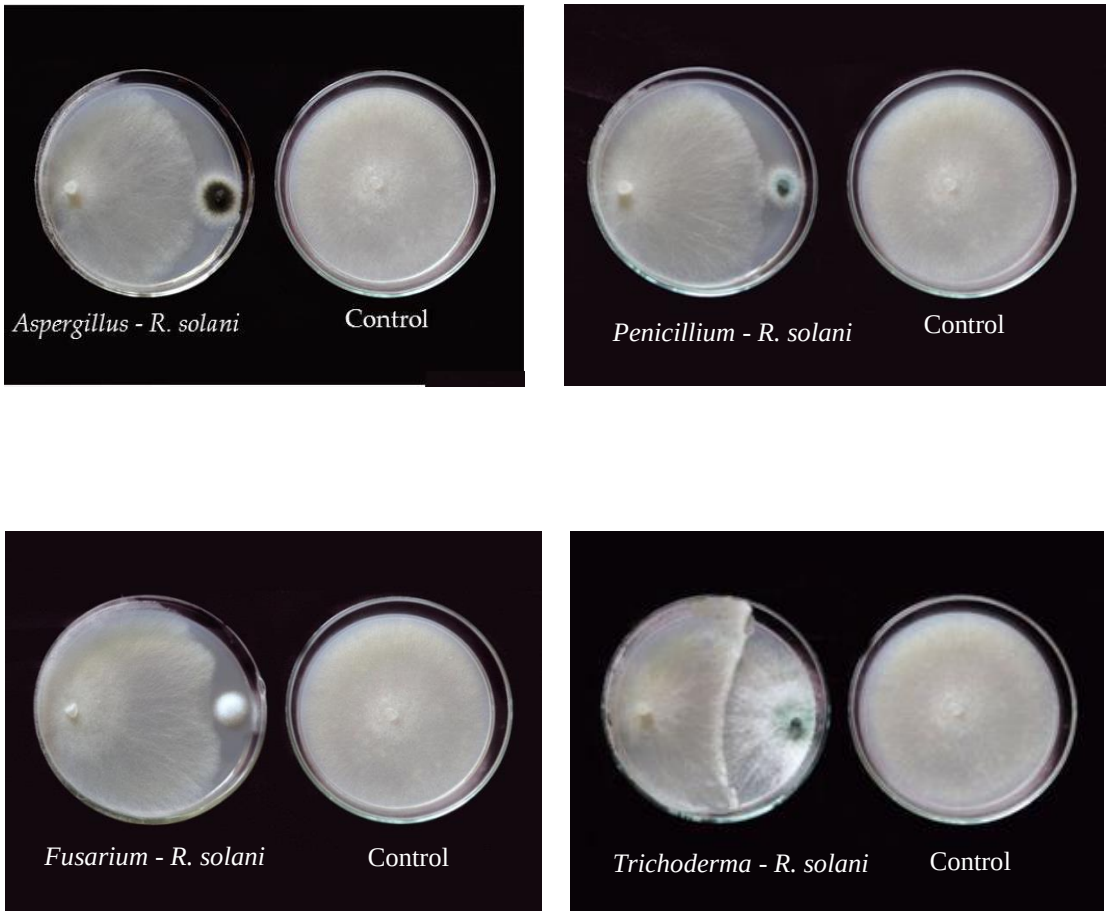


Plate 16. Suppression of web blight isolate of *R. solani* by saprophytic fungi under *in vitro* condition

4.6. IDENTIFICATION OF SELECTED ANTAGONISTIC FUNGI

Based on morphological, cultural and sporulation characteristics, the selected antagonist, *Trichoderma* sp. was identified as *Trichoderma virens* (Miller, Giddens & Foster Von Arx= *Gliocladium virens*, Miller, Giddens & Foster).

Trichoderma virens

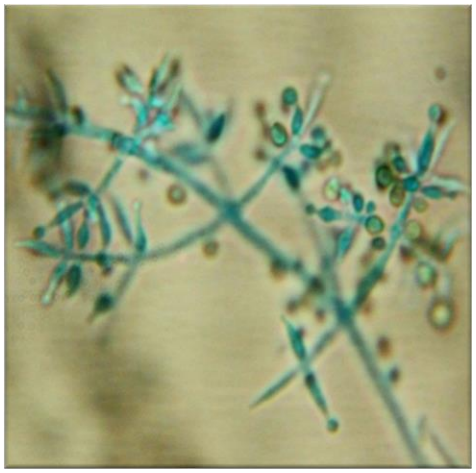
The isolate showed fast growth on PDA medium taking four days to cover full growth in petridish. It produced aerial mycelium with floccose texture of white to green colour (Plate- 17a). Conidiophores were sub- hyaline, measured 30- 300 µm in length and 2.5- 4.5 µm in diameter. Conidiophore branches arose at right angles (Plate- 17b). The base appeared unbranched for about half of the length, but irregular branching was noticed at the apex with each branch terminated by a cluster of three to six closely appressed phialides (Plate- 17c). Conidia were ellipsoidal to ovoid, 3.5 x 4.4 µm in size, dark green in colour (Plate- 17d). Conidia from adjacent phialides were found to form large gloeoid masses.

4.7. ASSESSMENT OF THE EFFECT OF COMPOSTS AND COMPOST TEAS FOR MANAGEMENT OF COLLAR ROT AND WEB BLIGHT

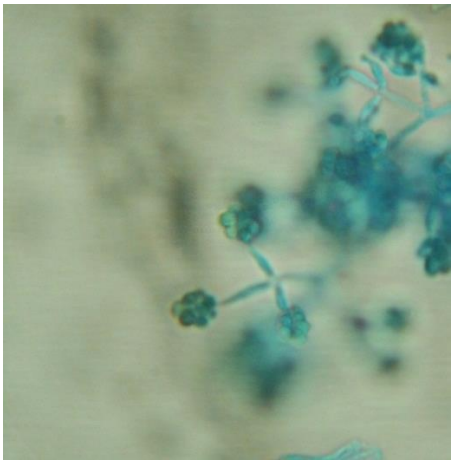
The effect of various composts, compost teas and their enriched products on the suppression of collar rot and web blight of cowpea was studied by a pot culture experiment. The plants were inoculated with the virulent isolates of *R. solani* at the collar region and on the leaves. The composts and enriched composts were applied as basal whereas the compost tea and the enriched products were given as soil drench and foliar spray. All the treatments were repeated at fortnightly intervals. The effects of different composts and compost teas were compared with that of un-inoculated control, inoculated control and a chemical control (0.2 per cent COC). Observations on collar rot incidence and web blight index were recorded.

