ECOFRIENDLY MANAGEMENT OF COLLAR ROT AND WEB BLIGHT OF COWPEA

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DECLARATION

I hereby declare that this thesis entitled "**Ecofriendly management of collar rot and web blight of cowpea**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other University or society.

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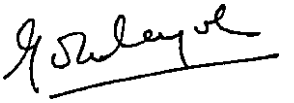
CERTIFICATE

Certified that this thesis entitled "**Ecofriendly management of collar rot and web blight of cowpea**" is a record of research work done independently by Ms. Bhadra Sree. S. (2004-11-46) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.



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Bhadra Sree. S

Bhadra Sree. S.

Dedicated to

My Parents and Brother

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

µl	Microlitre
µm	Micrometre
°C	Degree Celsius
CD	Critical difference
Cm	Centimetre
CRD	Completely Randomised Design
et al.	And others
g	Gram
h	Hour
i.e.,	That is
kg	Kilogram
l	Litre
m	Metre
mg	Milligram
min	Minute
ml	Millilitre
mm	Millimetre
mM	Millimolar
N	Normal
nm	Nanometre
rpm	Rotations per minute
sec	Second
spp.	Species
var	Variety
viz.	Namely
W/V	Weight/Volume
w/w	Weight/weight

INTRODUCTION

INTRODUCTION

Cowpea (*Vigna unguiculata* subsp. *sesquipedalis* (L) Verdcourt), an important vegetable crop of Kerala, is cultivated in the uplands throughout the year and in the rice fallows during summer season. The tender pods have very low calorific value and 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 yield.

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

R. solani, is a ubiquitous soil inhabiting plant pathogen with great diversity, wide host range and lack of sharp differentiation among its strains. Prolific growth and ability of pathogen to produce large number of sclerotia that may persist in the soil for several years and resistant to microbial attack makes elimination of soil borne inoculum through chemicals difficult and costly. Non availability of varieties resistant to this disease makes the problem more serious. Though the pathogen can be kept under check with elaborate and repeated sprays of fungicides (Upamanyu et al., 2002), the detrimental effect of these chemicals on the environment and human health as well as the huge cost involved, necessitates the disease to be managed by means of cheaper and environment friendly methods.

The continued search for better and ecofriendly methods of combating fungal pathogens has in the recent past moved away from traditional empirical search for new synthetic chemicals. The present trend is towards organic farming by which the farmers can fetch a premium price for their produce. The growing realization that plants and their products have fungitoxic potential has prompted a

renewed look at these natural biocides. This investigation was carried out with the objective of evolving an ecofriendly management practice for collar rot and web blight of vegetable cowpea using plant oils, oil cakes, biocontrol agents and indigenous materials. The study has made use of plant oils such as neem oil, castor oil, pongamia oil, calophyllum oil, samadera oil, odal oil and marotty oil, oil cakes such as neem, coconut, gingelly and groundnut cakes, biocontrol agents as *Trichoderma harzianum* and *Pseudomonas fluorescens* and indigenous materials like turmeric powder-baking soda and rice husk ash. The main objectives of the study are

- *In vitro* screening of plant oils and oil cakes to select the most effective plant oils and oil cakes for *in vivo* studies.
- To study disease suppression by plant oils, oil cakes, biocontrol agents and indigenous materials.
- To evolve a management practice for the disease using biocontrol agents, plant oils, oil cakes and indigenous materials as rice husk ash and turmeric powder - baking soda under *in vivo* conditions.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 Importance and yield loss

Cowpea has long been recognized as a promising crop as its foliage, green pods and seeds are edible with low fat, high to complex carbohydrate and moderate protein content. Tender pods of cowpea are consumed as a vegetable and the seeds at maturity is an important pulse. Cowpea can be grown in almost all types of soils and its cultivation is gaining momentum in the State. In the different growth stages, the crop is affected by a multitude of diseases, of which collar rot and web blight is one of great concern in Kerala.

Rhizoctonia solani is a soil borne fungus able to survive in the soil for more than a year as sclerotia during off season or as mycelium in soil or in plant remains depending on temperature and moisture conditions (Kannaiyan and Prasad, 1976). Among *Rhizoctonia* spp., *R. solani* is the most widely distributed, destructive and attacks over 287 genera of plants (Ahiya, 1977). Dubey and Mishra (1990) observed web blight caused by *R. solani* on horse gram (*Macrotyloma uniflorum*) that caused 10-60 per cent reduction in yield. Mishra and Dubey (1991) reported the incidence of web blight disease of groundnut as well as soybean, yard long bean and field bean during kharif season. The disease manifested in different stages on all the aerial parts and pods. Widespread occurrence of web blight disease caused by *R. solani* in *Albizia falcatoria*, a fast growing leguminous tree was reported in 1982 (Sharma and Sankaran, 1991). High rainfall coupled with high soil moisture, relative humidity and soil temperature (21-25°C) favoured the development of the disease (Mathew and Gupta, 1996; Sharma and Tripathi, 2001). They further reported that severity of web blight of French bean caused by *R. solani* ranged from 4.3 to 62.1 per cent. 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 (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 *R. solani* causing foliar blight of mungbean at early stage of crop growth causing premature defoliation and reduction in size of pods and grains with a disease intensity ranging from 6.66 to 75.35 per cent. Singh and Sinha (2005) reported that the diseases caused by *R. solani* due to the seed and soil borne nature needs constant monitoring.

2.2 Pathogen

The perfect stage of *Rhizoctonia solani* was reported to be *Thanatephorus cucumeris* (Frank) Donk (Talbot, 1970). The basidial stage on rice was first reported from India by Saksena and Chaubey (1972).

Several workers have attempted to group the isolates of *R. solani* (*Thanatephorus cucumeris*) according to various cultural, physiological or pathological criteria (Houston, 1945; Exner, 1953; Vijayan,1986) which were inconsistent due to diversity in pathogenicity and other characters. Townsend and Willets (1954) stated that the sclerotial development in *R. solani* did not follow any pattern of definite organization of the hyphae with the result that the sclerotia were loosely constructed.

R. solani is a wide spread and destructive plant pathogen comprising of isolates differing in various characters (Flentje et al., 1970). 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, formation of septa near the point of origin of the branch, septal pore apparatus and multinucleate cells in young hypha. Further they noted that characters like the presence of monilioid cells, sclerotia without differentiated rind and medulla, hyphae greater than 5 μ in diameter, rapid growth rate and pathogenicity are usually associated with *R. solani*, and that occasionally one or more of these characters might be lacking in individual isolates.

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). 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. 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 microsclerotia which were oval in shape, light to dark brown and flat. However, they could obtain macrosclerotia on repeated subculturing of the fungus on PDA. Morphological characters of the isolates from cowpea resembled those of *Rhizoctonia microsclerotia.*

Lakshmanan et al. (1979) were successful in inducing formation of perfect stage of *R. solani* in cowpea. The basidia were barrel shaped measuring 8.49 x 4.92 µm with long, narrow and horn-shaped with two to four sterigmata tapering towards the tip with a size of 4.92 µm having thin walled hyaline basidiospores, oval to pyriform in shape with a size of 6.2 µm x 3.57 µm. Similar observations were recorded by Tiwari and Khare (2002). They further 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 as a means of survival of the pathogen.

2.3 Symptomatology

Weber (1939) gave a detailed account of web blight of beans caused by *R. microsclerotia.* He recorded that the pathogen produced two types of mycelia, superficial and sub epidermal. The former having twice the diameter of the latter and spreading out in a fan like manner over the leaf blade. Small superficial sclerotia are characteristic of *R. microsclerotia* by means of which the fungus is

disseminated in soil. Host range studies conducted through artificial inoculation of microsclerotia showed that several other crop plants like tomato, carrot, beet root, egg plant, cucumber, water melon, snap and lima beans also developed similar symptoms within three days of incubation under congenial humid conditions. However typical web blight symptoms were observed only on members of the family Leguminosae.

Development of varying symptoms ranging from definite lesions to total destruction of leaves, petioles, pods and young stems along with defoliation has been reported to be associated with Rhizoctonia aerial blight of soy beans (Atkins and Lewis, 1952).

However Lambe and Dunleavy (1967) described the symptoms of Rhizoctonia root and stem rot of soy bean as a rotting or decay of the root system along with development of large red oval lesions at the soil line. The above ground symptoms of diseased plants included chlorosis, wilting, drying and premature leaf drop. High temperature and high humidity were necessary for disease development and spread whereas the development was limited by dry weather or low temperature. Grau and Martinson (1979) reported inhibition of that hypocotyl elongation of soy bean by *R. solani.* The inhibition of hypocotyl elongation differed with soy bean cultivars, isolates of *R. solani* and temperature.

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.

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 collar region girdling the basal portion of the stem. White mycelial growth, often studded with

small sclerotia was noticed in the affected area. The leaves turned yellow and finally dropped off. Symptoms of rotting was noticed and the root development was affected. Web blight symptoms appeared on leaves as small circular, light-greyish-brown spots surrounded by irregular water soaked area that enlarged to oblong or irregular shapes. Under congenial conditions, the spots coalesced covering major portion or entire leaf with mycelial growth, leading to shedding of affected leaves. Collar rot symptoms of cowpea caused by *R. solani* was described by Viswanathan and Viswambharan (1979). They recorded the first visible symptom of the disease as 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 sclerotial bodies appeared on the affected region. The final stages of infection witnessed yellowing of leaves with withering and drying off of the whole plant.

2.4 Use of plant oils for controlling pathogens

Plant oils have been explored for their fungitoxic properties (Mahadevan, 1982). Higher plants have been recognized to release volatile substances which keep the air remarkably free from pathogenic microorganisms. Muthusamy et al. (1988) reported 96.2 per cent inhibition of spore germination of groundnut rust by neem oil. Bhattacharya and Banerjee (1996) reported inhibitory effect of neem oil emulsion against *Macrophomina phaseolina* causing root rot of *Solanum melongena.*

Application of plant oils reduced the sheath blight of rice caused by *R. solani* (Shukla et al., 1990). Shahi et al. (1998) reported *in vitro* effect of neem oil on radial growth of *Sclerotium rolfsii* causing collar rot of chick pea. Kak (2000) reported that seed treatment with neem oil controlled soil borne plant pathogens such as *R. solani.* Sivakumar and Sharma (2000) reported that neem oil 5 per cent was highly effective in reducing mycelial growth and sclerotial germination

of *R. solani* causing sheath blight of rice. Exposure of oils upto 160°C and storage upto 360 days do not affect fungitoxicity of oils (Mishra et al. 2003). Dhaliwal et al. (2003) described the method of making desired concentrations of different plant oils with the help of water and Tween-80 against *R. solani* causing black scurf of potato. Sharma and Gupta (2003) reported that pongamia oil was more effective than neem oil against *R. solani* for the management of root rot and web blight of French bean. Raji (2004) reported inhibitory effect of plant oils *viz.*, marotty, neem, castor, samadera, pongamia and odal oils on *Helminthosporium oryzae* causing brown leaf spot of rice. Sethuraman et al. (2005a, b) used neem oil, pongamia oil and mahua oil at different concentrations to assess their efficacy against mycelial growth, sporulation and spore germination of *Sarocladium oryzae* causing sheath rot of rice both under *in vitro* and glass house conditions. They also reported that neem oil was most effective whereas pongamia oil and teepol were least effective against sheath rot of rice. Sharma and Sharma (2005) reported use of neem and castor oil against *Dematophora necatrix*, *Phytophthora cactorum* and *S. rolfsii* causing white root rot, collar rot and seedling blight in apple. Use of pongamia oil in managing storage diseases of onion as blue mould and black mould caused by *Penicillium digitatum* and *Aspergillus niger* respectively was advocated by Raju and Naik (2006). *In vitro* experiments revealed that pongamia oil gave least reduction of 0.58 and 8.19 per cent respectively against blue and black mould. Also least efficacy of disease reduction of 29.34 per cent was recorded by pongamia oil at five per cent concentration.

2.5 Effect of oil cakes in controlling pathogens

Ezhilan et al. (1994) reported use of gingelly and calophyllum cakes to suppress mycelial growth and sclerotial production *in vitro* at 10 per cent concentration on *R. solani*. Shahi et al. (1998) reported *in vitro* effect of neem cake on radial growth of *Sclerotium rolfsii* causing collar rot of chick pea. Dubey and Patel (2000) reported that among neem, til and groundnut cakes, groundnut

cake gave maximum suppression against *Thanatephorus cucumeris* causing web blight of urd and mung bean under *in vitro* condition. Groundnut cake suppressed *R. solani* by 38.9 per cent in comparison to til (30.8 per cent) and neem cake (10 per cent). . Sharma and Gupta (2003) reported that neem kernel extract gave good inhibition of *R. solani in vitro* causing root rot and web blight of French bean. Kaviyarasan et al. (2005) studied *in vitro* effect of oil cakes *viz.*, gingelly, groundnut, neem, castor and coconut at ten per cent concentration on radial mycelial growth of *Pythium aphanidermatum.* Rajesh et al. (2005) reported *in vitro* suppression of mycelial growth of *M. phaseolina* causing root rot of cowpea using neem cake at one per cent concentration. Bharadwaj (2005) reported maximum suppression of *R. solani* using neem cake *in vitro* at 20, 30, 40 and 50 per cent concentration. He also reported that neem cake showed good suppression of root rot and web blight of strawberry caused by *R. solani* and increased plant growth parameters over control.

One of the cheapest and effective methods of alteration of soil environment is the amendment of soil with decomposable organic matter. Kannaiyan and Prasad (1981) reported addition of ground nut, gingelly, neem and coconut cakes to soil to suppress saprophytic survival of *R. solani.* Lakshmanan and Nair (1984) reported loss of viability of sclerotia of *Rhizoctonia solani* in rice soils by the application of powdered neem, groundnut, gingelly and coconut cakes as soil amendments.

Jeyrajan et al. (1987) reported the effect of neem cake in reducing pre and post emergence mortality of cotton seedlings caused by *R. solani.* They also reported that in flooded conditions gingelly and neem cake were effective in reducing the disease. Use of groundnut and neem cake was found to control root knot disease and decline of ganoderma wilt of coconut (Vaish and Nayak, 1995).

Prasad et al. (1998) reported effect of neem, groundnut and sesamum cake against survival of sclerotia of *Rhizoctonia solani* in rice soils. Disease

suppression by neem, coconut and groundnut cakes was reported by Meena and Muthusamy (1999) against rice sheath blight caused by *R. solani*. Padmodaya and Reddy (1999) reported that use of neem cake improved seedling stand of tomato affected by *Fusarium oxysporum* f. sp. *lycopercisi*.

Ehteshamul – Haque et al. (1998) reported effect of neem cake against *Rhizoctonia solani* in reducing infection of sun flower. Kak (2000) reported that neem cake can suppress several soil borne pathogens like *R. solani*. Dubey (2002) reported use of groundnut cake at 5 and 2.5 per cent against web blight of urd and mung bean caused by *Thanatephorus cucumeris*. Jakhar et al. (2002) reported the effectiveness of sesamum cake against *Rhizoctonia* spp causing root rot of cotton. Upamanyu et al. (2002) reported effective use of neem cake in reducing root rot of French bean caused by *R. solani* in both glass house and field condition and also increased green pod yield. Gurjar et al. (2003) recommended neem cake for the management of *S. rolfsii* induced collar rot of chilli and for the improvement of green fruit yield. Sharma et al. (2003) reported groundnut and neem cake to be most effective in reducing the propagules of *Fusarium solani* causing wilt of mango. Azam and Tiyagi (2005) reported effective use of neem and groundnut cake for the management of the soil borne fungus, *R. solani* infecting tomato.

2.6 Use of indigenous materials for controlling pathogens

Use of indigenous materials as rice husk ash (RHA) and turmeric powder-baking soda (TP-BS) can be considered as an alternate approach in plant disease management. Indigenous materials such as RHA are experimented as supplements for containing pests and diseases with an added effect of growth promotion (Joshi, 2002). Dodan et al. (1991) reported that burnt rice husk ash reduced paddy neck blast significantly and on incorporation with tricyclazole it proved most effective. Laha et al. (2000) reported use of rice husk ash both as seed treatment, seedling root dip and soil amendment in the management of

sheath blight of rice. This treatment reduced disease severity and increased grain yield. Joshi (2002) reported the use of rice husk ash for management of rice blast caused by *Pyricularia oryzae*. RHA treated plants inoculated with *Pyricularia oryzae* showed complete resistance to blast. Priyadarsini (2003) reported the use of three concentrations of RHA *viz.*, 500 gm^{-2}, 750 gm^{-2} and 1000 gm^{-2} and found 1000 gm^{-2} treatment to be most effective in disease suppression and growth promotion, in the management of *R. solani* causing foliar blight of amaranthus.

Williams and Williams (1985) reported that researchers in Japan obtained control of mildew on cucumbers, egg plants and strawberries by weekly sprays of one-fourth ounce of baking soda per gallon of water. Ziy and Zitter (1992) found that a single spray of 0.50 per cent baking soda and ultrafine spray oil, a refined petroleum distillate almost completely inhibited powdery mildew on heavily infected pumpkin foliage. Williams and Williams (1993) found spraying of one ounce baking soda per gallon of water controlled powdery mildew on climbing roses, Urocladium leaf spot in cucumber, Alternaria leaf blight and stem blight in musk melon. All concentrations of essential oil of turmeric (1-5 per cent) inhibited growth of *R. solani in vitro* by 78 per cent (Saju et al., 1998). Gangopadhyay (1998) advocated use of turmeric powder – baking soda in 10:1 ratio to manage soil borne rice diseases by *in vitro* seed inoculation and foliar spray. Priyadarsini (2003) tried the combinations of turmeric powder – baking soda in the ratio 10:1, 6:4 and 8:2 and she found 10:1 ratio to be the most effective for the management of *R. solani* causing foliar blight of amaranthus. Turmeric and turmeric curcuminoids have a number of beneficial properties including antimicrobial properties (Jain et al., 2006).

2.7 Biological control of *R. solani*

Biological control has been reported to provide protection against many foliar diseases in crop plants (Blakeman and Fokkema, 1982). Several fungal and bacterial antagonists like *Trichoderma* spp and *Pseudomonas fluorescens* have been found to be effective in checking diseases caused by *R. solani* on crops such

as rice, pea and cotton (Elad et al., 1982). Recently direct application of antagonistic microorganisms to control foliar and root infecting pathogens has gained momentum (Whipps, 1992). Biological control of soil borne plant pathogens has emerged as an important alternative in plant disease management (Whipps, 1997; Bowen and Rovira, 1999). Hyakumachi (1999) observed that there was rapid decline in inoculum potential and increase in soil suppressiveness of *R. solani*. He also observed that enhanced competitive pressure of soil microbes also contributed to increase in soil suppressiveness.

