INTEGRATED MANAGEMENT OF FUSARIUM WILT OF VEGETABLE COWPEA
(*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt)

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DECLARATION

I hereby declare that this thesis entitled **"Integrated management of Fusarium wilt of vegetable cowpea (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt)"** 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 to me 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 **"Integrated management of Fusarium wilt of vegetable cowpea (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt)"** is a record of research work done independently by Mr.Senthil Kumar, E. (2001-11-54) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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INTRODUCTION

1. INTRODUCTION

Vegetable cowpea, also known as yard long bean (*Vigna unguiculata* sub sp. *sesquipedalis* (L.) Verdcourt), is a leguminous vegetable crop grown in different parts of the world. Due to the favourable agro-climatic conditions, this crop has gained wide popularity in Kerala, and has come to occupy a prime position among the vegetable crops raised in the State. This crop is cultivated throught the year, either in the upland or in the wetland fallows in summer as irrigated crop. The tender green pods used as vegetable are rich in protein, minerals, vitamins and dietary fibre.

Diseases and pests are the major constraints in the production of vegetable cowpea. Off late, *Fusarium* incited wilt has emerged as one of the serious disease problems affecting the crop in Kerala (Reghunath *et al.*, 1995).

Considering the soil borne and pliable nature of the pathogen, one single method of control may not be adequate to bring effective suppression of the disease. Very little information is available on the different aspects of the disease as well as its management. Hence, there is a need to explore and identify the chemical, biological and cultural methods of disease management, and to evaluate the efficacy of the combinations so as to develop an eco-friendly and multi dimensional integrated disease management (IDM) strategy for wilt of cowpea. Hence, the study was undertaken with the following objectives.

- Identification and characterization of the pathogen of the wilt disease of cowpea
- Pathogenicity testing and screening for virulence of *Fusarium* isolates.
- Isolation and *in vitro* screening of fungal and bacterial antagonists of the wilt pathogen.

- Selection, characterization and identification of effective biocontrol agent for *in vivo* suppression of Fusarium wilt.
- Testing and selection of effective fungicide, compatible with biocontrol agents, for suppression of Fusarium wilt.
- Selection of effective and compatible soil amendment for wilt suppression
- Testing combinations of the effective antagonist, fungicide and soil amendment for wilt disease suppression and development of an integrated disease management package.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 Importance and Yield loss

Cowpea is cultivated both as grain legume and vegetable crop in India. In Kerala, the vegetable cowpea (*Vigna unguiculata* sub sp *sesquipedalis* (L) Verdcourt) is widely cultivated especially in the wetland fallows. An array of diseases affect the crop at various stages of growth, among which, Fusarium wilt causes considerable reduction in yield.

Fusarium wilt is considered to be one of the most destructive soil borne diseases of pulses. The yield loss due to fusarial wilt vary with the stage at which the disease occurs. Severe incidence of the disease during early reproductive stage induce flower and pod abortion which drastically decrease the seed number and yield. The incidence of wilt occurs to the extent of 93 per cent and on an average causes 10 per cent yield loss annually in chickpea in India (Singh and Dahiya, 1973).

Haware and Nene (1980) recorded an yield loss of 77-94 per cent in chickpea when wilt occurred during early pod filling stage and 57-82 per cent at pod maturity and 23-65 per cent at harvest. Yield loss on account of Fusarium wilt in chickpea approached 100 per cent when wilt occurred at the pre-pod stage, about 67 per cent when wilt occurred at maturity and 30 per cent when it occurred at the pre harvest stage (Kannaiyan and Nene, 1981). Heavy yield loss upto 60 per cent has been reported in pea when epiphytotic occurred (Kumar, 1993).

Host resistance also decides the extent of yield loss due to Fusarium wilt. Srivastava *et al.* (1984) found that incidence of wilt varied from 12 to 50 per cent and yield loss from 22 to 53 per cent in different chickpea varieties.

Fusarium wilt has been first reported in cowpea from USA (Orton. 1902). Allen (1983) reported involvement of *F.solani* in dry root rot of cowpea. Younger cowpea plants were reported to be severely affected by wilt due to *Fusarium oxysporum* (Shihata *et al.*, 1989).

In India, this disease was first recorded in cowpea by Singh and Sinha (1955). The wilt of cowpea was noticed in farmer's fields in Thiruvananthapuram district of Kerala State since 1996-1997 (Reghunath *et al.*, 1995).

2.2 ETIOLOGY

The genus *Fusarium* was erected to include fungi with fusiform macroconidia with foot cell. Booth (1971) reported that the perfect stage of the fungi when present, belonged to the order Hypocreales, *Fusarium* spp. cause seed rot, damping off and vascular wilts of vegetable pulses, ornamental and fibre crops.

Cotton wilt noticed in desi cotton was identified as due to infection of *F.oxysporum* (Butler, 1926). Subramanian (1971) placed the incitant of wilt of pigeon pea in the Elegans section of Synder and Hansen (1940) based upon production of thin walled, 5-septate macroconidia with foot cell and curved or hooked tip. *F.oxysporum* f. sp. *udum* incited pigeon pea wilt which occurred as a serious disease in vertisols of Central India (Kannaiyan *et al.*, 1984).

F.oxysporum f. sp. *psidii* was reported to be the causal agent of wilt of guava (Prasad *et al.*, 1952) which occurred as a severe problem in U.P. Booth (1971) identified the *Fusarium* involved in wilt of chickpea as *F.oxysporum* f. sp. *ciceri*. The yellow disease of ginger caused by *F.oxysporum* f. sp. *zingiberi* was reported for the first time by Haware and Joshi (1973).

Vasudeva and Srinivasan (1952) found association of *F.oxysporum* f. sp. *lentis* with the wilt of lentil. Fusarium wilt (Panama wilt) of banana has been reported from most of the banana growing areas. Four races of *F.oxysporum* f.sp. *cubense* were reported to be involved in this disease (Ploetz, 1992). Verma and Dohroo (2003) reported that considerable, morphological cultural and pathogenic variability existed among races of *F.oxysporum* f. sp. *pisi*.

F.solani have been reported to be involved in root rots of chickpea, soybean, sunflower, cucurbits and melon etc in India. Bhatnagar and Prasad (1966) reported incidence of withering and dieback of twigs and sudden drooping of leaves in lime due to infection of *F.solani*.

F.moniliforme was reported as the Bakanae or foot rot pathogen of rice (Yabuta and Hayashi, 1939). Summanwar *et al.* (1966) reported involvement of *Fusarium* with mango malformation.

Narain *et al.* (1989) reported incidence of groundnut seed rot caused by *F.pallidoroseum* which occurred in Orissa during 1983-88. Sharma *et al.* (2001) reported the involvement of *F.pallidoroseum* in wilt disease of horse gram.

2.3 SYMPTOMATOLOGY

Fusarium wilt is characterized by yellowing of leaves followed by defoliation, drying of vines and root decay. Sometimes, there is also swelling of the basal part of the plant including the lower part of the stem and upper part of the tap root forming a tuber like structure which later gets disintegrated (Reghunath *et al.*, 1995).

Kurmut *et al.* (2002) observed that the root rot of *Vicia faba* caused by *F.nygamai* showed black root rot and decay of the lateral root system. Severely infected plants showed black neck canker at the soil level. These symptoms were usually accompanied by loss of the leaf turgor, followed by browning and eventually death of intact leaves.

2.4 MANAGEMENT OF FUSARIAL DISEASES

Fusarium wilt poses severe threat to cowpea cultivation and hence steps have to be taken to control the disease systematically taking into consideration the knowledge available on management of Fusarium wilt of other pulse crops. Information on cultural, biological, chemical and integrated management of *Fusarium* incited diseases provide guidelines

for envisaging a practical solution to this vexing problem in cowpea cultivation.

2.4.1 Biological control

Boswell (1965) described biological control as "landmark in great renaissance of interest and research in micro ecological balance in relation to soil borne plant diseases, and in the development of more enduring, profitable and wiser farming practices".

Biocontrol agents are alternatives to conventional chemicals for management of plant disease in an eco-friendly manner. Several fungal antagonists, such as *Trichoderma* spp. (Papavizas, 1985), *Aspergillus niger* (Sen, 2000), *Penicillium* (Cook and Baker, 1983), *Chaetomium* (Walther and Gindrat, 1988) etc and plant growth promoting rhizobacteria such as *Pseudomonas fluorescens* (Kloepper *et al.*, 1980) and *Bacillus* (Campbell, 1989) have been reported to be promising in controlling various plant pathogens.

2.4.1.1 Fungal antagonists

A large number of antagonistic fungi have been identified and are exploited for management of plant pathogens. Among these, *Trichoderma* spp. have been widely and successfully used to suppress aerial, seed and soil borne pathogens throughout the world. Many commercial biopesticides have been developed based on this Genus (Whipper, 1992).

2.4.1.1.1 Trichoderma spp.

Trichoderma spp are fast growing saprophytic fungi which can successfully compete with many plant pathogens. They produce antibiotics and other toxic metabolites and parasitise the target pathogen (Weindling, 1934). All these attributes contribute to their success in suppressing an array of pathogens.

The *in vitro* studies reveal the underlying mechanism of biocontrol of *Fusarium* spp. using *Trichoderma*. Asalmol and Sen (1992) while studying the antagonistic activity of *T. viride* on *F. solani*, the mutant of muskmelon wilt pathogen, found that the antagonist parasitised pathogen through appressoria like structures. Spore germination and radial growth of *F. solani* were inhibited by the cell free culture filtrate of *T.virens* (Mishra and Narain, 1992). There was also restriction of disease symptoms on plants pretreated with the pathogen. Isolates of *Trichoderma* were found inhibitory to *F. oxysporum* f. sp. *lycopersici* ,the incitant of wilt of tomato. Among the *Trichoderma* isolates, *T. viride* was highly effective in reducing radial growth of the pathogen which was accomplished by production of non-volatile compounds (Padmodaya and Reddy, 1996). *Trichoderma* spp. formed inhibition zone against chickpea root rot pathogen and reduced its colony diameter by 46.6 to 62.2 per cent (Selvarajan and Jeyarajan, 1996). *In vitro* studies showed that *T. virens* exhibited antagonistic effect on *F. oxysporum* f. sp. *phaseoli* involved in the wilt complex of french bean (Mukherjee and Tripathi, 2000). They attributed the suppression to the effect of volatile and non-volatile metabolites produced by the antagonist. *T. viride* and *T. harzianum* showed coiling around the hyphae of *F. oxysporum* f. sp. *capsici*, causing wilt of chilli, resulting in the formation of appressorium and penetration of hyphal cells leading to death (Naik *et al.*, 2000). *T. viride* isolated from healthy safflower rhizosphere inhibited *F. oxysporum* f. sp *ricini* causing wilt of castor (Chattopadhyay and Varaprasad 2001). Godwin-Egein and Arinzae (2001) investigated the interaction between *T. harzianum* and *F. oxysporum* and reported mechanisms such as lysis and hyper parasitism to be operating in the suppression of the pathogen. Ten day old cell free culture filtrate of *T.virens* significantly inhibited the spore germination of *F. solani* causing wilt of tomato (Narain and Behera, 2000). Vyas and Mathur (2002) reported that *Trichoderma* spp. under *in vitro* conditions effectively inhibited the growth and sporulation of *F.*

oxysporum, which was the effect of volatile and non-volatile antibiotics produced by antagonist. *Trichoderma* spp. produce β1-3 glucanase and chitnase enzymes. These enzymes have been indicated to play a major role in inhibiting the cell wall synthesis of *F. culmorum* and *F. oxysporum* (Witkowska and Maj, 2002).

Introduction of.*T. harzianum* to soil one month before sowing of soybean, resulted in significant reduction of wilt disease caused by *F.oxysporum* f.sp. *glycines* (Mousa, 1994). Somasekhara *et al.* (1996) found that *T.viride* significantly reduced the number of propagules of *F.udum* and incidence of wilt of pigeon pea caused by it. Siddiqui and Mahmood (1996) observed that *T.harzianum* effectively reduced the wilt disease of pigeon pea in field conditions and also resulted in increased plant height, shoot dry weight and number of nodules. However, they noticed that *T.harzianum* when applied along with *Glomus mosseae* exerted an adverse effect on mycorrhizal root colonization. Seed treatment with *T.viride* resulted in 27.62 per cent reduction in wilt of pigeon pea (Pandey and Upadhyay, 1999). They also reported that *T.viride* and *T.harzianum* effectively reduced the wilt of pigeon pea caused by *F.udum* in field conditions by 57 and 56 per cent, respectively. Jha and Singh (2000) obtained effective reduction in Fusarium wilt of chickpea through seed treatment as well as soil drenching with *T.viride* and *T.virens*. When chickpea seeds were coated with *T.virens*, it reduced the wilt disease occurrence both in glasshouse and field conditions (Tewari and Mukhopadhyay, 2001).

Sivan and Chet (1982) have found the incorporation of *Trichoderma* spp. in field gave 60-83 per cent control of Fusarium wilt of tomato. *Trichoderma* spp. have been employed for management of fusarial diseases of other crops also. *T. viride* (10^6 cfu/ml) along with 500 g wheat bran when applied 3 months after planting effectively reduced the Fusarium wilt of banana and also produced high yield (Raguchander *et al.*, 1997). Nagesh *et al.* (1998) reported the *T. harzianum* and *T. viride*

controlled the wilt disease of gladiolus caused by *F. oxysporum* f. sp. *gladioli* in the presence of *Meloidogyne incognita*. *T. harzianum* isolated from rhizome rot suppressive soils effectively checked *Fusarium* spp. and increased plant stand and rhizome yield of ginger (Ram *et al.*, 1999). *T. viride* and *T. harzianum* reduced the wilt of coriander caused by *F. oxysporum* f. sp. *corianderii*. According to Kumar and Ranganathan (2000) combination of *T. viride* and *P. fluorescens* was the most ideal treatment for promoting shoot and root length, dry matter production; as well as for suppression of wilt in coriander. Pre-treatment of seeds and seedlings of tomato with *T. virens* resulted in reduction or complete suppression of *Fusarium* incited wilt (Narain and Behera, 2000). When *T. viride* was inoculated to forest nurseries of *Acacia nilotica*, it gave protection against damping off pathogens including *Fusarium* (Arya and Kaushik, 2001). Jahagirdar *et al.* (2001) reported that soil application of *T. viride* at the rate of 90 g/plant in banana, effectively reduced the panama wilt disease caused by *F. oxysporum* f. sp. *cubense*. Application of *T. harzianum* three times as pre transplant root immersion and as stem drip 15 and 30 days after transplanting reduced Fusarium wilt of tobacco. (Zapata and Vecchietti, 2001).

2.4.1.1.2 Piriformospora indica

P. indica is a newly described growth promoting cultivable endophyte of roots of several plants (Varma *et al.*, 1998). It resembled arbuscular mycorrhizal fungi (AMF) with respect to morphology, physiology and intracellular invasion of the root (Varma *et al.*, 1998). Hyphae of *P. indica* developed appressoria and colonized intercellularly forming coils, branches or round bodies (Singh *et al.*, 2000).

Application of *P. indica* and its culture filtrate promoted the plant growth and biomass production (Varma *et al*, 1998). *P. indica* tremendously improved the growth and overall biomass production of different monocots and dicots such as maize, tobacco, paddy, sorghum,

wheat, soybean and chickpea and medicinal plants (Varma *et al.*, 1999). However, the roots of several plants belonging to family Brassicaceae, a mutant of *Glycine max* and *Pisum sativum* were not colonized by the fungus (Varma *et al.*, 2001).

P. indica was proved to be promising as a biological hardening agent of micro propagated tissue culture plantlets and several medicinal plants (Sudha, 1998). Similarly, regenerated plantlets of tobacco when subjected to *P. indica* treatment showed 88 to 94 per cent survival and helped them to overcome transplanting shock (Sahay and Varma, 1999).

2.4.1.2 Bacteria as biocontrol agents

Several rhizosphere bacteria have been proven to be effective as biocontrol agents. Weller (1988) listed bacteria belonging to several genera showing antagonism against several phytopathogens. Three genera of bacteria *viz. Agrobacterium, Bacillus* and *Pseudomonas* have been widely tested in both laboratory and field conditions.

Fluorescent pseudomonads have emerged as the largest and potentially the most promising group of plant growth promoting rhizobacteria (PGPR) which can effectively control many soil borne plant pathogens (Kloepper and Schroth, 1978). This unique group comprising of bacteria which are predominantly rhizosphere inhabitants, can suppress many plant diseases due to their general biological activities including competition for space and nutrients, production of antibiotics, volatiles and antimicrobial substance and chelating compounds such as siderophores and HCN.

Fusarium spp. which incite seed rot, seedling death, damping off, root rot or wilt have been reported to be suppressed by application of rhizobacteria, especially the fluorescent pseudomonads.

Sakthivel *et al.* (1986) reported that native strains of *Pseudomonas fluorescens* showed *in vitro* inhibitory activity against *F. oxysporum* f. sp. *vasinfectum*, *F. oxysporum* f. sp. *cubense* and several other soil borne

plant pathogens. *Bacillus* sp. showed significant inhibition of tomato wilt pathogen *F. oxysporum* f. sp. *lycopersici* (Kapoor and Kar, 1989). They also demonstrated the role of antibiotics in the inhibition of the pathogen. Another plant growth promoting rhizobacteria, *Azotobacter* spp. failed to inhibit the wilt pathogen *P. cepacia* strain 526 and 406 and *Enterobacter agglomerans* strain 621. The predominant rhizobacteria on the roots of maize, exhibited *in vitro* antagonism towards *F. moniliforme* (Hebbar *et al*, 1992). *P.fluorescens* exhibited inhibition against *F. oxysporum* f. sp. *cubense* under *in vitro* conditions (Raguchander *et al.*, 1997).

Bacillus spp. have been considered as important antagonists of many pathogens (Campbell, 1989). Vasudeva and Roy (1950) did pioneering work on the management of pigeon pea wilt pathogen, *F. udum* and explained the role of *B. subtilis* in disease suppression. Further, their work revealed the production of the antibiotic 'bulbiformin' by the bacterium into the rhizosphere soil (Vasudeva *et al.*, 1958). Seed treatment of groundnut with *B.subtilis* improved seed emergence and reduced incidence of chickpea wilt (Kumar and Dube, 1992). Hwang (1994) found that *B. subtilis* could reduce root rot of lentil caused by *F avenacearum*. Similarly, Zhang *et al.* (1995) reported the inhibitory effect of *B. subtilis* on *F. oxysporum* causing wilt of cotton. Seven different strains of plant growth promoting *Bacillus* sp. provided protection against *F.moniliforme* associated with seed rot and seedling wilt in okra (Mashooda Begum *et al.*, 2003).

Fluorescent pseudomonads have revolutionized the field of biological control of soil borne pathogen like *Fusarium*. Colonization of chickpea roots by herbicide tolerant strains of *P. fluorescens* and *P. putida* significantly reduced the wilt caused by *F. oxysporum* f. sp. *ciceri* and increased dry weight of chickpea plants (Gupta *et al*, 1996). Banana suckers dipped into a suspension of *P. fluorescens* (10^6 cfu/ml) effectively reduced the Fusarium wilt incidence and also produced higher yield (Raguchander *et al.*, 1997). Duijff *et al.* (1997) reported that fluorescent

pseudomonads suppressed Fusarium wilt of tomato by microbial antagonism and induced resistance and attributed this to systemic acquired resistance associated with the synthesis and accumulation of PR-proteins. Fluorescent pseudomonads isolated from the tomato rhizosphere inhibited the growth of *F. oxysporum* f. sp. *lycopersici* and several other fungal pathogens of the crop (Varshney and Chaube, 1999). They also showed plant growth promoting activity and biocontrol potential under *in vitro* conditions. Marjan de Boer *et al.* (1999) reported that the different native strains of *Pseudomonas* spp. suppressed the wilt disease of radish caused by *F. oxysporum*. *P. fluorescens* NBR 11303 strain was found highly inhibitory to *F. oxysporum* f. sp. *ciceri* on chickpea. It also significantly increased the shoot length, dry weight and grain yield of chickpea (Nautiya, 2000). The enhancement of growth of tomato plants was significantly more with fluorescent pseudomonads isolates when compared with that of VAM and *Trichoderma* spp. However, *P. fluorescens* was more effective in growth promotion and disease suppression when it was applied along with VAM (Varshney and Chaube, 2000). . Yeole and Dube (2001) working with fluorescent pseudomonads from rhizosphere of different crops such as chilli, cotton, groundnut and soybean found that these isolates reduced soil borne pathogens by production of siderophores and also promoted the growth of the parent crops.

2.4.2 Soil amendments and suppression of plant diseases

One of the cheapest and effective methods of alteration of soil environment is amendment of soil with decomposable organic matter. It is one of the methods of biological control of plant diseases. The decomposition of the organic matter helps in alteration of the physical, chemical and biotic conditions of the soil. The altered conditions reduce the inoculum potential of the soil dwelling pathogen. In addition, it improves soil structure which in turn promotes root growth of the host.

Fusarium being a soilborne pathogen, incorporation of soil amendments helps in reducing the incidence of the diseases incited by it.