The per cent disease incidence of collar rot and per cent disease index of web blight were calculated based on standard procedures (Table- 11). The

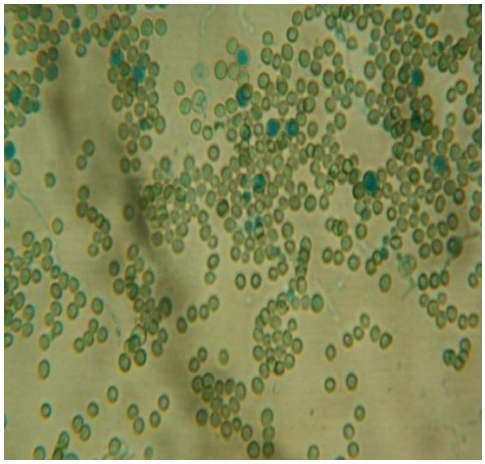
a. Mycelial growth and sporulation



b. Conidiophore branching



c. Phialides and gleoid mass



d. Conidia of *T. virens*

Plate 17 a-d. Characteristics of *Trichoderma virens*

percent disease suppression of web blight was maximum in chemical control (89.82 percent) followed by *Trichoderma* enriched leaf compost tea (82.75 per cent) with respect to inoculated control. Among the different composts and compost teas tested, treatment T 12 (*Trichoderma* enriched leaf compost tea) with lowest web blight index of 14.83 was significantly different from inoculated control (86.02) in which highest per cent disease was recorded. The result also revealed that there was no significant difference between the treatment T12 (soil incorporation of enriched leaf compost tea + *R. solani*) and T15 (chemical control-COC 0.2%). Similarly, treatment T1 (soil incorporation of vermicompost), T2 (soil incorporation of enriched vermicompost), T8 (spraying and drenching with enriched coirpith compost tea) and T11 (spraying and drenching with leaf compost tea) was statistically on par. No natural incidence of web blight disease was recorded.

In case of collar rot disease, the treatments T9 (soil incorporation of leaf compost) and T12 (spraying and drenching with *Trichoderma* enriched leaf compost tea) exhibited lowest disease incidence (5.86) which was statistically on par with that of chemical check. Percentage disease suppression of 92.39 was recorded in these cases which was significantly different from inoculated control (77.03 per cent). The highest incidence of collar rot was obtained in treatment T5 (soil incorporation of coirpith compost) which was in the order of 66.66. A percentage disease suppression of 13.46 was obtained in this case followed by 29.50 in treatment T6 (soil incorporation of enriched coirpith compost) which was statistically on par. Among vermicompost and vermiwash, highest percentage disease suppression was obtained with treatment T3 (spraying and drenching with vermiwash) and T4 (spraying and drenching with enriched vermiwash). These were in the order of 77.96 each. No natural incidence of collar rot disease was observed.

Table 11. Effect of composts and compost teas on the incidence of collar rot and web blight of cowpea

Treatments	*Disease incidence (Collar rot)	% disease suppression	*Disease index (Web blight)	% disease suppression
T1	43.19^{abc} (6.64)	43.93	30.36^{de} (5.60)	64.70
T2	24.28^{abcd} (5.02)	68.47	28.66^{de} (5.44)	66.68
T3	16.97^{bcd} (4.23)	77.96	39.24^{bcd} (6.34)	54.38
T4	16.97^{bcd} (4.23)	77.96	43.94^{bcd} (6.70)	48.91
T5	66.66^{ab} (8.22)	13.46	52.22^{bc} (7.29)	39.29
T6	54.30^{ab} (7.43)	29.50	46.06^{bcd} (6.86)	46.45
T7	43.19^{abc} (6.64)	43.93	52.82^{b} (7.33)	38.59
T8	32.83^{abc} (5.81)	57.38	41.63^{bcd} (6.52)	51.60
T9	5.86^{cd} (2.61)	92.39	36.05^{bcd} (6.08)	58.09
T10	24.28^{abcd} (5.02)	68.47	30.95^{cd} (5.65)	64.01
T11	33.33^{abc} (5.85)	56.73	38.10^{bcd} (6.25)	55.70

(Contd…)

(Table 11. Contd…)

T12	5.86[cd] (2.61)	92.39	14.83[ef] (3.97)	82.75
T13	77.03[a] (8.83)	_	86.02[a] (9.32)	_
T14	0[d] (1.00)	100	0[g] (1.00)	100
T15	5.86[cd] (2.61)	92.39	8.75[f] (3.12)	89.82
SE m(±)	1.41	_	0.57	_
CD (0.05)	4.09	_	1.65	_

(Figures given in the parenthesis 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.

T1-vermicompost + *R. solani*, T2- enriched vermicompost + *R. solani*, T3- vermiwash + *R. solani*, T4 - enriched vermiwash + *R. solani*, T5- coirpith compost + *R. solani*, T6- enriched coirpith compost + *R. solani*, T7- coirpith compost tea + *R. solani*, T8 - enriched coirpith compost tea + *R. solani*, T9- leaf compost + *R. solani*, T10- enriched leaf compost + *R. solani*, T11- leaf compost tea + *R. solani*, T12- enriched leaf compost tea + *R. solani*, T13- inoculated control, T14- uninoculated control, T15- chemical check (COC 0.2%).

Plate 18. General view of pot culture experiment

4.8. POPULATION DYNAMICS OF SAPROPHYTIC FUNGI IN SOIL AT MONTHLY INTERVALS

The population of saprophytic fungi in soil has showen an increasing trend from first to third month and thereafter during the fourth month, the population showed a general decline. On comparison with the inoculated control, the population of saprophytic fungi in the treated soil was higher. The highest fungal population of 76.66×10^4 cfu/ g was recorded in soil treated with spraying and drenching of neem leaf compost tea (T 11). During the second month treatment T11 (spraying and drenching of neem leaf compost tea) recorded highest fungal population of 85×10^4 cfu/ g soil which was significantly higher than that of inoculated control (T 13). These were in the order of 18.33×10^4 and 29.88×10^4 cfu/g of the soil respectively. During the third month, the population of saprophytic fungi was highest in treatment T12 (soil incorporation of enriched leaf compost). It was recorded as 99.11×10^4 cfu /g of the soil which was significantly different from inoculated control (38.88×10^4 cfu/g of the soil) (Table- 12).

During the first month, the lowest population of saprophytic fungi (28.66×10^4 cfu/g soil) was recorded in the treatment T7 (spraying and drenching with coirpith compost tea) which was on par with inoculated control (18.33×10^4 cfu/g). In the second third and fourth month, treatment T1 (soil incorporation of vermicompost) showed lowest population count which was, however, statistically significant over inoculated control. The population recorded in these treatments was 40.88×10^4 cfu /g, 50.44×10^4 cfu/g and 57.22×10^4 cfu/g of soil respectively.

4.9. BIOMETRIC OBSERVATIONS

The results on biometric observations revealed that there exists significant difference between all the parameters. 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.

Table 12. Population dynamics of saprophytic fungi in soils amended with composts, compost tea and *Trichoderma* enriched composts and compost teas at monthly intervals

Treatments	First month	Second month	Third month	Fourth month
T1	30.00^{fgh}	40.88^{i}	57.22 ^{g}	50.44^{f}
T2	43.66^{ef}	57.88^{fgh}	61.55^{fg}	67.11^{de}
T3	56.77^{bcd}	66.77^{def}	78.00^{de}	76.11^{bcd}
T4	63.22^{abc}	70.66^{bcd}	83.66^{cde}	78.55^{abc}
T5	42.77^{efg}	51.77^{h}	73.55^{ef}	63.77^{e}
T6	44.77^{de}	59.77^{efgh}	89.55^{abcd}	75.55^{bcd}
T7	28.66^{gh}	54.22^{gh}	78.55^{de}	68.55^{cde}
T8	54.77^{bcde}	68.77^{cde}	91.00^{abc}	79.66^{ab}
T9	54.22^{cde}	64.00^{defg}	85.33^{bcde}	73.33^{bcde}
T10	69.22^{ab}	78.11^{abc}	93.66^{abc}	83.66^{ab}
T11	76.66^{a}	85.00^{a}	97.44^{ab}	89.11^{a}
T12	68.22^{abc}	81.00^{ab}	99.11^{a}	88.33^{a}
T13	18.33^{h}	29.88^{j}	38.88^{h}	38.77^{g}
T14	24.66^{h}	39.22^{ij}	56.55^{g}	48.11^{fg}
T15	25.66^{h}	41.33^{i}	59.77^{g}	40.22^{fg}
SE m(±)	5.02	3.58	4.28	3.68
CD (0.05)	14.50	10.34	12.37	10.64

*Mean of three replications.