2.7.1 Biocontrol potential of *Trichoderma* spp and *Pseudomonas* spp

Weindling (1932) first demonstrated that *T. viride* was parasitic on and antagonistic to *Rhizoctonia solani*. The inhibitory effect of fluorescent *Pseudomonas* spp to a wide variety of microorganisms and the plant growth promoting effect was reported by Kloepper and Schroth (1981). Mukherjee and Mukhopadhyay (1995) suggested mycoparasitism as one of the important mechanisms through which *T. virens* suppressed *R. solani*. *P. fluorescens* suppresses soil borne pathogens by producing antifungal metabolites as pyrrolnitrinol, pyoluteorin, phenazines and 2,4-diacetyl phloroglucinol (Dwivedi and Johri, 2003). *Trichoderma* spp and *Pseudomonas* spp are extensively used for managing soil borne diseases and is effective against managing diseases in vegetable crops caused by *R. solani* (Pandey et al., 2006).

Padmakumary (1989) found *T. harzianum* and *T. viride* to be antagonistic to *R. solani* under *in vitro* conditions. Khan and Hussain (1991) tested inhibitory effects of culture filtrates of a test fungus isolated from rhizosphere of cowpea against *R. solani*. The maximum inhibition of *R. solani* was obtained with the filtrate of *T. viride* and reduction of the mycelial weight was directly correlated with the concentration of the filtrate. *T. harzianum* was effective as a biocontrol agent *in vitro* and it reduced post-emergence root rot of French bean caused by *R. solani*. Three *Trichoderma* isolates ie., *T. harzianum, T. viride* and *T. virens*

significantly inhibited the mycelial growth and sclerotial production of *R. solani* causing web blight of horsegram (Dubey, 1998). Pandian and Singh (2000) reported *T. harzianum* and *P. fluorescens* as effective in suppressing mycelial growth of *R. solani* causing root rot of wheat. Dubey (2000) reported that among the biocontrol agents *T. viride, T. harzianum* and *T. virens* evaluated for their antagonism against *R. solani in vitro, T. virens* was found to produce maximum growth inhibition of 59.8 per cent and 70 per cent sclerotial production. Bunker and Mathur (2001) reported *in vitro* evaluation of *Trichoderma harzianum* against *R. solani* and recorded it to be most effective in causing significant suppression of *R. solani* causing dry root rot of chilli. Laha and Venkataraman (2001) reported that *P. fluorescens* was effective in managing sheath blight both under *in vitro* and *in vivo* conditions. Several strains of *P. fluorescens* producing siderophore showed antagonism *in vitro* (Leong, 1986). *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). Devi and Reddy (2002) reported *T. harzianum* to be the most potential antagonist among five isolates of *Trichoderma* spp., *P. fluorescens* and *Bacillus* spp. against *R. solani* causing damping off of groundnut.

Priyadarsini (2003) reported that *T. harzianum* exhibited 47.41 per cent inhibition against *R. solani in vitro* followed by *T. virens* (45.18 per cent). She further reported that *P. fluorescens* exerted significant inhibition on *R. solani in vitro.*

Sharma and Gupta (2003) reported *in vitro* and *in vivo* use of *Trichoderma* spp effectively for management of root rot and web blight of French bean caused by *R. solani.* Meena et al. (2003) recorded *in vitro* suppression of *R. solani* f. sp. sasakii causing banded leaf and sheath blight of maize by *T. harzianum* (80 per cent) and *P. fluorescens* (74 per cent). *T. harzianum* and *P. fluorescens* are able to check the growth of *R. solani* in dual culture within 120 and 72 h of incubation respectively (Saxena et al., 2004). Sen et al. (2006) reported the antagonistic effect of fluorescent pseudomonad BRL-1 against *S. rolfsii* in dual culture.

Hadar et al. (1979) reported that *T. harzianum* applied in the form of wheat bran culture to *R. solani* infested soil effectively controlled damping off of bean, tomato and egg plant seedlings. Incorporation of wheat bran preparation of *T. harzianum* in *R. solani* infested soil significantly reduced disease and increased growth of bean plants in non infested soil (Elad et al., 1980). Wu (1980) used *T. pseudokoningiii* and *T. harzianum* for seed treatment of soy bean to control pre emergence damping off caused by *R. solani. T. hamatum* effectively reduced seedling disease of radish and pea caused by *R. solani* under field conditions (Harman and Taylor, 1980). Elad et al. (1982) reported that several fungal and bacterial antagonists like *Trichoderma* spp., *Bacillus subtilis* and *P. fluorescens* have been found to be effective in checking the diseases caused by *R. solani* in rice, pea and cotton. Marshal (1982) reported that there was reduction in incidence of damping off of snap beans caused by *R. solani.* Selected strain used for bacterisation of rice enhanced growth upto 12-27 per cent (Sakthivel et al., 1986). Bacterisation of rice seeds with bacteria producing fluorescent and non-fluorescent pigments on King's B medium suppressed sheath blight caused by *R. solani* and promoted rice seed germination (Mew and Rosales, 1986). Seed bacterization with fluorescent pseudomonad, reduced the incidence of *R. solani* in cowpea (Barbosa et al., 1995).

Soil application of *T. virens* increased the germination percentage and decreased the seedling mortality of horsegram (Dubey, 1998). Out of seven biocontrol agents tested against *R. solani* causing root rot of French bean, *T. virens* and *T. harzianum* reduced the disease to 6.7 and 13.3 per cent as compared to 36.7 per cent in control (Mathew and Gupta, 1998). Prasad and Rangeshwaran (2000) found that a wheat bran kaolin formulation of *T. harzianum* controlled seed rot and damping off of chickpea caused by *R. solani* upto 85 per cent resulting in high plant stand. Sprays of *Trichoderma* spp at ten days interval against web blight of urd bean decreased the disease severity (Sharma and Tripathi, 2001). Latha and Narasimhan (2001) reported the seed treatment of *P.*

fluorescens and *T. viride* in the management of root rot and cyst nematode complex in black gram. *P. fluorescens* was effective in controlling sheath blight of rice both under glass house and field conditions (Laha and Venkataraman, 2001). Dubey (2002) reported foliar spray of *T. virens* and *T. viride* to be efficient in increasing the grain yield in urd and mung beans. A significant increase in root length and number of root nodules over control were also observed. Seed and soil treatment of *T. harzianum* was more effective than seed treatment alone leading to increased pod yield and minimized incidence caused by *Thanatephorus cucumeris* (Abhimanyu and Singh, 2002).

Upamanyu et al. (2002) reported that *T. harzianum* inhibited pre-emergence root rot of cowpea by *R. solani* by 48.89 per cent. Gaikwad and Nimbalkar (2003) reported use of *T. viride* and *T. harzianum* as seed treatment, nursery drench, root dip and soil application against collar and root rot of bell pepper caused by *R. solani* and increased fruit weight, yield and monetary returns. Dubey (2003) reported that seed treatment with slurry or water mixed with spores of *T. viride* gave best protection to germinating seeds of urd or mung bean against *R. solani.* Chakraborty et al. (2003a) reported protection of soy bean from *F. oxysporum* causing root rot of soy bean by *T. harzianum. T. virens* was found most effective followed by *T. harzianum* against *R. solani* causing banded leaf and sheath blight of maize (Singh et al., 2004b). *T. viride* was applied as seed treatment (2g/kg seed), soil amendment and foliar spray against *R. solani* causing web blight of urd and mung bean (Dubey and Singh, 2004).

Khan and Sinha (2005) reported the efficacy of *T. harzianum* in the management of sheath blight of rice. Das et al. (2006) reported lowest disease index and highest percentage seed germination in soy bean stem rot caused by *R. solani* when seeds were treated with *T. harzianum* formulation. Mathur et al. (2006) recorded less disease severity for plants treated with *T. harzianum* for the management of root rot of chilli caused by *R. solani.* Bhattacharya and Ghosh

(2006) reported antifungal properties of fluorescent pseudomonads in managing *Macrophomina phaseolina* causing root rot of *Solanum melongena.*

2.8 Use of fungicide

Fungicidal application even today remains as the easiest and best proven practical method to manage diseases caused by *R. solani.* Jhooty and Bains (1972) reported that nonsystemic fungicide, brassicol and the systemic fungicide benomyl effectively managed pre and post emergence damping off of mung bean caused by *R. solani.* Bristow et al. (1973) observed significantly less severity of disease caused by *R. solani* in bean plants containing PCNB when compared to control plants. Malhan et al. (1975) reported that at pH 7.6 and 20°C *R. solani* isolated from roots of urd bean were highly sensitive to benomyl. Seed treatment with benomyl and chloroneb gave good results in cowpea upto 30 days and in radish upto 15 days. 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. Shatla et al. (1976) reported management of lentil root rot caused by *R. solani* in Egypt by soil treatment with thiophanate and quintozene. Kotasthane and Agrawal (1978) found that the fungicidal mixture carbendazim + thiram + brassicol (1:1:1) at the rate of 3g/kg of seed effectively managed pre-emergence mortality in bengal gram caused by *R. solani*, *R. bataticola* and *S. rolfsii.* Oyekan (1979) reported that captafol at the rate of 1.64 kg ai/ha and oxycarboxin at the rate of 0.16 kg ai/ha effectively reduced web blight and leaf spot of cowpea caused by *R. solani* in Nigeria. Bavistin 0.2% as seed treatment significantly reduced the incidence of root rot of cowpea to the extent of 57.5 and 58.9 per cent in case of *R. solani* and *R. bataticola* respectively. Gambhir and Khairnar (1986) found that *Penicillium brefeldianum* infecting groundnut was unable to tolerate even 500 ppm of copper oxychloride.

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). Gaikwad and Nimbalkar (2003) reported use of copper oxy chloride 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 . 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. (2004a) applied different concentrations of copper oxychloride as soil drench to betel vine for the management of collar rot disease caused by *Sclerotium rolfsii*. Bhatnagar et al. (2004) reported use of copper oxychloride to manage cumin blight caused by *Alternaria burnsii*. Tiwari et al. (2004) reported use of copper oxychloride to manage leaf spots caused by *Cercospora arachidicola* and *Cercosporidium personata* and rust caused by *Puccinia arachidis* in groundnut. Rahman and Bhattiprolu (2005) reported the use of copper oxychloride for management of damping off diseases of tomato, chilli and brinjal in nurseries. 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*.

2.9 Biochemistry of defense to plant disease

Several biochemical reactions take place inside the host plants to ward off the invading pathogens. Presence of phenolics in high concentration in cells of plants contribute to disease resistance.

2.9.1 Phenols

Mahadevan (1966) reported high amounts of phenols in cotton varieties resistant to wilt disease than susceptible ones. Accumulation of phenolic compounds due to infection by pathogens were reported in crops like ragi (Vidyasekharan, 1974), mung (Arora and Bajaj, 1978; Arora, 1983), tea (Borah et al., 1978) and rice (Chatopadhyaya and Bera, 1980). Sindhan and Jaglan (1988) reported that both resistant and susceptible genotypes of groundnut infected with *Cercospora arachidicola* and *Phaeoisariopsis personata* exhibited higher quantity of phenols and it was noted that on comparison with the susceptible genotypes the resistant ones had more quantity of phenols (Reddy and Ravindranath, 1988). Mitter et al. (1997) reported that in both resistant and susceptible genotypes of chickpea on inoculation with *Botrytis cinerea* causing grey mould of chickpea there was reduction in phenol content . Kalim et al. (2000) reported higher amounts of total phenols in cowpea plants susceptible to *Rhizoctonia* spp. raised from seeds treated with 0.2 per cent bavistin. Beckman (2002) related physiological aspect of disease resistance and phenol as due to rapid oxidation of phenolic compounds which resulted in lignification and suberisation of cells and cell death that sealed off further infection at the site of cellular penetration by pathogen. Sukhwal and Purohit (2003) reported higher accumulation of phenols in resistant varieties of maize infected with *Helminthosporium* spp. than in the susceptible ones. Priyadarsini (2003) observed significant increase in total phenol content by soil application of *Trichoderma* and also its foliar spray for Amaranthus leaf blight caused by *R. solani.* Saravankumar et al. (2005) reported increased accumulation of phenolics in *Bacillus* amended with chitin bioformulation pretreated plants challenge inoculated with *Macrophomina phaseolina.* Prakash and Mohan (2005) reported that in all bacterial treated plants appreciable amount of phenol was noticed when compared to control. Todkar et al. (2005) reported that the roots of cotton cultivars resistant to *Fusarium oxysporum* f. sp. *vasinfectum* causing wilt of cotton were found to contain higher levels of total phenols. Thakker et al. (2005) reported greater production of

phenols in banana plantlets with Fusarium wilt disease treated with elicitors of *Fusarium oxysporum* f. sp. *cubense*.

2.9.2 Proteins

In early stages of pathogenesis of *R. solani* in mung bean there was an increase in amino acids (Wu, 1973). Mohan (1976) found reduction in aromatic amino acids in a moderately resistant variety of rice infested with *Acrocylindrum oryzae*. Sundaram (1980) recorded that amino nitrogen content in plant decreased due to *R. solani* inoculation and potassium application. Charya and Reddy (1981) reported that protein content of mung bean seeds inoculated with seed borne fungi including *R. solani* decreases on storage. Similar findings were reported in arhar by Sinha et al. (1981). Girija (1993) reported that amino acid content progressively decreased with increase in lesion development of rice plants inoculated with *R. solani*. Ushamalini et al. (1998) reported reduction in protein content in cowpea seeds inoculated with *Macrophomina phaseolina*. Khan and Ashraf (2005) reported lower protein content in resistant varieties to white rust of Indian mustard as compared to susceptible ones.

2.9.3 Carbohydrates

Dhanapal (1975) reported increase in reducing and non- reducing sugars immediately after inoculation with *Helminthosporium oryzae*. Sundaram (1980) reported that inoculation of *R. solani* in rice resulted in depletion of reducing sugar content. Padhi and Chakrabarti (1984) observed that susceptible rice cultivars on infection with *Pyricularia oryzae* had higher levels of reducing sugars but lower levels of non reducing sugars than resistant ones. Mitter et al. (1997) reported that in both resistant and susceptible genotypes of chickpea sugars decreased after inoculation of *Botrytis cinerea* causing grey mould. Ushamalini et al. (1998) reported reduction of total sugars in cowpea seeds inoculated with *Macrophomina phaseolina*, *Fusarium oxysporum* and *Aspergillus*

niger. Priyadarsini (2003) observed reduction in carbohydrate content due to soil application of rice husk ash and the root endophyte, *Piriformospora indica*.

2.9.4 Defense related enzymes

Various oxidative enzymes alter the concentration of defense related substances in plants and yield products with enhanced disease resistance properties (Mahadevan and Sridhar, 1982).

2.9.4.1 Polyphenol oxidase

Role of polyphenol oxidase (PPO), a copper containing enzyme which oxidized phenolics to highly toxic quinones in plant disease resistance was observed by Umaerus (1959), Kasuge (1969) and Yamamoto et al. (1978). Increased polyphenol oxidase activity in rice tissues following infection with *Helminthosporium oryzae* has been reported by Oku (1967), Mukherjee and Ghosh (1975) and Chattopadhyay and Sammadar (1980). Rao et al. (1988) explained increase in polyphenol oxidase activity due to latent host enzyme. Avdiushko et al. (1993) obtained enhanced PPO activity in cucumber leaves in the vicinity of lesions caused either by *Colletotrichum lagenarium* or TNV. The specific activity of PPO and PO in cowpea plants raised from bavistin treated seeds was higher in comparison with that of untreated seeds (Kalim et al., 2000). The specific activity of peroxidase was several times more than that of polyphenol oxidase. In amaranthus plants, Priyadarsini (2003) noted significant increase of PPO activity for the treatments *T. harzianum* and bacterial isolates B_3 and KK_{16} (*Pseudomonas fluorescens* isolates) over the control plants. Todkar et al. (2005) reported that the roots of cotton cultivars resistant to *F. oxysporum* f. sp. *vasinfectum* causing wilt of cotton were found to contain higher levels of PPO and PAL.

2.9.4.2 Peroxidase

Bonner (1950) reported that peroxidase was a key enzyme involved in plant disease resistance as it played an important role in the biosynthesis of lignin and oxidation of mono and diphenolic compounds and aromatic amines to highly toxic quinines in the presence of hydrogen peroxide. The level of peroxidase activity in potato tubers before infection was positively correlated with resistance to *Phytophthora infestans* (Fehrman and Dimond, 1967). Kasuge (1969) observed that peroxidase activity was frequently increased in plants infected by pathogens which was correlated with disease resistance. Vir and Grewal (1972) found that the gram variety 1-13 resistant to Ascochyta blight showed highest activity of PO after 12 days of inoculation whereas the susceptible varieity Pb-7 showed relatively less increase at eight days after inoculation. Hammerschmidt et al. (1982) found enhanced peroxidase activity to be associated with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. Wasfy et al. (1984) reported that the number and concentration of peroxidase enzymes in bean hypocotyls increased in response to fungal infection *by R. solani*. Arora and Bajaj (1985) observed fluctuating activity of peroxidase enzyme in hypocotyls of mung bean inoculated with *R. solani*. Yedidia et al. (1999) reported increase of chitinase peroxidase activities both in leaf and root tissues of cucumber grown in the prescence of *T. harzianum* strain 203. Shamina and Sarma (2000) reported the activity of peroxidase, polyphenol oxidase and ß-1,3 glucanase. They showed an earlier and greater increase in root length and number of leaves of resistant selection of pepper than the susceptible plant due to *Phytophthora capsici*. Howell et al. (2000) studied the biochemistry of biocontrol of damping off of cotton by *T. virens* and observed an increase in host resistance due to enhanced peroxidase activity and terpenoid synthesis. Chakraborty and Gupta (2001) reported that PO activity was significantly lower in pigeonpea seedlings inoculated with *Fusarium udum* than their noninoculated counterparts. Priyadarsini (2003) noted that in amaranthus plants, infected with *R. solani* PO activity was significantly increased in plants which received treatments *T.*

harzianum and bacterial isolate B3 over control. An increase in activity of PO and PPO was observed in maize varieties infected with *Helminthosporium* spp. (Sukhwal and Purohit, 2003). Chakraborty et al. (2003b) reported higher activity of PO and PAL in roots infected with *Fusarium oxysporum* causing root rot of soybean in comparison with their healthy counterparts. They further reported that PAL activity was higher while PO activity was lower in soybean plants treated with *Bradyrhizobium japonicum* challenge inoculated with *Fusarium oxysporum*. In all bacteria treated plants appreciable amount of PO, PAL and PPO were detected as compared to control (Prakash and Mohan, 2005). Thakker et al. (2005) reported greater production of PO and PPO in Fusarium wilt affected banana plantlets treated with elicitors of *Fusarium oxysporum* f. sp. *cubense*. Bhatnagar (2005) reported PO and PPO enzymes in stem and seeds of cumin plants affected by *Alternaria burnsii* causing blight as compared to its healthy counterparts.