Fusarium wilt of tomato was reduced by raising the soil pH to 7.5 with hydrated lime in conjunction with nitrate N and mulch. (Jones and Overman, 1985). Fusarium wilt of cumin was effectively checked by amending soil with neem cake followed by soil solarization (Lodha. 1995). Meena and Mariappan (1993) observed that neem extract inhibited the mycelial growth and spore germination of *Fusarium* sp. infecting pea seeds. Diyora and Khandar (1995) tested the effectiveness of mustard cake, groundnut cake, castor cake, neem cake and mahua cake for managing cumin wilt caused by *F. oxysporum* f. sp. *cumini*. Among these mustard cake was the most effective, followed by groundnut cake. Banana leaves, bagasse, synthetic mushroom compost, paddy straw and spent mushroom compost applied into the soil, reduced Fusarium wilt disease of tomato. Neemcake and farmyard manure application were reported to enhance antagonistic fungi in soil and help in bringing suppression of wilt of cowpea (Ushamalini et al., 1997). Organic amendments such as neemcake, pongamia cake, fresh and dried leaves of pongamia,dry leaves of eucalyptus and farmyard manure significantly reduced the seedling disease of tomato caused by *F. oxysporum* f. sp *lycopersici*. As the concentration of amendments increased, healthy seedling stand also increased (Padmodaya and Reddy, 1999). Organic amendments applied at the rate of 100g/pot at 15 days prior to planting significantly reduced the Sclerotium wilt of jasmine. Among the organic amendments, coirpith compost and farmyard manure were least effective on Sclerotium wilt of jasmine (Ramamoorthy *et al.*, 2000). On the contrary, Fugro (2000) reported that organic manures such as farmyard manure, neem cake and vermicompost treated chilli plants showed higher die back disease caused by *Colletotrichum capsici*.

2.4.3 Fungicides

Fungicide application even today remains as the easiest and the best means to combat fusarial diseases. Dwivedi and Pathak (1980) reported that *F. oxysporum* f. sp. *lycopersici*, the tomato wilt pathogen, was suppressed by carbendazim treatment under laboratory conditions. The chemicals also caused deformation of hyphae and reduction in sporulation. Shugha *et al.*, (1995) evaluated twelve fungicides against *F.oxysporum* f. sp. *ciceri* causing wilt of chickpea, and found that carbendazim and thiram alone, or, in combination were highly effective in inhibiting mycelial growth. As a result of this, suppression of wilt incidence was noticed under green house and field conditions. *F. moniliforme* causing sugarcane wilt was inhibited by carbendazim (100 to 2000 ppm) and chlorothalonil (200-3000 ppm) (Gohil and Vala, 2000). However, carbendazim at concentration as low as 5 ppm was sufficient to completely check mycelial growth of *F. moniliforme*,the stalk rot pathogen of maize (Bohra *et al.*, 2001). Similarly, *Fusarium* spp. causing post harvest losses in bell pepper could be successfully controlled with this fungicide at 500 µg/ml concentration (Shukla and Sharma, 2002) Bavistin, Kri-Benomyl and TBZ at 25 ppm completely suppressed the growth of *F. oxysporum* f. sp. *pisi*, the pea root rot pathogen, under *in vitro* conditions. (Verma and Dohroo, 2002). Singh and Goswami (2003) obtained significant suppression of mycelial growth of *F. moniliforme* causing wilt of sugarcane using carbendazim at 500, 1000 and 2000 ppm

Several other fungicides are also effective in checking *Fusarium* spp. Chakrabarty (1993) reported that mancozeb effectively suppressed the growth of *F. equiseti* involved in the curd rot complex of cauliflower. Pushpavathi *et al.*, (1998) found that mancozeb (500 ppm) completely inhibited *F. oxysporum* f. sp. *ricini*, the casual agent of castor wilt. Mancozeb and captafol were found to be almost on par with carbendazim in checking the fusarial wilt pathogen of castor. Mancozeb and several other fungicides such as thiram, copper oxychloride, iprodione,

carbendazim, metalaxyl, chlorothalonil, benomyl and captan at 500, 1000 and 1500 ppm, significantly inhibited the growth of *F. oxysporum* f. sp. *lini* (Sharma *et al.*, 2002).

F. solani failed to grow and sporulate in solid and liquid media amended with fungicides such as Baynate, Blitox 50, Captan, Bavistin, Contaf and Indofil M-45 at various concentrations (Singh *et al.*, 2000). Inhibitory activity of mancozeb and captafol towards *F. oxysporum* f. sp. *carthami* was significant and next only to that of carbendazim (Chavan *et al.*, 2000). Bohra *et al.* (2001) found that mancozeb at a dose of 800 ppm significantly suppressed *F. moniliforme*, whereas similar suppression was obtained with lower doses of fungicide such as carbendazim, bayleton, kitazin, captafol etc. Trivedi *et al.* (2002), reported the mancozeb totally inhibited *F. pallidoroseum* at 1000 ppm, whereas, carbendazim, thiram etc were effective at 500 ppm concentration.

Application of carbendazim and thiram alone or in combination were highly effective in reducing wilt incidence in chickpea under green house and field conditions (Sugha *et al.*, 1995). Drenching carbendazim 0.1 per cent @ 100 ml/plant along with application of carbofuran (1 g/plant) reduced fusarial wilt of brinjal to 10.6 per cent. This was closely followed by application of copper oxychloride (0.2 %) and carbofuran (Jayasekhar, 1995). Intensity of wilt complex of banana caused by *F. oxysporum*, *Pseudomonas solanacearum* and *Meloidogyne incognita* was reduced by dipping the healthy suckers in 0.2 per cent solution of Bavistin. (Roy *et al.*,1998). Dipping corms or drenching soil with carbendazim and thiabendazole at 0.2 per cent effectively controlled *F. solani* in saffron (Sud *et al.*, 1999).

Pandey and Upadhay (1999) reported that Bavistin is highly effective in controlling pigeon pea wilt caused by *F. udum*. Seed treatment with carbendazim, triademefon at 0.1 per cent and captan at 0.25 per cent suppressed *F. oxysporum* in pea. Verma and Dohroo (2002) also observed that wet seed treatment of autumn pea with 0.1 per cent of Bavistin or Kri-

Benomyl could reduce the incidence of pre- emergence rot and wilt caused by *F. oxysporum* f. sp. *pisi* both under greenhouse and field conditions. Similarly, Singh *et al.*, (2002) reported the seeds of *Dipterocarpus* returns treated with 0.2 per cent aqueous solution of Captan or Bavistin for 30 min. effectively suppressed Fusarium wilt and also increased seedling emergence. Vrinda (2002) reported that fungicides hexaconazole and propiconazole completely inhibited the growth of *F. solani* and several other fungi. She also found that mancozeb was more effective at higher concentrations in inhibiting of pathogen. Singh and Goswami (2003) reported that carbendazim application to sugarcane helped in checking Fusarium wilt as well as in improving sugarcane sprouting.

2.4.4 Compatibility of fungicides with biocontrol agents

The biocontrol agents known today cannot replace chemical fungicides in managing fusarial diseases. So, the selected biocontrol agents should be compatible with fungicides if they should become integral part of integrated disease management strategy.

Henis *et al.*, (1978) reported that PCNB at 4 µg/g soil applied along with *T. harzianum* had an additive effect on control of damping off of radish and synergistic effect on decreasing the inoculum density of *Rhizoctonia solani* propagules. Biotypes of *T. harzianum* tolerant to Captan, Captafol, Chlorothalonil, Iprodione and BAS 352 were obtained by exposing conidia to increased concentrations of the fungicides (Papavizas, 1980). He further proposed that biological approach could be successful only if antagonists are compatible with fungicides and biopesticides. Papavizas (1985) attributed the differential response of antagonistic flora to various fungicides to their inherent ability to degrade chemicals. Indu and Mukhopadhyay (1990) reported that there was no inhibition of radial growth of *T. harzianum* by metalaxyl at concentration as high as 50 ppm. Sawant and Mukhopadhay (1990) also studied the tolerance of *T. harzianum* to metalaxyl and found that the fungus could

tolerate the fungicide upto 50 ppm. Mukhopadhyay *et al.* (1992) reported the insensitivity of *Trichoderma* species against Vitavax and Captan.

Singh *et al.* (1993) reported that *T. koningii* was compatible with the fungicide carboxin at 200 and 500 ppm. The growth of *T. hamatum* and *T. viride* was poor indicating their incompatible response to carboxin. Sharma and Mishra (1995), Singh et al. (1995) observed that captan showed comparatively low inhibition of *T.harzianum* than thiram.

Mukherjee *et al.*, (1995) obtained vinclozolin tolerant isolates of *T. viride* by selecting on the fungicide amended medium. Similarly, benomyl tolerance has been obtained in mutants of *T. viride* isolate T-15 used in integrated control of *Botrytis* grey mold of chickpea (Mukherjee *et al.*, 1997).

Gupta *et al.*, (1999) found that *T. pseudokoningii* was compatible with Dithane M-45 under *in vitro* conditions, and opined that they could well be integrated for reducing rotting of mulberry caused by *F. solani*. Somasekhara *et al.*, (2000) reported that Captan had no inhibitory action on certain strains of *Trichoderma* spp. Siltuang *et al.*, (2000) reported that the populations of *T. harzianum* were higher in triademefon treated medium. Upamanyu *et al.*, (2002) while screening various biocontrol agents for management of root rot and web blight of French bean found that they were all sensitive to carbendazim though they showed maximum tolerance to fungicides such as carboxin and tebuconazole. Vrinda (2002) found that hexanconazole completely inhibited *Trichoderma* at all the concentration tested, where as, mancozeb caused only slight inhibition of mycelial growth and delay in sporulation. She also observed that *P. fluorescens* was not inhibited by these fungicides. Girija and Umamaheswaran (2003) reported total suppression of *T.virens* by carbendazim at 1000 ppm., though it was compatible at lower dose of 100 ppm.

2.4.5 Integrated management of *Fusarium* wilt of cowpea

Integration of biological, chemical and cultural methods in a compatible manner is essential to arrive at a long lasting solution for management of many important soil borne plant pathogens.

Integration of biological control agents with chemical fungicides has high potential for success. Mukhopadhyay (1996) showed that integration of biocontrol agents with fungicides gave significantly higher disease control in crops such as tomato, chickpea etc, than that obtained by the biocontrol agents or fungicide alone. Integration of bioagents *viz.*, *T. viride*, *T.virens* and *T. harzianum* C and D isolates with thiram benefited pigeon pea by decreasing wilt incidence incited by *F. udum*. However, combination of the bioagent *Trichoderma* with Bavistin was not effective in disease suppression (Pandey and Upadhyay, 1999). Rajb *et al.* (2000) reported that lentil wilt caused by *F. oxysporum* f. sp. *lentis* could be managed by seed treatment with *Bacillus subtilis* and Vitavax. In this treatment, wilt suppression of 66 per cent and subsequent yield increase of 64.5 per cent were recorded. Combined application of *P. fluorescens*, *T. viride* and Vitavax decreased the wilt incidence by 65 per cent. Integration of propagules of *T virens*, leaf powder of *Allium alliceae* and Bavistin (0.1%) effectively controlled the pigeon pea wilt caused by *F. udum* (Singh and Rai, 2000).

Soil application of *P.fluorescens* along with fungicidal rhizome treatment (Bavistin + Ridomil) effectively reduced the rhizome rot of ginger caused by *Fusarium* sp. (Ram *et al*, 1999). Seeds treated with Captan (1g/kg of seed) and *T. harzianum* (10^6 spores/10 g seed) reduced the collar rot disease of pea caused by *F.solani* f. sp. *pisi*. This also helped in improving germination and plant stand (Kumar and Dubey, 2001). Integration of Contaf (0.025 %) with *T. virens* (0.1 %) reduced the wilt complex of french bean caused by *Sclerotium rolfsii*, *R. solani* and *F. oxysporum* f. sp. *phaseoli* under field conditions. Seed treatment with

chemical and biological control agents were also effective in controlling wilt of french bean (Mukherjee *et al.*, 2001).

Combined application of cultural, biological and chemical treatments have been attempted by several workers for effective management of plant diseases. Narendrappa and Gowda (1995) reported that Panama wilt of banana caused by *F. oxysporum* f. sp. *cubense* was managed by using disease free planting material, pre planting dipping in 0.2 per cent carbendazim for 45 minutes application of lime or neem cake at 1 kg/pit before planting or the application of urea (200 g/plant) plus sugarcane trash mulch at five and seven months. Integration of soil solarization (6 weeks) and seed treatment with carbendazim (0.25 w/w) along with carbosulfan (3% w/w) reduced the wilt disease complex of chickpea caused by *Meloidogyne incognita – F. oxysporum* f. sp. *ciceri* (Patel *et al.*, 2000).

Chattopadhyay and Sen (1996) reported that integration of *T. viride* isolate T4, 0.1 per cent carbendazim and 186.7 kg/ha of KCl as soil amendment reduced 74.14 per cent wilt disease of muskmelon caused by *F. oxysporum.* Soil amendment with pine needles, rhizome treatment with mancozeb + thiophanate methyl at 0.25 per cent and carbendazim at 0.1 per cent for 60 minutes, combined with soil application of *T. harzianum* controlled the ginger yellows caused by *F. oxysporum* f sp. *zingiberi* in ginger (Sharma and Dohroo, 1997). Combination of *Paecilomyes lilacinus*, *T. harzianum* or *T. viride* and neem cake reduced wilt disease complex in gladiolus caused by the infection of *Meloidogyne incognita* and *F. oxysporum* f. sp. *gladioli* (Nagesh *et al.*, 1998). *T. viride*, *P. fluorescens*, *B. subtilis* and neem cake when applied together effectively controlled the wilt disease of pigeonpea caused by *F. udum* and also increased the yield. (Madhukeshwara and Seshadri, 2001). Siddiqui *et al.* (2001) studied the efficacy of 2 strains of *P.aeruginosa* and *B.subtilis*, combination of antagonist with neem cake or *Datura fastuosa* at 0.5 per cent and 1 per cent, respectively reduced the root disease of urdbean caused by

Macrophomina phaseolina, F. solani and *R. solani*. Soil amendment with Bavistin and neem cake was effective in suppressing the seed pathogens of *Dipterocarpus retusus viz. Penicillium, Fusarium* and *Phoma* (Singh *et al*, 2002). Bhaskar (2000) found that integration of deep ploughing, seed treatment with Thiram 0.25 per cent + Bavistin 0.2 per cent + carbofuran 1 per cent (w/w) and *T. harzianum* gave complete control of root rot disease of Egyptian clover caused by *R.solani, F. semitectum* and a nematode *Tylenchorrhynchus vulgaris*. The biomass production of the crop was increased, and hence the fodder yield also registered increase from 46.6 to 71.1 t/ha. Soil amendment with neem cake immediately followed by soil solarization seed treatment with *T.viride* and soil drenching with Bio-wonder (multi-bacterial culture) twice at an interval at 20 days after sowing effectively reduced the Fusarium wilt of watermelon and also increased the yield (Fugro *et al.*, 2000).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 ISOLATION AND CHARACTERISATION OF PATHOGEN ASSOCIATED WITH WILT DISEASE

3.1.1 Collection of samples for isolation

Cowpea plants showing yellowing and wilting were collected from Vellayani, Kalliyoor, Ookodu, Sasthankoil, Sasthavatam, Peringamala and Pallichal of Thiruvananthapuram District of Kerala State.

3.1.2 Isolation of the pathogen

The root of wilted cowpea plants were washed in water and cut into small bits. The pieces were then surface sterilized in 0.1 per cent mercuric chloride solution for one minute followed by washing in sterile water 2-3 times. The pieces were transferred into sterile petridishes containing Potato Dextrose Agar (PDA), under aseptic conditions and incubated at room temperature. When the fungal growth was visible, mycelial bits were transferred to PDA slants and labelled.

3.1.3 Purification

Eight fungal isolates obtained from cowpea roots were purified by single spore technique and pure cultures were maintained on PDA slants for further studies.

3.1.4 Pathogenicity

The pathogenicity of the eight isolates was proved following Koch's postulates. Cowpea seedlings were grown in small plastic cups. Ten to fifteen days old seedlings were inoculated with isolates of pathogen by placing the mycelial bit from five day old culture of the pathogen on root portion after giving injury by pinpricking. Three replications were maintained. The isolates capable of producing symptom of the disease were taken up for further studies the number of days taken for based on

the initiation of wilt after inoculation virulence rating was done. On the basis there virulent isolates of *Fusarium* were selected.

3.1.5 Characterisation of pathogens

3.1.5.1 Morphology and cultural characters

The morphology and cultural characters of different isolates of pathogen were studied by growing them on potato sucrose agar medium. Four to ten day old cultures were used for the study. Colony characters such as colour of the colony (both upper slower side of the petridish), texture, and growth rate were recorded.

3.1.5.2 Slide culture

The morphological characteristics of the isolates were studied by following slide cultures as described by Riddel (1950).

Sterile plain agar medium was poured in sterilized petridishes to a thickness of 2 mm. After solidification, 6 mm square pieces were cut using a sterile needle. One square disc was placed at the centre of a sterile slide and each of the four sides of the agar block was inoculated with mycelial bits of the pathogen. A cover slip was placed on the top of the inoculated agar disc and the slides were placed in moist petridish chambers (sterile petridish and wet filter paper in the bottom on which two glass rods kept as support for the slides). The dish with the slide was then incubated at room temperature for two to three days. After this, the cover slip was lifted off gently and mounted on another slide using lactophenol stain. The square of agar was removed from the culture slide and another mount was prepared on it without disturbing the fungal growth on the slide. The slides were examined under low and medium power objectives of compound microscope, and micro morphological characters of conidia and conidiophores were recorded.

3.1.5.3 Identification of pathogen

Based on the morphological, cultural and microscopic characters. The identification of the isolates were identified and confirmed at Indian Type Culture Collection (I.T.C.C.), I.A.R.I., New Delhi.

3.2 ISOLATION OF ANTAGONISTIS

3.2.1 Isolation of antagonistic fungi

Soil was collected from the rhizosphere of healthy cowpea plants at various locations. Fungi present in these soils were isolated using dilution plate technique (Waksman, 1922).

One gram of thoroughly mixed rhizosphere soil sample was transferred aseptically into a 250 ml conical flask containing 100 ml of sterile water and shaken thoroughly for 20 minutes. From the dilution, one ml was transferred into 99 ml of sterile water in a 250 ml conical flask under aseptic conditions, so as to get 10^{-4} dilution.

One ml of this dilution was transferred into sterile petridish and plated with Rose Bengal agar medium. Petridish were then incubated at room temperature for 48-72 h. The fungal colonies were examined and transferred to PDA slants. The fungal culture was purified by the hyphal tip method (Parmeter *et al.*, 1969). The purified cultures were then stored for subsequent studies on antagonism.

3.2.2 Isolation of saprophytic bacteria

Isolation of bacteria from soil was done by following the method of Waksman (1922) and Johnson and Curl (1972). One gram of thoroughly mixed rhizosphere soil sample was transferred aseptically into 250 ml conical flasks containing 100 ml of sterile water and shaken thoroughly. One ml of this suspension was transferred to another flask containing 99 ml of sterile water using sterile pipette so as to get a dilution of 10^{-4}. The process was repeated to get 10^{-6} dilution. One ml of aliquot from this dilution was transferred to sterile petriplates. Melted and cooled King's B medium as well as nutrient agar at the rate of 20 ml per dish were used for

plating. The petridish were incubated at 28±1^{0}C for 48 hours. Fluorescent colonies on King's B and other bacterial colonies on Nutrient agar were transferred and maintained on King's B slants / nutrient agar slants.

3.3 *IN VITRO* SCREENING OF MICROORGANISM FOR SUPPRESSION OF *FUSARIUM*

3.3.1 Mass screening

The fungi and bacteria isolated from dilution plates were tested for their antagonism to *Fusarium* by cross culture method (Henis *et al.*, 1979). Six mm diameter mycelial discs of the antagonist fungi and pathogen, and twenty four hour old culture of bacteria were used for this purpose.

Bacterial isolates were spotted at the rate of four isolates per petridish at a distance of 3 cm from the centre and near to the periphery at equidistant points. Similarly, four discs of different antagonistic fungi were placed in a similar way in separate petridish. Actively growing test pathogen was introduced at the centre of the dish. The replications were maintained and the dishes were kept at 28 ± 1^{0}C and examined for inhibition of growth of pathogen.

3.3.2 Screening of antagonistic fungi

The saprophytic fungi isolated from healthy cowpea rhizosphere were tested for their antagonism to *Fusarium* by dual culture technique (Skidmore and Dickinson, 1976). Agar disc of 5 mm diameter cut from seven day old culture of the saprophytic fungus and the pathogen were placed on two opposite sides of petriplates containing sterilized PDA and incubated at room temperature. Three replications were maintained for the experiment. The paired cultures were examined at regular intervals and the radial growth of the pathogen was recorded. Petridishes inoculated with pathogen alone served as the control.

The percent inhibition of mycelial growth was calculated using the formula

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

Where

I- Inhibition of mycelial growth

C- Growth of pathogen in control plates (cm)

T – Growth of pathogen in dual cultures (cm)

(Vincent, 1927)

3.3.3 Screening of antagonistic bacteria

Isolates of *P. fluorescens* and bacteria were isolated from healthy cowpea rhizosphere. Isolates were tested to determine their antagonistic potential against the wilt pathogen of cowpea. King's B medium and nutrient agar medium was used for the study. King's B and nutrient agar medium were melted and poured into sterile petriplates. After solidification, culture bits of 5 mm size of the pathogen was placed at the centre of each dish. The respective bacterial isolate was then streaked 2 cm away from pathogen at the centre in a triangular pattern. Each treatment was replicated thrice. Plates inoculated with *Fusarium* alone served as the control. Radial growth of the pathogen was taken from bacteria treated dishes.