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

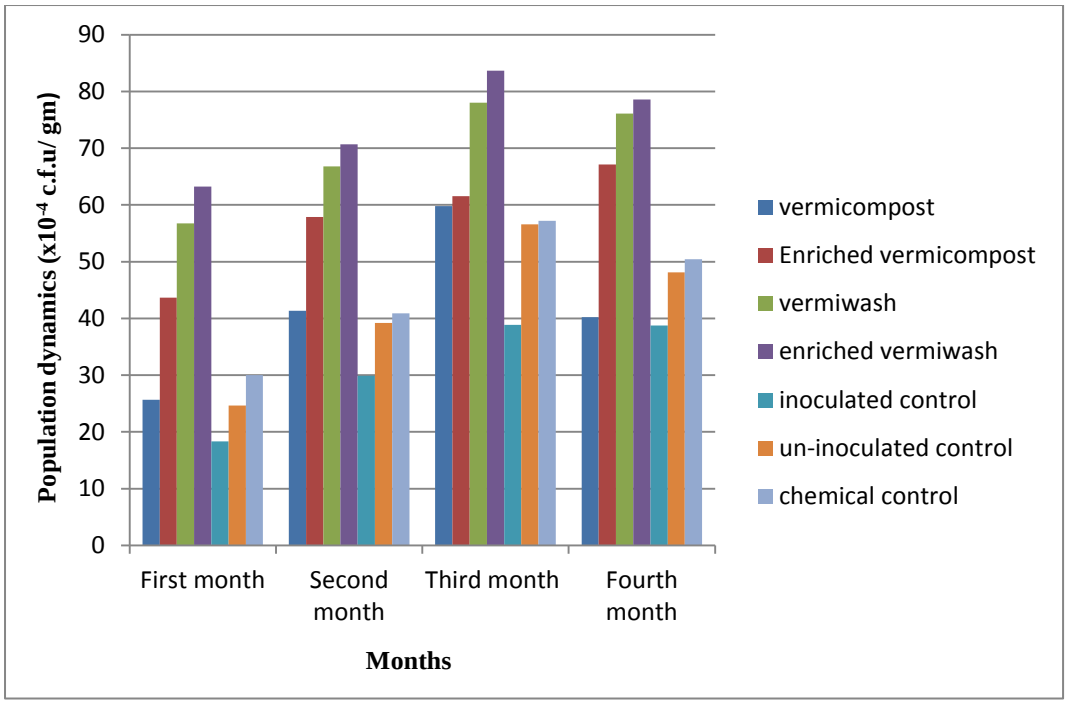


Fig.2. Effect of soil amendment with vermicompost and its different forms on the population of saprophytic fungi

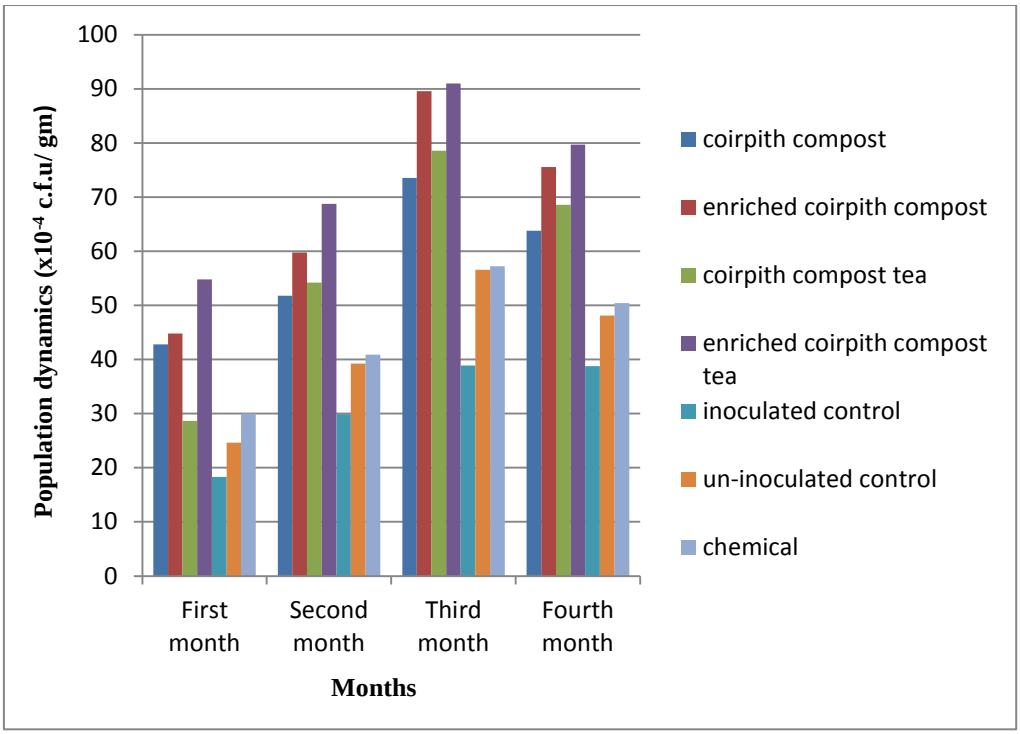


Fig.3. Effect of soil amendment with coirpith compost and its different forms on the population of saprophytic fungi

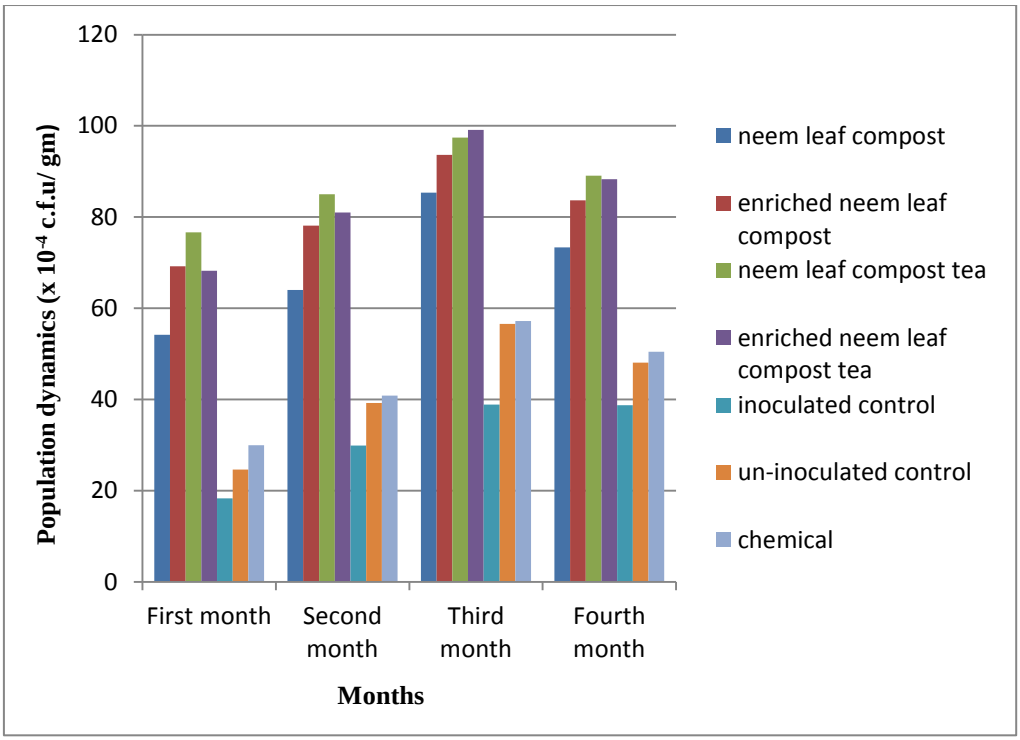


Fig.4. Effect of soil amendment with neem leaf compost and its different forms on the population of saprophytic fungi