2.9.4.3 Phenyl alanine ammonia lyase

Phenyl alanine ammonia lyase is the key enzyme in the biosynthesis of phenolics. Dixon and Fuller (1976) got a positive correlation between increased PAL activity and biosynthesis of phaseolin. Treatment with *P. fluorescens* caused increase in activities of PAL and PO isozyme in tobacco while chitinase activity was limited (Schneider and Ullrich, 1994). Spraying *P. fluorescens* increased PAL activity one day after treatment in rice plants (Meena et al., 1999). Priyadarsini (2003) noted significant increase in PAL activity due to soil application of bacterial isolates B_3 and KK_{16} and *T. harzianum* in the case of foliar blight of amaranthus caused by *R. solani*. Sivakumar and Sharma (2000) observed a higher activity of PAL, peroxidase, polyphenol oxidase and phenols in maize plants raised from seeds bacterized with *P. fluorescens* after inoculation with *R. solani*. PAL activities showed initial increase and then declined after inoculation by *Cercospora moricola* in mulberry (Nagaveni et al., 2005). Seed treatment with ß amino butyric acid induces resistance to *P. halstedi* accompanied

by activation of PAL and PO (Kumar et al., 2005), Chitra et al. (2005) reported increased activity of PAL, PO and PPO in plants treated with plant products and challenge inoculated with *Alternaria alternata*. Increased accumulation of PAL, PO and PPO were observed in *Bacillus* spp. amended with chitin bioformulation pretreated plant which was challenge inoculated with *M. phaseolina* (Saravanakumar et al., 2005).

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 Isolation of the pathogen

Cowpea plants showing typical collar rot and web blight symptoms caused by *Rhizoctonia solani* were collected from the Crop Museum of College of Agriculture, Vellayani. The collar region and leaves of infected cowpea plants showing rotting and blighting symptoms were washed with 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 minute followed by two to three washings in sterile water. The pieces were then transferred into sterile petridishes containing potato dextrose agar (PDA), under aseptic conditions and incubated at room temperature. When fungal growth was visible, mycelial bits were transferred to PDA slants and labelled.

3.1.2 Purification

The two isolates obtained from collar region and the leaf were purified by hyphal tip method and pure cultures were maintained on PDA slants by serial sub culturing for further studies.

3.1.3 Pathogenicity

Pathogenicity of the two isolates were proved following Koch's postulates. Cowpea seedlings were grown in earthen pots. Ten to fifteen days old seedlings were inoculated with collar rot pathogen on collar region after giving injury by pin pricking. To provide moisture a thin layer of moist cotton was placed over inoculated region. To ensure humidity the plant was covered with a

polypropylene cover sprinkled with water and having sufficient holes. The pathogen isolated from leaf region was inoculated separately on leaves of 10 to 15 days old seedlings. For application on the leaves the mycelial suspension of *R. solani* was prepared by harvesting mycelial mats from a five day old culture raised in potato dextrose broth (PDB). Mats were suspended in sterile distilled water (SDW) and homogenized in a warring blender for one minute and strained through a double layered muslin cloth and diluted with SDW in such a manner to contain 15-20 mycelial bits per microscopic field (200X). Then it was sprayed using a hand sprayer on to the leaves. To ensure humidity the plant was covered with a polypropylene cover sprinkled with water and having sufficient holes. Three replications were maintained for each. Both the isolates were capable of producing symptoms of disease on plants. Reisolation of pathogen was done from leaves and collar region showing typical web blight and collar rot symptoms and the identity of the pathogen was established. These cultures were used for further studies.

3.2 *In vitro* studies

3.2.1 Plant oils

Seven plant oils *viz.*, Neem oil (*Azadirachta indica* A. Juss.), Castor oil (*Ricinus communis* Linn.), Pongamia oil (*Pongamia glabra* Vent.), Calophyllum oil (*Calophyllum inophyllum* Linn.), Samadera oil (*Samadera indica* Gaerb.), Odal oil (*Balinitis roxburghii* Wall.) and Marotty oil (*Hydnocarpus pentandra* Blume.) each at five per cent concentration were used for *in vitro* studies by poisoned food technique.

Potato dextrose agar (PDA) medium was amended separately with five per cent of each oil, emulsified with 0.1 per cent emulsifier *viz.*, Polysorbate (tween 80) by sterilizing 100 ml media each in 250 ml Erlenmeyer flasks. The media amended with 0.1 per cent emulsifier alone was used to study the effect of polysorbate (tween – 80). Unamended PDA served as control. After autoclaving

at 15 lb pressure for 20 minutes melted and cooled media were poured to petridishes and inoculated with culture discs of five mm dia cut from actively growing edges of five to seven days old culture. Plates containing PDA with *R. solani* inoculated at center served as control. Dishes were maintained at 28 ± 2°C and examined for inhibition of growth of the pathogen. Radial growth of the pathogen was taken and per cent inhibition of mycelial growth was calculated using the formula,

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

(Vincent, 1927)

I = Inhibition of mycelial growth of the pathogen

C = Radial growth of the pathogen in control plates (cm)

T = Radial growth of the pathogen in treated plates (cm)

3.2.2 Oil cakes

Four oil cakes each at ten per cent concentration were used for *in vitro* studies.

Neem	-	*Azadirachta indica* (A. Juss)
Coconut	-	*Cocos nucifera* (Linn.)
Gingelly	-	*Sesamum indicum* (Linn.)
Groundnut	-	*Arachis hypogaea* (Linn.)

Oil cakes were dried at room temperature (30°C) for three days, broken into small pieces and powdered thoroughly with pestle and mortar, sieved through a screen (2 mm porosity) and put in sterilized distilled water in the ratio 1:2 (W/V) for soaking. After 24 h cake extract was obtained by squeezing the cake in water through four folds of muslin cloth. The extract was centrifuged (5000 rpm for 10 min) and supernatant was decanted into a 250 ml flask. From this 10 ml

was added to 90 ml potato dextrose agar and autoclaved at 15 lb pressure for 20 minutes.

Media was melted, cooled and poured to petridishes. Discs of 5 mm diameter cut from edges of actively growing five to seven days old culture was used to inoculate the plates. Four replications were maintained for each treatment and observations recorded. Plates containing PDA with *R. solani* inoculated at center served as control. Dishes were maintained at 28±2°C and examined for inhibition of growth of the pathogen. Radial growth of the pathogen was taken and percentage inhibition of mycelial growth was calculated using formula described under 3.2.1.

3.2.3 Indigenous materials

3.2.3.1 Turmeric powder – baking soda

Three different ratios of turmeric powder – baking soda (TP-BS) as per the quantities mentioned below were added to 200 ml PDA taken in 500 ml conical flasks before autoclaving. After sterilization, the media containing three different concentrations of turmeric powder – baking soda were poured into sterile petridishes. Five mm discs were cut out from edges of five to seven day old mycelial growth of *R. solani* with a cork borer of 0.5 cm dia. The discs were then placed at the center of each dish. Three replications were maintained for each treatment and unamended PDA served as control. Dishes were incubated at 28±2°C and examined for inhibition of growth of the pathogen. Radial growth of the pathogen was taken and the inhibition of mycelial growth was calculated using the formula described under 3.2.1.

Ratios of turmeric powder – baking soda	Quantity of turmeric powder in 200 ml PDA (g)	Quantity of baking soda in 200 ml PDA (g)
3 : 2	0.48	0.32
4 : 1	0.64	0.16
10 : 1	0.727	0.073

3.2.3.2 Rice husk ash

The effect of three concentrations of rice husk ash (RHA) *viz.*, 0.5, 0.75 and 1.00 per cent on *R. solani* were tested by growing the fungus on PDA incorporated with RHA. The experiment was conducted as described under 3.2.3.1 and observations recorded.

3.2.4 Mycelial growth on sclerotial germination

Mycelial growth on germination of sclerotia of *R. solani* was tested in five per cent oils, 10 per cent cake extracts, different concentrations of turmeric powder – baking soda *viz.*, 3:2, 4:1 and 10:1 and rice husk ash 0.75 per cent, 0.5 per cent and one per cent. Five per cent oils were prepared by taking five ml oil along with 0.1 per cent emulsifier along with 95 ml sterile distilled water to get 100 ml of solution. 10 per cent cake extracts were prepared as described in 3.2.2. The indigenous materials *i.e.*, TP-BS as per the quantity described in 3.2.3.1 and three concentrations of RHA were dissolved in sterile distilled water. All the solutions were autoclaved at 15 lb pressure for 20 min. Sclerotia were inoculated on PDA after treatment for 0, 24, 48 and 72 h in respective solutions. Sterile distilled water served as control and three replications were maintained for each treatment and the radial growth of pathogen was taken after incubating the dishes at 28±2°C and examined for inhibition of growth of pathogen. Inhibition of mycelial growth was calculated using the formula described under 3.2.1.

3.3 *In vivo* studies on pathogen suppression

3.3.1 Effect of plant oils, oil cakes, biocontrol agents and indigenous materials on plant growth, disease intensity and disease incidence

3.3.1.1 Treatments

T_1 - Foliar spray of 2 per cent odal oil + soil drench

T_2 - Foliar spray of 2 per cent pongamia oil + soil drench

T_3 - Soil application of coconut oil cake (10 g/kg soil)

T_4 - Soil application of gingelly oil cake (10 g/kg soil)

T_5 - Soil application of neem cake (10 g/kg soil)

T_6 - Foliar spray of turmeric powder – baking soda 10:1 (4 g/l)

T_7 - Foliar spray of turmeric powder – baking soda 4:1 (4 g/l)

T_8 - Foliar spray of turmeric powder – baking soda 3:2 (4 g/l)

T_9 - Soil application of RHA (500 g/m^2) + two per cent foliar spray

T_{10} - Soil application of RHA (750 g/m^2) + two per cent foliar spray

T_{11} - Soil application of RHA (1000 g/m^2) + two per cent foliar spray

T_{12} - Soil application of *Trichoderma harzianum* talc based formulation (20 g/kg soil) + one per cent foliar spray

T_{13} - Soil application of talc based formulation of *Pseudomonas fluorescens* P_1 (20 g/kg soil) + two per cent foliar spray

T_{14} - Soil application of talc based formulation of *Pseudomonas fluorescens* P_{22} (20 g/kg soil) + two per cent foliar spray

T_{15} - Prophylactic copper oxychloride drench + 0.4 per cent foliar spray

T_{16} - Inoculated control

Design - CRD

Replications - Four

Variety - Malika

Cowpea plants were maintained as pot culture as per the package of practice recommendations : Crops (KAU, 2002) by giving timely application of fertilizers and adopting eco-friendly protection measures.

3.3.1.2 Preparation of pathogen inoculum

Rhizoctonia solani was mass multiplied in rice bran. Rice bran was mixed with water in the ratio 2:1 and 250g of mixture was filled in polypropylene bags, sealed and sterilized at 121.1°C at 1.02 kg cm^{-2} for two h.

Mycelial discs were cut from seven day old PDA cultures of collar rot pathogen. Each bag was inoculated with three five mm mycelial discs and was incubated at 28±2°C for two weeks.

For the multiplication of the web blight pathogen potato dextrose broth was prepared and sterilized in conical flasks. These were inoculated with mycelial discs of five mm dia cut from edges of an actively growing five to seven day old culture of *R. solani* and incubated at room temperature for one to two weeks.

3.3.1.3 Application of Inoculum

For application at collar region 250 g of inoculum was mixed with soil in the ratio 1:2 and the rice bran – soil mixture was applied on the collar region of seedlings after five days of germination of seeds. Three seedlings were maintained per pot.

For application on the leaves the mycelial suspension of *R. solani* was prepared by harvesting mycelial mats from a five day old culture raised in PDB medium. Mats were suspended in sterile distilled water (SDW) and homogenized in a warring 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). Then it was sprayed using a hand sprayer on to the leaves when the plant had sufficient leaves *i.e.*, 40 days after sowing.

3.3.1.4 Application of oil cakes

Based on results of *in vitro* studies three oil cakes exhibiting maximum antagonistic activity against the pathogen *i.e.*, coconut, gingelly and neem were selected for *in vivo* studies. These oil cakes were finely powdered using a mixer grinder and mixed with unsterilised potting mixture contained in pots of 20 cm dia at the rate of 100 g/kg soil. Oil cakes were then allowed to decompose for a week after which seeds were sown.

3.3.1.5 Application of Indigenous Materials and Biocontrol Agents

Turmeric powder and baking soda at three different levels (10:1, 4:1 and 3:2) were prepared by dissolving them in water. This was then filtered using muslin cloth and used for soil drenching and foliar spray. The application was done two days before inoculation of the pathogen and two, five and ten days after inoculation of the pathogen.

Two per cent of the three recommended dosages of RHA (500 gm^{-2}, 750 gm^{-2} and 1000 gm^{-2}) were prepared by suspending the required quantity in water. This was filtered using muslin cloth and used for foliar spray. Foliar spray was given two days before inoculation of the pathogen and two, five and ten days after inoculation of pathogen. Different quantities of rice husk ash, *i.e.*, 15.7 g, 23.55 g and 31.4 g respectively for the treatments 500 gm^{-2}, 750 gm^{-2} and 1000 gm^{-2} were used for mixing with top soil just after germination of seeds.

One per cent aqueous suspension of formulated product of the fungal antagonist *Trichoderma* was used for foliar spray and 200 g of talc based formulation per kilogram of soil was used for soil application in pots of 20 cm dia just after emergence of seedlings.

For the bacterial antagonists, two per cent aqueous suspension of the formulation was used for foliar spray. Talc based formulation was applied in soil at the rate of 20 g/kg soil in pots of 20 cm dia. The formulations were thoroughly mixed with soil just after the emergence of seedlings. Foliar spray was given two days before inoculation of the web blight pathogen and two, five and ten days after inoculation of the web blight pathogen. Fungal and bacterial antagonists were also used for seed treatment. *Trichoderma* was applied at the rate of 4 g/kg seed and *Pseudomonas* at the rate of 10 g/kg seed. The seeds were sprinkled with a small quantity of water over which the formulation was applied. The treated seeds were dried in shade for half an hour and then used for sowing.

3.3.1.6 Application of fungicide

Copper oxychloride (0.4 per cent) was prepared by suspending the required quantity of fungicides in appropriate quantity of water. It was thoroughly mixed and used for soil drenching and as foliar spray in cowpea plants. Foliar application was done as mentioned in 3.3.1.5.

3.3.1.7 Observations

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

3.3.1.7.1 Disease incidence (%)

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

Disease incidence was calculated using the formula,

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

3.3.1.7.2 Disease intensity

Observations on web blight disease intensity were recorded before inoculum application and five and 11 days after inoculum application. Scoring of the disease was done using the disease scale developed for the purpose after careful study of the disease and disease development. The extent of infection was estimated based on the parts of the plant affected. Size of the lesion, yellowing and drying of infected leaves were taken into account for devising the scale. Based on this a 0-9 scale has been devised (Plate-1).

Grade	Description
0	No infection
1	1-10 per cent of leaf area infected
3	11-25 per cent of leaf area infected
5	26-50 per cent of leaf area infected
7	51-75 per cent of leaf area infected
9	> 75 per cent of leaf area infected

Percentage disease index was calculated using the formula:

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

(Mayee and Datar, 1986)

Plate 1 0-9 scale for the scoring of web blight of cowpea

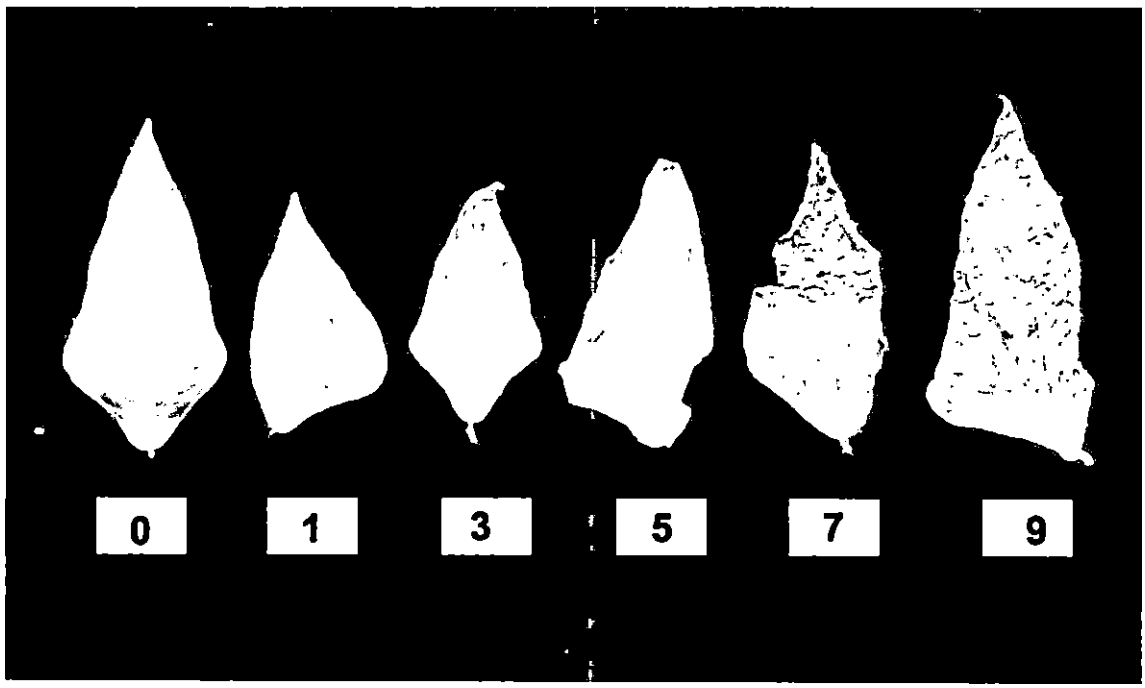


Plate 1

3.3.1.7.3 Shoot length (cm)

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

3.3.1.7.4 Root length (cm)

Length of longest root of each plant was measured after depotting.

3.3.1.7.5 Fresh weight of plants (g)

Fresh weight of plants was taken in an electronic balance immediately after depotting.

3.3.1.7.6 Dry weight of plants (g)

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

3.3.1.7.7 Yield (g/plant)

Number of pods per plant and pod weight are recorded.

3.3.1.8 Biochemical studies

Leaf samples of the different treatments were collected for estimating changes in activity of carbohydrates, proteins, phenols and defense related enzymes such as phenylalanine ammonia lyase, peroxidase and polyphenol oxidase. Wherever the treatments were incorporated in soil, leaf samples were

taken ten days after application while in treatments involving foliar spray samples were taken one week after their application.

3.3.1.8.1 Total Phenols

Total phenol content was estimated following the procedure described by Bray and Thorpe (1954).

One gram leaf sample was ground in 10 ml of 80 per cent ethanol. The homogenate was centrifuged at 10,000 rpm for 20 min, supernatant was saved and residue extracted with five times the volume of 80 per cent ethanol and centrifuged. The supernatant was evaporated to dryness and the residue was dissolved in a known volume of distilled water (five ml). An aliquot of 0.3 ml was pipeted out and made up to three ml with distilled water. Folin – ciocalteau reagent (0.5 µl) was added and 2.0 µl of 20 per cent sodium carbonate solution was added to each tube after three min. This was mixed thoroughly and kept in boiling water for one min. This was cooled and absorbance was measured at 650 nm against reagent blank. The standard curve was prepared using different concentrations of catechol and expressed in catechol equivalents as microgram per gram leaf tissue on fresh weight basis.