The per cent inhibition of mycelial growth was calculated using the formula

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

(Vincent, 1927)

3.4 SCREENING OF BIOCONTROL AGENTS FOR WILT SUPPRESSION AND PLANT GROWTH PROMOTION

The antagonistic fungi and bacteria selected through the *in vitro* screening procedure was further evaluated for their efficiency in reducing wilt in cowpea. A pot culture experiment in CRD was conducted at the College of Agriculture, Vellayani. The newly reported endophytic biocontrol fungus, *Piriformospora indica* and the cultures of *Trichoderma viride* and *Pseudomonas fluorescens* released from the Department of Plant Pathology, College of Agriculture, Vellayani, Kerala Agricultural University were also included. Three replications were maintained. The treatments were as follows:

Treatments

T_1 – (A_7) *T. viride* + *Fusarium*

T_2 – (A_{15}) *T. virens* + *Fusarium*

T_3 – *Trichoderma* (KAU culture) + *Fusarium*

T_4 – (P_5) *P. fluorescens* + *Fusarium*

T_5 – (P_9) *P.fluorescens* + *Fusarium*

T_6 – *P.fluorescens* (KAU culture) + *Fusarium*

T_7 – *P. indica* + *Fusarium*

T_8 – Control (without *Fusarium*)

T_9 – Control (with *Fusarium*)

Earthern pots were filled with ½ kg sterilised soil mixture consisting of sterilized soil and well decomposed farmyard manure. Three cowpea seeds (var. Sarika) were sown in each pot.

3.4.1 Preparation of inoculum of antagonists

3.4.1.1 Preparation of talc based formulation of bacterial antagonist

The cultures of P5 and P9 were grown in King's B broth in conical flasks and incubated at room temperature. The bacterial population was estimated after 48 hours of inoculation.

The ingredients of the talc based formulation *viz.*, 100 g of talc, 4 g of calcium carbonate and 1 g of carboxy methyl cellulose (CMC), were mixed thoroughly and kept in polypropylene bags. The bags were then sealed and autoclaved at 1.5 kg cm^{-2} for one hour on two successive days. After sterilization, 40 ml of two day old broth culture of the bacterium was mixed with the carrier in each polypropylene bag under aseptic condition. This formulation was used for mass delivery of the antagonist in the pot culture.

3.4.1.2 Preparation of antagonistic fungal inoculum

3.4.1.2.1 Trichoderma sp.

Selected *Trichoderma* spp. (A_7 and A_{15}) were mass multiplied on sand-rice bran mixture using the modified method of Lewis and Papavizas (1984). Rice bran was mixed with sand in the ratio 1:9. The mixture was moistened with water sufficient enough to promote fungal growth. This mixture was taken in 750 ml conical flasks and sterilized at 1.02 kg cm^{-2} for one hour. Actively glowing culture disc of the *Trichoderma* was aseptically transferred into the flasks and incubated at room temperature for 10 days to develop fungal growth.

3.4.1.2.2 Piriformospora indica

The culture of *P.indica* was obtained from School of Life Sciences, Jawaharlal Nehru University, New Delhi. *P.indica* was mass multiplied on potato dextrose broth medium. PD broth was prepared and autoclaved at 1.02 kg cm^{-2} for 15 minutes. Actively growing culture disc of *P.indica* was aseptically transferred into the flasks and incubated at room temperature for two weeks to develop fungal growth. The mycelium and culture filtrates were used in pot culture experiment.

3.4.2 Preparation of pathogen inoculum

Fusarium was mass multiplied on sand-rice bran mixture using the modified method of Lewis and Papavizas (1984) as described under 3.4.1.2.1.

3.4.3 Inoculation of antagonists and pathogen

Fifteen days after sowing mass multiplied *Fusarium* culture was applied at the root zone of the plant and thoroughly incorporated into the soil.

After 45th day, the antagonists *Trichoderma*, *P. fluorescens* were applied to the soils of respective pots. Mycelial mat of *P. indica* was applied at the root zone of the crop and culture filtrate was poured at the root zone. The cowpea was maintained as per the Package of Practices Recommendations: crops (KAU, 2002) by giving timely application of manures and fertilizers.

Biometric observations like height of the plant, number of pods / plant, pod weight, fresh and dry weight of plant and root, fresh and dry weight nodules were recorded at the time of harvest of plants wilt incidence was reckoned the number of plant units infected *ie.*, whole plants and expressed percentage of diseased entities within sampling unit (James, 1974).

Wilt incidence was calculated using the formula

$$\text{Disease incidence (\%)} = \frac{\text{No. of plants wilted}}{\text{Total no. of plants}} \text{ X } 100$$

3.5 USE OF FUNGICIDES FOR SUPPRESSION OF *FUSARIUM*

3.5.1 Bioassay of commonly used fungicides against the wilt pathogen

Fungicides, commonly used for vegetable disease control were evaluated *in vitro* against wilt pathogen (*Fusarium*) at the recommended concentrations

Fungicide	Concentration (%)
carbendazim	0.1
mancozeb	0.3
copper oxychloride	0.2
chlorothalonil	0.1

The test was performed following the poisoned food technique (Zentmeyer, 1955). The required quantity of fungicide was thoroughly mixed with 50 ml of sterile water in a 250 ml conical flask. The fungicidal suspension was poured into another 250 ml conical flask containing 50 ml of double strength melted PDA and mixed thoroughly. The medium was poured into sterile petridishes. After solidification of the medium each plate was inoculated at the centre with 5 mm agar disc of the pathogen. Plates containing non-poisoned media served as control. Three replications were maintained for each concentration of the chemical. The petridishes were incubated at room temperature and radial growth of pathogen was recorded.

The per cent inhibition of mycelial growth was calculated using the formula

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

(Vincent, 1927)

3.5.2 Compatibility of fungicides with antagonistic microorganisms

The experiment was conducted to test the compatibility of selected fungicides and biocontrol agents that are to be used for the field study. The cultures of fungal isolates (A_7 and A_{15}) and bacterial isolates (P5 and P9) which showed maximum biocontrol potential against the *Fusarium* under *in vitro* condition were selected for the compatibility test.

The fungicide and their respective concentrations used for the study are as follows

Fungicide	Concentration (%)
carbendazim	0.1
mancozeb	0.3
copper oxychloride	0.2
chlorothalonil	0.1

3.5.2.1 Compatibility of fungicides with Trichoderma spp.

The experiment was conducted by the poison food technique as described in 3.5.1. Observations on the radial growth and per cent inhibition of *Trichoderma* were recorded.

3.5.2.2 Compatibility of fungicides with *Pseudomonas fluorescens*

The effect of fungicides such as carbendazim, mancozeb, copper oxychloride and chlorothalonil on growth of *Pseudomonas fluorescens* was studied under *in vitro* conditions by the method of Bandopadhyaya *et al.* (1979).

Sterile filter paper discs of 10 mm diameter were dipped in appropriate concentrations of fungicides listed under 3.5.2. Kings'B medium was melted and allowed to cool to 40^0C. This was seeded with 48 hours old culture of *P.fluorescens* isolates P_5 and P_9 separately. Medium was poured into the sterile petridish and allowed to solidify. Four filter paper discs dipped in appropriate fungicidal solution were placed at equidistant places 1 cm from the periphery of the dish. Three replications were maintained for each treatment. Observations on the zone of growth inhibition was noted after 72 h of incubation at room temperature. Plates seeded with *P.fluorescens* and having sterile filter discs dipped in sterile water served as control.

3.5.3 *In vivo* screening of fungicides for suppression of wilt

Details of experiment were as follows:

Design – CRD

Treatments – 5

Replication – 5

Treatments

T_1 – Soil drenching of mancozeb (0.3%)

T_2 – Soil drenching of copper oxychloride (0.2%)

T_3 – Soil drenching of carbendazim (0.1%)

T_4 – Control (without *Fusarium)*

T_5 – Control (with *Fusarium)*

Cowpea seeds of the variety Sarika were sown in pots. Two-week after sowing mass multiplied culture of the *Fusarium* was applied into the soil.

Fortyfifth day after sowing, the fungicides were suspended in water at the required concentration and used for drenching soil for the respective treatments. Cowpea plants were maintained as per the Package of Practice Recommendations: crops (KAU, 2002) by giving timely application of fertilizers and adopting need based plant protection measures. Onset of wilt and symptoms were recorded. Plants biometrics observations *viz.*, plant height, fresh and dry weight of shoots and roots, number of pods, pod weight, number of nodules, nodule fresh and dry weight were recorded at harvest.

The percentage wilt intensity was calculated using score chart (Fig 1). The individual plants in each treatment were scored by assigning scores of 0-4, where

0-healthy plants

1-slight yellowing of leaves

2-yellowing and necrosis of leaves

3-Basal swelling, yellowing and necrosis of leaves

The individual plants in each treatment were scored by assigning scores of 0-4, where

0 – healthy plants

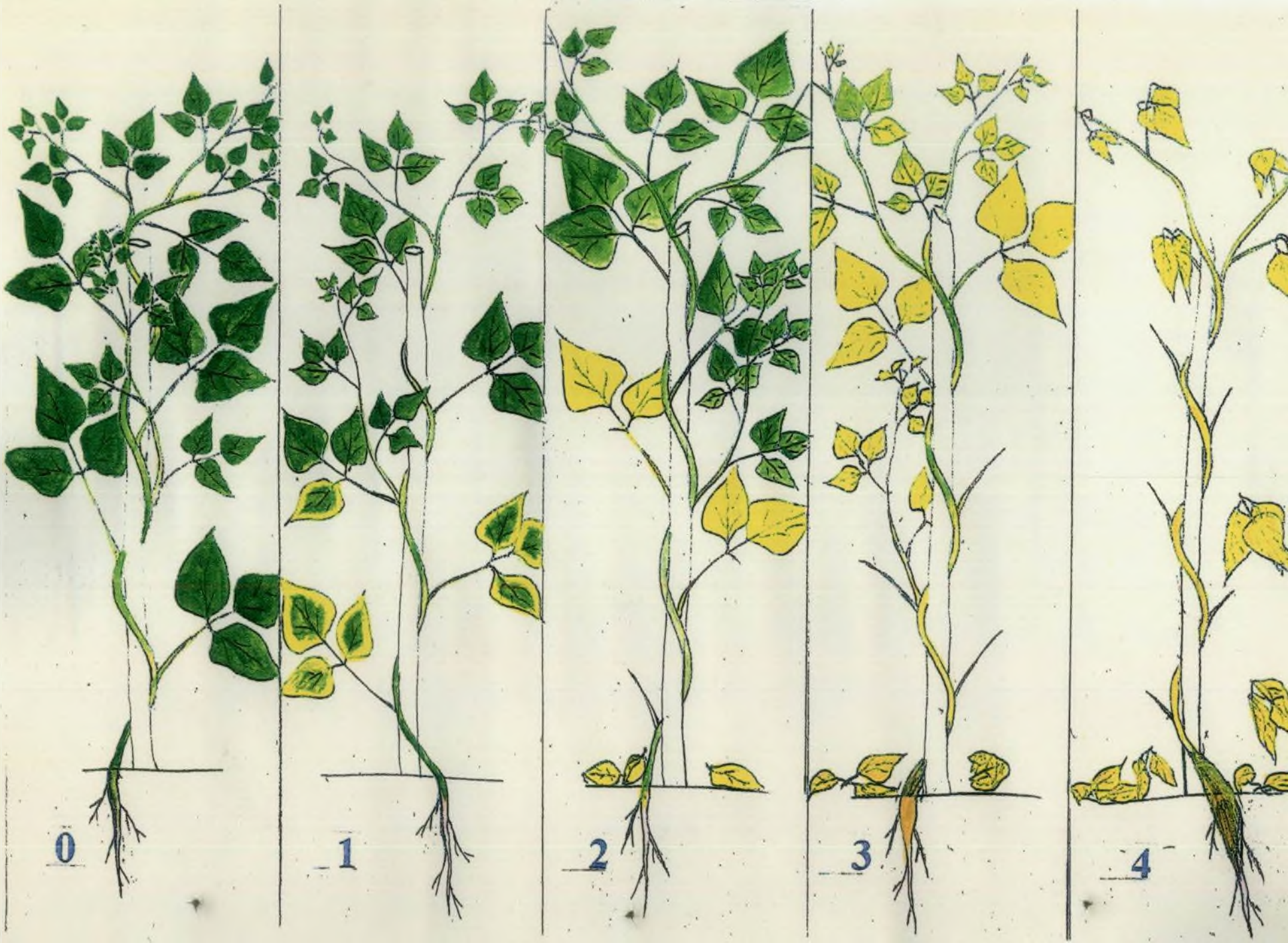
1 – slight yellowing of leaves

2 – yellowing and necrosis of leaves

3 – Basal swelling, yellowing and necrosis of leaves

4 – Basal swelling, distortion, yellowing and necrosis of leaves (total wilting)

SCORE CHART



4-Basal swelling, distortion, yellowing and necrosis of leaves (total wilting)

Percentage disease intensity was calculated by using the modified formula of Chattopadhyay and Sen (1996).

$$\text{Disease intensity} = \frac{\text{Sum total of scores}}{\text{Total number of plants assessed}} X \frac{100}{\text{Maximum grade}}$$

3.6 USE OF AMENDMENTS FOR SUPPRESSION OF FUSARIUM

3.6.1 Bioassay of amendments for suppression of *Fusarium*

Soil amendments were evaluated under *in vitro* condition against *Fusarium* following the method of Grover and Moore (1962). The amendments and concentration used are given below.

Amendments	Concentrations %
Neem cake	5, 10
Vermicompost	5, 10
Coirpith compost	5, 10
Lime	0.1

Hundred gram of organic amendment was transferred into 250 ml conical flask containing 100 ml of distilled water. This was allowed for overnight soaking. This was filtered through two layers of muslin cloth to get 100 per cent standard solution of the extract. From this 5 ml and 10 ml of extract was transferred to 95 ml and 90 ml of PDA and autoclaved. This was used for the further studies.

The medium containing extract was melted and poured into sterile petridishes in aseptic conditions. Five day old culture of the *Fusarium* grown in PDA in petridishes was used for the study. Five mm mycelial disc of the fungus was introduced into center of each dish. PDA without extract was used as control. The radial growth of the pathogen was recorded and per cent inhibition was calculated using the formulae.

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

(Vincent, 1927)

3.6.2 Compatibility of amendments with antagonistic microorganisms

The experiment was conducted to test the compatibility of selected amendments with biocontrol agents that were to be used for the field study. The *Trichoderma* isolates (A_7 and A_{15}) and *P.fluorescens* isolates (P_5 and P_9) which showed maximum biocontrol potential against the *Fusarium in vitro* were selected for the compatibility test.

The amendments and their respective concentrations used for the study are as follows.

Amendments	Concentrations, %
Neem cake	5, 10
Coirpith compost	5, 10
Vermicompost	5, 10
Lime	0.1

3.6.2.1 Compatibility of amendments with *Trichoderma spp.*

The extract was prepared as described in 3.6.1. The medium containing extract was melted and poured into sterile petridishes in aseptic conditions. Five day old culture of the *Trichoderma* spp grown on PDA in petridishes was used for the study. 5 mm mycelial disc of the fungus were transferred into centre of each dish. PDA without extract of amendments was used as control. The radial growth of the *Trichoderma* was recorded.

Per cent inhibition of mycelial growth over control was calculated using the following formula,

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

(Vincent, 1927)

3.6.2.2 **Compatibility of amendment with *Pseudomonas fluorescens***

The organic amendment extract was prepared as described in 3.6.1. and compatibility of the extract against *P. fluorescens* was studied as described under 3.5.2.2.

3.6.3 *In vivo* screening of amendments for suppression of wilt

A pot culture experiment was conducted to evolve management practices by using soil amendments. Details of experiment were as follows:

Design – CRD

Treatments – 7

Replication – 5

Treatments

T_1 – Soil application of vermicompost (6.25 t/ha)

T_2 – Soil application of coirpith (6.25 t/ha)

T_3 – Soil application of neemcake (150 kg/ha)

T_4 – Soil application of lime

T_5 – Soil drenching with Copper oxychloride (0.2 %)(Chemical check)

T_6 – Control (without *Fusarium)*

T_7 – Control (with *Fusarium*)

Earthern pots were used for this study.

For treatments T_1, T_2 and T_3 the respective quantity of amendment was thoroughly mixed with soil and filled into the pots. These pots kept 7 – 10 days for decomposition of organic amendments.

Cowpea seeds of the variety Sarika were sown in pots. Two-week after sowing, mass multiplied culture of the *Fusarium* was applied into the soil.

Forty-fifth day after sowing, copper oxychloride (0.2 %) was suspended into water at the required concentration and used for drenching

soil into the respective treatments. Cowpea plants were maintained as per the Package of Practice Recommendations: Crops (KAU, 2002) by giving timely application of fertilizers and adopting need based plant protection measures. Plants biometric observations like plant height, fresh and dry weight of shoot and roots, number of pods, pod weight, number of nodules, nodule fresh and dry weight were recorded at the time of harvest of the crop. The percentage wilt intensity was recorded as detailed in 3.5.3.

3.7 INTEGRATED MANAGEMENT PRACTICE OF FUSARIUM WILT

The pot culture experiment was conducted to evolve an integrated management practice by incorporating the most effective fungicide, biocontrol agent and organic amendments selected through experiment 3.4, 3.5.3 and 3.6.3 respectively in all possible combinations.

Details of experiment was follows

Design – CRD

Treatments – 9

Replication – 3

Treatments

T_1 – *Trichoderma* (seed treatment 4 g/kg seed+ soil application 2.5 kg/ha 30 DAS

T_2 – Soil application of neem cake (150 kg / ha) at the time of potting mixture preparation + Soil application of *Trichoderma* (2.5 kg / ha) 30 DAS

T_3 – Soil application of neem cake (150 kg /ha) at the time of potting mixture preparation

T_4 – Soil drenching of mancozeb (0.3 %) 45 DAS

T_5 – Soil application of neem cake (150 kg/ha) at the time of potting mixture preparation + Soil drenching of mancozeb (0.3 %) 45 DAS

T_6 – *Trichoderma* (seed treatment 4 g/kg seed + soil application 2.5 kg/ha at 30 DAS) + Soil drenching of mancozeb (0.3 %) 45 DAS

T_7 – Soil application of neem cake 150 kg/ha + soil application of *Trichoderma* 2.5 kg/ha 30 DAS + Soil drenching of mancozeb (0.3 %) 45 DAS

T_8 – Control (without *Fusarium*)

T_9 – Control (with *Fusarium*)

3.7.1 Preparation and application of *Trichoderma* for seed treatment

Trichoderma was inoculated into sterile petridishes containing Potato Dextrose Agar (PDA) medium and incubated for five days. The spores of the fungus were scraped from the surface of the medium and suspended in sterile water to which 0.1 per cent carboxymethyl cellulose (CMC) was added. Cowpea seeds were treated with this mixture. Seeds were dried under shade and used for sowing in pots.

3.7.2 Preparation of *Trichoderma* for soil application

The selected *Trichoderma* spp. were mass multiplied on sand-rice bran mixture as described under 3.4.1.2.1 and applied as in 3.4.3.

3.7.3 Application of soil amendments

Soil amendments were incorporated at the time of pot mixture preparation. Neem cake was thoroughly mixed with sterilized soils filled into the earthern pots and incubated ten days, after which cowpea seeds were sown.

3.7.4 Application of fungicides

Mancozeb (0.3 %) was prepared by suspending the required quantity of fungicides in appropriate quantity of water. It was thoroughly mixed and used for drenching in the cowpea plants at 45 days after sowing.

3.7.5 Application of pathogen

Fusarium was mass multiplied on sand-rice bran mixture as described under on 3.4.2 was incorporated into the soil two days after sowing.

Cowpea plants were maintained as per the Package of Practice Recommendations: crops (KAU, 2002) by giving timely application of fertilizers and adopting eco-friendly protection measures.

Plant biometric observations such as plant height, fresh and dry weight of stem and root, number of pods per plant, pod weight, number of nodules per plant, and fresh and dry weight of nodules were recorded at the time of harvest of the crop. The percentage wilt intensity was calculated as detailed in 3.5.3.

3.8 STATISTICAL ANALYSIS

The data obtained from the studies conducted under laboratory and field conditions were statistically analysed and interpreted.

RESULTS

4. RESULTS

4.1 SYMPTOMATOLOGY

The natural incidence of cowpea wilt was observed in farmers' fields and the sequence of events leading to final death of the plants were recorded. The symptoms started as yellowing of foliage followed by defoliation (Plate 1). Lower stem portion showed rotting leading to drying of vines above. The roots showed rotting. In severe cases, the lower part of the stem and the upper part of the taproot together formed a swollen tuber like structure which gradually became shredded and disintegrated (Plate 2).

4.2 COLLECTION OF SAMPLES IN ISOLATION

Cowpea plants showing yellowing and wilting symptoms were collected from different cowpea growing areas of Thiruvananthapuram district. The list of *Fusarium* isolates obtained from the diseased samples and their locations are presented in Table 1.

4.2.1 Pathogenicity test

The pathogenicity studies using the *Fusarium* isolates showed that all isolates except F_3, F_4 and F_6 produced symptoms of wilt (Table 2). Re-isolation from infected seedlings also yielded *Fusarium* identical to the respective original culture (Plate 3).

4.2.2 Identification of Fusarium isolates

Based on the morphological and cultural characters presented in Table 4, the isolates of *Fusarium* were tentatively identified (Table 3 and Plate 4, 5) as *F.pallidoroseum*, *F.oxysporum* and *F.solani*.