4.9.1. Shoot length

Maximum shoot length of 42.03 cm was recorded for the plants treated with enriched leaf compost tea (T12) followed by those treated with vermiwash (40 cm). These treatments were on par with un-inoculated control (40 cm) and chemical control check (40.80 cm). All the treatments were significantly different from that of the inoculated control. When compared to that of the inoculated control, all other treatments showed an increase in shoot length.

4.9.2. Root length

The highest root length was recorded in plants treated with enriched leaf compost (T10-11.96 cm) followed by plants treated with Trichoderma enriched leaf compost tea (T12- 10.60 cm) and Trichoderma enriched vermiwash (T2-10.33 cm). These treatments were on par with chemical control check (T15-12.23cm) and un- inoculated control (T14- 11.93 cm). All other treatments except T5 (soil incorporation of coir pith compost), T6 (soil incorporation of enriched coirpith compost) and T7 (spraying and drenching with coirpith compost tea) showed significant difference over inoculated control (T13- 5.3 cm). All the treatments recorded an increased root length when compared to that of the inoculated control.

4.9.3. Fresh weight of shoot

Plants in all the treatments showed an increase in fresh weight of the shoot and were significantly different from that of the inoculated control. All the treatments except Trichoderma enriched coir pith compost tea (67.13g) was on par with that of chemical control (78.12g) and un-inoculated control (81.82g).

4.9.4. Dry weight of shoot

Highest shoot weight on drying was obtained in plants treated with Trichoderma enriched leaf compost tea (11.82g) followed by leaf compost (10.07g) which was significantly different from inoculated control and on par with un-inoculated control (12.78g) and chemical control (10.49g). All the

treatments showed increase in shoot weight on drying when compared over the inoculated control.

4.9.5. Fresh weight of root

Highest fresh weight of roots was recorded in plants treated with *Trichoderma* enriched leaf compost tea (21.82g) followed by *Trichoderma* enriched vermiwash (21.16g), leaf compost (21.09g), *Trichoderm*a enriched leaf compost (19.75g) and vermiwash (19.63g). These differed significantly when compared to that of inoculated control.

4.9.6. Dry weight of root

All the treatments showed an increase in root weight on drying than that of the inoculated control. The highest root weight on drying was recorded in plants treated with *Trichoderma* enriched leaf compost (5.58g) followed by soil incorporation of *Trichoderma* enriched leaf compost tea (5.22g) and soil incorporation of leaf compost (5.17g). These treatments showed significant difference when compared with that of inoculated control (1.91g). The treatments T2 (soil incorporation of enriched vermicompost), T8 (spraying and drenching with enriched coirpith compost tea), T7 (spraying and drenching with coirpith compost tea) and T5 (soil incorporation of coirpith compost) was on par with inoculated control.

4.9.7. Number of root nodules/ plant

All the treatments recorded higher no: of root nodules on comparing with that of inoculated control. The maximum number of root nodules was recorded in plants treated with leaf compost tea (23.33) followed with spraying and drenching with Trichoderma enriched vermiwash (23.00) which was significantly different from inoculated control (5.00). The result revealed that all the treatments were significantly different over the inoculated control.

Table 13. Effect of different composts and compost teas on growth, nodulation and yield characters 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	37.60^{abc}	9.26^{c}	73.63^{ab}	8.96^{abc}	15.99^{fg}	3.92^{bcd}	17.33^{abc}	22.33^{ab}	177.64^{d}
T2	36.23^{abc}	10.33^{abc}	69.33^{ab}	8.48^{bc}	16.08^{efg}	3.75^{bcde}	19.33^{abc}	16.60^{bc}	194.31^{c}
T3	40.00^{abc}	9.86^{c}	74.42^{ab}	9.09^{abc}	19.63^{abcde}	4.17^{abcd}	20.66^{abc}	24.33^{ab}	166.67^{de}
T4	38.13^{abc}	9.93^{c}	76.69^{ab}	9.60^{abc}	21.16^{abc}	5.00^{abcd}	23.00^{a}	24.20^{ab}	215.67^{ab}
T5	33.76^{bc}	6.76^{de}	72.88^{ab}	6.74^{cd}	14.71g	3.23^{de}	14.66^{bc}	17.20^{bc}	128.00^{h}
T6	38.26^{abc}	6.73^{de}	77.81^{ab}	8.62^{abc}	16.20^{defg}	4.09^{abcd}	13.00^{c}	11.42^{c}	145.97^{g}
T7	33.00^{c}	7.03^{de}	70.02^{ab}	7.83^{bcd}	18.42^{bcdef}	3.24^{de}	17.66^{abc}	17.42^{bc}	122.67^{h}
T8	34.73^{bc}	7.23^{d}	67.13^{b}	7.79^{bcd}	18.00^{cdefg}	3.51^{cde}	19.00^{abc}	21.00^{abc}	151.00^{fg}
T9	39.73^{abc}	10.13^{bc}	79.35^{ab}	10.07^{abc}	21.09^{abc}	5.17^{abc}	17.33^{abc}	25.30^{ab}	161.00^{ef}
T10	37.13^{abc}	11.96^{ab}	71.30^{ab}	8.73^{abc}	19.75^{abcd}	5.89^{a}	19.66^{abc}	18.80^{abc}	175.33^{d}
T11	38.53^{abc}	10.13^{bc}	72.35^{ab}	7.56^{cd}	17.88^{cdefg}	4.20^{abcd}	23.33^{a}	20.33^{abc}	162.64^{ef}
T12	42.03^{a}	10.60^{abc}	78.79^{ab}	11.82^{ab}	21.82^{ab}	5.22^{abc}	22.66^{ab}	26.00^{ab}	223.28^{a}

Contd…

(Table 13. Contd…)

T13	26.23^{d}	5.30^{e}	46.89^{c}	3.82^{d}	14.65^{g}	1.91^{e}	5.00^{d}	10.66^{c}	36.95^{i}
T14	40.00^{abc}	11.93^{ab}	81.82^{a}	12.78^{a}	22.63^{a}	5.50^{ab}	23.00^{a}	28.30^{a}	213.67^{ab}
T15	40.8^{ab}	11.23^{a}	78.12^{ab}	10.49^{abc}	20.89^{abc}	5.59^{ab}	23.66^{a}	26.60^{ab}	207.67^{b}
SE	2.45	0.66	4.67	1.44	1.25	0.65	2.88	3.59	6.53
CD (0.05)	7.07	1.91	13.50	4.17	3.61	1.90	8.32	10.39	12.48

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

T1-vermicompost + *R. solani*, T2- enriched vermicompost + *R. solani*, T3- vermiwash + *R. solani*,
T4 - enriched vermiwash + *R. solani*, T5- coirpith compost + *R. solani*, T6- enriched coirpith compost + *R. solani*, T7- coirpith compost tea + *R. solani*, T8 - enriched coirpith compost tea + *R. solani*,
T9- leaf compost + *R. solani*, T10- enriched leaf compost + *R. solani*, T11- leaf compost tea + *R. solani*,
T12- enriched leaf compost tea + *R. solani*, T13- inoculated control, T14- uninoculated control,
T15- chemical check (COC 0.2

4.9.8. Number of root pods/ plant

The highest number of pods was recorded in plants treated with enriched leaf compost tea (26) which was significantly different from inoculated control (10.66). All the plants showed significant increase in number of pods on comparison with that of the inoculated control.