3.3.1.8.2 Estimation of Protein

The total soluble protein content was estimated as per the procedure described by Bradford (1976). One gram leaf sample was homogenized in 10 ml, 0.1 M sodium acetate buffer (pH 4.7) and centrifuged at 5000 rpm for 15 minutes at 4°C. Supernatent was saved for estimation of soluble protein. The reaction mixture consisted of 0.5 ml of diluted (5 times) dye solution. Absorbance was read at 595 nm in a spectrophotometer against reagent blank. Bovine serum albumin was used as the protein standard. The protein content was expressed as microgram albumin equivalent of soluble protein per gram on fresh weight basis.

3.3.1.8.3 Carbohydrates

Total carbohydrates was estimated by Anthrone method (Hedge and Hofreiter, 1962).

Hundred milligram sample was taken in boiling tubes and hydrolysed by keeping it in boiling water bath for three h with 5.0 ml of 2.5 N hydrochloric acid and cooled to room temperature. Sodium carbonate was added to neutralize it until the effervescence ceased and the volume made up to 100 ml and centrifuged. 0.5 ml of aliquot of supernatant was taken for analysis. The standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of working standards from which the standard zero served as blank. The volume was made upto one ml in all tubes including sample tubes by adding distilled water. Four ml anthrone reagent was then added and heated for eight min in a boiling water bath, cooled rapidly and read at 630 nm. Standard graph was prepared and from the graph the amount of carbohydrate was calculated.

3.3.1.8.4 Phenyl alanine ammonia lyase (PAL)

PAL activity was analysed using the procedure described by Dickerson et al. (1984).

The enzyme extract was prepared by homogenizing one g leaf sample in five ml of 0.1 M sodium borate buffer (pH 8.7) (Appendix-II B) containing 0.05 g of poly vinyl pyrolidone using chilled pestle and mortar. The homogenate was centrifuged at 10,000 rpm for 20 min at 4°C and the supernatant was used for the assay. The reaction mixture contained 3 ml of 0.1 M sodium borate buffer, 0.2 ml enzyme extract and 0.1 ml of 12 mM L-phenyl alanine prepared in the same buffer. The blank contained three ml of 0.1 M sodium borate buffer and 0.2 ml enzyme extract. The reaction mixture and blank were incubated at 40°C for 30

min and reaction was stopped by adding 0.2 ml of 3 N hydrochloric acid. The absorbance was read at 290 nm in a spectrophotometer.

PAL activity was expressed as microgram of cinnamic acid produced per min per gram on fresh weight basis.

3.3.1.8.5 Peroxidase (PO)

Peroxidase activity was determined according to procedure described by Srivastava (1987).

Leaf samples of 200 mg were homogenized in 1.0 ml of 0.1 M sodium phosphate buffer (pH 6.5) (Appendix-II A) to which 0.05g of polyvinyl pyrrolidone was added. Homogenisation was done at 4°C using pestle and mortar. The homogenate was filtered through a muslin cloth and centrifuged at 5,000 rpm for 15 minutes at 4°C. The supernatant was used as enzyme extract. The reaction mixture consisting of one ml of 0.05 M pyrogallol and 50 µl enzyme extract was taken in both reference and sample cuvettes, mixed and placed in the spectrophotometer with reading adjusted to zero at 420 nm. The enzyme reaction was started by adding one ml of one per cent hydrogen peroxide into sample cuvettes and change in absorbance was measured at 30 seconds interval.

3.3.1.8.6 Estimation of Polyphenol Oxidase (PPO)

PPO was determined as per the procedure given by Mayer et al. (1965).

Leaf samples of 200 mg was homogenized in one ml of 0.1 M sodium phosphate buffer (pH 6.5) to which 0.05g poly vinyl pyrrolidone was added. Homogenisation was done at 4°C using chilled pestle and mortar. The homogenate was filtered through a muslin cloth and centrifuged at 5,000 rpm for 15 minutes at 4°C and the supernatant was used as enzyme extract. The reaction

mixture contained one ml of 0.1 M sodium phosphate buffer and 1.0 ml of 0.01 M catechol. Cuvettes were placed in spectrophotometer and absorbance was set at zero. The reaction was started after adding 50 µl of enzyme extract. The change in absorbance was recorded at 495 nm and PPO activity expressed as change in absorbance of reaction mixture per milliliter per gram on fresh weight basis.

3.4 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

4.1 Symptomatology

Incidence of collar rot and web blight of cowpea plants and the sequence of events leading to the death of diseased plants were observed. Collar rot symptoms 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. Further, girdling of basal portion of stem occurred resulting in yellowing and shedding of leaves and ultimately drying up of the entire plant. Root development was poor and showed symptoms of rotting of the tap as well as the lateral roots. White mycelial growth, often studded with small sclerotia, was seen on the basal part of the affected stem. (Plate-2).

Web blight symptoms appeared as small circular, light greyish brown spots 0.1 cm in dia that soon enlarged and became oblong or irregular in shape. The affected regions were surrounded by an irregular water-soaked area. Under high atmospheric humidity the spots coalesced rapidly and covered larger areas sometimes extending throughout the entire leaf lamina. There was mycelial growth of the organism accompanied by sclerotial formation over the affected areas. Cobweb like symptoms were also noticed in the leaves. Mycelial strands held the adjacent infected leaves together in a web like fashion. In severe cases the affected leaves became yellow and senescent and were shed in large numbers (Plate-3).

4.2 Pathogen

4.2.1 Isolation of the pathogen

R. solani, the incitant of collar rot and web blight was isolated from the diseased cowpea plants collected from the Crop Museum of the College of Agriculture, Vellayani, Thiruvananthapuram. Mycelial growth of the cultures of collar rot and web blight pathogen were cream in colour initially which later

Plate 2 Symptoms of collar rot disease on cowpea plants

Plate 3 Symptoms of web blight disease on cowpea leaves



Plate 2



Plate 3

Table 1 Comparison of radial growth of collar rot and web blight isolates

Pathogen	*Mycelial growth (cm)
Collar rot	8.09 (3.02)
Web blight	6.83 (2.80)
CD (0.05)	0.18

*Mean of four replications

Table 2 Effect of plant oils on mycelial growth of collar rot isolate of *R. solani*

Oils	Collar rot	
	*Mycelial growth (cm)	Percentage inhibition
Neem	5.46 (2.54)	39.24 (38.77)
Castor	9 (3.16)	0.00 (1.00)
Pongamia	1.63 (1.62)	81.87 (64.77)
Calophyllum	7.73 (2.96)	14.03 (21.99)
Samadera	4.43 (2.33)	50.73 (45.40)
Odal	0 (1)	100.00 (90.00)
Marotty	5.37 (2.52)	40.37 (39.43)
Tween	5.89 (2.63)	14.33 (22.24)
Control	9 (3.16)	
CD (0.05)	0.0761	-

(Figures given in parenthesis are transformed values)
*Mean of four replications

turned brown. Zonations were also noticed in culture plates. The collar rot isolate of *R. solani* produced sclerotia rarely, whereas, the web blight isolate produced sclerotia profusely on completion of mycelial growth in the petriplates. However, the sclerotia formed in the former case were large and dark brown in colour as compared to the minute lighter brown coloured sclerotia of the latter. The fungal cultures were maintained on PDA slants with periodic sub-culturing.

4.2.2 Pathogenicity test

Koch's postulates were proved for confirming the pathogenicity of the isolates of *R. solani.* Artificial inoculation of the isolates on collar region (Plate-4) of cowpea seedlings produced collar rot symptoms. Similarly artificial inoculation of the isolates on leaf lamina of cowpea seedlings produced web blight (Plate-5) symptoms. In both the cases symptoms were produced in three to four days time. Re-isolation from infected seedlings also yielded *R. solani* identical to the respective original cultures.

4.2.3 Comparison of growth of collar rot and web blight pathogen

Collar rot and web blight isolates exhibited significant difference in their radial mycelial growth. The collar rot pathogen exhibited a faster growth of 8.09 cm, while web blight isolate had a mycelial growth of 6.09 cm. Collar rot isolate took three days to complete its growth in petridish while web blight isolate took four days (Table-1). Collar rot isolate produced sclerotia rarely, while web blight isolate produced sclerotia profusely on completion of its growth in the petridish.

4.3. *In vitro* studies on inhibition of fungal growth

4.3.1 *In vitro* screening of plant oils

Seven plant oils *viz.*, odal oil, marotty oil, calophyllum oil, samadera oil, neem oil, pongamia oil and castor oil along with Tween-80 were tested by

poisoned food technique on PDA for their toxicity against the two isolates of *R. solani.*

A comparison of the effect of plant oils on the collar rot isolate and web blight isolate reveals that odal oil is highly effective in causing *in vitro* suppression of both the isolates. On the contrary, Pongamia oil that exerted 81.87 per cent inhibition of the collar rot isolate caused only 22.53 per cent inhibition of the web blight isolate. Samadera oil caused two times more suppression of the web blight isolate than the collar rot isolate.

4.3.1.1 *In vitro* screening of plant oils on inhibition of collar rot isolate of *R. solani*

Plant oils, in general, exerted suppressing effect on the pathogen. However, castor oil caused no inhibition of *R.solani.* The highest inhibition of 100 per cent inhibition was obtained on incorporation of odal oil into potato dextrose agar medium which was followed by Pongamia oil which caused 81.87 per cent inhibition. The other oils *i.e.*, samadera oil, marotty oil and neemoil also caused suppression of the collar rot isolate to the tune of 50.73, 40.37 and 39.24 per cent, respectively. The wetting agent, Tween also exerted inhibition to the extent of 14.33 per cent which was on par with Calophyllum oil (14.03 per cent) (Plate-6, Table-2).

4.3.1.2 *In vitro* screening of plant oils for inhibition of web blight isolate of *R. solani*

Plant oils had a suppressing effect on the web blight pathogen also. Oils of samadera and odal caused complete suppression of the fungus while neem oil caused only 44.78 per cent suppression. The other plant oils exhibited varying levels of suppression among which Pongamia oil (28.32 per cent) surpassed the other two oils *viz.*, Calophyllum (19.45 per cent) and marotty (21.55 per cent)

Table 3 Effect of plant oils on mycelial growth of web blight isolate of *R. solani*

Oils	Web blight	
	*Mycelial growth (cm)	Percentage inhibition
Neem	4.53 (2.35)	49.64 (44.78)
Castor	9.00 (3.16)	0.00 (1.00)
Pongamia	6.9 (2.81)	22.53 (28.32)
Calophyllum	8.00 (3.00)	11.10 (19.45)
Samadera	0.00 (1)	100.00 (90.00)
Odal	0.00 (1)	100.00 (90.00)
Marotty	7.77 (2.96)	13.51 (21.55)
Tween	9.00 (3.16)	0.00 (1.00)
Control	9.00 (3.16)	
CD (0.05)	0.79	55.55

(Figures given in parenthesis are transformed values)
*Mean of four replications

Table 4 Inhibition of mycelial growth of collar rot isolate of *R. solani* by oil cakes

Oil cake	Collar rot	
	*Mycelial growth (cm)	Percentage inhibition
Gingelly	5.74 (2.60)	34.17 (35.76)
Coconut	3.96 (2.23)	56.32 (48.61)
Neem	9 (3.16)	0.00 (1.00)
Groundnut	5.00 (2.45)	43.49 (41.24)
Control	9.0 (3.16)	
CD (0.05)	0.79	55.35

(Figures given in parenthesis are transformed values)
*Mean of four replications

Plate 4 Proving of Koch's postulates for collar rot pathogen

Plate 5 Proving Koch's postulates for web blight pathogen

Plate 4

Plate 5

Plate 6 *In vitro* inhibition of Collar rot isolate of *R. solani* by plant oils

T_1 - Neem oil
T_2 - Castor oil
T_3 - Pongamia oil
T_4 - Calophyllum oil
T_5 - Samadera oil
T_6 - Odal oil
T_7 - Marotty oil
T_8 - Tween

Plate 7 *In vitro* inhibition of Collar rot isolate of *R. solani* by oil cakes

T_1 - Neem cake
T_2 - Coconut cake
T_3 - Gingelly cake
T_4 - Groundnut cake

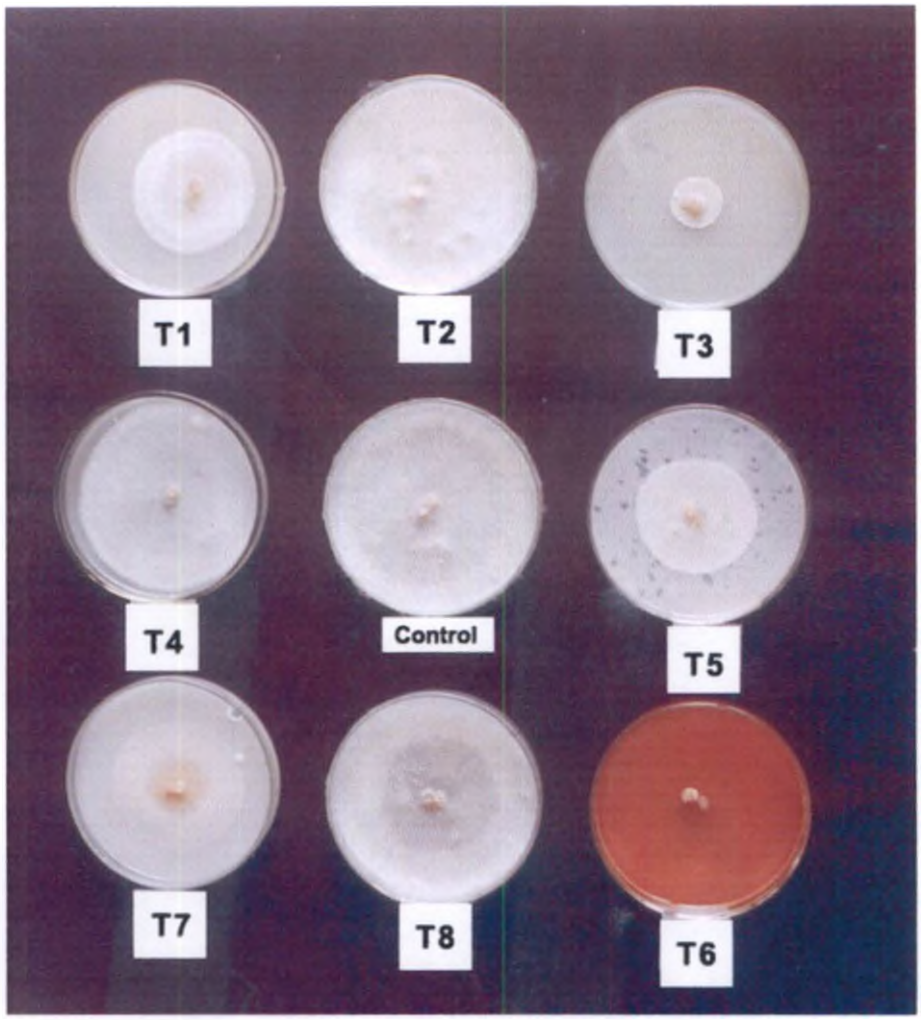


Plate 6

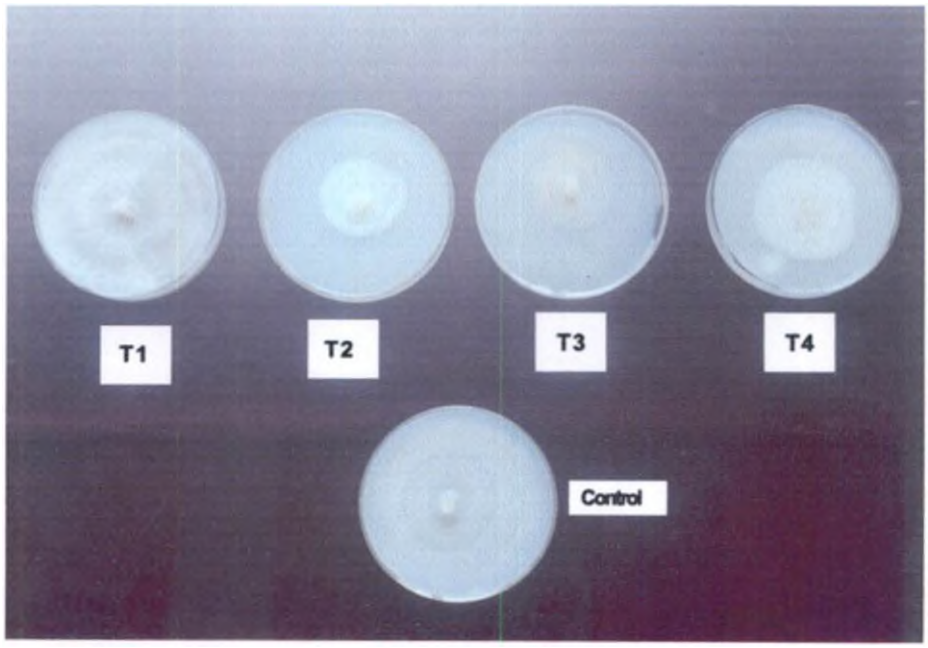


Plate 7

which were on par, in the *in vitro* suppression of the web blight isolate. However, neither castor oil nor Tween 80 had any inhibitory effect on the fungus (Table-3).

4.3.2 *In vitro* screening of oil cakes

Four locally available oil cakes *viz.*, neem, groundnut, gingelly and coconut cake were evaluated *in vitro* to check suppression of radial growth of *R. solani* isolates. Some of the oil cakes caused suppression of collar rot isolate whereas they did not affect the growth of web blight isolate.

4.3.2.1 In vitro screening of oil cakes for inhibition of collar rot isolate

All the oil cakes tested except neem cake significantly suppressed the mycelial growth of the collar rot isolate of *R.solani*. Among them, the maximum suppression of the collar rot pathogen was caused by coconut oilcake (48.61 per cent) followed by groundnut oil cake(41.24 per cent) and gingelly oil cake (35.75 per cent). The suppression caused *in vitro* by incorporation of coconut oil cake was significantly higher than all the other treatments. There was also significant difference in the inhibitory effect of groundnut oil cake and gingelly oil cake .Neem cake did not have any inhibitory effect on the test fungus (Plate-7, Table-4).