The characters of species described are as follows

Table 1. *Fusarium* isolates and their location

Sl. No.	Isolate	Location
1.	F_1	Vellayani
2.	F_2	Ookodu
3.	F_3	Kalliyoor
4.	F_4	Sasthavattam
5.	F_5	Pallichal
6.	F_6	Sasthankoil
7.	F_7	Peringamala
8.	F_8	Kalliyoor

Table 2. Comparative virulence of Fusarium isolates on cowpea plants

Sl. No.	Isolates	Time taken for symptom development (days)	Virulence rating
1	F_1	8	+++
2	F_2	40	+
3	F_3	-	-
4	F_4	-	-
5	F_5	15	++
6	F_6	-	-
7	F_7	36	+
8	F_8	12	++

+ pathogenic
++ virulent
+++ highly virulent
-- not pathogenic

Table 3. Identification of *Fusarium* isolates

Sl. No.	Isolate	Location
1.	F_1	*F.pallidoroseum*
2.	F_2	*F.solani*
3.	F_3	*F.solani*
4.	F_4	*F.oxysporum*
5.	F_5	*F.pallidoroseum*
6.	F_6	*F.solani*
7.	F_7	*F.oxysporum*
8.	F_8	*F.pallidoroseum*

Plate 1. Wilt affected cowpea plant

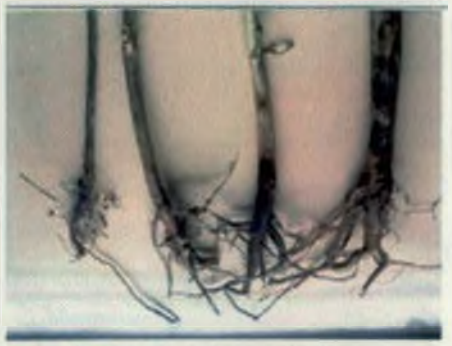


Plate 2. Wilt affected cowpea roots

Plate 3. Pathogenicity testing

4.2.2.1 Fusarium pallidoroseum

The isolates F_1, F_5 and F_8 had fast rate of growth on PDA and took only 5-6 days to complete growth in 9 cm diameter petridishes. The colony was floccose in texture and white in colour, and which changed to peach or light violet on upperside and violet or peach colour on lower surface. Conidiophores arose singly from aerial mycelium and later became loosely irregular and rarely verticillately branched. They were mostly monophialidic or polyphialidic. The phialides were slender and cylindrical to sub cylindrical. The macroconidia were hyaline, fusoid in shape, 0-7 septate and measured 27.5-36.06 x 3.0-3.5 µm. Sometimes, conidia had typical heeled and non-pedicillate foot cell. Wedge shaped basal cell were also found. Microconidia were ovoid in shape. Chlamydospores were sparse. They were mostly intercalary, hyaline and formed singly, paired or in chains or clusters. The perfect stage was not detected.

4.2.2.2 Fusarium oxysporum .

The isolate, F_4 and F_7 had medium growth rate taking 7-8 days for full growth in petridish. Colonies appeared floccose in texture and white on upper surface, reddish brown or faint pink on lower side of petridish. Conidiophores contained single phialides, arising laterally on the verticillately branched hyphae or compactly packed short branches. Single, obclavate phialides arose from primary and secondary conidiophores. Macroconidia were 1-4 septate, 13.6-17.6x 1.5-2.3µm size, thin walled, sub-cylindrical/fusoid with pointed ends, occasionally falcate with hooked terminal cells, and not pedicellate or with pointed basal cell. Microconidia were abundant and ovoid in shape. Chlamydosores, when present, were intercalary and in chains.

4.2.2.3 Fusarium solani

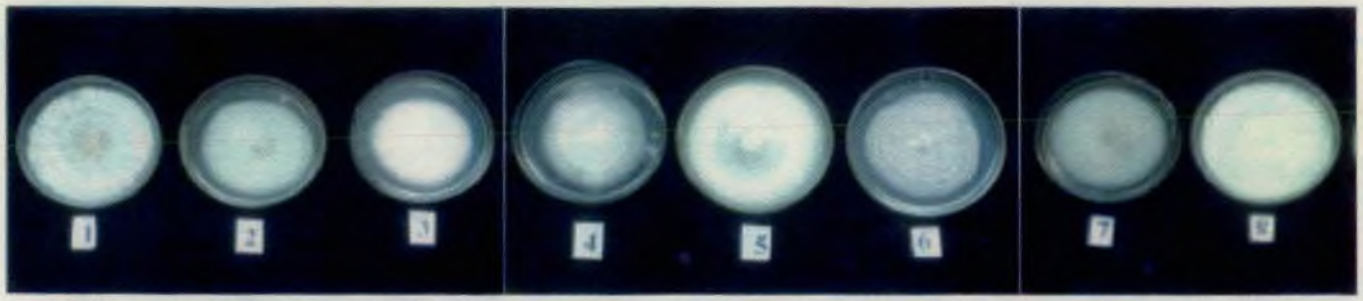
The isolates F_2, F_3 and F_6 had medium growth rate and took 7-8 days to attain full growth in petridish. Colony appeared fluffy in texture,

Table 4. Cultural and morphological characteristics of *Fusarium* isolates

Isolate No.	No.of days taken to cover 9 cm petridish	Average rate of growth, cm/day	Colony texture	Colour of colony		Conidiophore arrangement			Phialides	
				Upper side	Lower side	Single/ group	Loose / compact	Branching	Number	Shape
F_1	5	1.8	Floccose	White	Peach	Single	Loose	Irregular	Mono/poly	Cylindrical/ subcylindrical
F_2	7	1.28	Fluffy	White	Dirty white	Single to many	Compact	Lateral	Single	Subcylindrical
F_3	8	1.12	Fluffy	Violet	Dark brown	Groups	Compact	Lateral	Single	Subcylindrical
F_4	8	1.12	Floccose	White	Faint pink	Dense group	Compact	Verticillate	Single	Obclavate
F_5	6	1.50	Floccose	White	White	Single	Loose, divergent	Verticillate	Single	cylindrical
F_6	7	1.28	Fluffy	White	Reddish brown	Groups	Compact sporodochia	Lateral	Single	cylindrical
F_7	7	1.28	Floccose	White	Reddish brown	Groups	Compact	Sparse verticillate	Single	Obclavate
F_8	5	1.80	Floccose	Light violet	Violet	Singly rarely many	Loose	Irregular	Mono	Cylindrical / subcylindrical

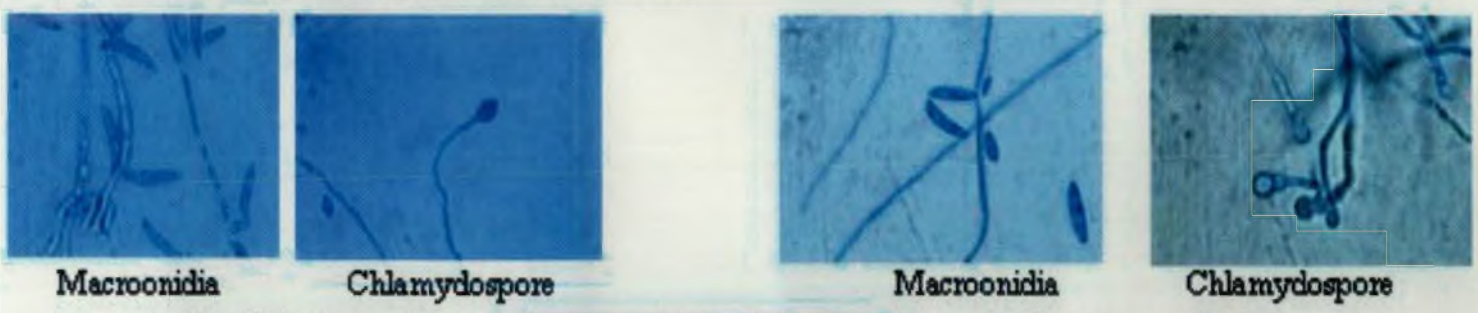
Table 4. Continued...

Isolate No.	Macro conidia						Micro conidia shape	Chlamydospores	
	Number of septa		Average length (µm)	Average breadth (µm)	Basal cell type	Shape		Position	Formation (single/chain/ cluster)
	Range	Average							
F1	2-7	5	27.5	3.5	Not pedicillated, but heeled	Fusoid	Oviod	Intercalary	Paired/ single
F2	3-5	3	24.6	3.0	Pointed	Falcate and bend apicaly	Ovoid straight	Terminal	Single / paired / chains
F3	3	3	26.6	2.5	Pedicillated	Falcate and hooked	Ellipsoid al to curved	Terminal/i ntercalary	Single / paired / chain
F4	1-4	2	17.6	2.3	Pointed end	Subcylindrical	Ovoid	Intercalary	Chains
F5	0-5	3	28.0	3.0	Not pedicillated	Fusoid	Ovoid	Terminal/ intercalary	Paired / chains
F6	4	4	17.11	1.0	Pedicillated	Subcylindrical	Ovoid and straight	Not formed	-
F7	3	3	13.64	1.5	Not pedicillated	Falcate/Fusoid with hooked terminal cells	Ovoid	Not formed	-
F8	2-6	4	36.06	3.0	Wedge shaped	Fusoid	Ovoid	Intercalary	Chains



F_1 F_2 F_3 F_4 F_5 F_6 F_7 F_8

Plate 4. Growth of *Fusarium* isolates on PDA



Macroonidia Chlamydospore Macroonidia Chlamydospore

a. *F.pallidoroseum* (F_1) b. *F.oxysporum* (F_4)

Macroonidia

c.*F.solani* (F_3)

Plate 5. Microphotographs of macroconidia and chlamydospore of *Fusarium* spp.

white or violet on upper side and dirty white, reddish brown or dark brown on lower side of the petridish. The primary conidiophores arose laterally from hyphae on aerial mycelium which was unbranched or sparsely branched and were found in sporodochia. The sub-cylindrical/cylindrical phialides produced long macroconidia. The macroconidia were abundant in number and thick walled, sub-cylindrical/falcate in shape pointed at base, pedicillated slightly curved and bent apically. Macroconidia were 3-5 septate and measured 17.1-26.6 x 1.0-2.5 µm in size. Microconidia were ovoid and straight or rarely ellipsoidal to curved. Chlamydospores were present at terminal or intercalary position. These were found in singles, pairs or chains.

4.2.3 Virulence testing of *Fusarium* isolates

The pathogenicity and comparative virulence of eight isolates of *Fusarium* in causing wilt was assessed by inoculating them on two week old cowpea seedlings. Yellowing and wilting symptoms were recorded. The time taken for symptom expression of isolates was recorded and presented in Table 2. F_1 isolate obtained from Vellayani was highly pathogenic and it took only eight days for development of the disease. On the contrary, the maximum time for the disease development was noticed with F_2 isolate, which took 40 days to cause wilt. The *Fusarium* isolates, F_3, F_4 and F_6 did not produce any symptoms. Based on the virulence, *Fusarium* isolate F_1 was selected as the test pathogen for further studies.

4.2.4 Confirmation of identity of F_1 isolate

F_1 isolate, tentatively identified as *F. pallidoroseum* by studying the cultural and morphological features, was sent to Indian Type Culture Collection, I.A.R.I., New Delhi for confirming the identification. It was confirmed and identified as *Fusarium pallidoroseum* (Cke) Sacc. and was allotted the I.T.C.C. No.5429.

4.3 ISOLATION OF ANTAGONISTS

4.3.1 Isolation of antagonistic fungi

A total of 82 fungal isolates were made from the rhizosphere of healthy cowpea plants. The most frequently isolated fungi from the rhizosphere belonged to the genus *Trichoderma*.

4.3.2 Isolation of antagonistic bacteria

A total of 115 bacterial isolates were isolated from healthy cowpea rhizosphere. The most frequently isolated bacterium from the rhizosphere was *Pseudomonas fluorescens*.

4.4 *IN VITRO* SCREENING OF MICROORGANISMS FOR SUPPRESSION OF *FUSARIUM*

4.4.1 Fungi

4.4.1.1 Mass screening of antagonistic fungi

The fungal isolates obtained by dilution plate were screened for antagonism to *F. pallidoroseum* under *in vitro* conditions. The inhibition of growth of the pathogen by the different isolates was visually examined. Out of 82 fungal isolates, 18 showing antagonism to the pathogen were selected for further study. These were serially numbered from A_1 to A_{18}.

4.4.1.2 Dual culture studies for antagonism

In general, all the fungi tested showed suppression of *F. pallidoroseum* to varying extents. The maximum inhibition was noticed with the isolate A_7, which inhibited colony growth of the pathogen by 73.33 per cent (Table 5 and Plate 6). Colony diameter of the pathogen, when dual cultured with A_7 was only 2.4 cm as compared to 9.0 cm as control (Table 5). This was followed by the co-inoculation of the isolate, A_{15} which gave an inhibition of 68.88 per cent of the pathogen (Table 5 Plate 6). All the other isolates produced lesser inhibition to *Fusarium*.

Table 5. Inhibition of mycelial growth of *F.pallidoroseum* by fungal antagonists

Sl. No.	Fungal isolates	Colony diameter of *F.pallidoroseum* after 5 days (cm)	Inhibition of mycelial growth of *F.pallidoroseum* (%)
1	A_1	5.6	37.77
2	A_2	5.9	33.33
3	A_3	5.5	38.88
4	A_4	3.6	58.88
5	A_5	3.0	66.66
6	A_6	3.0	66.66
7	A_7	2.4	72.33
8	A_8	4.3	48.88
9	A_9	6.8	24.44
10	A_{10}	5.1	43.33
11	A_{11}	5.4	40.00
12	A_{12}	5.4	40.00
13	A_{13}	5.3	41.11
14	A_{14}	5.1	43.33
15	A_{15}	2.8	68.88
16	A_{16}	4.0	55.56
17	A_{17}	3.8	57.77
18	A_{18}	3.6	58.88
	Control	9.0	
	CD(5%) - 20.63		

Table 6. Inhibition of mycelial growth of *F.pallidoroseum* by antagonistic bacteria

Sl. No.	Bacterial isolates	Mycelial growth of *F.pallidoroseum*, cm	Inhibition of mycelial growth of *F.pallidoroseum*, %
1	P_1	4.4	51.50
2	P_2	3.9	56.66
3	P_3	3.9	56.66
4	P_4	3.9	56.66
5	P_5	3.1	65.55
6	P_6	4.6	48.88
7	P_7	3.9	56.66
8	P_8	4.3	52.22
9	P_9	3.5	61.11
10	P_{10}	4.2	53.33
	Control	9.0	

Hence, A_7 and A_{15} were selected for further *in vivo* studies. Both these antagonists belonged to the genus *Trichoderma*.

4.4.1.3 Identification of antagonistic fungi

Based on the morphological and cultural characteristics the A_7 and A_{15} isolates are identified as *Trichoderma viride* Per. ex. S.F. Gray (Plate) and *Trichoderma virens* (Miller, Giddens and Foster) Von Arx = *Gliocladium virens* Miller, Giddens and Foster (Plate 8).

4.4.1.3.1 Description of the Trichoderma spp.

T. viride

The isolate showed fast growth on PDA medium taking 4 days to cover 9 cm diameter petridish. The colony surface was smooth, laterally becoming hairy and dark green. The typical coconut odour was emitted in old cultures. Mycelium was hyaline, smooth, septate and much branched. Chlamydospores were noticed and positioned intercalary. They were globose or ellipsoidal measuring 10-15 µm in diameter. Conidiophores were seen arising in loose tuft, main branches produced several side branches in groups of 2-3, standing at wide angles. Phialides were noticed in loose whorls. The conidia were green in colour, globose or broadly ellipsoidal with minutely roughened wall, 3.5–4.5 µm in size, accumulated at the tip of each philalides. They were pale green in colour.

T. virens

The isolate showed fast growth on PDA medium taking 4 days to cover full growth in petridishes. It produced aerial mycelium with floccose texture and white to grey coloured colonies. Conidiophores were subhyaline, measured 30-300 µm in length and 2.5-4.5 µm in diameter. The base appeared unbranched for about half of the length, but irregular branching was noticed at the apex with each branch terminated by a cluster of 3-6 closely appressed phialides. Conidiophore branches arose at right angles. Conidia were ellipsoidal to oval, 3.5 x 4.4 µm in size, dark

green in colour. Conidia from adjacent phialides were found to form large gloeoid masses.

4.4.2 Bacteria

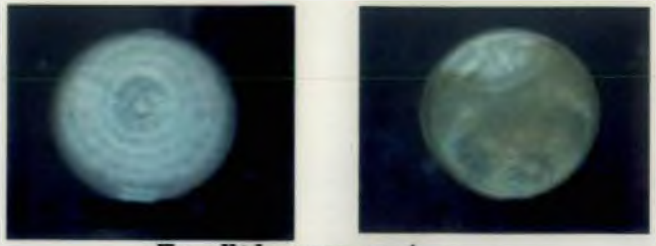
4.4.2.1 Mass screening of saprophytic bacteria

The bacterial isolates obtained by dilution plate were screened for antagonism to *F.pallidoroseum* by cross culture method. The inhibition of growth of the pathogen by the different isolates were visually examined. Out of 115 bacterial isolates, ten isolates that showed antagonism to *F.pallidoroseum* were selected for further studies. These isolates were serially numbered from P_1 to P_{10}.

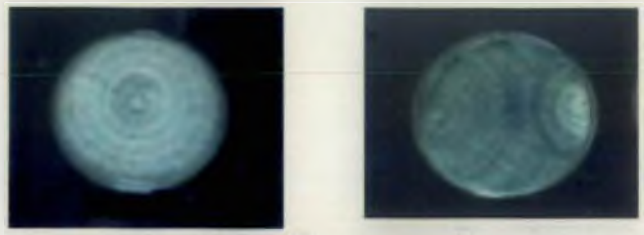
4.4.2.2 Screening of antagonistic bacteria by dual culturing

The efficacy of the ten selected isolates in inhibiting the growth of the pathogen under *in vitro* condition was studied by dual culturing on King's B medium. The bacterial isolates varied in their ability to inhibit *F.pallidoroseum*.

The bacterial isolate P_5 produced the highest inhibition of 65.55 per cent when co-inoculated with the pathogen, *Fusarium* showed mycelial growth of only 3.1 cm as against 9.0 cm in control (Table 6 and Plate 7). The isolate P_9 caused a reduction of 61.11 per cent of colony growth of *Fusarium* by restricting the growth of pathogen to 3.5 cm (Table 6 and Plate 7). The least inhibition percentage was observed with P_1 isolates (51.50 %) which corresponded to 4.4 cm of growth of *Fusarium* (Table 6). Based on the extent of inhibition exerted on mycelial growth of the pathogen, two bacterial isolates P_5 and P_9 were selected for further pot culture studies. Based on the fluorescent pigment production on King's B Medium, these bacterial isolates were found to belong to *Pseudomonas fluorescens*.

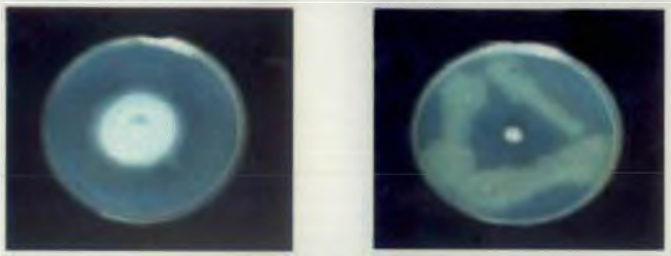


F. pallidoroseum x A_{13}

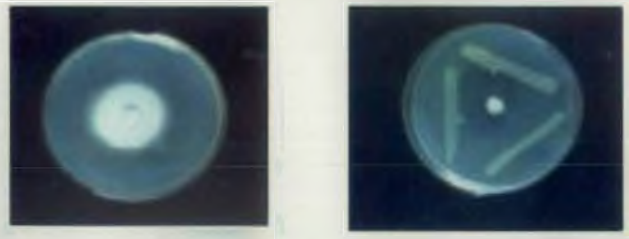


F.pallidoroseum x A_7

Plate 6. Inhibition of *F.pallidoroseum* by *Trichoderma* spp.

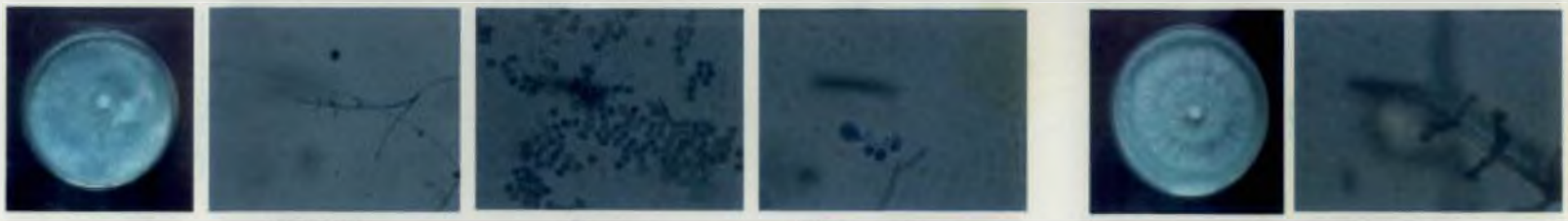


F. pallidoroseum x P_3



F.pallidoroseum x P_9

Plate7. Inhibition of *F.pallidoroseum* by *P.fluorescens*



Phialides Spores Chlamydospores Phialides

a. *T.viride* b. *T.virens*

Plate 8. Cultural, morphological characters of *T.viride* and *T.virens*

4.4.3 *Piriformospora indica*

The antagonisitic activity of *P.indica* Varma, Rexer, Kost and Franken sp. nov. was studied by dual culturing under *in vitro* conditions. When compared to *F.pallidoroseum* growth of, *P.indica* was slow. When co-inoculated, *P.indica* did not inhibit the mycelial growth of *F. pallidoroseum*. *F. pallidoroseum* was found to cover the entire dish, though there was no overgrowth on the colonies of *P.indica*.

4.5 *IN VIVO* SCREENING OF BIOCONTROL AGENTS

The efficacy of fungal antagonists A_7 (*T. viride*), A_{15} (*T. virens*) and bacterial antagonists P_5 and P_9 (*P. fluorescens*) was compared with *T. viride* and *P.fluorescens* released by KAU as efficient antagonist against several pathogens and *P.indica* an endophytic fungus both antagonistic property.