4.9.9. Yield/ plant

Highest pod weight was recorded in plants treated with enriched leaf compost tea (223.28g) followed by Trichoderma enriched vermiwash (215.67g) which was on par with un-inoculated control. All the treatments were significantly different from inoculated control and produced increased pod yield than inoculated control (36.95g).

Discussion

5. DISCUSSION

Vegetable cowpea (*Vigna unguiculata* subsp. *unguiculata* (L.) Verdcourt) is an important protein rich leguminous vegetable crop in Kerala. It is well adapted to cultivation in open fields and under protected cultivation. Collar rot and web blight caused by *Rhizoctonia solani* Kuhn is a major soil-borne disease crippling the cultivation of this remunerative crop. Prevalence of high temperature and humidity aggravates the situation and results in severe yield loss (Vavilapalli *et al*., 2014). In order to address the disease, a cafeteria of management strategies has been proposed earlier by many workers (Kumar *et al*., 2014; Mathew and Gupta, 1996; Sindhan *et al*., 1999; Sharma and Tripathi, 2001). Though the most reliable one seems to be the use of fungicides, the associated human health hazards and environmental safety concerns prompt the search for safer and effective alternate management strategies. The growing demand for organic produce also highlights the need for sustainable eco-friendly disease management options.

The use of composts and compost teas has been exploited worldwide for suppression of various plant diseases. Recognizing the scope of this ecofriendly technique, the study entitled “Management of collar rot and web blight of cowpea with composts and compost teas” was conducted in the Department of Plant Pathology, College of Agriculture, Vellayani, Thiruvananthapuram during the period 2012- 2014.

Collar rot and web blight incited by *R. solani* is a wide spread and destructive disease. Menon (1979) reported the incidence and etiology of this disease in cowpea in Kerala for the first time. In the present study, *R. solani* was found to induce two distinct phases of symptoms in cowpea; the web blight and root rot/collar rot which were separated in time and space. Collar rot was noticed at the seedling stage at the stem near the soil level whereas the web blight appeared at the later stages of crop growth on the foliage. Vavilapalli *et al*. (2014) observed that collar rot is most severe at the seedling stage and web blight is severe at the vegetative stage. In this study, it was observed that the collar rot

appeared as brownish black lesions at soil level near the collar region resulting in girdling at the basal portion of the stem where white cottony mycelial growth along with small sclerotia was noticed concomitant with foliar yellowing and defoliation. These observations are in tune with those described for the disease in cowpea by Lakshmanan *et al.* (1979). Lambe and Dunleavy (1961) observed that the symptoms of *Rhizoctonia* root rot and stem rot in soybean appeared as rotting or decay of the root along with the development of large red oval lesions at the collar region. Web blight phase of the disease was noticed as water soaking followed by necrosis and webbing of affected leaves by the white mycelia of the fungus wherein numerous mustard-like sclerotia developed. Varying symptoms ranging from water soaked lesions and cobwebby mycelia on the affected leaves have been reported by several workers (Nitzan *et al.,* 2012, Viswanathan and Viswambharan, 1979, Shailbala and Tripathi, 2007).

The causal organism was isolated in pure culture and identified as *Rhizoctonia solani* by the characters such as off- white to pale, rapidly growing mycelium with average diameter varying from 5- 11.2 µm with branching near the distal septum of hyphal cells, hyphal branches inclined to the direction of growth and constricted at the point of origin from the main hyphae, production of monilioid cells in chains, formation of septum in the branch near the point of origin, multinucleate hyphae and formation of small round or irregular sclerotia less than 1 mm in size. These observations conformed to the characters described by Parmeter and Whitney (1970) for *R. solani*. Dugger (1915) observed that in *R. solani*, young hyphal branches were inclined to the direction of growth and constricted at the point of union with the main hyphae. However, Palo (1926) reported that in certain cases the young branches arose at right angles to the main hyphae but they were later found to bend towards the direction of growth of the main filaments.

The pathogenicity of *R. solani* isolates was proved by following Koch's postulates. The collar rot isolate and web blight isolates were artificially inoculated on healthy cowpea seedlings. The collar rot isolate, C1 and the web

blight isolate, W2 produced typical symptoms of collar rot and web blight on the inoculated plants, whereas, the web blight isolate W1 did not produce any symptom when inoculated. This was in conformity with the findings of Shajahan *et al.* (1997) who reported that cultural characteristics and sclerotial production were related to virulence. Tan coloured mycelia with floccose nature and absence of sclerotia have been described as the characters of hypovirulent cultures by Castanho and Butler (1978). Girija (1995) and Ranjit (2000) while working with *R. solani* isolates of rice sheath blight disease also got similar results. In addition to morphological similarity, the collar rot and web blight isolates were found to be cross infective as well. Emechebe and Lagoke (2002) observed that web blight was induced by aerial types, usually belonging to AG-1, while the strains that induce root rots or seedling diseases were strongly soil-borne, in contrast to the aerial strain, which has only a transient association with the soil.

The use of plants with antifungal properties has been in vogue in organic farming system for the ecofriendly disease management of several plant diseases. Plants produce a great deal of secondary metabolites such as flavonoids, phenols and phenolic glycosides, unsaturated lactones, sulphur compounds, saponins, cyanogenic glycosides and glucosinolates which attributes to their antifungal activity(Agrios, 1997). In the present study, *in vitro* screening of plant extracts for the suppression of collar rot and web blight isolates were carried out to identify the most promising one for the preparation of leaf compost and to utilize the leaves of the same for suppression of the pathogen. Among the different leaf extracts tested, neem leaf extract was identified as the most effective one in suppressing both collar rot and web blight isolates of *R. solani* under *in vitro* condition. The percentage inhibition with neem leaf extract was found to be 60.70. The henna leaf extract caused an inhibition of 55.90 per cent followed by clerodendron (39.06 per cent), tulsi (34.73 per cent) and pongamia (38.70 per cent). These results throw light on the potential use of neem leaf extract and henna leaf extract for the management of *Rhizoctonia* induced diseases. Leaf

compost was prepared from neem leaf based on the *in vitro* results using standard procedures.

Compost technology is a valuable tool to boost the yield of crops. In addition, scientists have also observed the disease suppressive nature of composts and compost extracts or teas against many soil-borne pathogens (*Pythium ultimum, Phytophthora sp., Fusarium oxysporum and R. solani*) and against few foliar pathogens (Scheuerell and Mahaffee, 2006). Disease control with compost has been attributed to four possible mechanisms (1) successful competition for nutrients by beneficial micro-organisms (2) antibiotic production by beneficial micro-organisms (3) successful predation against pathogens by beneficial micro-organisms and (4) activation of disease- resistant genes in plants by composts. An attempt was made to scientifically ascertain the impact of various composts such as vermicompost, coirpith compost and neem leaf compost and compost teas based on these composts for the suppression of collar rot and web blight of cowpea.