4.3.2.2 *In vitro* screening of oil cakes for inhibition of web blight isolate of *R. solani*

The oil cakes incorporated in the medium did not have much inhibitory effect on the web blight isolate of *R. solani*. In this case, only the coconut oilcake exerted suppressive effect which was to the tune of 6.29 per cent and was significant over the control. None of the other oilcakes tested had any inhibitory

Table 5 Inhibition of mycelial growth of web blight isolate of *R. solani* by oil cakes

Oil cake	Web blight	
	*Mycelial growth (cm)	Percentage inhibition
Gingelly	9.0 (3.16)	0.00 (1.00)
Coconut	8.16 (3.03)	6.29 (14.52)
Neem	9.00 (3.16)	0.00 (1.00)
Groundnut	9.00 (3.16)	0.00 (1.00)
Control	9.00 (3.16)	
CD (0.05)	0.10	

(Figures given in parenthesis are transformed values)
*Mean of four replications

Table 6 Effect of different ratios of Turmeric powder – Baking soda on mycelial growth of the collar rot isolate of *R. solani*

Turmeric powder – Baking Soda	Collar rot	
	*Mycelial growth (cm)	Percentage inhibition
10:1	8.67 (3.11)	2.47 (0.04)
4:1	7.61 (2.93)	15.44 (23.11)
3:2	8 (3.00)	11.10 (19.45)
Control	9.0 (3.16)	
CD (0.05)	0.19	

(Figures given in parenthesis are transformed values)
*Mean of four replications

Table 7 Effect of different ratios of Turmeric powder - Baking soda on the mycelial growth of the web blight isolate of *R. solani*

Turmeric powder – Baking Soda	Web blight	
	*Mycelial growth (cm)	Percentage inhibition
10:1	2.31 (1.82)	74.14 (59.41)
4:1	5.98 (2.64)	52.24 (46.26)
3:2	4.09 (2.25)	36.54 (37.17)
Control	9.0 (3.16)	
CD (0.05)	0.58	5.55

(Figures given in parenthesis are transformed values)
*Mean of four replications

Table 8 Effect of different concentrations of Rice husk ash on mycelial growth of the collar rot and web blight isolates of *R. solani*

Rice Husk Ash	Collar rot		Web blight	
	*Mycelial Growth (cm)	Percentage Inhibition	*Mycelial Growth (cm)	Percentage Inhibition
1 %	9.0	0.00 (1.00)	9.0	0.00 (1.00)
0.75 %	9.0	0.00 (1.00)	9.0	0.00 (1.00)
0.5 %	9.0	0.00 (1.00)	9.0	0.00 (1.00)
Control	9.0		9.0	
CD (0.05)				

(Figures given in parenthesis are transformed values)
*Mean of four replications

Plate 8 Effect of turmeric powder – baking soda on the *in vitro* growth of Collar rot isolate of R. solani

1. 10:1 combination
2. 4:1 combination
3. 3:2 combination

Plate 9 Effect of rice husk ash on the *in vitro* growth of Collar rot isolate of *R. solani*

1. 0.50 per cent
2. 0.75 per cent
3. 1.00 per cent

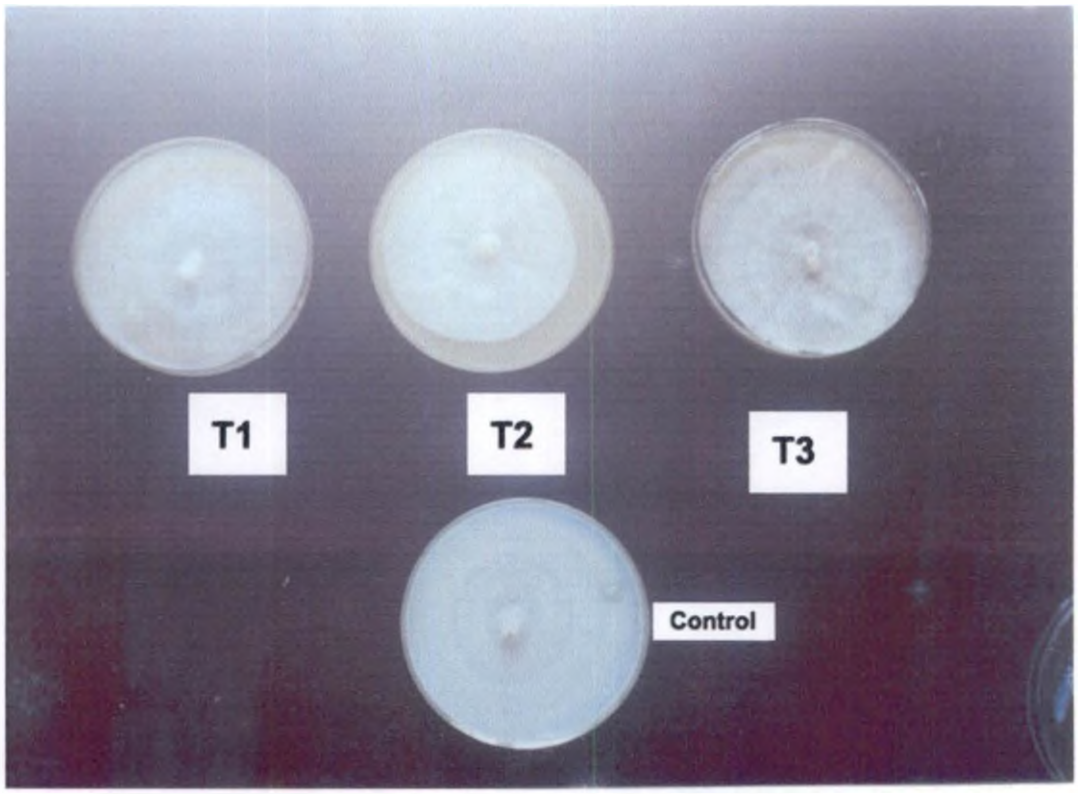


Plate 8

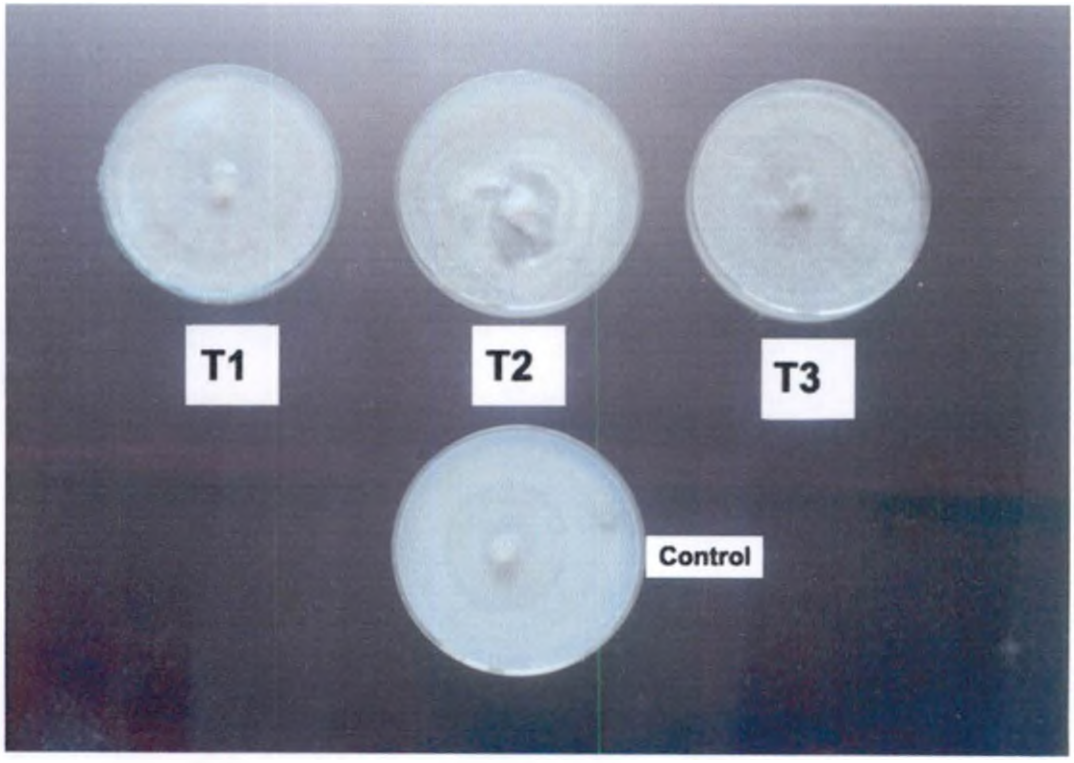


Plate 9

effect on the web blight isolate. The pathogen isolate attained full growth of 9.0 cm in media amended with these oilcakes (Table-5).

4.3.3 *In vitro* screening of Turmeric powder – baking soda(TP-BS)

Three concentrations of turmeric powder – baking soda *viz.*, 10:1, 4:1 and 3:2 were tried against the radial growth of *R. solani*.

4.3.3.1 *In vitro* screening of TP-BS for inhibition of collar rot isolate

All the three proportions of turmeric powder and baking soda caused significant suppression of the growth of the collar rot isolate of *R. solani* as compared to control. However, the 4:1 proportion of turmeric powder and baking soda was superior to the other combinations and caused a suppression of 15.44 per cent. In the media amended with this combination the mycelial growth of the collar rot isolate was only 7.61cm as compared to 8.67cm and 8.0 cm respectively in 10:1 and 3:2 combination of turmeric powder and baking soda (Table-6, Plate-8).

4.3.3.2 *In vitro* screening of TP-BS for inhibition of web blight isolate

Amending the medium with combinations of turmeric powder and baking soda caused significant suppression of the web blight isolate of *R. solani.* The effect of all the three combinations on the mycelial growth of the isolate differed significantly. The 10:1 proportion of turmeric powder and baking soda caused very high suppression of 74.14 per cent and the mycelial growth of the pathogen in the amended medium was only 2.31cm as compared to full growth or 9.0 cm. in the untreated control. The other combinations *i.e.*, 4:1 and 3:2 also considerably reduced the growth of the isolate *in vitro.* They resulted in suppression of 52.24 per cent and 36.54 per cent, respectively (Table-7).

Table 9 *Effect of plant oils on mycelial growth on sclerotial germination at different time intervals

Oils	0 h		24 h		48 h		72 h	
	Mycelial growth	Percentage inhibnition	Mycelial growth (cm)	Percentage inhibition	Mycelial growth	Percentage inhibition	Mycelial growth	Percentage inhibition
Marotty	9.0 (3.16)	0.00 (1.0)	7.5 (2.91)	11.95 (3.6)	2.47 (1.86)	72.56 (8.58)	0.00 (1.0)	100.00 (10.05)
Odal	5.07 (2.46)	41.19 (6.5)	0.00 (1)	100.00 (10.05)	0.00 (1)	100.00 (10.05)	0.00 (1.0)	100.00 (10.05)
Cal	8.97 (3.15)	0.32 (1.15)	9.00 (3.16)	0.00 (1.00)	9.00 (3.16)	0.00 (1.00)	9.00 (3.16)	0.00 (1.00)
Sam	4.93 (2.43)	43.34 (6.66)	0.00 (1)	100.00 (10.05)	0.00 (1.00)	100.00 (10.05	0.00 (1.0)	100.00 (10.05)
Tween	9.00 (3.16)	0.00 (1.00)	9.00 (3.16)	0.00 (1.00)	9.00 (3.16)	0.00 (1.00)	9.00 (3.16)	0.00 (1.00)
Neem	6.67 (2.77)	23.38 (4.94)	0.93 (1.38)	100.00 (10.05)	0.00 (1)	67.37 (8.27)	0.00 (1.0)	100.00 (10.05)
Castor	8.33 (3.05)	4.16 (2.27)	8.00 (3.00)	11.10 (3.48)	9.00 (3.16)	0.00 (1.00)	8.33 (3.05)	4.16 (2.27)
Pongamia	9.00 (3.16)	0.00 (1.00)	7.50 (2.91)	9.77 (3.28)	9.00 (3.16)	0.00 (1.00)	8.57 (3.09)	4.01 (2.24)
Control	9.00 (3.16)		9.00 (3.16)		9.00 (3.16)		9.00 (3.16)	
CD	2.11		1.14		0.19		0.72	

(Figures given in parenthesis are transformed values)

* Mean of three replications

4.3.4 *In vitro* screening of Rice husk ash(RHA)

4.3.4.1 *In vitro* screening of TP-BS for inhibition of collar rot and web blight isolates

Three ratios of RHA *viz.*, one per cent, 0.5 per cent and 0.75 per cent were tested for their effects on *R. solani.* None of the concentrations inhibited the growth of the collar rot pathogen *in vitro.* Similar results were obtained for web blight pathogen also (Table-8, Plate-9) except for 29 per cent suppression at 0.75 per cent concentration.

4.4. Studies on mycelial growth on sclerotial germination

Effect of different plant oils at 5 per cent concentration, oil cakes at 10 per cent concentration, three ratios of turmeric powder baking soda *viz.*, 10:1, 4:1 and 3:2 and three concentrations of RHA *viz.*, 0.5 per cent, 0.75 per cent and 1 per cent on mycelial growth on sclerotial germination at different time intervals *viz.*, 0, 24, 48 and 72 hours were recorded. As the culture of collar rot pathogen produced sclerotia rarely, the culture of web blight pathogen which produced sclerotia profusely on completion of mycelial growth in the petriplates was used to determine the effect of the above said treatments on sclerotial germination of *R. solani.*

4.4.1 Plant oils

The effect of treating the sclerotia of web blight causing isolate of *R.solani* varied with plant oils. Samadera oil, odal oil and neem oil treatment showed suppression of sclerotial germination at different time intervals. The effect of marotti oil, calophyllum oil, castor oil and Tween was more or less similar to the untreated control. Among the oils with suppressive effect on germination, samadera oil and odal oil produced appreciable effect even at 0 h *i.e.*,

Figure 1. Effect of plant oils on mycelial growth on sclerotial germination at different time intervals

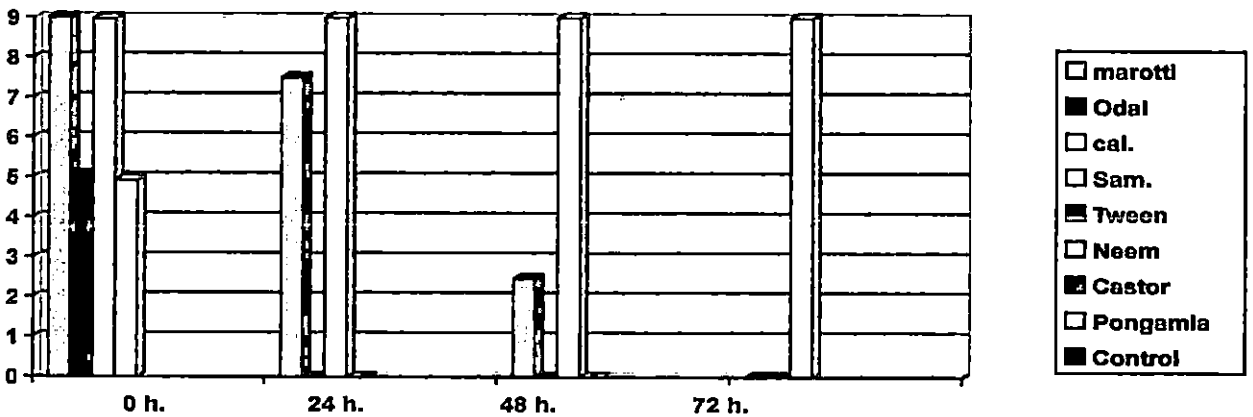


Figure-2 Effect of oil cakes on mycelial growth on sclerotial germination at different time intervals

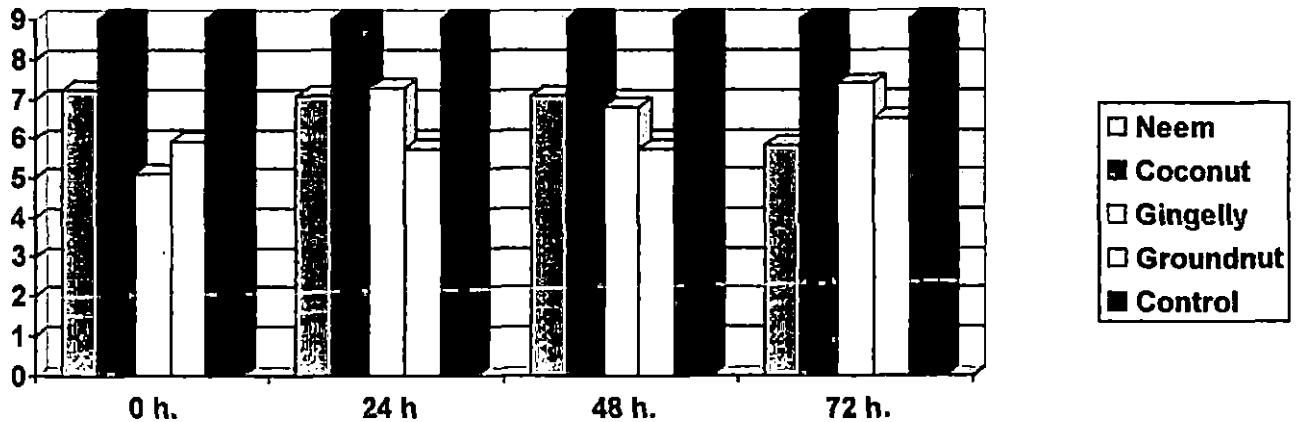


Table 10 *Effect of oil cakes on mycelial growth on sclerotial germination at different time intervals

Oil cakes	0 h		24 h		48 h		72 h	
	Mycelial growth	Percentage inhibition	Mycelial growth (cm)	Percentage inhibition	Mycelial growth	Percentage inhibition	Mycelial growth	Percentage inhibition
Neem	7.23 (2.86)	21.78	7.07 (2.84)	21.44	7.07 (2.84)	21.44	5.83 (2.61)	35.22
Coconut	9.00 (3.00)	0.00	9.00 (3.00)	0.00	9.00 (3.00)	0.00	9.00 (3.00)	0.00
Gingelly	5.10 (2.46)	43.32	7.27 (2.87)	19.22	6.80 (2.79)	24.44	7.40 (2.89)	17.7
Groundnut	5.93 (2.63)	32.54	5.73 (2.59)	40.64	5.73 (2.59)	40.64	6.50 (2.73)	27.65
Control	9 (3.00)		9 (3.00)		9 (3.16)		9 (3.00)	
CD	2.73	-	2.05	-	1.24	-	-	-

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

immediately after dipping in this oils. Neem oil treatment showed gradual progress in the inhibition of mycelial growth on sclerotial germination with advent of time. At 72 h of incubation in neem oil there was no mycelial growth on germination of the sclerotia (Table-9, Figure-1).

4.4.2 Oil cakes

The mycelial growth on sclerotial germination was variously affected by treatment with different oil cakes. Treatment of the sclerotia of the collar rot pathogen with the oilcakes, in general, caused mild suppression of germination. Among the oil cakes gingelly cake, groundnut cake and neem cake caused suppression of mycelial growth on germination of sclerotia at different time intervals. Gingelly cake produced maximum suppression of 24.44 per cent at 48 h of incubation. Maximum suppression of 40.64 per cent was obtained with groundnut cake at 24 h of incubation which was significant over control. Neem oil cake exerted inhibition to the tune of 21.78 per cent to 35.22 per cent. Coconut oil cake did not, however, have any effect on sclerotial germination and was on par with control at all levels of incubation studied (Table-10, Figure-2).