The results showed all the biocontrol agents, in general, suppressed of wilt due to *Fusarium* (Table 7 and 8, Plate 9). The isolates of *Trichoderma* were more effective than other antagonists. All the *Trichoderma* isolates *ie.*, the native A_7 and A_{15} as well as the KAU culture fully protected the plants against the pathogen.

Plants treated with *P.fluorescens* showed symptoms of wilt to varying extent (Fig.2). The per cent mortality of cowpea plants inoculated with P_9 was 33.33 compared to 66.66 per cent, with the KAU culture. *P. indica* inoculated plants showed wilt incidence of 33.33 per cent. All the control plants inoculated with *Fusarium* alone succumbed to the disease. The wilt incidence in the pathogen inoculated control was noticed within 25 days of inoculation. The bacterial application protected the plants for a longer period. The protection lasted for 35-40 days, when few of the plants showed yellowing and wilting. *P.indica* treated plants showed the symptoms of disease in 40 days (Table 7).

In general, the biocontrol agents promoted growth of cowpea plants (Table 8). This was more pronounced with inoculation of *Trichoderma*

Table 7. Wilt disease incidence as affected by biocontrol agents

Treatments	No. of days for onset of wilt	Disease incidence, %
T_1	-	-
T_2	-	-
T_3	-	-
T_4	-	-
T_5	40	33.33
T_6	35	66.66
T_7	40	33.33
T_8	25	100.00
T_9	-	-

isolates than that of *P.fluorescens* isolates. *P.indica* showed variable results for the different characters studied. Statistical analysis showed that the various treatments did not differ significantly with respect to characters like plant height, dry weight of plant, root length and dry weight of root. Significant difference was however noticed for the fresh weight of the plant, number of nodules, nodule fresh weight and nodule dry weight.

The maximum plant height was noticed in plants inoculated with *Trichoderma* isolate A_7. It produced height of 380 cm whereas the untreated control showed a height of only 207.33 cm. *viz.*, 88.33 per cent increased over control. Plant grown in soil incorporated with *Trichoderma* isolates A_{15} and KAU had 350 and 282 cm height respectively, which were 68.81 per cent and 36.01 per cent more than control. Among *P.fluorescens* isolates, treatment T_4 with P_5 gave the highest value for plant height (280 cm) which was 35.05 per cent more than the control. *P.indica* decreased the height of plants by 42 per cent as compared to control.

Plants inoculated with A_7 isolate of *T. viride* had a fresh weight of 321.22 g and dry weight of 60.33 g which were to 52.46 and 13.88 per cent more than the control. This was closely followed by T_2 and T_3 producing increase of 13.64 per cent and 3.64 per cent for fresh weight and 10.05 and 11.32 g for dry weight. *P. fluorescens* P_5 produced 10.72 per cent of more shoot fresh weight and 7.35 per cent shoot dry weight over untreated control. T_5 treatment (*P. fluorescens*, P_9) showed decreased fresh shoot weight of 16.48 per cent over control and recorded increased dry weight of 10.24 per cent over control.

The root length of 48.68 and 48.66 cm were registered in plants inoculated with *Trichoderma* isolate A_{15} and *P.indica* which was 56.98 and 56.96 per cent more than the control, respectively. The plants in treatments T_6 (*P.fluorescens* KAU culture) and T_1 (*Trichoderma* A_7) also had 45.16 and 46.22 per cent more root length than control.

Table 8. Effect of biocontrol agents on growth promotion and nodulation of cowpea

Treatments	Height of the plant		Fresh weight of shoot		Dry weight of shoot		Root length		Fresh weight of root		Dry weight of root	
	cm	% ↑/↓	g	% ↑/↓	g	% ↑/↓	cm	% ↑/↓	g	% ↑/↓	g	% ↑/↓
T_1	380	88.33	321.22	52.46	60.33	13.88	45.33	46.22	35.33	130.46	9.33	86.6
T_2	350	68.81	240.15	13.64	58.33	10.05	48.68	56.98	27.00	76.12	9.00	80.0
T_3	282	36.01	219.02	3.64	59.00	11.32	41.00	32.25	22.33	45.66	6.16	23.2
T_4	280	35.05	237.12	10.72	57.21	7.33	36.66	18.25	19.00	23.93	5.20	4.0
T_5	255	22.99	176.50	16.48	58.43	10.24	40.00	29.03	16.00	5.00	5.10	2.0
T_6	250	20.58	238.42	12.81	59.61	12.47	45.00	45.16	20.00	30.46	5	-
T_7	220	-42.00	109.00	-48.42	47.21	-11.32	48.66	56.96	16.21	5.7	5	-
T_8	207.33		211.33		53		31		15.33		5	-
CD (5%)	113.16		73.36		28.72		17.28		14.02		5.14	

↑/↓ increase or decrease over control

Table 8. continued...

Treatments	No.of nodules		Nodule fresh weight		Nodule dry weight	
		% ↑/↓	g	% ↑/↓	g	% ↑/↓
T1	199.66	88.67	1.89	100.00	0.52	23.80
T2	166.00	56.60	1.42	10.93	0.49	8.80
T3	104.33	-1.57	1.15	-10.15	0.29	-35.55
T4	134.66	27.03	1.51	18.08	0.57	28.38
T5	146.50	38.20	0.84	-34.37	0.22	-51.11
T6	132.00	24.52	2.64	106.52	-0.46	4.40
T7	62.00	41.50	0.60	-53.1	0.10	-77.77
T8	106.00		1.28		0.45	
CD (5%)	52.40					

T_1- *T.viride* (A_7) T_2-*T.virens* (A_{15}) T_3-*Trichoderma* KAU culture T_4-*P.fluorescens* (P_5)
T_5- *P.fluorescens* (P_9) T_6-*P.fluorescens* KAU culture T_7-*P. indica* T_8-Control

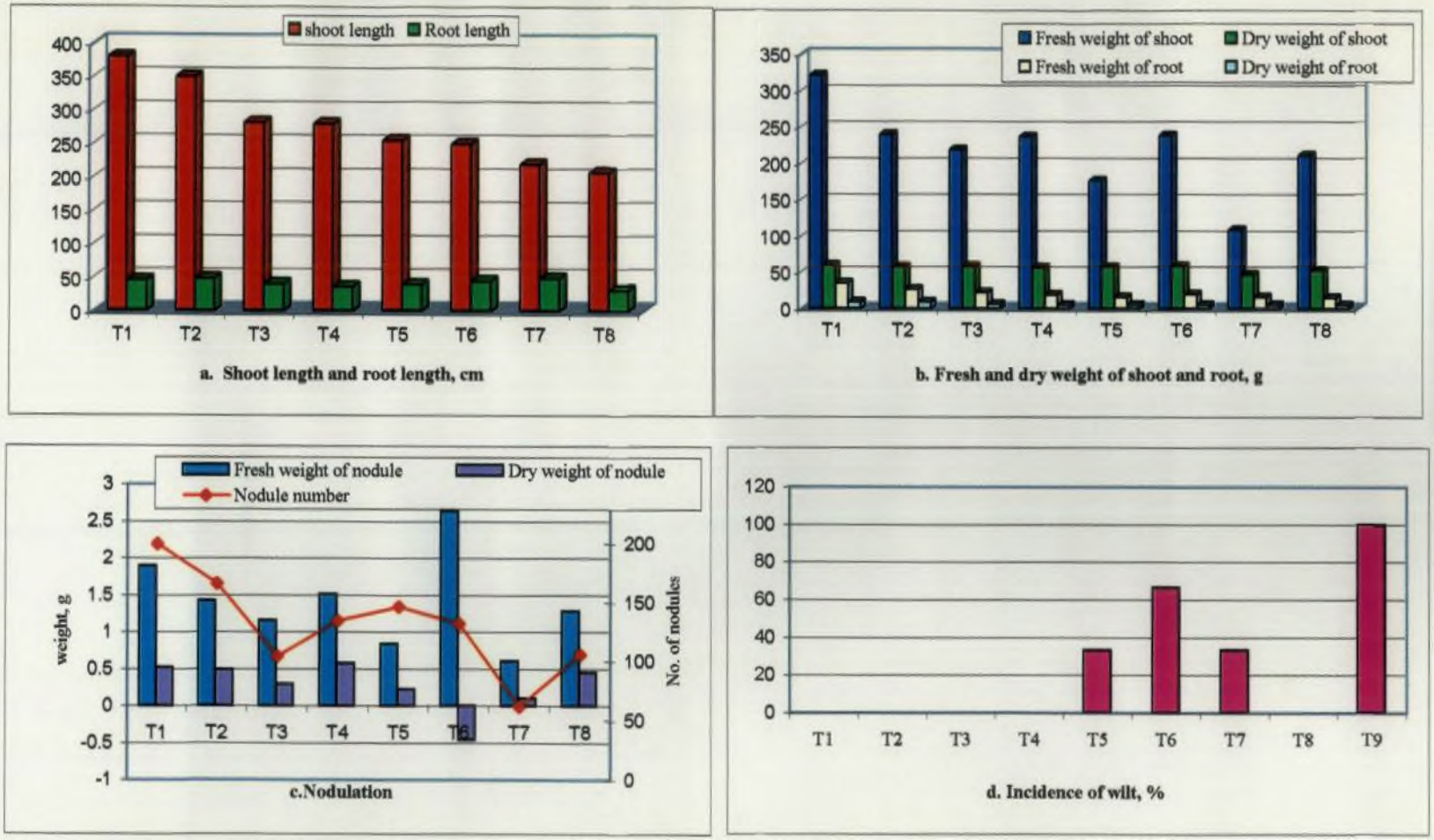


Fig 2. Effect o f biocontrol agents on plant growth promotion, nodulation and wilt incidence

The fresh and dry weight of roots were higher in inoculation with *T. viride* isolate A_7. It produced 35.33 g and 9.33 g respectively for fresh and dry weight which in comparison to control showed increase of 130.46 and 86.60 per cent, respectively. The A_{15} isolate of *T. virens* also gave increase of 76.12 per cent and 80 per cent, respectively of fresh and dry weight of root over control. The *P.fluorescens* isolates P_5 and P_9 produced 23.93 and 5.00 per cent respectively of increased fresh weight of root and 4 per cent and 2 per cent respectively of increased dry weight of root. *P. indica* produced 5.7 per cent in fresh weight of root over control though the dry weight was similar to that of uninoculated control.

Nodulation increased significantly on inoculation of *T. viride* isolate A_7. This isolate produced nodule number of 199.66, nodule fresh weight of 1.89 and dry weight of 0.52 g, which was the highest value recorded. The per cent increase over control was 88.67 per cent of nodule number, 100 per cent for nodule fresh weight and 23.80 per cent for nodule dry weight. *T. virens* A_{15} treated plants ranked closely behind and produced 166 number of nodules, 1.42 g of nodule fresh weight and 0.49 g of nodule dry weight. The nodule fresh weight and dry weight showed comparatively lower value on inoculation with *P.fluorescens* isolates. Among these the isolates, P_5 and KAU culture influenced nodulation better. P_5 inoculated plants produced 18.08 per cent, 28.88 per cent increased nodule fresh weight and dry weight respectively over control, 106.52 per cent and 4.4 per cent of increased in nodule fresh and dry weight was recorded in KAU released *P.fluorescens* inoculated plants. *P.indica* inoculated plants showed 53.10 per cent and 77.77 per cent of decreased in nodule fresh and dry weights, respectively. Nodule number was increased by 41.50 per cent over control.

4.5.1 Selection of biocontrol agent

Based upon the suppression of wilt incidence and the promotion of growth of plants and nodulation, the isolate A_7 (*T.viride*) was selected as the best biocontrol candidate (Table 9 and Fig. 2) for further studies.

4.6 BIOASSAY OF COMMONLY USED FUNGICIDES AGAINST *F.PALLIDOROSEUM*

The fungicides such as carbendazim, copper oxychloride, chlorothalonil and mancozeb were tested against *F.pallidoroseum* at various concentrations. All the fungicides inhibited the growth of *F.pallidoroseum* at the recommended concentrations (Table10 and Plate 10). carbendazim (0.1 %) and mancozeb (0.3 %) completely inhibited the growth of pathogen. Copper oxychloride and chlorothalonil caused a suppression of 82.22 per cent and 56.66 per cent respectively over control. Based on the higher extent of inhibition of the growth of pathogen, three fungicides *viz.*, copper oxychloride, carbendazim and mancozeb were selected for further *in vivo* studies.

4.6.1 Compatibility of fungicides with antagonistic microorganisms

4.6.1.1 Compatibility of fungicides with Trichoderma spp.

The growth of *Trichoderma* spp. was not inhibited by using as mancozeb (0.3 %) copper oxychloride (0.2 %) and chlorothalonil (0.1 %) (Table 11 and Plates 11). However, carbendazim (0.1 %) caused inhibition of the growth of *Trichoderma* isolates A_7 and A_{15} by 46 and 48 per cent, respectively (Table 11 and Plate 11).

4.6.1.2 Compatibility of fungicides with Pseudomonas fluorescens

The growth of *P.fluorescens* was not inhibited by mancozeb (0.3 %), copper oxychloride (0.2 %), chlorothalonil (0.1 %) and carbendazim (0.1 %) (Plate 12).

Plate 9. Disease suppression and growth promotion of cowpea by biocontrol agents

Plate 10. Inhibition of *F.pallidoroseum* by fungicides

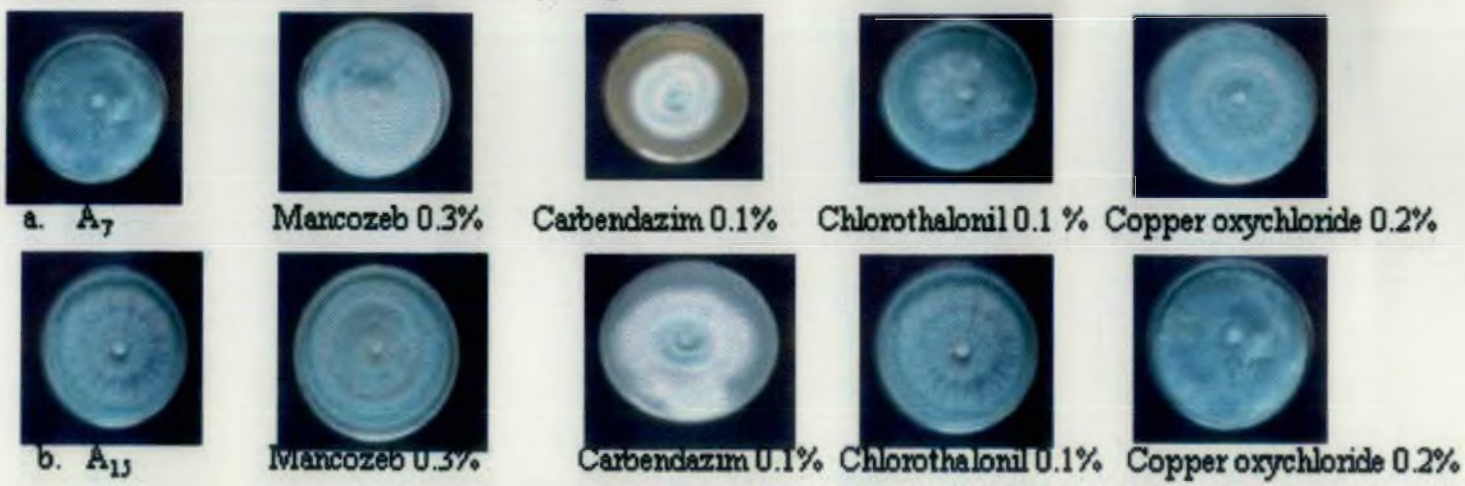


Plate 11. Compatibility of fungicides with *Trichoderma* spp.

Table 9. Summary of performance of biocontrol agents for *in vivo* disease suppression, plant growth promotion and nodulation of cowpea

Sl. No.	Character	Best performing biocontrol agent	Medium performing biocontrol agent	Least performing biocontrol agent
1	*In vitro*			
	i. Suppression of *F.pallidoroseum*	A_7	A_{15}	*P.indica*
2	Growth characters			
	i.Height of the plant	A_7	A_{15}	*P.indica*
	ii.Fresh weight of shoot	A_7	A_{15}	*P.indica*
	iii.Dry weight of shoot	A_7	T_6	*P.indica*
	iv. Root length	A_{15}	*P.indica*	P_5
	v.Fresh weight of root	A_7	A_{15}	P_9
	vi.Dry weight of root	A_7	A_{15}	*P.f.*(KAU)
3	Nodulation			
	i.Number of nodules	A_7	A_{15}	T_3
	ii.Nodule fresh weight	P.f.(KAU)	A_7	*P.indica*
	iii.Nodule dry weight	P_5	A_7	*P.indica*

Table 10. Effect of fungicides on *in vitro* suppression of *F.pallidoroseum*

Sl. No.	Fungicide	Dose (%)	Colony diameter of *F. pallidoroseum*, cm	Inhibition on growth of *F.pallidoroseum*, %
1	carbendazim	0.1	-	100.00
2	copper oxychloride	0.2	1.6	82.22
3	chlorothalonil	0.1	3.9	56.66
4	mancozeb	0.3	-	100.00
	Control		9.0	

4.7 *IN VIVO* SCREENING OF FUNGICIDES AGAINST FUSARIUM WILT

Soil drenching of mancozeb (0.3 %), copper oxychloride (0.2 %) and carbendazim (0.1 %) were effective in suppressing the disease (Table 13). The pathogen inoculated control plants showed yellowing and drooping within 45 DAS and after two weeks initial symptom appearance, all the plant perished while wilt incidence was not recorded in any of the plants treated with fungicides. However, yellowing symptom was noticed in copper oxychloride applied plants towards the flowering time.

Disease index of 25.00 and 16.66 per cent were recorded in plants treated with copper oxychloride (0.2 %) carbendazim (0.1 %), respectively. The mancozeb (0.3 %) treated plants showed lowest disease index of 8.33 per cent when compared to other fungicide treatment (Table 12).

The application of chemical fungicides also helped in improving the growth of the plants. The biometric parameters were increased to varying extent by fungicides application (Table 13 and Fig. 3). However, statistical analysis showed that there is no significant difference between treatments in case of characters such as root fresh weight, dry weight of root, nodule fresh weight and nodule dry weight. Significant difference was, however, observed in height of plants, fresh weight of shoot, dry weight of shoot, root length and number of nodules.

Carbendazim 0.1 per cent as soil drenching significantly improved the height of the plant. The fungicide treated plants registered 313.33 cm of height, which was 39.25 per cent more than untreated control. Copper oxychloride 0.2 per cent, treated plants also recorded 17.77 per cent increase in height over untreated control, which was statistically on par to that carbendazim. Soil drenching of mancozeb (0.3 %) increased the height of the plant only marginally *ie.*, 4.29 per cent more than untreated control.

The maximum fresh weight was recorded in mancozeb 0.3 per cent treated plants *ie.*, 314 g, This was 17.45 per cent more than untreated control. This was followed by carbendazim (0.1 %) with 13.58 per cent

Table 11. Mycelial growth of *Trichoderma* isolates in fungicide amended media

			A_7		A_{15}	
Sl. No.	fungicide	Concentration, %	Colony diameter, cm	Inhibition, %	Colony diameter, cm	Inhibition, %
1	Mancozeb	0.3	9.0	-	9.0	-
2	Chlorothalonil	0.1	9.0	-	9.0	-
3	Copper oxychloride	0.2	9.0	-	9.0	-
4	Carbendazim	0.1	4.8	46	4.6	48
	Control		9.0		9.0	

Table 12. Disease index of fungicide treated plants

Sl. No.	Fungicide	Disease index, %
1	mancozeb (0.3 %)	8.33
2	copper oxychloride (0.2 %)	25.00
3	carbendazim (0.1 %)	16.66
4	Control	100.00

Table 13. Effect of fungicides on plant growth promotion and nodulation in cowpea

Treatments	Height of plant		Fresh weight of plant		Dry weight of shoot,		Root length		Fresh weight of root		Dry weight of root	
	cm	%↑/↓	g	%↑/↓	g	%↑/↓	cm	%↑/↓	g	%↑/↓	g	%↑/↓
T_1	234.66	4.29	314	17.45	52.66	31.65	50.66	5.54	33.66	18.81	4.62	32.75
T_2	265	17.77	285	6.60	63.33	58.32	66.00	37.5	32.33	14.11	4.50	29.31
T_3	323.33	39.25	303.66	13.58	70	75	48.33	0.68	41.66	47.05	6.08	74.71
T_4	225		267.33		40		48.00		28.33		3.48	
CD (5%)	40.25		70.38		20.33		12.79		NS		NS	

%↑/↓ increase or decrease over control

Table 13. continued...