Aerated and non- aerated compost teas can be used in disease suppression. A potential problem associated with non-aerated compost tea is that there are chances for regrowth of human pathogens (Yohlem *et al.*, 1994). But Ingham (2003) stated that facultative anaerobes do not grow well under conditions of high oxygen concentration and nutrient competition. The human pathogens cannot compete in highly aerated compost teas that contain high numbers of beneficial organisms. Hence, aerated compost tea was selected for the present investigation. Ingham (2005) proposed different methods for the preparation of compost teas. Among these, the bucket bubbler technique developed by Ingham (2005) was modified for developing a simplified method for the preparation of compost tea. The different components of modified bucket bubbler technique used in this study included an aquarium type aerator, power supply, stone filters, suitable vessel and compost tied in a muslin bag.

Different dilutions ranging from 1:5 to 1:10 and brewing time has been proposed by several workers. Hence, an experiment was conducted to standardize the dilution and brewing time for the preparation of compost tea. The result of the study revealed that 1:5 ratio of compost to water when brewed for 24 h resulted in maximum microbial population. The population in 1:5 dilution after a brewing time of 24 h corresponded to 72.52×10^4 c f u/ ml compost tea. Thereafter the population decreased drastically and reached to the level of 1.96×10^4 cfu/ ml in 48 h. This result was in conformation with the studies of Ingham (2005) who proposed that optimal brew time of 18-24 h coincides with maximum activity of microbial population in aerated compost tea. On the contrary, the studies of non-aerated compost teas by Scheuerell and Mahafee (2002) showed that brewing time of 8-16 days was optimal for disease control. According to Scheuerell (2003) longer brewing period promoted greater extraction of nutrients from the compost in anaerobic situation and also enabled accumulation of antibiotics that activated natural plant defense responses resulting in disease suppression.

It is well established that compost contains a diverse group of organisms dominated by bacteria and fungi participating in the decomposition of organic matter many of which also have pathogen suppressive capabilities (Brinton and Droffner, 1995). Scientists have enhanced the natural ability of compost to suppress diseases by enriching it with specific micro-organisms with antagonistic properties, or with other amendments that promote the growth of microflora. In this study, it was observed that the predominant fungal genera isolated from the various composts and compost teas were *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., and *Trichoderma* sp. Among these, *Trichoderma* sp. caused significant suppression of both collar rot and web blight isolates of *R. solani.* The percentage inhibition of 44.73 and 44.42 was obtained with *Trichoderma* sp. for collar rot and web blight isolates, which was significantly superior to the other antagonistic fungi. Rehman *et al.* (2008) found that several *Trichoderma* species inhibited the mycelial growth of *R. solani* under *in vitro* conditions.

Trichoderma sp. that was effective in pathogen suppression in the present study was identified as *Trichoderma virens* based on morphological, cultural and sporulation characteristics. This was used for enrichment of composts and compost teas for further studies on disease management. The inoculation of mature composts with efficient biological control agents has been reported to improve the efficiency of composts (Hoitink *et al*., 1997; Noble, 2011).

The effect of various composts, compost teas and their enriched products on the suppression of collar rot and web blight of cowpea was studied by a pot culture experiment using susceptible cowpea variety, Bhagyalekshmy. The plants were inoculated with the virulent isolates of *R. solani* at the collar region and on the leaves. The composts and enriched composts were applied as basal, whereas, the compost teas and the enriched compost teas were given as soil drench and foliar spray. In general, all the composts and their respective compost teas caused disease suppression. Tuitert *et al.* (1998) observed that peat amended with composts in potting mixtures used for growing woody ornamental nursery stock reduced several soil-borne pathogens including *Rhizoctonia*.

The percent disease suppression of web blight was maximum in chemical control (89.82 percent) followed by *Trichoderma* enriched leaf compost tea (82.75 per cent) with respect to inoculated control (Fig.5). Among the different composts and compost teas tested, treatment T12 (*Trichoderma* enriched leaf compost tea) with lowest web blight index of 14.83 was significantly superior to the inoculated control (86.02) in which highest per cent disease was recorded. Alfano *et al*. (2007) reported that the compost colonised by biocontrol agents after peak heating stimulated natural suppression to *Rhizoctonia* damping off. Plants grown in the compost mix amended with *Trichoderma* had significantly less foliage disease than the control. Researchers at the Ohio State University of U. S. A. recently concluded that composts used as one component of growing media in container production, when “fortified” with *Trichoderma hamatum* strain 382, suppressed many foliar plant diseases such as leaf blight of cucumber caused by

Phytophthora capsici **(**Khan *et al.,* 2004), bacterial leaf spot on vegetables caused by *Xanthomonas campestris* (Al-Dahmani *et al*., 2005) and leaf blight of begonia caused by *Botrytis cinerea* (Horst *et al*., 2005). They have also indicated that biocontrol agent fortified compost-based mixes could be useful for organic transplant production where the use of pesticides is limited.

In case of collar rot disease, the treatments T9 (soil incorporation of leaf compost) and T12 (spraying and drenching with *Trichoderma* enriched leaf compost tea) exhibited the lowest disease incidence (5.86%) which was statistically on par with that of chemical check (Fig.5). Nazaire and Albert (2012) found that use of compost tea differed significantly from the use of solid compost to suppress potato diseases. The difference between the use of soil applied compost and compost tea has been reported to be that compost tea application gives immediate control of surface spreading pathogens, while soil compost acts more slowly over a longer period of time against soil-borne pathogens.

Application of vermicompost and vermiwash when used alone or enriched with *Trichoderma* caused significant suppression of collar rot and web blight disease. Soil application of *Trichoderma* in vermicompost along with 20 per cent neem cake gave significant control of collar rot or root rot caused by *R. solani* in cowpea under field conditions (Pan and Das, 2011). This has been attributed to the greater multiplication of the antagonists in the rhizosphere and phyllosphere (Pan and Bhagat, 2007). In the present study, percentage suppression of 68.47 was obtained for collar rot and 66.68 percent for web blight on the application of *Trichoderma* enriched vermicompost. Disease suppressiveness of vermicompost produced from agricultural wastes on damping-off of cucumber (*Cucumis sativus* cv. Cevher) seedlings infected by *R. solani* has been already reported by Simsek *et al*. (2009). Compost amendments can modify the microbial community composition and as a result, enhance the competition or antagonism among microbes, leading to a decrease in activity of plant pathogens (Hoitink and Boehm, 1999; Steinberg *et al*., 2004).

The population dynamics of saprophytic fungi in different compost treated soils were assessed. The results revealed that the population of saprophytic fungi in treated soils was higher and significantly different from inoculated control. Harender and Kapoor (1997) reported that addition of composts to soil increased the soil microflora leading to reduction in the soil population of *Fusarium oxysporum* causing wilt of tomato. The present findings are in tune with the findings of Boehm *et al*. (1993) who noticed that microbiostasis contributed to the suppressive effect of certain composts and compost teas.