4.4.3 Indigenous materials

The performance of indigenous materials in suppressing the mycelial growth on sclerotial germination of *Rhizoctonia solani* at the four different time intervals are presented in Table-11. Highly varying and low percentage suppression was exhibited by the different treatments. Except the observations at 48 h time interval all the others were statistically significant.

The mycelial growth on sclerotial germination was affected by treatment with RHA one per cent and 0.75 per cent and turmeric powder- baking soda in the ratio 4:1 immediately after treating the sclerotia with these indigenous materials. The suppression caused by RHA – one per cent was initially very high

Figure-3 Effect of Indigenous materials on mycelial growth on sclerotial germination at different time intervals

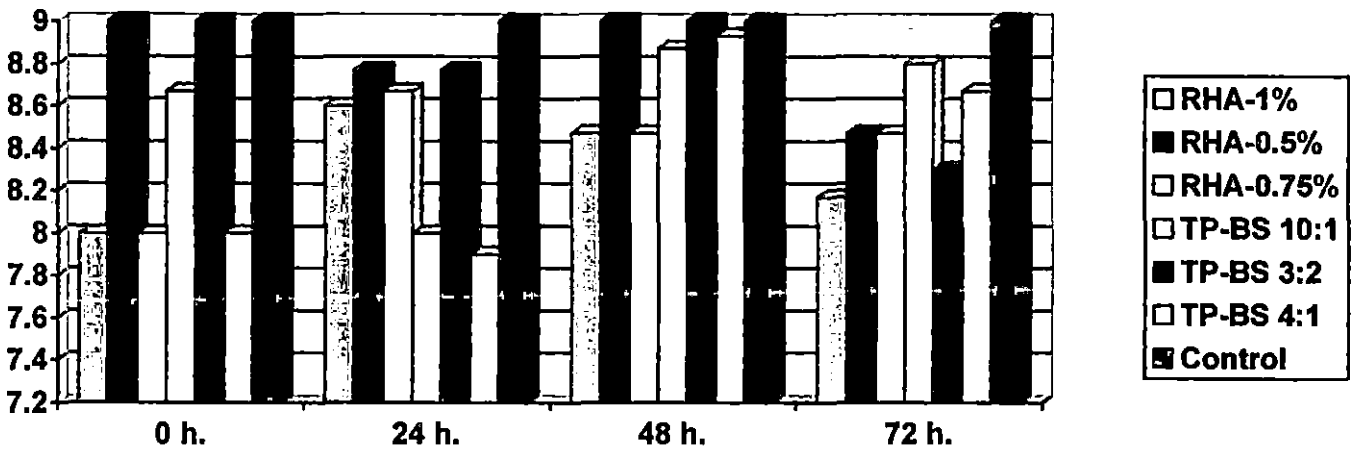


Table 11 *Effect of indigenous materials on mycelial growth on sclerotial germination at different time intervals

Turmeric powder Baking soda	0 h		24 h		48 h		72 h	
	Mycelial growth	Percentage inhibition	Mycelial growth (cm)	Percentage inhibition	Mycelial growth	Percentage inhibition	Mycelial growth	Percentage inhibition
RHA - 1%	8.00 (3.00)	11.1 (3.48)	8.60 (3.09)	5.37 (2.52)	8.17 (3.02)	0.00 (1.00)	8.17 (3.02)	9.2 (3.19)
0.5 %	9.00 (3.16)	0.00 (1.00)	8.77 (3.12)	2.55 (1.88)	9.00 (3.16)	0.00 (1.00)	8.47 (3.07)	5.82 (2.61)
0.75%	8.00 (3.00)	11.1 (3.48)	8.67 (3.10)	3.44 (2.11)	8.47 (3.07)	0.00 (1.00)	8.47 (3.07)	5.82 (2.61)
TP-BS 10:1	8.67 (3.10)	2.33 (1.83)	8.00 (3.00)	11.1 (3.48)	8.87 (3.14)	1.27 (1.51)	8.80 (3.13)	1.94 (1.72)
TP-BS 3:2	9.00 (3.16)	0.00 (1.00)	8.77 (3.12)	2.44 (1.85)	9.00 (3.16)	0.00 (1.00)	8.30 (3.04)	7.42 (2.90)
TB-BS 4:1	8.00 (3.00)	11.1 (3.48)	7.90 (2.98)	15.21 (4.03)	8.93 (3.15)	0.60 (1.26)	8.67 (3.1)	1.63 (1.62)
Control	9.00 (3.16)		9.00 (3.16)		9.00 (3.16)		9.00 (3.16)	
CD	0.47		0.31		-		0.44	

(Figures given in parenthesis are transformed values)

* Mean of three replications

(11.1 per cent) as compared to other time intervals. However the decrease in suppression at 48 h incubation improved slightly after further incubation. The effect of this treatment on mycelial growth on sclerotial germination was similar to that of RHA - 0.75 per cent at 72 h of incubation. Turmeric powder- baking soda in the ratio 4:1 caused an appreciable reduction in mycelial growth on sclerotial germination at initial period *i.e.*, immediately after treatment (Figure-3).

4.4.1. Sclerotial formation

Sclerotial formation was observed for the treatments as indigenous materials and also in control treatments in the case of web-blight isolate. For treatments with oils and oil cakes sclerotial formation was not observed. In the case of RHA six sclerotia were produced on completion of mycelial growth in the petriplates and for TP-BS five sclerotia were produced on completion of mycelial growth in petriplates which was less than that in control plates which recorded production of fourteen sclerotia on completion of mycelial growth. As the culture of the collar rot isolate produced sclerotia rarely sclerotial formation was not noticed in the control or treatment plates in the case of any of the treatments mentioned above.

4.5. *In vivo* studies

4.5.1. Collar rot incidence

There was minimum incidence of collar rot disease in the cowpea plants treated with copper oxychloride, fluorescent pseudomonad (P22), *Trichoderma*, rice husk ash 750 gm^{-2}, pongamia oil, odal oil and turmeric powder - baking soda at 3:2 proportion. There was complete suppression of the collar rot disease by the application of gingelly oil cake. All the other treatments recorded varying levels of collar rot almost in the same level as that seen in the inoculated control. The application of oils and oil cakes, in general, caused reduction in disease incidence of collar rot as compared to control. There was absolutely no disease incidence in

Table 12 *In vivo* studies on incidence of collar rot of cowpea

Treatment		*Percentage disease incidence
T_1	Odal oil	1.10
T_2	Pongamia oil	1.10
T_3	Coconut cake	6.56
T_4	Gingelly cake	0.00
T_5	Neem cake	9.64
T_6	TP-BS 10:1	4.37
T_7	TP-BS 4:1	4.37
T_8	TP-BS 3:2	2.35
T_9	RHA 500 g/m^2	9.64
T_{10}	RHA 750 g/m^2	1.10
T_{11}	RHA 1000 g/m^2	4.37
T_{12}	*Trichoderma*	2.35
T_{13}	P-1	6.56
T_{14}	P-22	1.10
T_{15}	Copper oxychloride	1.53
T16	Inoculated control mean	6.56
	CD	-NS

* Mean of four replications

Plate 10 Effect of odal oil on collar rot of cowpea

Plate 11 Effect of pongamia oil on collar rot of cowpea

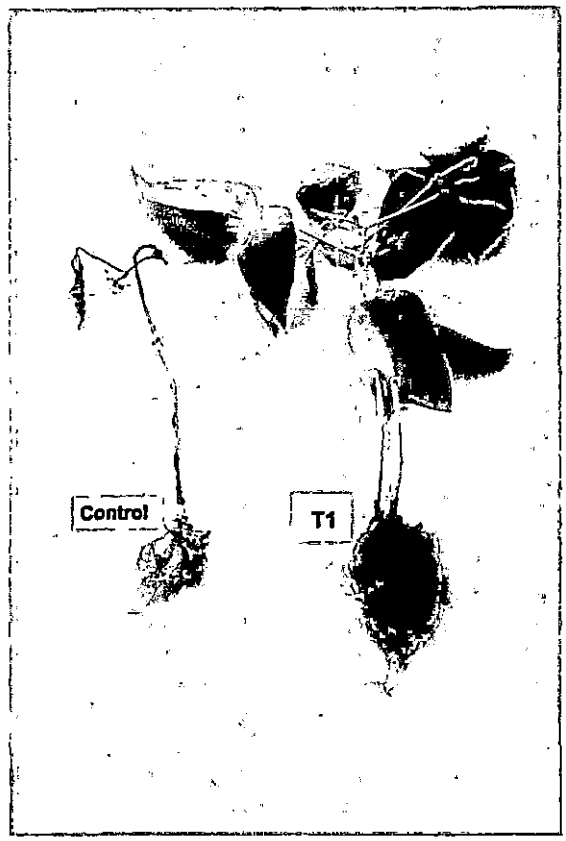


Plate 10

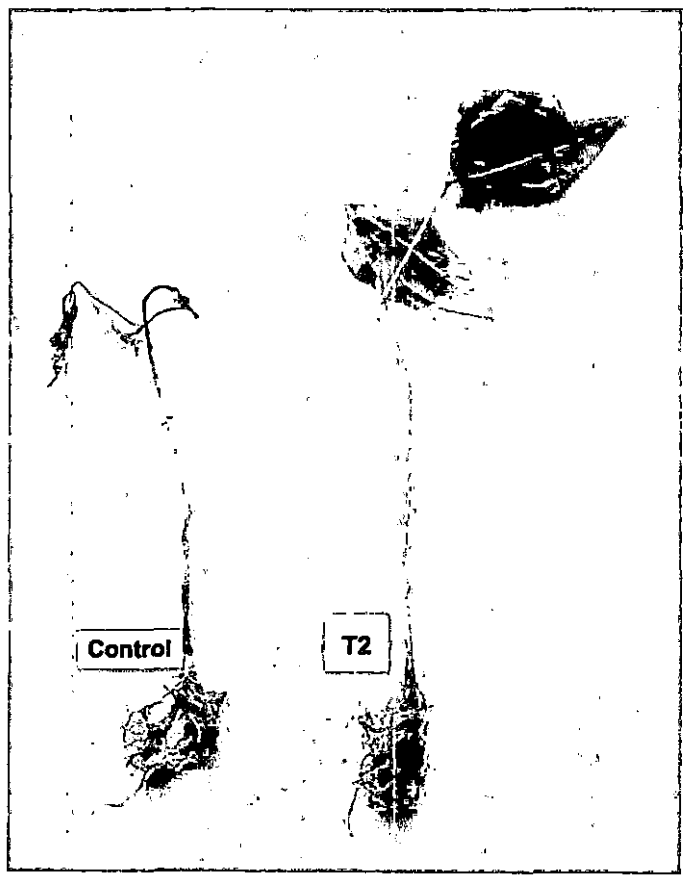


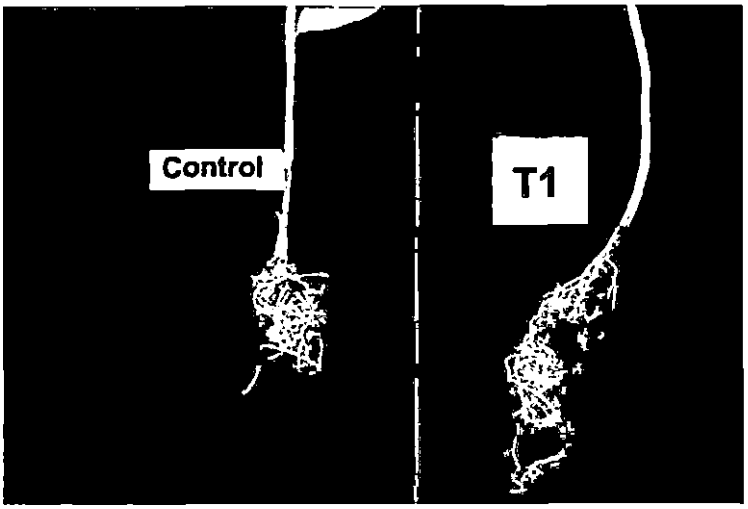
Plate 11

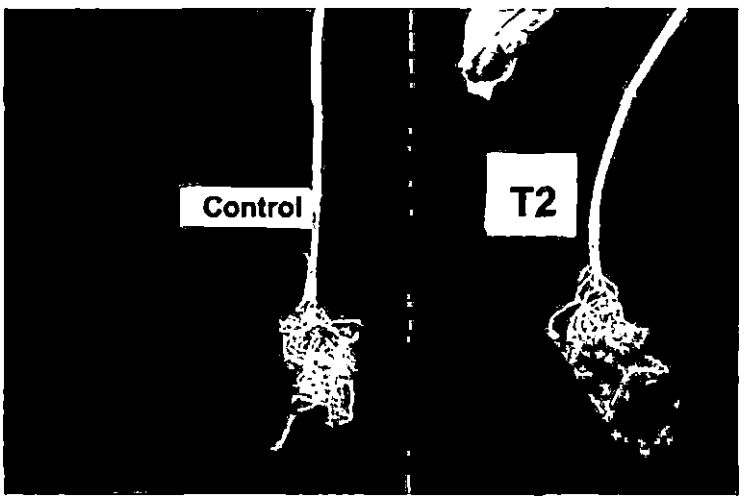
Plate 12 Effect of Turmeric Powder – Baking soda on collar rot of cowpea

T_1 - 10:1 combination

T_2 - 4:1 combination

T_3 - 3:2 combination





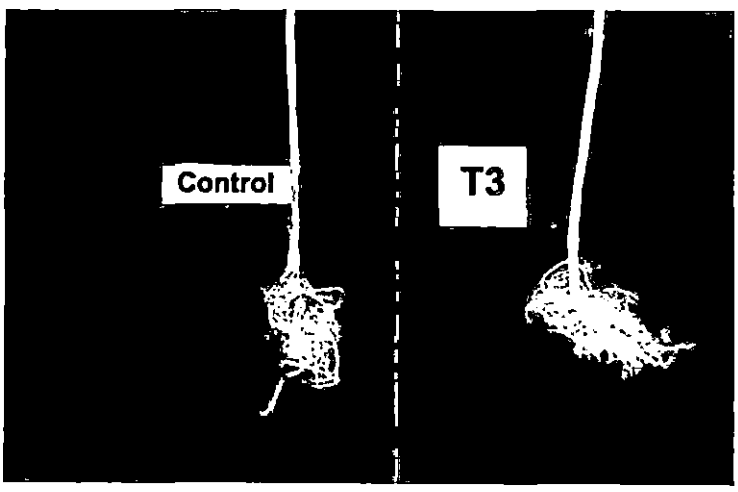


Plate 12

Table 13 *Effect of different treatments on disease intensity of web blight of cowpea

Treatment		Disease index before treatment application	Disease index after 1st treatment application	Disease index after 2nd treatment application
T_1	Odal oil	25.33 (5.15)	28.74 (5.45)	26.27 (5.22)
T_2	Pongamia oil	24.15 (5.01)	24.81 (5.08)	25.97 (5.19)
T_3	Coconut cake	34.01 (5.92)	25.67 (5.16)	12.99 (3.74)
T_4	Gingelly cake	14.59 (3.95)	12.79 (3.71)	12.17 (3.63)
T_5	Neem cake	24.07 (5.01)	11.69 (3.56)	16.02 (4.13)
T_6	TP-BS 10:1	4.76 (21.65)	34.17 (5.93)	19.38 (4.51)
T_7	TP-BS 4:1	28.14 (5.40)	23.74 (4.97)	19.70 (4.55)
T_8	TP-BS 3:2	20.89 (4.68)	25.91 (5.19)	23.17 (4.92)
T_9	RHA 500 g/m2	16.44 (4.18)	23.94 (4.99)	26.58 (5.25)
T_{10}	750 g/m2	27.55 (5.34)	17.77 (4.33)	24.18 (5.02)
T_{11}	1000 g/m2	33.68 (5.89)	38.01 (6.25)	21.30 (4.72)
T_{12}	Trichoderma	32.19 (5.76)	32.06 (5.75)	21.30 (4.72)
T_{13}	P-1	26.61 (5.25)	14.22 (3.90)	18.35 (4.40)
T_{14}	P-22	19.72 (4.55)	9.87 (3.30)	16.89 (4.23)
T_{15}	Copper oxychloride	27.89 (5.38)	13.09 (3.75)	16.11 (4.14)
	Inoculated control mean	19.77 (4.51)	36.75 (6.17)	15.92 (4.09)
	CD	-	1.86	-

(Figures given in parenthesis are transformed values)

* Mean of four replications

the treatment involving application of gingelly oil cake. Odal oil and Pongamia oil also caused reduced disease incidence to the tune of 1.10 per cent. This was superior to the effect of application of the chemical fungicide, copper oxychloride. There was no difference in the disease incidence in coconut oil cake applied and the pathogen treated control.

Application of turmeric powder - baking soda at various proportions caused reduction in the disease incidence. Among the three proportions, the 3:2 combination of turmeric powder and baking soda resulted in lower disease incidence (2.35 per cent). Value of 4.37 per cent was recorded in the other combinations.

Incorporation of rice husk ash at 750 gm^{-2} reduced the incidence of collar rot in cowpea. The effect was better than that of the fungicidal check. Rice husk ash at 1000gm^{-2} also resulted in lowered disease incidence (4.37 per cent) whereas application of lesser quantity *i.e.*, 500 gm^{-2} did not have any suppressive effect.

Pseudomonas fluorescens isolate P-22 caused reduction in disease incidence. The disease incidence in this treatment was only1.10 per cent which was more than that in chemical control.

However, the isolate P-1 did not reduce the disease incidence and was similar to the treated control (Table-12, Plate-10, 11 and 12).

4.5.2 Disease Index

The disease index was calculated based on the damage caused by web blight pathogen to the foliage. Data on disease index before inoculum application, after first treatment application (two days after inoculum application) and after second treatment application (five days after inoculum application) are given in Table-13.

Gingelly cake recorded lowest disease index for the three phases. Coconut cake also succeeded in reducing disease index. Neem cake showed reduction in disease index after first treatment application.

In the case of plants treated with odal oil the disease index after first treatment application showed an increase over the untreated plants. But with the receipt of second application of treatments there was a gradual reduction in the disease index parameter (26.27 per cent). But for pongamia oil the disease index which was low before treatment application went on increasing after each dosage of treatments.

In the case of turmeric powder – baking soda 4:1 ratio showed gradual decrease in disease index with the two dosages of treatment application. 3:2 ratio also performed better while 10:1 ratio exhibited poor performance.

750 gm^{-2} and 1000 gm^{-2} dosage of RHA can be considered equally effective in reducing disease index while 500 gm^{-2} concentration only increased the disease index.

Trichoderma and *Pseudomonas fluorescens* can be considered equally effective in reducing disease index after application of treatments. Copper oxychloride also exhibited superior performance in reducing disease index.

4.6. Biometric observations

Statistical analysis showed that there was no significant difference between treatments on shoot length, root length and number of nodules.