Treatments	No. of nodules		Nodules fresh weight		Nodule dry weight	
	g	%↑/↓	g	%↑/↓	g	%↑/↓
T_1	71	-56.79	2.23	-5.5	0.77	-7.22
T_2	128	-22.1	3.03	28.38	0.78	6.02
T_3	213.33	29.63	4.85	105.5	1.37	65.66
T_4	164.33		2.36		0.83	
CD (5%)	60.81		NS		NS	

T_1-Soil drenching of mancozeb (0.3 %)
T_2-Soil drenching of copper oxychloride (0.2 %)
T_3-Soil drenching of carbendazim (0.1 %)
T_4-Control

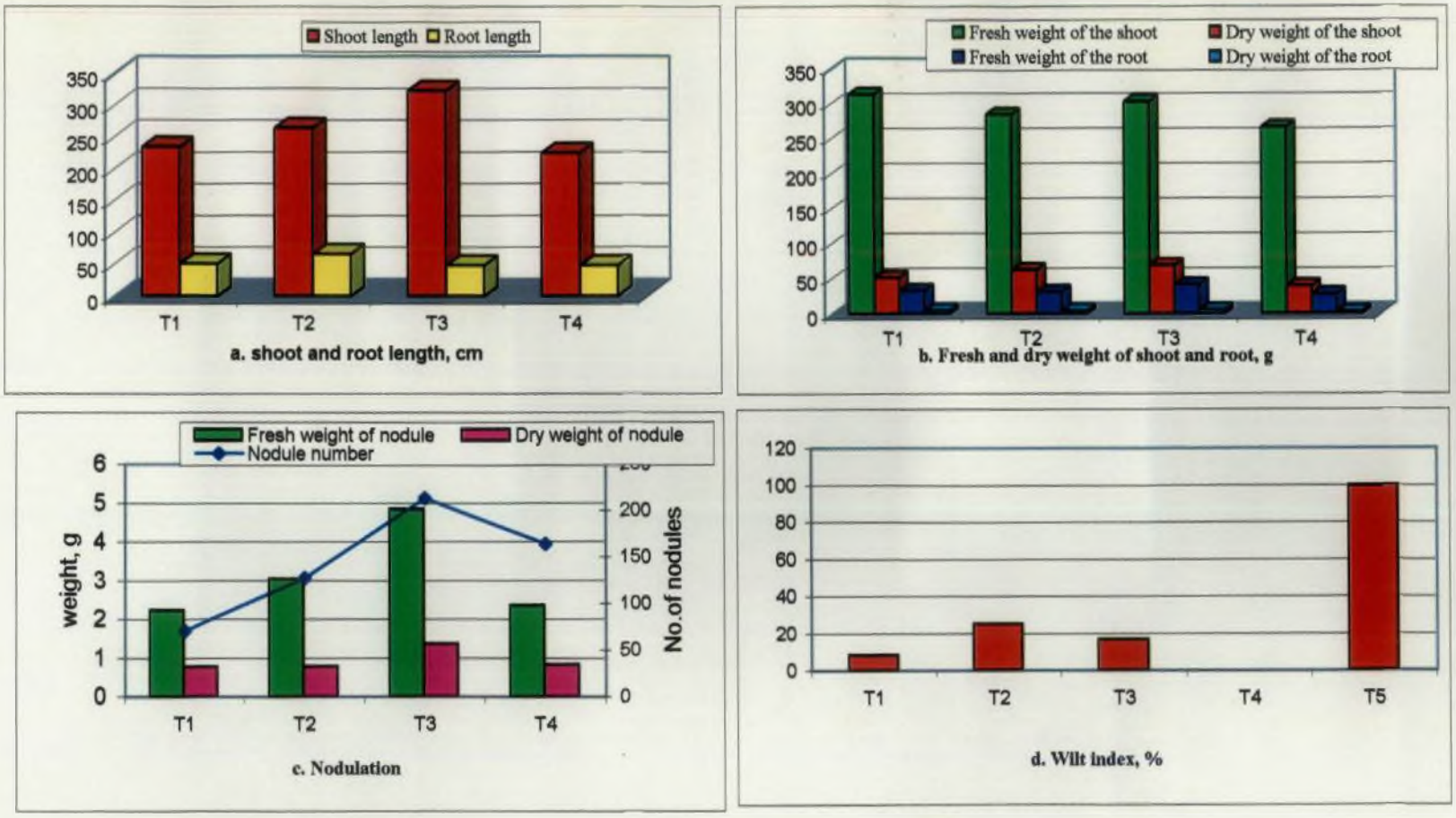


Fig. 3. Effect of fungicides on plant growth characters, nodulantion and wilt index

increase over untreated control. The increase in fresh weight in copper oxychloride (0.2 %) treated plant was only 6.6 per cent more than untreated control.

The highest dry weight of shoot was recorded in plants treated with carbendazim (0.1 %), which was 75 per cent more than over untreated control. Copper oxychloride (0.2 %) and mancozeb (0.3 %) also exhibited 58.32 per cent and 31.65 per cent increase in dry weight of shoot, respectively over untreated control.

Significantly longer roots were produced in cowpea plants treated with copper oxychloride (0.2 %) *ie.*, 37.50 per cent more than untreated control. Increase in root length in mancozeb (0.3 %) and carbendazim (0.1 %) treated plants were only less than six per cent over the untreated control.

Plants drenched with carbendazim (0.3 %) showed a root weight of 41.66 g, which was 47.05 per cent more than untreated control. Mancozeb (0.3 %) and copper oxychloride (0.2 %) also registered an increase of 18.81 per cent and 14.11 per cent over untreated control. A similar trend was noticed in both the treatments for dry weight of roots also.

Nodulation was, in general, adversely affected by application of fungicides to soil. However, this was not true of carbendazim application. The plants drenched with carbendazim showed a significant increase in nodule number, nodule fresh and nodule fresh weight. The value for this parameters were 29.63 per cent, 105.5 per cent and 65.06 per cent higher than that of the untreated control. The nodule number was significantly less in plants drenched with mancozeb (0.3 %) and copper oxychloride (0.2 %), and showed value of 71 and 128, respectively. The application of mancozeb also caused a decrease in nodule fresh weight and dry weight by 5.5 per cent and 7.22 per cent over control. Copper oxychloride (0.2 %) treated plants showed 28.33 per cent of increase nodule fresh weight over control, but registered 6.02 per cent of decrease nodule dry weight over control.

4.8 SELECTION OF FUNGICIDES

Carbendazim (0.1 %) and mancozeb (0.3 %) were found effective in suppression of wilt incidence, the promotion of growth of cowpea plants and nodulation. Though carbendazim (0.1 %) showed better performance than mancozeb (0.3 %) with respect to these characters, it was not compatible with *Trichoderma* and hence, mancozeb (0.3 %) was selected as fungicide component for integrated management studies (Table 14 and Plate 13).

4.9 BIOASSAY OF AMENDMENTS FOR SUPPRESSION OF *F. pallidoroseum*

The aqueous extracts of neem cake, coirpith and lime caused inhibition of the growth of pathogen at five per cent and ten per cent concentrations (Table 15 and Plate 15). The results showed that media amended with five and ten per cent concentrations of neem cake caused 33 and 44 per cent inhibitions, respectively. Lime at 0.1 per cent concentration caused 24 per cent inhibition over control and the rate of growth recorded was 1.36 cm/day. The media amended with coirpith ten per cent gave inhibition of 4.4 per cent which showed 1.72 rate of growth per day. Coirpith five per cent concentration did not inhibit the growth while vermicompost promoted the growth of pathogen. Vermicompost five per cent and ten per cent extracts showed 5.2 per cent and 25 per cent of increased growth of the pathogen, respectively (Table 14).

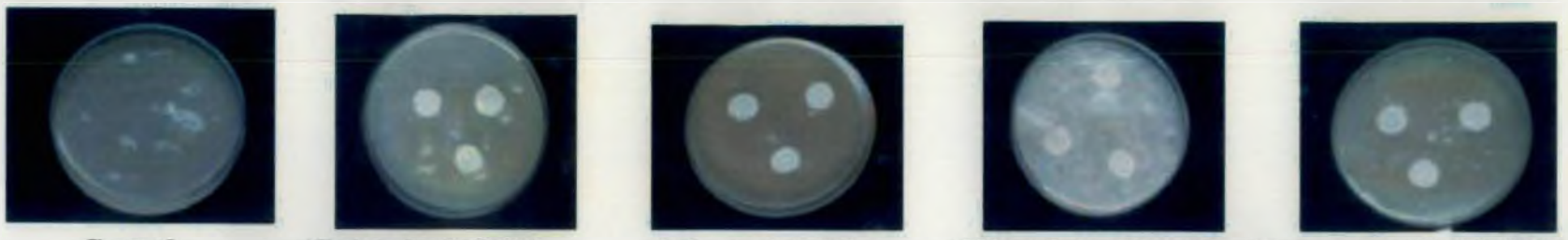
4.9.1 Compatibility of amendments with antagonistic microorganisms

4.9.1.1 Compatibility of Trichoderma spp. with amendments

The growth of *Trichoderma* spp. was in general promoted by addition of organic amendments into the medium (Plate 18). The growth of *Trichoderma* spp. A_7 and A_{15} was not inhibited by neemcake 5 per cent and 10 per cent, vermicompost 5 per cent and 10 per cent, coirpith compost 5 per cent and 10 per cent and lime 0.1 per cent. However, neem

Table 14. Summary of performance of fungicides for disease suppression, growth promotion and nodulation

Sl. No.	Characters	Best performing fungicide	Medium performing fungicide	Least performing fungicide
1	*In vitro*			
	i.Suppression of *Fusarium*	mancozeb and carbendazim	copper oxychloride	chlorothalonil
	ii.Compatibility with *Trichoderma*	mancozeb all the fungicides are compatible	copper oxychloride	carbendazim
	iii.Compatibility with *P.fluorescens*			
2	*In vivo*			
	i.Disease suppression	carbendazim, mancozeb	copper oxychloride	
	2.Growth characters			
	i.Height of the plant	carbendazim	copper oxychloride	mancozeb
	ii. Fresh weight of the plant	mancozeb	carbendazim	copper oxychloride
	iii.Dry weight of the shoot	carbendazim	copper oxychloride	mancozeb
	iv. Root length	copper oxychloride	mancozeb	carbendazim
	v.Fresh weight of root	carbendazim	mancozeb	copper oxychloride
	vi. Dry weight of root	carbendazim	mancozeb	copper oxychloride
3	Nodulation			
	i. Number of nodules	carbendazim	mancozeb	copper oxychloride
	ii.Fresh weight of nodules	carbendazim	copper oxychloride	mancozeb
	iii. Dry weight of nodules	carbendazim	copper oxychloride	mancozeb

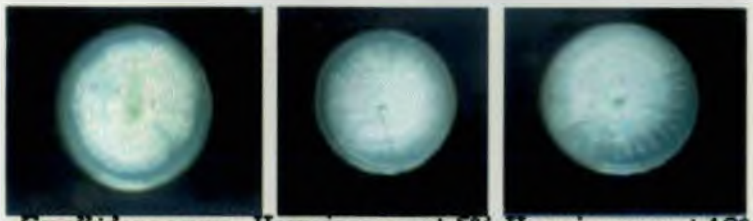


Control Carbendazim 0.1% Mancozeb 0.3% Chlorothalonil 0.1 % Copper oxychloride 0.2%

Plate 12. Compatibility of fungicides with *P.fluorescens*

Plate. 13. Effect of mancozeb incorporation on Fusarium wilt suppression and growth of cowpea

Plate. 16. Effect of neem cake incorporation on Fusarium wilt suppression and growth of cowpea



F.pallidoroseum Vermicompost 5% Vermicompost 10%

Plate 14. Effect of vermicompost on growth of *F.pallidoroseum*

F.pallidoroseum Neemcake 10% Lime 0.1%

Plate 15. Effect of amendments on growth of *F.pallidoroseum*

Table 15. Effect of soil amendments on *in vitro* suppression of *F. pallidoroseum*

Sl. No.	Amendment	Concentration, %	Colony diameter of *F.pallidoroseum*, cm		Rate of growth /day	% inhibition on growth of *F.pallidoroseum*
			4th day	5th day		
1	Neemcake	5	4.8	6.0	1.2	33
		10	4.0	5.0	1.0	49
2	Coirpith	5	7.2	9.0	1.80	-
		10	6.8	8.6	1.72	4.4
3	Vermicompost	5	7.8	9.0	1.80	Increased 5.2%
		10	9.0	9.0	2.25	Increased 25 %
4	Lime	0.1	5.4	6.8	1.36	24
	Control		7.6	9.0		

Table 16. Effects of amendments on mycelial growth of *Trichoderma viride* (A_7)

Sl. No.	Amendment	Concentration, %	Colony diameter, cm		
			3rd day	4th day	5th day
1	Neem cake	5	-	9.0	-
		10	9.0	-	-
2	Coirpith	5			
		10		9.0	
3	Vermicompost	5			
		10		9.0	
4	Control				9.0

Table 17. Effect of soil amendments on suppression of Fusarium wilt of cowpea

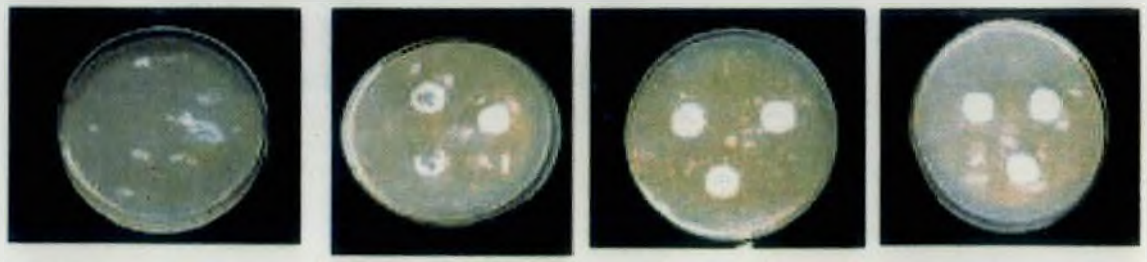
Sl. No.	Amendment	Disease index, %
1	Neem cake	25.00
2	Coirpith	33.33
3	Vermicompost	75.00
4	Lime	87.50
	Control	100.00

Control A_7 Neemcake 10 % Neemcake 5% Control A_{13} Neemcake 10 % Neemcake 5 %

Plate 17. Increased growth of *Trichoderma* with neem cake incorporated media

Control A_7 Vermicompost 10% Coirpith 10% Lime 0.1% Control A_{13} Vermicompost 5% Coirpith 5% Lime 0.1%

Plate 18. Growth of *Trichoderma* with amendment incorporated media



Control (*P.fluroscens*) Neemcake 10 % Coirpith 10 % Vermicompost 10%

Plate 19. Growth of *P.fluorescens* as affected by amendments

cake extract incorporation increased growth of the antagonist medium amended with neem cake extract 10 per cent recorded full colony growth of *Trichoderma* with in three days compared to five days in control (Table 16 and Plate 17).

4.9.1.2 Compatibility of P. fluorescens with amendments

The growth of *P. fluorescens* (P_5 and P_9) was not inhibited by the different concentrations of amendments used (Plates 19).

4.10 *IN VIVO* SCREENING OF AMENDMENTS FOR FUSARIUM WILT SUPPRESSION AND PLANT GROWTH PROMOTION

The data (Tables 17 and Fig. 4) showed that addition of amendments except lime to the soil reduced the wilt index. Soil application of neem cake (150 kg/ha) reduced the disease and gave lowest wilt index of 25 per cent. The highest disease index of 87.5 per cent was noticed in lime application. The coirpith and vermicompost exhibited disease index of 33.33 and 75 per cent, respectively. The vermicompost treated plants also showed yellowing symptoms.

The statistical analysis showed that there is no significant difference between treatments on height of plant, root length and nodule dry weight. However, significant difference was noticed for fresh weight of the shoot, dry weight of plant, root fresh weight, dry weight of root, number of nodules and nodule fresh weight (Table18).

The soil application of neemcake (150 kg/ha) gave 31.11 per cent increase in plant height over untreated control which was also 11.32 per cent more than that recorded in plants treated with copper oxychloride (0.2 %). Vermicompost application increased the growth of plant by 5.4 per cent, while coirpith decreased it by 2.2 per cent over control.

Fresh weight of the shoot was maximum 385 g in soil incorporated with neemcake and exhibited 144.7 per cent of increase over untreated control. This was also superior to fungicide treated check by 30.06 per cent. Soil application of coirpith and vermicompost recorded only less than 10 per

cent increase in fresh weight of the shoot over control. The lowest shoot weight was noticed in soil amended with lime.

Neemcake amendment resulted in 50 per cent increase in dry weight over untreated control and 1.2 per cent over copper oxychloride (0.2 %) application. When the effect of neemcake and coirpith compost application were compared, the former was found to increase dry weight by 12.95 per cent over the latter.

The root length was significantly enhanced due to soil application of neemcake. It produced 42.22 per cent increase over untreated control but it was 3 per cent less in comparison with fungicidal check. Soil application of coirpith and vermicompost increased root length by 20.73 and 17.02 per cent, respectively over control. Lowest root length was noticed in lime amended soil.

Addition of neem cake to soil increased fresh weight of roots by 84.71 per cent and dry weight by 111.49 per cent more than untreated control. Soil application of neemcake was even better than copper oxychloride in increasing root biomass. Neemcake soil application enhanced the fresh and dry weight of root by 65.91 and 62.11 per cent over fungicidal application. Among the amendments lime treated soil supported the least fresh and dry weight of roots. Soil application of vermicompost and coirpith compost enhanced fresh and dry weight of root, which were 29.4 per cent and 31.50 per cent and 77.87 per cent and 1.14 per cent, respectively over untreated control.

Soil application of coirpith compost increased the number of nodules in plants by 3 per cent over control. This was followed by the application of neemcake. Both lime and copper oxychloride reduced in the nodule number compared to control, while vermicompost gave an increase by 2.6 per cent.

Vermicompost and coirpith amendments increased the fresh weight of nodule by 50.84 and 68.00 per cent respectively over control compared to 15.25 per cent in neem cake amended plants. However, this was significantly less effective than the fungicidal control in improving nodule fresh weight.

Table 18. Effect of soil amendments on plant growth promotion and nodulation in cowpea

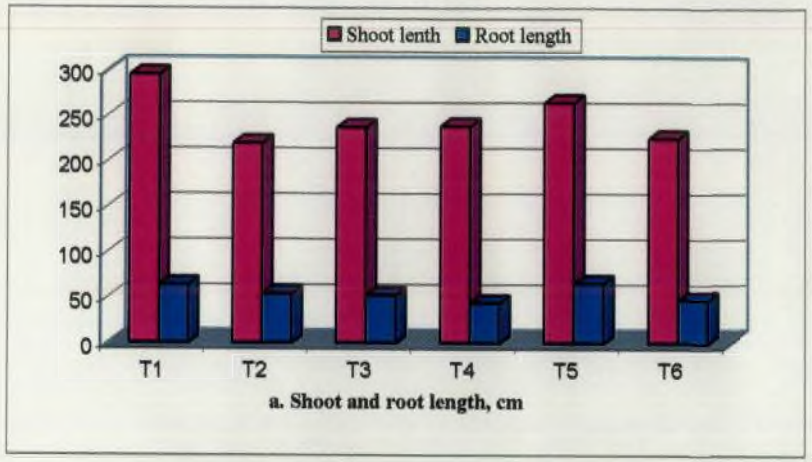
Treatments	Height of plant		Fresh weight of shoot		Dry weight of shoot		Root length		Fresh weight of root		Dry weight of root	
	cm	%↑/↓	g	%↑/↓	g	%↑/↓	cm	%↑/↓	g	%↑/↓	g	%↑/↓
T_1	295	31.11	385	144.70	64.00	50	64	42.22	52.33	84.71	7.36	111.49
T_2	220	-2.2	290	8.61	56.66	41.65	54.33	20.73	29.33	31.50	3.54	1.14
T_3	237.33	5.4	286	7.36	51.66	29	52.66	17.02	36.66	29.40	6.19	77.87
T_4	239.00	6.22	110	-55.05	32.50	-18.75	44.50	-7.1	15.00	-47.05	0.56	-83.90
T_5	265.00	17.7	296	10.86	63.33	58.32	66	46.66	31.54	11.33	4.54	30.45
T_6	225		267		40.00		48		28.33		3.48	
CD (5%)	NS		76.81		18.07		NS		9.8		3.64	

%↑/↓ increase or decrease over control

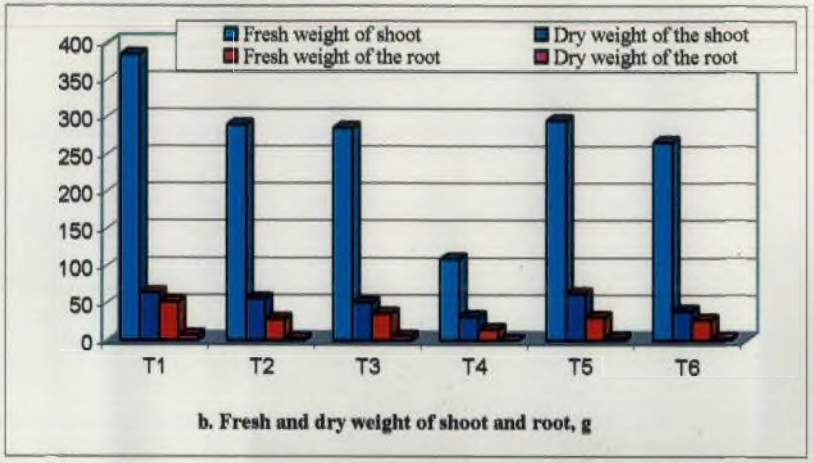
Table 18. continued...