The influence of different composts and compost teas on the growth, nodulation and yield characters of cowpea was assessed. The results revealed that even though there was not much significant difference between the different treatments, *Trichoderma* enriched neem leaf compost tea was found to be superior. The biometric parameters such as shoot length (cm), dry shoot weight (g), fresh weight of root (g), number of pods per plant and yield per plant was recorded to be highest in *Trichoderma* enriched neem leaf compost tea (Treatment- T12) (Fig 6 &7). This result was in consonance with the report of increased shoot and root length on the application of compost prepared from bagasse, banana leaves and spent mushroom beds in tomato affected by *Fusarium* wilt by Harender and Kapoor (1997). The desirable effect of compost tea may be attributed to its content of diverse microorganisms and soluble nutrients (Klock-Moore, 2001). Microorganisms in compost tea are believed to fight disease by competing with pathogens for colonization sites and nutrient supplies, secreting antibiotic or anti-fungal substances or directly parasitizing the pathogens, while soluble nutrients improve plant health and enhance natural defense mechanisms (Scheuerell, 2003).

The present study has brought out the suppressiveness of composts and compost teas on *Rhizoctonia* incited collar rot and web blight of cowpea. The highlight of the study is the enhanced disease suppression by neem leaf compost tea enriched with the specific disease fighting antagonistic fungus, *T. virens*. The positive effects derived by using amended or “tailored” compost on crop

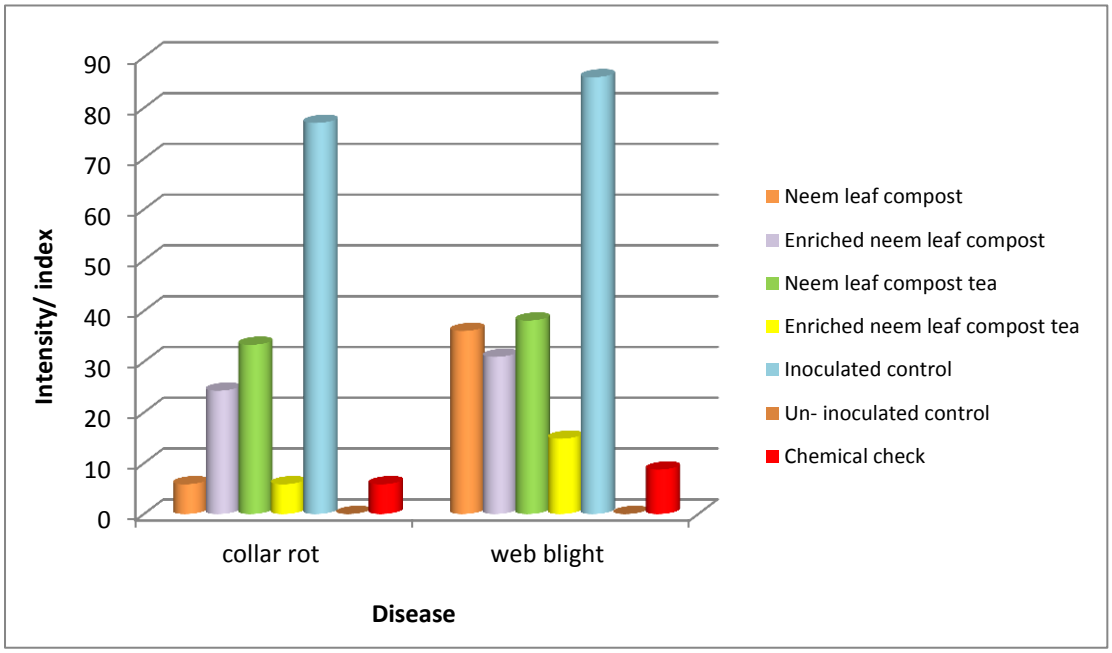


Fig.5. Comparison of the effect of neem leaf compost and its different forms on the suppression of collar rot and web blight of cowpea

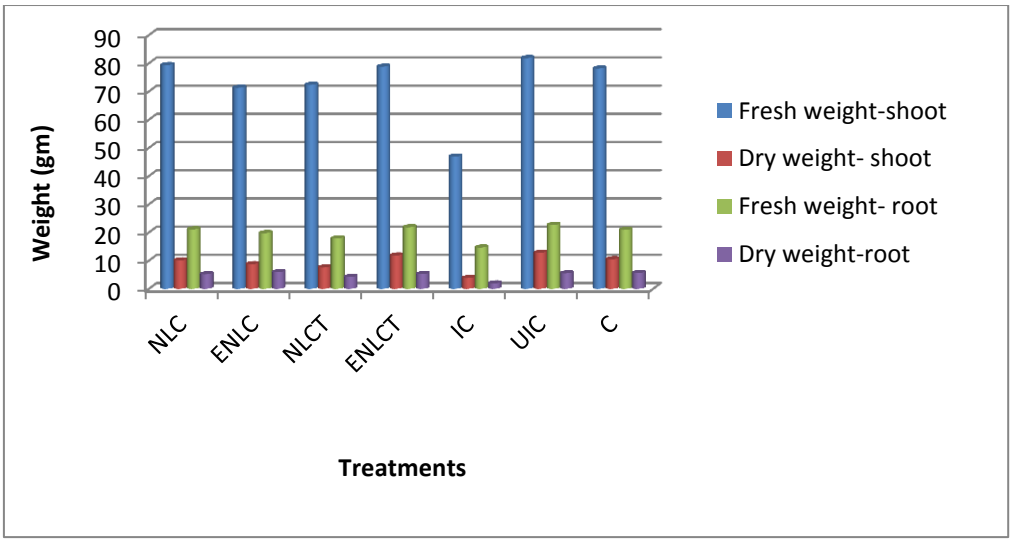


Fig.6. Comparative effect of neem leaf compost and its different forms on growth related attributes of cowpea

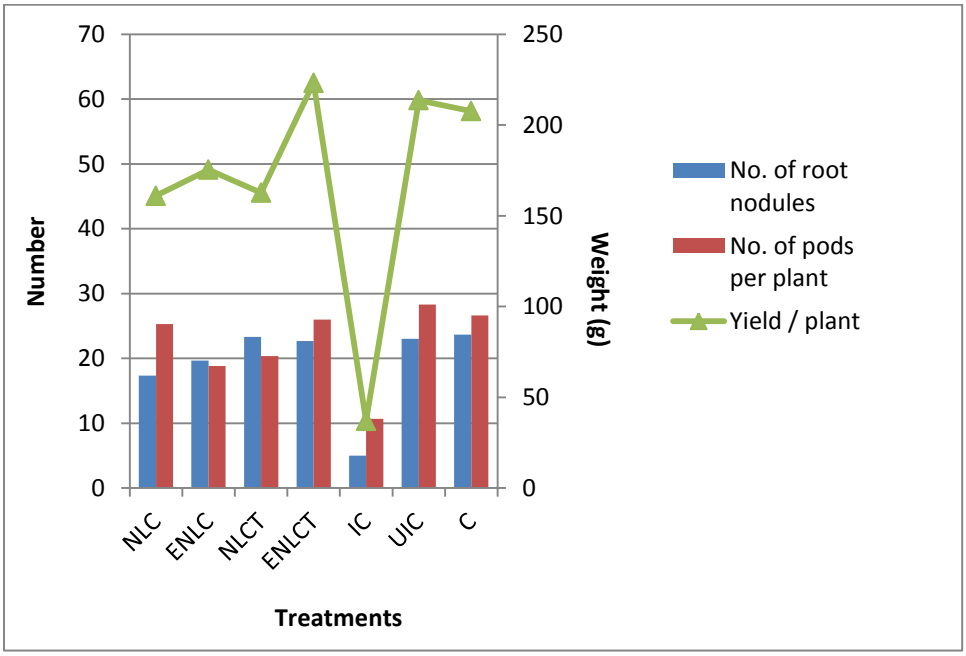


Fig.7. Comparative effect of neem leaf compost and its different forms on nodulation and yield attributes of cowpea

NLC- Neem leaf compost, ENLC- Enriched neem leaf compost, NLCT- Neem leaf compost tea, ENLCT- Enriched neem leaf compost tea, IC- Inoculated control, UIC- Un- inoculated control, C- Chemical check.

protection and crop growth promotion offer immense scope. In addition, the neem leaf compost, its compost tea and their enriched forms afforded significant reduction of collar rot and web blight symptoms as well as toned up the growth and boosted yield compared to other treatments (Fig.5, 6 &7).