Table 14 Biometric parameters of cowpea

Treatments		*Biometric parameters				
		Shoot length (cm)	Root length (cm)	Number of pods	Pod weight (g)	Number of Nodules
T_1	Odal oil	188.25	23.88	20.50	149.43	23.25
T_2	Pongamia oil	189.50	27.38	25.50	197.19	19.50
T_3	Coconut cake	220.25	23.38	40.00	297.68	38.63
T_4	Gingelly cake	224.25	27.75	36.25	286.15	61.50
T_5	Neem cake	220.25	33.25	32.75	244.26	54.25
T_6	TP-BS 10:1	140.00	26.50	24.50	205.45	59.00
T_7	TP-BS 4:1	198.63	24.38	30.00	279.17	42.63
T_8	TP-BS 3:2	195.25	28.13	22.25	176.33	22.25
T_9	RHA 500g/m^2	190.00	32.50	22.00	189.93	16.75
T_{10}	RHA 750 g/m^2	154.88	24.13	32.00	273.99	44.00
T_{11}	RHA 1000 g/m^2	134.75	23.50	22.00	179.19	68.88
T_{12}	*Trichoderma*	208.50	28.25	14.50	109.27	55.13
T_{13}	*P.fluorescens* P-1	186.50	32.88	19.75	122.31	44.75
T_{14}	*P.fluorescens* P-22	148.13	26.13	20.25	165.70	89.13
T_{15}	Copper oxychloride	190.38	27.75	24.50	173.45	20.25
T_{16}	Inoculated control	199.125	24.75	22.25	133.54	21
	CD (0.05)	-	-	10.52	100.43	-

*Mean of four replications

4.6.1. Shoot length

The maximum increase in shoot length was observed for treatment with gingelly cake (224.25 cm) followed by coconut cake and neem cake (220.25 cm). Shoot length was found to be enhanced for plants treated with *Trichoderma* (208.50 cm) and TP-BS 4:1 ratio (198.63 cm) in comparison to inoculated control (199.13 cm). There was reduction in shoot length of plants treated with odal oil (188.25 cm), pongamia oil (189.50 cm), TP-BS 10:1 ratio (140 cm), TP-BS 3:2 ratio (195.25 cm), RHA 500 gm^{-2} (190 cm), RHA 750 gm^{-2} (154.88 cm), RHA 100 gm^{-2} (134.75 cm), P-1 (186.50 cm), P-22 (148.13 cm) and copper oxychloride (190.38 cm) over the controls (Table-14).

4.6.2. Root length

Longest root length was noted in the case of plants treated with neem cake (33.25cm) ,P-1 (32.88 cm), RHA 500 gm^{-2} (32.50 cm) *Trichoderma* (28.25 cm), TP-BS 3:2 ratio (28.13 cm), gingelly cake (27.75 cm), copper oxychloride (27.75 cm), pongamia oil (27.38 cm), TP-BS 10:1 ratio (26.5 cm) and P-22 (26.13 cm) recorded longer root length on comparison with inoculated control (24.75 cm). The treatments odal oil (23.88cm) and coconut cake (23.38 cms) exhibited almost the same root length. TP-BS 4:1 ratio (24.38 cm), RHA 750 gm^{-2} (24.13 cm) and RHA 1000 gm^{-2} (23.50 cm) gave almost same value as that exhibited by inoculated control (Table-14).

4.6.3. Number of pods

Highest number of pods were obtained from plants treated with coconut cake (40) followed by gingelly cake (36.25). RHA 500 gm^{-2} (22), RHA 750 gm^{-2} (32), RHA 1000 gm^{-2} (22), copper oxychloride (24.50) and inoculated control (22.25) showed significant increase in number of pods. In general there was significant increase in number of pods in almost all the treatments. Plants treated

with odal oil (20.5), pongamia oil (25.5), neem cake (32.75), TP-BS 10:1 ratio (24.5), TP-BS 4:1 ratio (30) and TP-BS 3:2 ratio (22.25) recorded significant increase in number of pods on comparison with inoculated control (20.5). P-1 (19.75) and P-22 (20.25) recorded lower number of pods than the control (Table-14). Lowest number of pods was noticed in the case of *Trichoderma* (14.5) treated plants.

4.6.4. Pod weight

Pod weight also showed statistical significance with respect to the different treatments. Highest pod weight was recorded for coconut cake (297.68 g) followed by gingelly cake (286.15 g). Significant increase in pod weight was recorded for pongamia oil (197.19 g), neem cake (244.26 g), TP-BS 10:1 (205.45 g), TP-BS 4:1 ratio (279.17g), RHA 500 gm^{-2} (189.93g), RHA 750 gm^{-2} (273.99 g), RHA 1000 gm^{-2} (179.19 g) ,copper oxychloride (173.45 g), P-22 (165.70 g), TP-BS 3:2 ratio (176.33 g) and odal oil (149.43 g) showed significant increase in pod weight over inoculated control (133.54 g). *Trichoderma* (109.27 g) and P-1 (122.31 g) showed reduction in pod weight over control (Table-14).

4.6.5. Number of nodules

Data in Table-14 reveals that plants treated with P-22 (89.13) recorded the highest number of nodules. Almost all other treatments as odal oil (23.25), coconut cake (38.63), gingelly cake (61.50), neem cake (54.25), TP-BS 10:1 ratio (59), TP-BS 4:1 (42.63); TP-BS 3:2 ratio (22.25), RHA 750 gm^{-2} (44), RHA 1000 gm^{-2} (68.88), Trichoderma (55.13) and P-1 (44.75) showed higher root nodules when compared to inoculated control (21). Plants treated with pongamia oil (19.50), RHA 500 gm^{-2} (16.75) and copper oxychloride (20.25) recorded reduction in root nodules over the control.

Table 15 *Root and shoot weight

Treatments		Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)
T_1	Odal oil	20.12	6.43	6.28	1.66
T_2	Pongamia oil	43.51	9.96	11.37	2.58
T_3	Coconut cake	34.97	8.97	9.13	2.84
T_4	Gingelly cake	42.01	11.79	5.71	2.01
T_5	Neem cake	56.51	13.49	15.91	3.42
T_6	TP-BS 10:1	36.81	11.40	9.30	2.66
T_7	TP-BS 4:1	38.55	8.15	12.88	2.17
T_8	TP-BS 3:2	41.95	9.92	10.26	2.51
T_9	RHA 500g/m^2	40.15	8.20	11.38	3.60
T_{10}	750 g/m^2	39.26	8.73	8.74	2.10
T_{11}	1000 g/m^2	36.64	6.96	9.16	1.71
T_{12}	Trichoderma	48.76	11.71	10.55	2.91
$_{13}$	P-1	39.39	9.42	10.02	2.50
T_{14}	P-22	32.60	7.87	6.50	1.61
T_{15}	Copper oxy chloride	53.59	9.06	7.34	2.26
T_{17}	Inoculated control	34.35	7.18	8.54	1.99
	CD (0.05)	-	-	-	-

* Mean of four replications

4.6.6. Fresh shoot weight

Highest fresh shoot weight was noticed for plants treated with neem cake (56.51 g) followed by copper oxychloride (53.59 g). Increase in fresh shoot weight was observed for plants treated with pongamia oil (43.51 g), coconut cake (34.97 g), gingelly cake (42.01), TP-BS 10:1 ratio (36.81 g), TP-BS 4:1 ratio (38.55 g), TP-BS 3:2 (41.95 g), RHA 500 gm^{-2} (40.15 g), RHA 750 gm^{-2} (39.26 g), RHA 1000 gm^{-2} (36.64 g), *Trichoderma* (48.76 g) and P-1 (39.39g) over inoculated control(34.35 g). However, the increase of shoot weight were not statistically significant. Treatments such as P-22 (32.6 g) and odal oil (20.12 g) showed lower shoot weight over control (Table-15).

4.6.7. Dry shoot weight

Neem cake recorded highest dry shoot weight (13.49 g) though not statistically significant. Higher shoot weight on drying was observed for gingelly cake (11.79 g), *Trichoderma* (11.71 g), TP-BS 10:1 ratio (11.40 g), pongamia oil (9.96 g), TP-BS 3:2 ratio (9.92 g), P-1 (9.42 g), copper oxychloride (9.06 g), coconut cake (8.97 g), RHA 750 gm^{-2} (8.73 g), RHA 500 gm^{-2} (8.20 g), TP-BS 4:1 ratio (8.15 g) and P-22 (7.87 g) over inoculated control (7.18 g). Lower dry shoot weight was noticed in the case of RHA 1000 gm^{-2} (6.96 g) and odal oil (6.43 g) over the control (Table-15).

4.6.8. Fresh root weight

Highest fresh root weight in the case of treatments was observed for neem cake (15.91 g) followed by RHA 500 gm^{-2} (11.38 g) and pongamia oil (11.37 g). Comparatively higher fresh root weights were observed for the treatments TP-BS 10:1 ratio (9.30 g), RHA 1000 gm^{-2} (9.16 g), coconut cake (9.13 g) and RHA 750 gm^{-2} (8.74 g) over inoculated control (8.54 g). The treatments were not statistically significant. Treatments such as copper oxychloride (7.34 g), P-22

(6.50 g), odal oil (6.28 g) and gingelly cake (5.71 g) gave lower fresh root weight on comparison with control (Table-15).

4.6.9. Dry root weight

Of the treatments highest dry root weight was observed with RHA 500 gm^{-2} (3.60 g) followed by neem cake (3.42 g). Higher values were noticed for *Trichoderma* (2.91 g), coconut cake (2.84 g), TP-BS 10:1 ratio (2.66 g), pongamia oil (2.58 g), TP-BS 3:2 ratio (2.51 g), P-1 (2.50 g), copper oxchloride (2.26 g), TP-BS 4:1 ratio (2.17 g), RHA 750 gm^{-2} (2.10 g) and gingelly cake (2.01 g) on comparison with inoculated control (1.99 g). Lower dry root weight was seen for treatments RHA 1000 gm^{-2} (1.71 g), odal oil (1.66 g) and P-22 (1.61 g) when compared to control (Table 15).

4.7 Biochemical analysis

4.7.1. Protein

Data (Table-16) on protein content on statistical analysis revealed that the highest protein content was observed for plants treated with TP-BS 10:1 ratio (148 µg) followed by T-BS 4:1 ratio with a protein content of 120 µg. There was increase in protein content over the inoculated control (80.67 µg) for plants treated with gingelly cake (93.33 µg)and neem cake (89 µg). Plants which received treatments as odal oil (78.33 µg), *Trichoderma* (75.33 µg), P-22 (74 µg) and TP-BS 3:2 ratio (73 µg) proved to contain lower protein content. Treatments as P-1 (65.67 µg), RHA 750 gm^{-2} (64.33 µg), RHA 1000 gm^{-2} (60.67) and pongamia oil (60.33 µg) gave lower protein content than both controls. Same protein content was noticed in plants which received the treatments, coconut cake (55 µg) and RHA 500 gm^{-2} (55 µg) . Lowest protein content of 10.47 µg was observed for plants which were treated with copper oxychloride.

Table 16 *Effect of different treatments on changes in protein, carbohydrate, phenol, PO, PPO and PAL activity in cowpea leaves inoculated with *R. solani*

Treatments		Protein	Carbohydrate	Phenol	PO	PPO	PAL
T_1	Odal oil	78.33	11.67	34.33	0.49	0.19	0.62
T_2	Pongamia oil	60.33	25.33	37.67	0.29	0.03	0.59
T_3	Coconut cake	55.00	12.50	30.00	0.36	0.36	0.60
T_4	Gingelly cake	93.33	25.40	28.67	0.73	0.30	0.62
T_5	Neem cake	89.00	11.60	35.61	0.54	0.20	0.57
T_6	TB-BS 10:1	148.00	8.47	37.00	4.50	0.13	0.60
T_7	TP-BS 4:1	120.00	9.73	30.00	4.36	0.29	0.60
T_8	TP-BS 3:2	73.00	8.47	31.33	1.94	0.43	0.59
T_9	RHA 500 g/m^2	55.00	9.60	34.33	2.01	0.30	0.59
T_{10}	750 g/m^2	64.33	9.67	27.33	2.29	0.10	0.60
T_{11}	1000 g/m^2	80.67	9.47	33.33	1.67	0.25	0.62
T_{12}	Trichoderma	75.33	11.93	32.00	2.59	0.14	0.51
T_{13}	P-1	65.67	9.13	37.00	2.28	0.52	0.62
T_{14}	P-22	74.00	11.10	42.00	0.57	0.12	0.59
T_{15}	Copper oxychloride	10.47	9.87	31.33	0.70	0.20	0.61
T_{16}	Inoculated control	80.67	7.28	39	0.32	0.20	0.60
	CD (0.05)	-	5.69	-	1.39	-	-

*Mean of three replications

4.7.2. Carbohydrate

Significant difference was noticed for the various treatments on analysis of carbohydrate content in the leaves. Significantly high carbohydrate was noticed in plants treated with gingelly cake (25.40) followed by pongamia oil (25.33). Lowest carbohydrate content was observed in plants which served as inoculated control (7.28). Same carbohydrate content was observed for plants treated with TP-BS 10:1 (18.47) and TP-BS 3:2 ratio (8.47) which was the lowest carbohydrate content noticed among the different treatments. Plants treated with coconut cake (12.53), *Trichoderma* (11.93), odal oil (11.67), neem cake (11.60) and P-22 (11.00) recorded higher carbohydrate content . Higher carbohydrate content was also observed for plants treated with copper oxychloride (9.87), TP-BS 4:1 ratio (9.73), RHA 500 gm^{-2} (9.60), RHA 750 gm^{-2} (9.07) over inoculated control (Table-16).

4.7.3 Phenol

Phenol content was highest (42) for plants treated with P-22 culture followed by inoculated control with phenol content of 39. Phenol content of 37.67 was recorded for pongamia oil. Lower phenol content was observed with TP-BS 3:2 ratio (31.33), copper oxychloride (31.33), odal oil (34.33) and RHA 500 gm^{-2} (34.33). Lowest phenol content was noticed for plants treated with RHA 750 gm^{-2} (27.33). All other treatments recorded lower phenol content when compared with control (Table-16).

4.7.4 Peroxidase

Significantly wide variation was observed for peroxidase content of cowpea plants for the different treatments with TP-BS 10:1 ratio recording highest peroxidase content of 4.5 followed by TP-BS 4:1 ratio having a value of 4.36. Lowest peroxidase activity was noticed for plants treated with pongamia oil

(0.29). Significantly higher values were noticed for plants treated with RHA 750 gm^{-2} (2.29), *Trichoderma* (2.59), P-1 (2.28), RHA 500 gm^{-2} (2.01), TP-BS 3:2 ratio (1.94) and RHA 1000 gm^{-2} (1.67). Plants treated with gingelly oil cake (0.73), copper oxychloride (0.70), neem cake (0.54), P-22 (0.57), odal oil (0.49) and coconut cake (0.36) showed higher peroxidase content over inoculated control having a value of 0.32 (Table-16).

4.7.5 Polyphenol oxidase

Highest polyphenol oxidase activity was recorded for P-1 culture with a value of 0.52. Lowest activity of polyphenol oxidase was observed for plants treated with pongamia oil (0.03). Higher peroxidase activity was noted for TP-BS 3:2 (0.43), coconut cake (0.36), TP-BS 4:1 (0.29), RHA 1000 gm^{-2} (0.29) over copper oxychloride (0.2) and inoculated control (0.2). Lower values were noted for *Trichoderma* (0.14), TP-BS 10:1 (0.13), P-22 (0.12) and RHA500 gm^{-2} (0.10). Cowpea plants which received the treatments, gingelly cake (0.3) and RHA 500 gm^{-2} (0.3) showed the same polyphenol oxidase activity (Table-16).

4.7.6 Phenyl alanine ammonia lyase (PAL)

Highest phenyl alanine ammonia lyase activity of 0.63 was observed for plants treated with gingelly cake followed by odal oil (0.62), RHA 1000 gm^{-2} (0.62) and P-1 (0.62). Higher PAL activity was observed for the treatment copper oxychloride (0.61), inoculated control (0.60), coconut cake (0.60), TP-BS 10:1 ratio (0.60), TP-BS 4:1 ratio (0.60) and RHA 750 gm^{-2} (0.60). Moderately higher PAL activity was noticed for plants treated with pongamia oil (0.59), TP-BS 3:2 ratio (0.59), RHA 500 gm^{-2} (0.59) and P-22 culture (0.59). Also lowest observation in the case of PAL activity was noticed for plants treated with neem cake (0.51) and *Trichoderma* (0.51) (Table-16).

DISCUSSION

5. DISCUSSION

The efficacy of ecofriendly management practices using plant oils, oil cakes, indigenous materials and biocontrol agents for the management of collar rot and web blight of cowpea, the most commonly cultivated vegetable crop of Kerala was investigated in the present study. Collar rot and web blight of cowpea was first reported from Kerala by Lakshmanan et al. (1979). *Rhizoctonia solani* was indicated as the causal agent of the disease. Collar rot is initially manifested in the collar region of the plants right from the seedling stage. Collar rot begins as brownish – black lesions at soil level near collar region girdling the base of the stem leading to yellowing and drooping of leaves and rotting of roots affecting the root development. White mycelial growth often studded with small sclerotia is characteristically seen on the affected regions. Web blight appears as small circular light greyish-brown spots on leaf lamina which enlarges to oblong or irregular water soaked areas. Later shot hole symptoms are produced or the spots coalesces to cover entire leaf area resulting in shedding of leaves.

The disease results in a loss of photosynthetic area or total collapse of the crop depending upon the region of pathogen attack. *R.solani* incidence incurred yield loss of 10-60 per cent in horse gram (Dubey and Mishra, 1990), 30 per cent in urdbean (Sharma, 1999) and 6.66 to 75.35 per cent in mung bean. Fungicidal application even today remains as the easiest and best proven practical method to manage diseases caused by *R. solani.* Chemical control has been reported to be effective against the disease by Upamanyu et al. (2002). However, development of residues in vegetables pose a serious threat to human and animal health as well as to the degradation of environment. These problems warrant the need for safer, eco-friendly approaches using plant products and biocontrol agents for the management of diseases affecting vegetables.

In the present study, isolates of the pathogen causing collar rot and web blight of cowpea were isolated and Koch's postulates were proved. Mycelial growth and sclerotial formation of these isolates were different. The web blight isolate produced sclerotia profusely as compared to the collar rot isolate. The sclerotia produced in the former case were large and dark brown in colour as compared to the minute lighter brown coloured sclerotia of the latter. The collar rot pathogen exhibited a faster growth of 8.09 cm while web blight isolate had a mycelial growth of 6.09 cm. Lakshmanan et al. (1979) reported that *R. solani*, the incitant of collar rot and web blight of cowpea produced only microsclerotia .