Treatments	No. of nodules		Nodules fresh weight		Nodule dry weight	
	g	%↑/↓	g	%↑/↓	g	%↑/↓
T_1	168.00	2.20	2.72	15.25	0.91	9.63
T_2	169.33	3.00	3.04	68.00	0.88	6.02
T_3	168.66	2.60	3.56	50.84	0.87	0.48
T_4	122.50	-25.75	0.68	-71.18	0.01	-98.70
T_5	128.00	-22.10	3.03	28.38	0.88	6.02
T_6	164.33		2.36		0.83	
CD (5%)	43.72		1.40		NS	

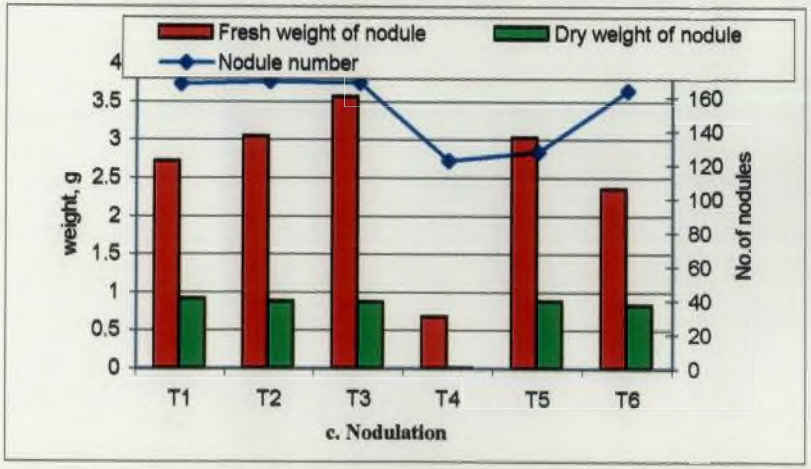
T_1- Soil application of vermicompost
T_2-Soil application of coirpith
T_3-Soil application of neem cake
T_4-Soil application of lime
T_5-Soil drenching with copper oxychloride



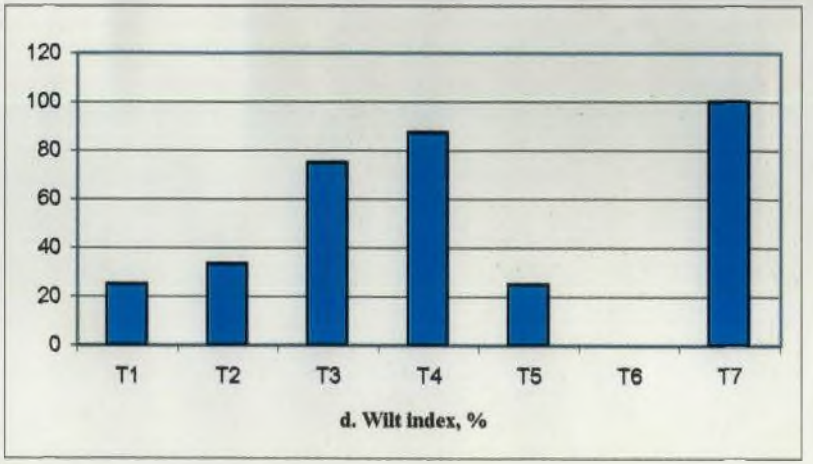
a. Shoot and root length, cm



b. Fresh and dry weight of shoot and root, g



c. Nodulation



d. Wilt index, %

Fig. 4. Effect of amendments on plant growth characters, nodualtion and wilt index

There was 10.23 per cent reduction of nodule fresh weight, in neemcake treatment plants over copper oxychloride treated plants. The lowest value of fresh weight of nodule was noticed with in lime application.

The dry weight of the nodule registered an increase with soil application of neemcake while it decreased on with amendment lime. The highest value of 0.91 g was noticed in neemcake application. This was significantly higher than that of untreated control and fungicidal application. Amendment of soil with coirpith and vermicompost increased nodule dry weight over control by 6.02 and 0.48 per cent, respectively. The nodule dry weight in plants was lower in lime amended soil.

4.11 SELECTION OF ORGANIC AMENDMENTS

Based upon the suppression of wilt incidence and the promotion of growth of plants and nodulation, the soil incorporation of neem cake (150 kg/ha) was selected as the best amendment (Table 19 and Plate 16) for integrated management studies.

4.12 INTEGRATED MANAGEMENT OF FUSARIUM WILT OF COWPEA

The results presented in Table 20 and Fig. 5, revealed that various combinations of biocontrol agent, amendment and fungicide differed for reducing wilt incidence increasing biometric parameters and yield of cowpea. The pathogen inoculated plants showed yellowing, drooping and wilt within 20 days after inoculation. Wilt incidence was not noticed in any of the combinations or their components. Based on the yellowing symptoms developed on leaves, disease index was calculated (Table 21). The least disease index of 16.66 per cent was recorded in combination of *T.viride* (Seed treatment + soil application) + neemcake 150 kg/ha (soil application) + mancozeb (0.3 %) (soil drenching). The highest disease index of 41.60 was noticed in *T.viride* alone and in treatment combination of *T.viride* + neemcake. Other combinations like mancozeb + neem cake, mancozeb + *T.viride* exhibited disease index of 25 per cent and 33.33 per cent

Table 19. Summary of performance of amendments for disease suppression and growth promotion

Sl. No.	Characters	Best performing amendment	Medium performing amendment	Least performing amendment
1	*In vitro*			
	i.Suppression of *Fusarium*	Neem cake	Lime	Coirpith
	ii.Promotion of growth of *Trichoderma*	Neem cake	Vermicompost .coirpith	-
	iii.Compatibility with *P.fluorescens*	All are compatible		
2	*In vivo*			
	1. Disease suppression	Neem cake	Coirpith	Lime
	2. Growth characters			
	i.Height of the plant	Neem cake	Vermicompost	Lime
	ii.Fresh weight of shoot	Neem cake	Coirpith	Lime
	iii.Dry weight of the shoot	Neem cake	Coirpith	Lime
	iv.Root length	Neem cake	Coirpith	Lime
	v.Fresh weight of root	Neem cake	Coirpith	Lime
	vi.Dry weight of root	Neem cake	Coirpith	Lime
3	Nodulation			
	i.Nodule number	Coirpith	Neemcake	Lime
	ii.Fresh weight of nodule	Vermicompost	Coirpith	Lime
	iii.Dry weight of nodule	Neem cake	Coirpith	Lime

respectively. Soil drenching of mancozeb (0.3 %) and soil application of neemcake alone produced disease index of 25 per cent and 25 per cent, respectively.

Significant difference was noticed between treatments for characters like height of the shoot, fresh weight of the shoot, root length, fresh weight of the root, dry weight of the shoot, nodule fresh weight, number of pods and pod weight.

The highest plant height was recorded in case of combined soil application of *T.viride*, mancozeb and neemcake. It produced height of 316.66 cm, which was 53.71 per cent more than untreated control. Soil application of *T.viride* and neemcake produced same effect as that of integrated application on height of the plant. This was followed by combined application of mancozeb and neemcake and soil drenching of mancozeb, which caused 44.0 per cent and 3.43 per cent increase respectively, over control. Combined application of mancozeb, neemcake and *T.viride* caused 6.7 per cent increase in plant height with respect to combined application of mancozeb and neemcake to soil. Individual application of neemcake to soil showed 38.34 per cent increase of height over control.

Application of *T.viride* (2.5 kg/ha) and combined application of *T.viride* with neemcake registered 60.20 and 42.85 per cent, respectively over control. Highest value for fresh weight of plant was recorded for T_7 (i.e. combined soil application of *T.viride*, mancozeb and neem cake) which was 89.79 per cent more than the untreated control. Combined application of fungicide, bioagent and amendment increased fresh weight of the shoot by 24.73 per cent over combined application of *T.viride* and neemcake. Other combinations like mancozeb and neemcake, mancozeb and *T.viride* also increased shoot fresh weight by 45.91 per cent and 56.12 per cent over untreated control, respectively. The individual application of treatments like mancozeb and neem cake produced 184.33 g and 203 g of fresh weight of shoot, which was 12.85 per cent and 24.44 per cent, respectively over untreated control.

Plate 20. Experiment on integrated disease management: overview

Plate 21. Plants treated with *T.viride* + mancozeb + neemcake

Table 20. Effect of combination of *T. viride*, neem cake and mancozeb on plant growth promotion and nodulation in cowpea

Treatments	Height of plant		Fresh weight of shoot		Dry weight of shoot,		Root length		Fresh weight of root		Dry weight of root	
	cm	%↑/↓	g	%↑/↓	g	%↑/↓	cm	%↑/↓	g	%↑/↓	g	%↑/↓
T_1	210	1.94	261.66	60.20	80.66	27.36	31.33	13.26	51.00	53.01	9.74	17.34
T_2	316.66	53.71	233.33	42.85	84.85	33.98	36.33	31.34	54.32	62.97	10.24	23.33
T_3	295	38.34	203	24.44	79.74	25.92	34.96	26.42	48.09	44.31	9.86	18.89
T_4	213.33	3.43	184.33	12.85	67.66	6.83	29.00	4.84	36.33	9.00	9.72	17.10
T_5	296.66	44.00	238.33	45.91	85.00	34.21	25.00	-9.61	41.66	24.99	10.11	21.80
T_6	206.66	0.32	255.00	56.12	78.33	23.68	21.33	-22.88	39.42	18.27	8.40	1.20
T_7	316.66	53.71	310.00	89.79	103.33	63.16	37.33	34.96	61.00	83.01	10.91	31.44
T_8	206.00		163.33		63.33		27.66		33.33		8.30	
CD (5%)	51.98		61.44		8.59		18.48		20.93		6.19	

%↑/↓ increase or decrease over control

Table 20. continued...

Treatments	No. of nodules		Nodule fresh weight		Nodule dry weight		Total no. of pods		Total pod weight	
		%↑/↓	g	%↑/↓	g	%↑/↓		%↑/↓	g	%↑/↓
T_1	139.66	9.10	2.71	4.63	1.07	10.30	33.43	7.83	281.00	8.07
T_2	147.66	15.62	3.53	36.29	1.14	17.52	36.33	17.19	360.00	38.46
T_3	142.61	11.42	3.22	24.54	1.09	12.43	34.88	12.52	334.17	28.52
T_4	74.33	-41.92	0.90	-65.25	0.98	1.03	32.24	4.00	279.00	7.30
T_5	74.00	-42.18	1.95	-24.71	1.01	4.10	34.94	12.70	324.15	24.67
T_6	100.33	-21.61	1.51	-41.69	1.00	4.10	33.64	8.51	280.00	7.69
T_7	162.66	27.07	3.61	39.38	1.15	18.55	41.00	32.25	399.66	53.37
T_8	128.00		2.59		0.97		31.00		260.00	
CD (5%)	56.49		1.66		0.66		14.04		120.78	

T_1-*Trichoderma* T_2-*Trichoderma* + neem cake T_3- Neem cake T_4-Mancozeb
T_5- mancozeb + neem cake T_6- Mancozeb + *Trichoderma* T_7-*Trichoderma* + mancozeb + neem cake

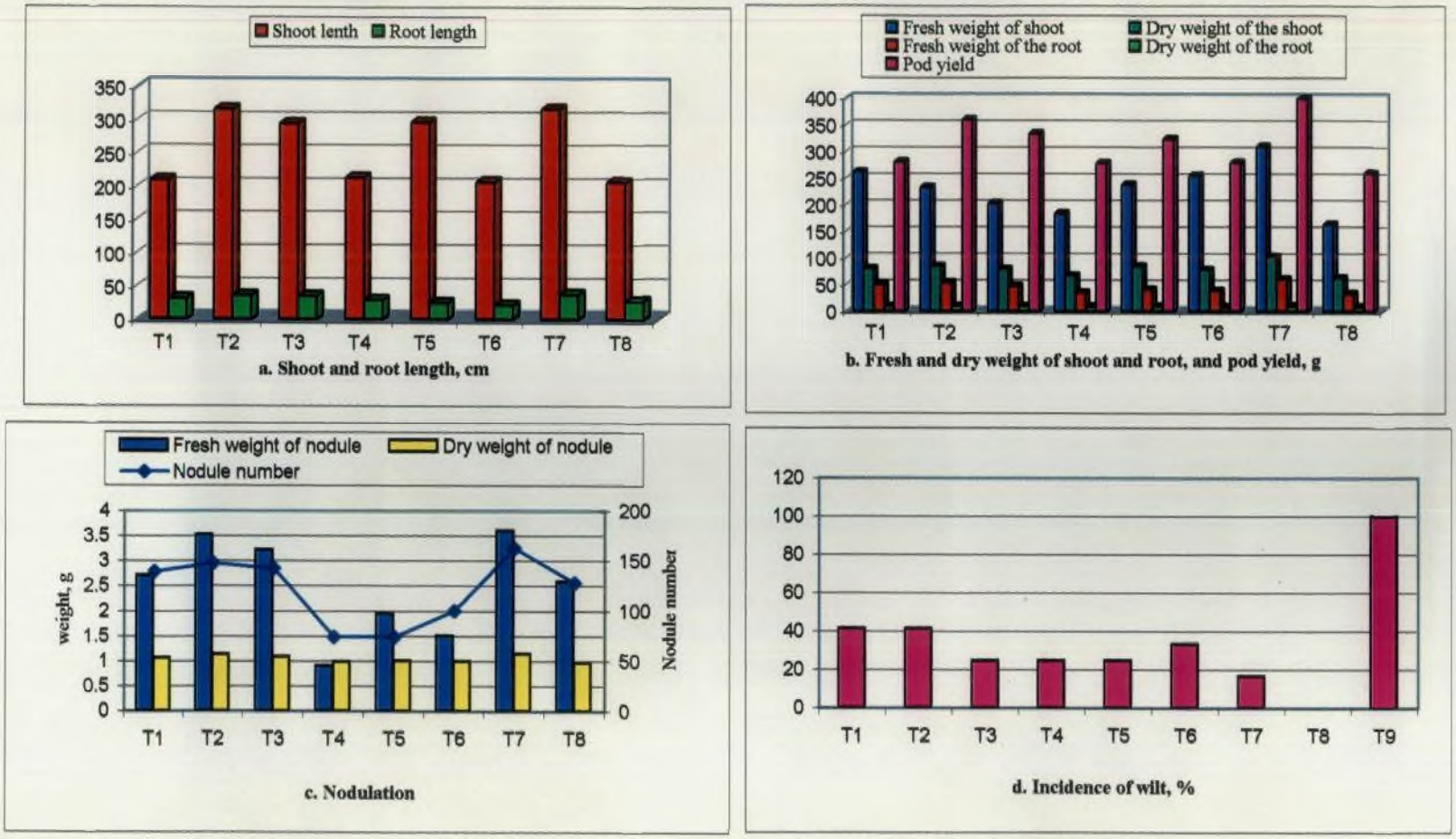


Fig. 5. Integration of *T. viride* , mancozeb, neem cake on plant growth characters, pod weight, nodulation and wilt index

Table. 21. Effect of integration of *T.viride*, neem cake and mancozeb on Fusarium wilt of cowpea

Sl. No.	Treatments	Disease index
1	T_1	41.66
2	T_2	41.66
3	T_3	25.00
4	T_4	25.00
5	T_5	25.00
6	T_6	33.33
7	T_7	16.66
8	T_8	-
	T_9	100.00

Dry weight of shoot on integration of *T.viride* (seed and soil treatments), neemcake application and soil drenching with mancozeb caused the highest dry weight of cowpea plants. The next in rank was combined application of neemcake and *T.viride*, which produced a dry weight of 84.85 g. The treatments involving combination of *T.viride* and mancozeb application followed closely behind but was significantly inferior to the above treatments. Individual treatments independently also gave higher values than control but were statistically on par.

Combined application of mancozeb, *T.viride* and neemcake to the soil supported maximum root length. which was 34.96 per cent more than control. Combinations of *T.viride* and neemcake produced 36.33 cm of plant root length, which was statistically on par with rest of the treatments. Mancozeb alone produced negligible increase of 4.84 per cent in root length over control. Combinations of *T.viride* with mancozeb, and mancozeb with neemcake caused reduction in root length by 22.85 per cent and 9.61 per cent respectively. Soil application of neemcake alone produced 34.96 cm of root length, which was 26.42 per cent more than untreated control.

The fresh and dry weight of roots were highest when mancozeb, *T.viride* and neemcake were applied together and it recorded 83.01 per cent and 31.44 per cent increase, respectively over control. This was followed by combined application of *T.viride* and neemcake. Combined application of mancozeb, neemcake and *T.viride* gave 12.29 and 6.54 per cent better performance of fresh and dry weight of shoot with respect to combined soil application of *T.viride* and neemcake. Individual application of neemcake to soil produced 48.09 g and 9.86 g of fresh and dry weight of root respectively, which were 44.31 and 18.89 per cent increase over untreated control. The least value for fresh and dry weight of root was recorded in soil drenching of mancozeb (0.3 %).

Nodulation increased significantly with combined soil application of mancozeb, neemcake and *T.viride*. It produced nodule number of 162.66, which was 27.07 per cent more over untreated control. *T.viride* and

neemcake combined application to the soil also recorded 15.62 per cent increase over control and showed nodule number of 147.66. Mancozeb, neemcake and *T.viride* gave a 10.51 per cent increase of number of nodules over combined soil application of *T.viride* and neemcake. Similarly highest value of nodule fresh and dry weight was registered in combined application of mancozeb, neemcake and *T.viride* to the soil. The increase in nodule fresh and dry weight over control was 39.38 per cent and 18.55 per cent. Combined soil application of *T.viride* and neemcake also produced higher fresh and dry weight of 36.29 per cent and 17.52 per cent over untreated control, respectively. Combined application of mancozeb, neemcake and *T.viride* caused increase of 2.2 per cent and 0.87 per cent of nodule fresh and dry weight over combined application of *T.viride* and neemcake to soil. Individual application of *T.viride*, mancozeb, neemcake produced 2.71 g, 3.22 g, 0.9 g and 1.07g, 1.09 g and 0.98 g of nodule fresh and dry weight, respectively.

Combined application of mancozeb, neemcake and *T.viride* showed 32.35 per cent increase of total number of pods over untreated control. The combined application of *T.viride* and neemcake ranked next in yield, this treatment registered 17.19 per cent increase over untreated control. Individual application of treatments such as *T.viride*, mancozeb and neemcake produced 32.43, 32.24, 34.88 number of pods, respectively. Combined application of this treatments showed higher pod number than individual application.

Pod weight increased by 53.37 and 38.46 per cent, respectively over control in combined application of *T.viride*, neemcake and mancozeb and also combined soil application of *T.viride* with neemcake. Combinations of mancozeb and neem cake and mancozeb and *T.viride* showed 24.67 and 7.69 per cent increase pod weight over control. The pod weight in plants treated with *T.viride* and neemcake were 8.07 and 28.52 per cent more than that of control, respectively. Application of mancozeb alone showed 7.30 per cent increase pod weight over untreated control.

Table 22. Summary of performance of integration of *T.viride*, neem cake and mancozeb for disease suppression, growth promotion and nodulation in cowpea plants

Sl. No	Characters	Best performing combination	Medium performing combination	Least performing combination
1	Disease suppression	N+T+M		
2	Biometric characters			
	i.Height of the plant	T+N+M and T+N	M + N	T+M
	ii.Fresh weight of the shoot	T+N+M	M+T	T+N
	iii.Dry weight of the shoot	T+N+M	M+N	T+N
	iv.Root length	T+N+M	T+N	T+M
	v. Fresh weight of the root	T+N+M	T+N	T+M
	vi. Dry weight of the root	T+N+M	T+N	T+M
	vii. No. of nodules	T+N+M	T+N	M+N
	viii. Nodule fresh weight	T+N+M	T+N	M+T
	ix. Nodule dry weight	T+N+M	T+N	M+N, M+T
	x. Total no. of pods	T+N+M	T+N	M+T
	xi. Total pod yield	T+N+M	T+N	M+T

T- *Trichoderma* M- Mancozeb 0.3 % N- Neem cake

DISCUSSION

5. DISCUSSION

Cowpea is an important legume vegetable cultivated in Kerala for meeting dietary protein requirements. Cultivation of this crop suffers from serious setback due to several constraints among which diseases caused by fungi play a major role. Fusarium wilt is widely distributed at present in all the cowpea growing regions of the State leading to severe yield loss (Reghunath *et al.*, 1995). Considering the diversity of the pathogen and its soil borne nature, cultural, biological and chemical management practices singly are inadequate to tackle the disease. This study was undertaken to identify effective biocontrol agents, fungicides and soil amendments and to incorporate these for developing an integrated management package for suppression of Fusarium wilt.

The characteristic symptoms of the disease includes yellowing of foliage followed by defoliation. Lower stem portion showed rotting leading to drying of vines above. The roots also showed rotting. These symptoms were similar to those described earlier for Fusarium wilt of other crops. Tu (1991) reported that pea plants infected with *Fusarium* showed yellowing, withering and drying, while those with Fusarium root rot showed various degrees of stunting and yellowing. In cowpea, under severe conditions of disease incidence, lower part of stem and upper part of the crown region together with taproot formed a swollen tuber like structure, which gradually shredded and disintegrated. Kurmut *et al.* (2002) working with root rot and wilt of *Vicia faba* caused by *Fusarium nygamai* showed that there was black root rot which included rotting and death of the lateral root system. Severely affected plants showed black neck canker at the soil level.

Fusarium spp. associated with wilt disease were isolated from different cowpea growing regions of Kerala. The results indicated that these isolates showed variability in cultural, morphological and

pathogenic attributes (Table 4). Padwick (1940) observed significant variation in growth rate, production of aerial mycelium, sporulation, pigmentation of substrate in *F.oxysporum*. Verma and Dohroo (2003) noticed similar variability in *F.oxysprorum* isolates from pea. Based on the morphological and cultural characteristics, the *Fusarium* spp. involved in wilt of cowpea were identified as *F.oxysporum, F.solani* and *F.pallidoroseum* (Table 3). Butler (1918) correlated the role of *F.oxysporum* f.sp.*ciceri* in the incidence of chickpea wilt. *F.solani* have been reported to be the causal agent of root rot of many crops (Burke, 1965; Booth, 1971). The involvement of several isolates of *Fusarium* with wilt of cowpea points to the complexity of the disease. *Fusarium* spp. showed wide variability with respect to their pathogenesis-related attributes. These observations foretell the chances of development of severe and more virulent strains through interspecific hybridisation, mutation etc, which in future could pave way for epiphytotics.