The umbrella of protection of Trichoderma enriched neem leaf compost and compost tea can also be extended to R. solani affected diseases of other crops after further detailed studies. The enriched composts can also be exploited to address the disease management in organic production. The use of tailored compost offers possibility for reducing or replacing the application of fungicides which could adversely affect water resources, food safety and worker safety.

Summary

6. SUMMARY

The present study was aimed at developing an eco-friendly management strategy for collar rot and web blight of cowpea using composts and compost teas and evaluating their efficacy in disease suppression. The salient findings of the study are given below.

1. The pathogen causing web blight and collar rot was isolated from leaf and collar region of the diseased cowpea plants from Crop Museum, College of Agriculture, Vellayani. Two web blight isolates and a collar rot isolate were obtained.

2. Characterization of the pathogen revealed its identity to the description given by Parmeter and Whitney (1970) and hence identified as *Rhizoctonia solani* Kuhn.

3. Pathogenicity was proved by following Koch's postulates and the virulent isolates were selected based on pathogenicity, virulence and growth rate.

4. The cross infectivity study of the selected isolates revealed that the isolates were cross infective in nature capable of producing both collar rot and web blight symptom when artificially inoculated.

5. Among the different plant extracts tested for their *in vitro* suppression against *R. solani*, 5% neem leaf extract was found have significant suppression on both the isolates of the pathogen and hence selected for the preparation of leaf compost and leaf compost tea.

6. Different composts and teas used in the study included vermicompost, coirpith compost, neem leaf compost, vermiwash, coirpith compost tea, neem leaf compost tea and their enriched forms.

7. Leaf compost was prepared by mixing four parts fresh neem leaves and one part fresh cowdung with adequate moisture which took 60 days to complete the entire process of composting.

8. A simplified method for preparation of compost tea by modifying the bucket- bubbler technique proposed by Ingham (2005).

9. The study on the standardization of dilution and brewing time for the preparation of compost tea indicated that 1:5 dilution brewed for 24 h was ideal for its preparation with maximum microbial population.

10. *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., and *Trichoderma* sp. were the predominant fungal genera isolated from composts and compost teas.

11. The *in vitro* antagonistic ability of *Trichoderma* sp. was significantly higher than that of other saprophytic fungi isolated from composts and compost teas.

12. *Trichoderma* sp. caused significant *in vitro* suppression of *R. solani* in comparison with other saprophytic fungi and was selected for enrichment of composts and compost teas.

13. *Trichoderma* sp. was identified as *Trichoderma virens* based on morphological and cultural characteristics.

14. *T. virens* was multiplied in potato dextrose broth and enriched composts and compost teas were prepared by incorporation of *T. virens* @ 100 ml broth/ kg compost.

15. The population dynamics of saprophytic fungi in different compost and compost tea treated soils was assessed for a period of four months. In general, the population of saprophytic fungi in soil treated with neem leaf compost tea and *Trichoderma* enriched neem leaf compost tea was found to be higher than that of untreated soil.

16. The population showed a general increase from first to third month and during the fourth month the population decreased.

17. The result of pot culture experiment revealed that soil incorporation of composts (100g/ plant), foliar spraying and soil drenching of compost teas (1 L/ pot) repeated at fortnightly interval can effectively suppress collar rot and web blight disease of cowpea.

18. Incidence of collar rot was found to be lower in soil incorporated with neem leaf compost and spraying and drenching with *Trichoderma* enriched neem leaf compost tea (5.86%). This was significantly higher than that of inoculated control (77.03%).

19. Percentage suppression of web blight was higher with the application of *Trichoderma* enriched neem leaf compost tea (82.75%).

20. Application of *Trichoderma* enriched neem leaf compost tea resulted in an increase in biometric and yield parameters.

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Appendices

APPENDIX – I

COMPOSITION OF DIFFERENT MEDIA

Potato Dextrose Agar

Potato - 200 g
Dextrose - 20 g
Agar - 20 g
Distilled water - 1 L

Potato Dextrose Broth

Potato - 200 g
Dextrose - 20 g
Distilled water - 1 L

Martin's Rosebengal Agar

Dextrose - 10 g
Peptone - 5 g
Potassium dihydrogen phosphate - 1 g
Magnesium sulphate - 0.5 g
Rose bengal - 33 mg
Streptomycin solution (1%) - 3 ml
Agar - 15 g
Distilled water - 1 L
P^H - 7

Water agar (2 per cent)

Agar - 20 g

Distilled water - 1 L

APPENDIX – II

COMPOSITION OF DIFFERENT STAINS

Lactophenol cotton blue

Anhydrous lactophenol - 67 ml

Distilled water - 20 ml

Cotton blue - 0.1 g

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

Management of Collar Rot and Web Blight of Cowpea with Composts and Compost Teas

by

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ABSTRACT OT THE THESIS

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ABSTRACT

The study entitled “Management of collar rot and web blight of cowpea using composts and compost teas” was undertaken at the Department of Plant Pathology, College of Agriculture, Vellayani with the objective of developing an eco-friendly management strategy. The pathogen causing the disease was isolated from the collar rot and web blight affected parts and identified as *Rhizoctonia solani* Kuhn. Pathogenicity was proved following Koch’s postulates and the virulent isolates were identified. Collar rot and web blight isolates of *R. solani* were found to be cross infective.

Neem leaf was found to significantly suppress the collar rot and web blight isolates of the pathogen and was selected for the preparation of leaf compost. Studies on the effect of dilution and brewing time of compost tea indicated that 1:5 dilution brewed for a period of 24 h showed maximum fungal population and was adopted for the preparation of compost teas. The dual culture technique to study the antagonistic potential of the saprophytic fungi isolated from composts against *R. solani* revealed that *Trichoderma* sp. exhibited the maximum inhibition. Based on the cultural and morphological studies, the antagonist was identified as *Trichoderma virens* and was selected for enrichment of composts by inoculating @ 100 ml/ kg compost. Enriched compost teas were prepared from the enriched composts. The effectiveness of composts, compost teas and their enriched forms on the incidence of collar rot and intensity of web blight were assessed in a pot culture experiment. The incidence of collar rot was lowest in soil incorporated with neem leaf compost and *Trichoderma* enriched neem leaf compost tea. Similarly, the percentage suppression of web blight disease was higher in soil sprayed and drenched with *Trichoderma* enriched neem leaf compost tea. The application of *Trichoderma* enriched neem leaf compost tea recorded a higher value for biometric parameters. The present study indicated that the composts and compost teas offer eco-friendly management of collar rot and web blight along with crop growth promotion. The effect can be further augmented by enriching with *Trichoderma.*