Plant oils can be utilized for managing *R. solani* incited diseases (Mahadevan, 1982). Shukla et al. (1990) tested the efficacy of these oils for managing sheath blight of rice. As a part of the present investigation, *in vitro* screening of plant oils were carried out to identify their efficacy in inhibiting the growth of the pathogen in order to select the suitable ones and utilize them for *in vivo* pathogen suppression. Among the plant oils, odal oil was identified as the most effective treatment in causing complete suppression of both the collar rot and web blight isolates under *in vitro* conditions. Pongamia oil resulted in 81.87 per cent suppression of the collar rot isolate. Samadera oil also caused complete inhibition of the web blight isolate while neem oil caused 44.78 per cent suppression. Inhibitory effect of these oils against plant pathogens has already been established by earlier workers (Raji, 2004; Sharma et al., 2003). Sharma and Gupta (2003) have reported the efficacy of Pongamia oil in the *in vitro* suppression of *R. solani.*

The addition of oil cakes to soil enhance the beneficial saprophytic micro-organisms in the soil and affect the survival and competitive saprophytic ability of *R. solani*. Four locally available oil cakes *viz.*, neem, groundnut, gingelly and coconut cake were evaluated *in vitro* to check suppression of radial growth of *R. solani* isolates. The maximum suppression of the collar rot pathogen was caused by coconut oilcake, followed by groundnut oil cake and gingelly oil cake. Studies

conducted by Kaviyarasan et al. (2005) also highlight the *in vitro* suppressive effect of coconut, neem and gingelly oil cakes on the radial growth of the damping off pathogen, *Pythium aphanidermatum.* In the present study, neem cake did not have any inhibitory effect on the test fungus. Many of the previous workers, however, obtained significant suppression of *R.solani.* The efficacy of gingelly oil cake and neem cake against *Thanatephorus cucumeris* has been demonstrated by the studies of Dubey and Patel (2000). Sharma and Gupta (2003) also observed that neem kernel extract gave good inhibition of *R. solani in vitro* causing root rot and web blight of French bean.

The oil cakes incorporated in the medium, except coconut oilcake, did not have much inhibitory effect on the web blight isolate of the pathogen. Lakshmanan and Nair (1984) reported loss of viability of sclerotia of *Rhizoctonia solani* in rice soils on use of powdered neem, groundnut, gingelly and coconut cakes as soil amendments.

Turmeric is well known for its anti-microbial properties. Similarly, weekly sprays of low doses of aqueous solution of baking soda has been reported to control soilborne and aerial plant pathogens (Williams and Williams, 1985; Ziy and Zitter, 1992). Gangopadhyay (1998) advocated use of turmeric powder – baking soda in 10:1 ratio to manage soil borne pathogens of rice. In the present investigation, the three combinations of turmeric powder – baking soda tested *in vitro* against both isolates of *R. solani* inhibited the mycelial growth of collar rot pathogen. Among these, the 4:1 proportion of the turmeric powder and baking soda was superior and caused a suppression of 15.44 per cent. Priyadarsini (2003) has also reported the lack of inhibition of turmeric powder – baking soda under *in vitro* conditions against *R. solani* affecting amaranthus. However, Gangopadhyay (1998) reported that turmeric powder – baking soda was effective both under *in vitro* and *in vivo* conditions in managing *R. solani* affecting rice plants. There was only slight inhibition of *Xanthomonas axonopodis* pv *dieffenbechiae,* the causal agent of bacterial blight of anthurium at three levels of turmeric powder – baking soda *viz.*,0.05, 0.10 and 0.15 (Dhanya, 2000). Studies with the web blight

pathogen showed 74.1 per cent suppression in media amended with turmeric powder and baking soda at 10:1 ratio Saju et al. (1998) stated that different concentrations (1-5 per cent) of essential oil of turmeric inhibited the growth of *R. solani in vitro* by 78 per cent. Jain et al. (2006) has reported antimicrobial properties of turmeric and the curcuminoids present in turmeric.

Use of indigenous materials as RHA can be considered as an alternate approach in plant disease management and for growth promotion (Joshi, 2002; Dodan et al., 1991). In the present study, the three concentrations of RHA did not exert inhibitory effects on the growth of the pathogen under *in vitro* conditions. Priyadarsini (2003) also reported the lack of inhibition of three concentrations of RHA *in vitro* on *R. solani,* the incitant of leaf blight of amaranthus. This can be attributed to the fact that the mode of action of RHA is silicification which leads to increased resistance in plants thus reducing the disease intensity and not by the direct action against the pathogen. Joshi (2002) also reported that seed treatment with RHA was effective in managing blast disease of rice, and that the increased resistance can be attributed to the increased silicification and not due to the inhibitory action on *Magnaporthe grisea.*

Studies were conducted on mycelial growth on germination of sclerotia using plant oils, oil cakes and indigenous materials at four time intervals 0, 24, 48 and 72 h. Samadera oil, odal oil and neem oil treatment showed suppression of mycelial growth on sclerotial germination at different time intervals. Samadera oil and odal oil produced appreciable inhibitory effect just after mere dipping in these oils. Treatment with neem oil showed progressive reduction in mycelial growth on sclerotial germination during the period of observation, ultimately leading to total loss of germination. Shahi et al (1998) in their studies on management of collar rot of chickpea found that neem cake extract at 500 ppm inhibited the growth and sclerotial production of *Sclerotium rolfsii* and completely checked these processes at higher concentration of 2000 ppm. Sivakumar and Sharma (2000) reported that neem oil five per cent was highly

effective in reducing sclerotial germination of *R. solani* causing sheath blight of rice.

Organic amendments with oil cakes have been found to decrease the losses caused by several soil borne plant pathogens. Mycelial growth on sclerotial germination was suppressed by treatment with various oil cakes except coconut oil cake. Maximum suppression was obtained with groundnut cake at 24 h of incubation. The inhibitory effect of neem oil cake increased with incubation and could suppress mycelial growth on sclerotial germination to the tune of 35.22 per cent at 72 h. Gingelly oil cake produced maximum suppression of 24.44 per cent at 48 h of incubation. Suppressive effect of neem, groundnut and gingelly cake against *Thanatephorus cucumeris* causing banded leaf blight of rice was highlighted in the studies conducted by Dubey and Patel (2000).

The indigenous materials like rice husk ash and turmeric powder in combination with baking powder generally boost up the defence mechanism of the host plant rather than directly targeting the pathogen. The application of such materials either strengthens the wall layers by additional silicification or through building up the biochemical resistance mechanisms. The indigenous materials tested failed to inhibit mycelial growth on sclerotial germination of *R. solani.* Priyadarsini (2003) also opined that indigenous materials failed to exert, inhibitory effect on the growth of *R. solani in vitro* causing foliar blight of amaranthus.

Pot culture experiments were conducted to assess the efficacy of various treatments as plant oils, oil cakes, indigenous materials and biocontrol agents *in vivo* on different aspects. Gingelly oil cake incorporated in the soil caused complete suppression of the collar rot disease . Copper oxychloride, *Pseudomonas fluorescens* isolate P22, *Trichoderma*, rice husk ash 750 gm^{-2}, pongamia oil, odal oil and turmeric powder-baking soda at 3:2 proportion also reduced the incidence of the disease. The web blight disease intensity in cowpea plants after the first application of the various treatments was relatively less in the treatment receiving

application of *Pseudomonas fluorescens* isolate P-22 (9.87 per cent) and neem cake (11.69 per cent). Gingelly oil cake incorporation, application of *Pseudomonas fluorescens* isolate P-1 and addition of rice husk ash 750 gm^{-2} also resulted in lesser disease index as compared to control. Pot culture studies conducted by Lakshmanan and Nair (1984) demonstrated the suppressive effect of gingelly cake against *R. solani* causing collar rot and web blight of cowpea. Jhakar et al. (2002) working on the management of root rot of cotton caused by *Thanatephorus cucumeris* using oil cakes found the effectiveness of gingelly cake. Rajan (1980) reported that the oil cakes and other organic materials used as soil amendments were equally efficient in suppressing sheath blight and sheath rot of rice. This may be due to the stimulation of soil saprophytes leading to a reduction in the pathogen population or to a better plant tolerance because of increased nutrition offered by the amendment.

Plants offer an excellent array of biologically active natural products. Natural and indigenous materials contain several active principles that target phytopathogens either directly or indirectly. Further, they are least toxic to the ecosystem and to the non-target flora and fauna. Since such natural products do not accumulate in the ecosystem, the chances of building up of resistance are ruled out. In addition, the plant growth is enhanced and defenses are structured through stimulation or modification of host metabolites.

Indigenous materials like turmeric powder - baking soda at various proportions and rice husk ash when applied caused reduction in collar rot and web blight. Among the three proportions tried, the 3:2 combinations of turmeric powder and baking soda resulted in lower collar rot incidence (2.35 per cent). Priyadarsini (2003) tried the combinations of turmeric powder – baking soda in the ratio 10:1, 6:4 and 8:2 for the management of *R. solani* causing foliar blight of amaranthus and found 10:1 ratio to be the most effective. In the context of the present study also it might well be stated that turmeric powder may be recommended as a plant product with potential for developing into organic non hazardous, cheap eco-friendly fungicide.

Application of wood ash and rice husk ash to protect seeds from insect attack and diseases is an age-old farmers' practice followed even today. The main ingredient of rice husk ash is Silica (Si O_2) accounting to 96 per cent of the total content. Rice husk ash at 750gm^{-2} caused reduction in collar rot incidence. Similarly, lesser web blight intensity (16.44 per cent) was noticed in pots to be treated with rice husk ash 500 gm^{-2}. Incorporation of RHA could probably have enhanced the uptake of higher doses of silica and potassium, the two elements that impart resistance to plants against many pathogens.

Fluorescent pseudomonads have been found as an ideal candidate in the effective management of soil borne pathogens. Application of P-22 culture of *P. fluorescens* recorded reduced percentage of disease index after first application of treatments. Smitha (2000) reported that soil application followed by foliar application of talc based formulation of *P. fluorescens* was effective in reducing the intensity of foliar blight of amaranthus. Similar result was obtained by Priyadarsini (2003) in the case of *R. solani* causing foliar blight of amaranthus. Many workers have reported that suppression of plant diseases by fluorescent pseudomonads can be due to their activities like competition for space and nutrients, production of antibiotics, volatiles and antimicrobial substances, siderophores and HCN (Dowling and O'Gara, 1994; Rosales et al., 1995; Dave and Dube, 2000; Mondal et al., 2000). Nandakumar et al. (2001) reported that *P. fluorescens* application as bacterial suspension or through seeds, roots, soil and foliar either alone or in combination reduced the intensity of sheath blight of rice caused by *R. solani.*

In the case of biometric observations maximum shoot length was observed with plants treated with gingelly cake. The number of pods and pod weight is highest in the case of plants treated with coconut cake. Fresh and dry shoot weight was highest for plants treated with neem cake. This growth parameters points to the growth promotion activity of organic amendments added to soil. Amendments incorporated in soil operate in a variety of ways such as improving

plant nutrition, soil structure as well as suppressing the pathogen (Lakshmanan and Nair, 1984). Soil amendments influence soil physical characters such as pore size, aeration, water retention, pH etc. which help in better solubilization of minerals and release of several nutrients through decomposition. This in turn, facilitates the rapid expansion of the root system, better absorption of nutrients and improved vigour of the plants (Kannaiyan and Prasad, 1981). The role of organic amendments in plant growth enhancement has been well documented (Padmodaya and Reddy, 1999).

P-22 culture of *P. fluorescens* recorded maximum nodulation giving evidence for the growth promoting activity of biocontrol agents. Root nodule bacteria are considered as a group of plant growth promoting rhizobacteria which through its nodulation and symbiosis with plant help in fixation of atmospheric nitrogen. Reports on plant growth promotion by root nodule bacteria were given by Muthamilan (1994). The growth promoting effect of fluorescent pseudomonads can be attributed to the production of hormones and yellow green fluorescent siderophores. The siderophores complex Fe^{2+} in the root zone and makes it unavailable to deleterious microorganisms (Leong, 1986). Many workers have noted the plant growth promotion activities of *P. fluorescens* (Dileepkumar, 2002; Priyadarsini, 2003; Kumar, 2003).

Changes in the activity of defense related enzymes – PAL, PO and PPO, total phenols, amino acids and carbohydrates due to various treatments were estimated. There was an increase in total phenols due to soil application followed by foliar spray of P-22 culture of *P. fluorescens* after inoculation with the pathogen. There was no increase in carbohydrate and amino acid content due to these treatments when compared to control. Carbohydrates and PAL activity was highest in the case of plants treated with gingelly cake. Increase in PAL activity can be correlated with increased resistance in plants. PAL is the first enzyme of the phenol propanoid pathway which leads to the synthesis of phenolics and is the most important enzyme in inducing disease resistance in crop plants. Meena et al.

(1999) found that spraying rice leaves with *P. fluorescens* increased PAL and phenol activity one day after treatment.

The treatment TP:BS 10:1 ratio exhibited highest protein and peroxidase content. Peroxidase enzyme oxidizes phenolics to highly toxic quinones resulting in disease resistance (Vidyasekharan, 1988).

Disease management using plant oils, oil cakes and indigenous materials has been reported by many (Raji, 2002; Kannayian and Prasad, 1981; Gangopadhyay, 1998; Joshi; 2002). In this study effective control of the disease and enhancement of growth could be obtained with the soil application of oil cakes such as gingelly cake and coconut cake and foliar application along with soil drenching odal / pongamia oil. Soil and foliar application of biocontrol agents like *P. fluorescens* (P-22) were also found to be effective in managing the disease and improving the growth of the plant. Indigenous materials like turmeric powder - baking soda at various proportions and rice husk ash also caused reduction in collar rot and web blight of cowpea. In the context of the present study also it might well be stated that soil and foliar application of turmeric powder combined with baking soda and rice husk ash could be exploited further to develop a plant based non- hazardous, cheap eco-friendly fungicide.

SUMMARY

6. SUMMARY

Collar rot and web blight of cowpea is one of the most important disease affecting the crop in Kerala. The climatic conditions prevailing in the state are congenial for the spontaneous occurrence and spread of the disease. The pathogen with its high variability and wide host range has made it difficult to develop disease resistance. The chemical control measures though proven effective is costly and poses severe threat to human health and environment. Therefore the present investigation was carried out with the objective of evolving an ecofriendly practice for the management of the disease using plant oils, oil cakes, indigenous materials like turmeric power-baking soda and rice husk ash and biocontrol agents like *Trichoderma harzianum* and *Pseudomonas fluorescens.*

The collar rot and web blight pathogens were isolated from the Crop Museum of College of Agriculture, Vellayani, Thiruvananthapuram and were made use of in the study. *In vitro* studies were carried out to test the effect of plant oils, oil cakes and indigenous materials on *R. solani.* Among the seven plant oils tested odal and pongamia oil gave maximum suppression of the pathogen *in vitro.* Of the four oil cakes coconut and gingelly cakes exhibited maximum suppression of the pathogen. The indigenous materials failed to produce significant suppression of growth of the pathogen. The different combinations of turmeric powder-baking soda did not inhibit the growth of the pathogen *in vitro.* The three concentrations of rice husk ash also did not exert any inhibitory effect on the growth of the pathogen *in vitro.*

Pot culture experiments were conducted to assess the efficacy of selected plant oils, oil cakes, indigenous materials and biocontrol agents in managing the disease. The plant oils along with an emulsifier were used for foliar spray and soil drenching and odal and pongamia oils were found successful in managing the disease. Oil cakes were powdered and applied to soil and gingelly cake gave the best performance in disease management and growth enhancement. Among three

combinations of turmeric powder-baking soda 10:1 combination was effective in improving growth and reducing disease incidence. Among the three concentrations of rice husk ash tested the highest concentration 1000gm^{-2} was found effective in enhancing growth and suppressing the disease. Among *T. harzianum* and two cultures of *P. fluorescens* (P-1 & P-22) tested, P-22 was found to be superior in suppressing the disease and enhancement of growth. The fungicide copper oxychloride used as a check was effective in reducing the disease incidence and intensity but the reduction was much less when compared to the oil cakes and biocontrol agents.

Changes in levels of total phenols, proteins, carbohydrates and activities of defense related enzymes – PAL, PO and PPO due to the above treatments were estimated. Soil application of oil cakes increased the activity of carbohydrate and PAL. Highest protein and PO content was noticed in the case of 10:1 ratio of turmeric powder – baking soda. Highest phenol content was noticed with P-22. However there was no significant increase in the case of PPO due to those treatments.

The application of odal/pongamia oil (soil and foliar), gingelly cake (soil application) and application of P-22 (soil and foliar) is recommended as an effective package in managing the collar rot and web blight of cowpea.

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APPENDICES

APPENDIX – I

Composition of different media

A) Potato dextrose agar

Potato	:	200.00 g
Dextrose	:	20.00 g
Agar	:	20.00 g
Distilled water	:	1 litre

B) Potato dextrose broth

Potato	:	200.00 g
Dextrose	:	20.00 g
Distilled water	:	1 litre

APPENDIX – II

Buffers for enzyme analysis

A) 0.1 M Sodium phosphate (pH 6.4)

Stock solutions

A - 1.56 g of Sodium dihydrogen phosphate in 100 ml.

B - 1.42 g disodium orthohydrogen phosphate in 100 ml

68.5 ml A is mixed with 31.5 ml B

B) 0.1 M Sodium borate (pH 8.8)

A - 0.2 M solution of boric acid (12.4 g in 1000 ml)

B - 0.05 M solution of borax (19.05 g in 1000 ml)

50 ml of A is mixed with 30 ml of B, diluted to a total of 200 ml.

ECOFRIENDLY MANAGEMENT OF COLLAR ROT AND WEB BLIGHT OF COWPEA

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Abstract of the
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ABSTRACT

The study "Ecofriendly management of collar rot and web blight of cowpea was conducted at the Department of Plant Pathology, College of Agriculture, Vellayani during 2004-2007. Different plant oils, oil cakes, indigenous materials like turmeric power – baking soda and rice husk ash and biocontrol agents like *Trichoderma harzianum* and *Pseudomonas fluorescens* were used in the study to manage *R. solani* causing the disease.

Among the seven plant oils tested odal and pongamia oil emerged as the most potential ones. Of the four oil cakes gingelly and coconut cakes were identified as the best treatments. The different combinations of turmeric powder – baking soda and rice husk ash did not inhibit the growth of *R. solani*.

Pot culture experiments were conducted to assess the efficacy of plant oils, oil cakes, biocontrol agents and indigenous materials in enhancing the growth of cowpea. Both the plant oils used in the study were successful in managing the disease. Of the three oil cakes used in the study gingelly cake exhibited best performance in disease management and growth enhancement. Of the indigenous materials used 10:1 combination of turmeric powder – baking soda and 1000gm^{-2} concentration of RHA were found to be excellent in enhancing growth and disease suppression. Biocontrol agents *T. harzianum* and P-22 culture of *P. fluorescens* were found to be superior in disease suppression and growth enhancement.

Changes in levels of total phenols, proteins, carbohydrates and activities of defense related enzymes like PO, PPO and PAL due to above treatments were estimated. The treatment 10:1 ratio of turmeric powder – baking soda recorded highest protein and PO content. Soil application of oil cakes gave increased activity of PAL and carbohydrate.