In the present study, it was found that *F.pallidoroseum* caused quick and severe induction of wilt. Inoculation of cowpea seedlings with the isolate resulted in wilt induction in eight days (Table 2). Narain *et al.* (1989) also reported that *F.pallidoroseum* was mainly responsible for groundnut seed rot disease which occurred in Orissa during 1983-88. Similarly, Sharma (2001) reported the involvement of *F.pallidoroseum* in wilt disease of horsegram (*Macrotyloma uniflorum*). He obtained white colonies with reddish pigmentation, this on inoculation into healthy plants took 10-15 days for disease development. In the present investigation, *F.pallidoroseum* produced white floccose colonies, which formed light peach colour on the lower side of the petridish. It also initiated wilt symptoms on cowpea in 8-15 days (Table 4).

Management of wilt of cowpea is necessary for improving the yield as it leads to total crop loss. Seed, soil and foliar applications of fungicide have been practiced for reducing loss caused by Fusarium wilt, but this is quite expensive and poses environmental and health hazards.

Biological control has been an attractive option for controlling *Fusarium* incited diseases.

To quote Boswell (1965) "Biological control is a landmark in a great renaissance of interest and research in micro ecological balance in relation to soil borne plant disease and in the development of more enduring profitable and wiser farming practices". The real potential of biocontrol of *Fusarium* as in case of other plant diseases can be harnessed only if locally adapted antagonists are identified. Accordingly, search was made for pathogen suppressing fungi and bacteria from the native rhizosphere of healthy cowpea plants in sickplots. Out of the fungi isolated only 14.22 per cent found antagonistic to *Fusarium*. Similarly 0.08 per cent of bacteria isolated from cowpea rhizosphere found to have antagonistic activity against *Fusarium*. Such a low percentage of recovery of antagonist has been frequently encountered in screening antagonists against other plant pathogens also (Leben, 1964 and Montesinos *et al.*, 1996).

Preliminary *in vitro* screening of these microorganisms showed highest rate of antagonism by two *Trichoderma* isolates and two isolates of *P.fluorescens*. *Trichoderma* isolates A_7 and A_{15} caused higher inhibition of 73.33 per cent and 68.88 per cent, respectively. Among the bacterial antagonists, two isolates of *P.fluorescens*, P_5 and P_9 showed highest inhibition of 65.55 per cent and 61.11 per cent respectively, under *in vitro* conditions (Table 5 and 6). Effectiveness of these two groups of microorganisms have been well documented against *Fusarium* incited diseases in pulses (Kumar *et al.*, 1996 and Mukherjee and Tripathi, 2000) and in other crops (Sakthivel *et al.*, 1986; Asalmol and Sen, 1992; Mishra and Narain., 1992; Reguchander *et al.*, 1997 and Chattopadhyay and Varaprasad, 2001).

Trichoderma spp. through the production of metabolites, cell wall degrading enzymes, mycoparasitism, competitive saprophytic ability etc, compete with soil borne pathogens and bring about their suppression

(Lewis and Papavizas, 1985). *Trichoderma* spp. have been proved to be a potential biocontrol agent for managing several soil borne diseases under both green house and field conditions (Hardar *et al.*, 1979). Based on the cultural and morphological characteristics the *Trichoderma* isolates A_7 and A_{15} found effective in suppressing *F.pallidoroseum* were identified as *T.viride* and *T.virens*, respectively. These two species have been widely reported to be efficient in control of fusarial diseases of pulses (Kaur and Mukhopadhyay, 1992; Mukhopadhyay and Mukherjee, 1992).

Biological control, in general, caused suppression of wilt due to *Fusarium*. Isolates of *Trichoderma* were more effective than fluorescent pseudomonads and *Piriformospora indica*, the newly reported endosymbiotic fungus.

Among the *Trichoderma* isolates A_7, identified as *T.viride*, showed significant promise in suppressing *Fusarium* wilt in cowpea. This isolate gave complete protection to cowpea plants against wilt disease from seedling to harvest. All the plants in pathogen inoculated control succumbed to the disease. The inhibitory effect of *T. viride* was on par with the KAU released culture of *Trichoderma* and isolate A_{15} (*T. virens*) obtained in the present study.

Earlier workers (Sivan and Chet, 1982; Somasekhara *et al.*, 1996; Nagesh *et al.*, 1998; Jahagirdar *et al.*, 2001) have reported that *T.viride* application to soil helped in suppressing fusarial diseases. Some workers have found that seed treatment with *T.viride* significantly reduced the Fusarium wilt diseases (De *et al.*, 1996; Chattopadhyaya and Sen, 1996; Pandey and Upadhyay, 1999).

In addition to wilt management, *T.viride* application to soil also resulted in increase in height of cowpea plant by 88.33 per cent, shoot dry weight by 13.50 per cent, fresh weight of shoot by 52.75 per cent, and number of nodules by 88.67 per cent over untreated control (Table 7). Thus it could be seen from the present study that *T.viride* apart from

disease management could also be used to improve biometric parameters of cowpea. Pallodhi (1979) reported that in *Fusarium* infection in vegetables, one of the predominant symptom was reduction of root and shoot length. From the present studies, it is clear that the stunting effect due to *Fusarium* infection could be nullified by the application of *T.viride* to soil. Siddiqui and Mahmood (1996) made similar observations in pigeonpea plant challenged with *Fusarium* and observed that soil application of *Trichoderma* sp. promoted plant height, shoot dry weight and number of nodules.

Nodulation increased significantly on cowpea treated with *Trichoderma* isolates. Treatment with *T. viride* A_7, isolate resulted in an increase of 88.67 per cent of nodule number, 100 per cent of nodule fresh weight and 23.80 per cent of nodule dry weight when compared to untreated control (Table 7). 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. Previous report also support this view (Muthamilan and Jeyarajan, 1996).

Fluorescent pseudomonads have been found as an ideal candidate for suppression of *Fusarium* induced wilt as well as for plant growth promotion of several pulses (Elad and Baker, 1985; Alabouvette *et al.*, 1998; Marjan De Boer *et al.*, 1999). In the present investigation, *P.fluorescens* isolates inhibited *Fusarium* spp. better under *in vitro* condition while its performance in reducing the Fusarium disease of cowpea under *in vivo* conditions was very poor. This may be due to the fact that *in vivo* suppression of the plant pathogen by *P.fluorescens* is influenced by several factors such as plant species, soil characters, soil reaction etc, which do not operate under *in vitro* conditions. Elad and Baker (1985) observed that soils suppressive to *Fusarium* have generally high pH (more than 7.0) which in turn is linked to high Ca content and low level of available Fe. In Kerala soils, with acidic pH and greater Fe

availability, the siderophore mediated antagonistic activity may be restricted. This may be one of the reason for lack of correlation between *in vitro* and *in vivo* performance of *P.fluorescens* isolates.

Knudsen *et al.* (1997) opined that the poor correlation between *in vitro* and *in vivo* results of *P.fluorescens* was due to the dilution and inactivation of antibiotics in natural environment. Similarly, Pengnoo *et al.* (2000) obtained erratic results for sheath blight disease suppression using fluorescent pseudomonads selected through laboratory screening. This raises doubt on the process of selection of biocontrol agents based on the *in vitro* antagonism without taking into consideration the ecological factors (Deacon, 1991).

For biological control agent to be included IDM, it should be compatible with other methods of control. Fungicide tolerant strain of *A.niger* was considered as important component of integrated disease management of muskmelon wilt (Chattopadhyay and Sen, 1996). Studies on fungicide compatibility revealed that *T.viride* (A_7) and *T.virens* (A_{15}) isolates were insensitive to most of the common fungicides tested. Several earlier reports also lend support their findings (Indu and Mukhopadhyay, 1990; Papavizas, 1980). However, in the present investigation, it was noticed that carbendazim exerted 46 per cent and 48 inhibition of *Trichoderma* isolates A_7 and A_{15} (Table 14). At the same time there was no inhibition of growth of *Trichoderma* by mancozeb even when it was exposed to 0.3 per cent concentration. Compatibility studies of fungicide with *P.fluorescens* showed no inhibitory effect. Similar insensitiveness of *P.fluorescens* to fungicides have been reported by Vrinda (2002). Thus, based on the compatibility test, it can be advocated that both *Trichoderma* and *P.fluorescens* could be integrated well with mancozeb for providing better disease management.

None of the organic amendments suppressed the growth of *T.viride*. On the contrary, increased growth of the fungus was noticed in PDA amended with neem cake, vermicompost and coirpith compost.

Maximum growth promotion was noticed in neem cake amended medium. *Trichoderma* spp. are generally benefited by organic amendment application (Ganeshan *et al.*, 2000). Contrary to this, Srivastava and Saksena (1968) reported incompatibility of saw dust with *Trichoderma* for management of Fusarium wilt of potato.

The organic amendments caused reduction in growth of pathogens. Among these, neem cake extract at 5 and 10 per cent concentrations exerted 33 per cent and 44 per cent inhibition of *F.pallidoroseum* (Table 15). Usaman *et al.* (1992) demonstrated that neem cake extract 5 per cent effectively suppressed leaf spot pathogens of groundnut. However, contradictory result was obtained with vermicompost amended media. The pathogen growth was promoted upto 25 per cent in 10 per cent vermicompost extract amended media (Table 15). Such stimulatory effect of extract of organic amendments was reported by Singh and Singh (1970) on *F. oxysporum* f. sp.*udum*.

Amendments incorporated in soil operate in a variety of ways such as improving soil structure, plant nutrition as well as suppressing pathogen. Several examples can be quoted on the benefits of soil amendments on suppression of *Fusarium* incited diseases and plant growth promotion (Khalis and Manoharachary, 1985; Chakrabarti and Sen, 1996; Padmodayay and Reddy, 1999).

Neem cake (150 kg/ha) application was found to reduce wilt and gave the lowest percentage disease index *ie.*, 25 per cent (Table 17). This shows that the observations on *in vitro* inhibition tallied with the performance under *in vitro* conditions. Chakrabarti and Sen (1991) and Padmodaya and Reddy (1999), also obtained similar correlation between the results of laboratory and field evaluation. There was no significant suppression of disease due to vermicompost amendment. This would be due to enhanced growth of *F.pallidoroseum* in presence of vermicompost as was noticed from *in vitro* studies (Table 15). Liming was shown to increase the disease. This observation is contradictory to that observed

by Jones and Overman (1981), who found that increasing soil pH by incorporation of hydrated lime resulted in a reduction in Fusarium wilt of tomato.

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 inturn, facilitates the rapid expansion of the root system, better uptake of nutrients and improved vigour of the plants. Incorporation of neem cake significantly improved plant height, fresh and dry weight of shoots, length, fresh and dry weight of roots over untreated control. Though nodule number was not enhanced the fresh and dry weight of nodules was higher in this treatment (Table 18). The role of organic amendments in plant growth enhancement has been well documented (Padmodaya and Reddy, 1999).

Fungicides at their recommended dose for field application inhibited *F.pallidoroseum* to varying extent. Carbendazim (0.1 %) and mancozeb (0.3 %) gave total inhibition of *F.pallidoroseum* (Table 11). Pant and Mukhopadhyay (2001) observed 68 per cent inhibition of *Fusarium* by carbendazim. Suppression of mycelial growth and sporulation of *Fusarium* by mancozeb has been reported by several workers (Chakraborty, 1993; Pushpavathi *et al.*, 1998; Sharma *et al.*, 2002; Vrinda, 2002). Though carbendazim and mancozeb produced similar inhibition of *Fusarium* their effect on the antagonists varied. When carbendazim exerted 46 per cent inhibition of *T.viride*, mancozeb did not adversely affect the antagonist. This is in agreement with the finding of Upamanyu (2002) and Gupta *et al.* (1999). *P. fluorescens* isolates were compatible with organic amendments and fungicides. Earlier results also support this Anitha and Tripathi (2001) and Vrinda (2002) also recorded subtle tolerance of the bacterial antagonists to all the fungicides tested.

The comparison of *in vivo* suppression of Fusarium wilt and growth promotion of cowpea by using fungicides revealed that the systemic fungicide carbendazim showed superior performance (Table 13). The effect of mancozeb was also pronounced with respect to these characters. Mancozeb was selected as the fungicidal component of IDM of Fusarium wilt of cowpea after considering its effect on the antagonists. Further, the treatment also enhanced nodule number and fresh and dry weight of nodules. This indirectly reflects upon the general improvement of nodule producing as well as plant growth promoting bacteria in cowpea rhizosphere, which inturn, benefits the health of the crop and gives saving on use of nitrogen fertilizers.

Integrated disease management (IDM) strategy is targeted to manage disease severity below the economic threshold level following ecologically safe, economically viable and easier application procedure. To achieve this, the selected components *ie.*, antagonist *T. viride*, the effective and compatible fungicide, mancozeb (0.3 %) and organic amendments, neem cake (150 kg/ha), were applied singly and in combination to study their effect on plant growth promotion, wilt suppression and yield increase. All the parameters registered increased effect in treatment combinations. Soil application of mancozeb (0.3 %), *T. viride* and neem cake (150 kg/ha) registered lowest disease index of 16.66 per cent compared to individual application (Table 21). Earlier results also showed combined application of fungicide, amendment and biocontrol agents reduced the wilt incidence. Narendrappa and Gowda (1995) reported that Panama wilt of banana was managed by using disease free planting material, pre-planting dipping in 0.2 per cent carbendazim for 45 minutes, application of lime or neem cake (1 kg/pit) before planting or the application of urea (200 g/plant) and sugarcane trash mulch at five and seven months. Chattopadhyay and Sen (1996) reported the integration of *T. viride* T_4, 0.1 per cent carbendazim and

potassium chloride (186.7 kg/ha) as soil amendment reduced the wilt of muskmelon by 74.14 per cent.

Combined application of mancozeb (0.3 %), *T. viride* and neem cake (150 kg/ha) increased the shoot length and root length over control. This combination was superior to individual application and also other combinations like *Trichoderma* and neem cake, mancozeb and neem cake and *Trichoderma* and mancozeb (Table 20). Chattopadhyay and Sen (1996) made similar observations. Bhaskar *et al.* (2000), Fugro *et al.*, (2000) reported that integration of fungicide, soil amendment and biological control agent increased yield of several crops. In the present investigation also, integration of mancozeb (0.3 %), neem (150 kg/ha) and *T.viride* applied to soil increased the yield of cowpea by 53.37 over untreated control (Table 20).

Bio-control agents applied to soil are not self supporting and therefore, cannot maintain their required population for long. Incorporation of organic amendment like neem cake, on one hand, nourish the beneficial microorganisms and, on the other hand, exert suppressive effect on the plant pathogen. Soil drenching with compatible fungicide like mancozeb give direct death blow to the pathogen making the way clear for the antagonistic fungus, *T. viride* which also compete for the same niche as that of the pathogen *ie. F. pallidoroseum*. Thus, through direct, indirect and concerted efforts, the three components selected bring about effective management of Fusarium wilt of cowpea.

SUMMARY

6. SUMMARY

Fusarium wilt is one of major disease affecting cultivation of vegetable cowpea in Kerala. The present investigation was undertaken to identify effective biocontrol agent, soil amendment and chemical fungicide compatible with each other, and to develop an integrated disease management strategy for combating this soil borne disease.

Fusarium spp. were isolated from roots of wilt affected cowpea plants collected from different locations of Thiruvananthapuram district. Eight isolates of *Fusarium* showed variability in morphological and cultural characters. Based on these characters, *Fusarium* spp. were identified as *F. solani, F. oxysporum* and *F. pallidoroseum*. *F. pallidoroseum* took eight days for symptom development on cowpea seedlings and was considered as the most virulent isolate.

Out of 82 saprophytic fungi isolates obtained from cowpea rhizosphere, 18 isolates exhibited antagonistic activity to *F. pallidoroseum*. In dual culture studies, fungal isolates A_7 and A_{15} were effective in reducing the growth of *F. pallidoroseum*. These isolates exerted 73.33 per cent and 68.88 per cent of inhibition of the pathogen, respectively. Based on the cultural and morphological characters, A_7 and A_{15} were identified as *T. viride* and *T. virens*, respectively.

One hundred and fifteen bacterial isolates obtained from the rhizosphere of healthy cowpea plants were mass screened against *F. pallidoroseum*, and only ten were found to be antagonistic to the pathogen. Fluorescent pseudomonad isolates P_5 and P_9 were found superior in inhibiting *F. pallidoroseum*.

Pot culture experiment was conducted to assess the efficacy of biocontrol agents selected through *in vitro* studies as well as the endosymbiotic fungus *Piriformospora indica* in suppressing fusarial wilt. Among these, *T. viride* (A_7) showed good suppression of disease. This isolate also caused

enhancement of the plant height, fresh and dry weight of shoot and root, root length as well as nodulation of treated cowpea plants.

Bioassay of fungicides such as carbendazim, mancozeb, copper oxychloride and chlorothalonil againt *F. pallidoroseum* showed that carbendazim 0.1 per cent and mancozeb 0.3 per cent totally inhibited the mycelial growth of *F. pallidoroseum*

Under *in vivo* condition, mancozeb (0.3 %) and carbendazim (0.1 %) drenching reduced the wilt disease of cowpea, and showed wilt index of 8.33 and 16.66 per cent respectively. Carbendazim (0.1%) drenching also significantly enhanced the growth. The plant biomass, as well as nodulation were also improved by the fungicides. Mancozeb was compatible with *Trichoderma* and *P. fluorescens,* whereas carbendazim at the recommended concentration for field use inhibited *Trichoderma*.

Neem cake, coirpith compost, vermicompost and lime were tested for their effect on suppression of *F. pallidoroseum* under *in vitro* conditions. Among the amendments, neemcake five per cent and ten per cent concentrations showed superior performance and inhibited the mycelial growth by 33 per cent and 44 per cent, respectively. All the amendments at the recommended concentrations were compatible with *Trichoderma* and *P. fluorescens*.

In pot culture experiment, neem cake (150 kg/ha) apart from reducing the wilt disease of cowpea enhanced the root and shoot biomass and nodulation significantly. Different combinations of the selected biocontrol agent *T. viride*, soil amendment neem cake (150 kg/ha) and fungicide mancozeb (0.3 %) were attempted to develop an integrated disease management strategy for cowpea wilt. *T. viride* was applied as seed treatment and soil application at 30 DAS. Neem cake was applied to soil at the time of potting mixture preparation and mancozeb was applied as soil drenching 45 DAS. Combined application of *T. viride*, mancozeb and neemcake was more effective than the components singly and their other combinations in protecting the cowpea plants against *F. pallidoroseum*. This combination increased the yield significantly over control.

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*Originals not seen

APPENDIX

APPENDIX-I

Potato Dextrose Agar

Potato	–	200 g
Dextrose	–	20 g
Agar	–	20 g
Distilled water	–	1 l

Martin's Rosebengal Agar

Dextrose	–	10 g
Peptone	–	5 g
Potassium dihydrogen phosphate	–	1 g
Magnesium sulphate	–	0.5 g
Rose Bengal	–	33 mg
Streptomycin solution (1 %)	–	3 ml
Agar	–	15 g
Distilled water	–	1 l
PH	–	7.0

Soil extract agar

Glucose	–	1 g
Dihydrogen potassium phosphate	–	0.5 g
Agar	–	15 g
Soil extract	–	100 ml
Tap water	–	900 ml

Adjust pH to 6.8

King's B medium

Peptone	–	20 g
Dipotassium hydrogen phosphate	–	1.5 g
Magnesium sulphate	–	1.5 g
Glycerol	–	10 ml
Distilled water	–	1 l

Adjust pH to 7.2

Nutrient agar

Beef extract	–	1 g
Yeast extract	–	2 g
Peptone	–	5 g
Sodium chloride	–	5 g
Agar	–	15 g
Distilled water	–	1 l

Adjust pH to 7.2-7.4

Potato Sucrose Agar

Potato	–	200 g
Sucrose	–	20 g
Agar	–	20 g
Distilled water	–	1 l

INTEGRATED MANAGEMENT OF FUSARIUM WILT OF VEGETABLE COWPEA
(*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt)

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ABSTRACT

The study "Integrated disease management of Fusarium wilt of cowpea (*Vigna unguiculata* sub sp. *sesquipedalis* (L.) Verdcourt)" was conducted at the Department of Plant Pathology, College of Agriculture, Vellayani, Kerala Agricultural University during 2001-2003. Three species of *Fusarium* were found associated with cowpea wilt in Thiruvananthapuram district. They were identified as *F.pallidoroseum*, *F.oxysporum*, *F.solani*. Of these, *F.pallidoroseum* was the most virulent pathogen.

Two fungal antagonists *viz.*, *Trichoderma viride* (A_7) and *T. virens* (A_{15}) isolated from cowpea rhizosphere were highly effective in inhibiting *F. pallidoroseum* under *in vitro* conditions. Two fluorescent pseudomonads, P_5 and P_9 showed significant suppression of the pathogen. Pot culture experiments using biocontrol agents revealed that *T. viride* was most effective in reducing the disease and in enhancing the plant biomass and nodulation.

Among the fungicides tested, mancozeb (0.3 %) and carbendazim (0.1 %) showed significant inhibition of the pathogen under *in vitro* conditions and suppression of wilt disease under *in vivo* conditions. There was also enhancement of plant growth and nodulation of cowpea. However, carbendazim caused suppression of *Trichoderma*. So, mancozeb was selected as the fungicidal component for development of integrated disease management package.

Neemcake supported the growth of the antagonistic fungus, *T. viride* and inhibited the mycelial growth of the pathogen, *F. pallidoroseum*. The soil application of neemcake significantly decreased the disease and increased plant growth.

Combination of seed treatments and soil application of *T. viride*, soil application of neemcake (150 kg/ha) and soil drenching with mancozeb (0.3 %) effectively suppressed Fusarium wilt of cowpea. It also appreciably increased the biomass and pod yield of the crop.