STUDIES ON THE EFFICACY OF *TRICHODERMA* IN CONTROLLING THE SEEDLING DISEASES OF IMPORTANT VEGETABLES

BY

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AFFECTIONATELY DEDICATED TO MY BELOVED PARENTS FOR BUILDING UP MY EDUCATIONAL CAREER AND EXCELLENT WISHES THROUGHOUT MY LIFE

CANDIDATE'S DECLARATION

I hereby declare that the dissertation or part thereof has not been previously submitted by me to any other university or Institution for a degree or diploma

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CERTIFICATE - I

This is to certify that **Shri KOKATE RAMAKANT DATTATRAYA** has satisfactorily prosecuted his course credits and research for a period of not less than four semesters and that the dissertation entitled **"STUDIES ON THE EFFICACY OF *TRICHODERMA* IN CONTROLLING THE SEEDLING DISEASES OF IMPORTANT VEGETABLES"** submitted by him is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the dissertation or part thereof has not been previously submitted by him for a degree of any university.

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Research Guide

CERTIFICATE - II

This is to certify that the dissertation entitled **"STUDIES ON THE EFFICACY OF *TRICHODERMA* IN CONTROLLING THE SEEDLING DISEASES OF IMPORTANT VEGETABLES"** submitted by **Shri KOKATE RAMAKANT DATTATRAYA** to the Marathwada Agricultural University, Parbhani in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE (Agriculture)** in the subject of **PLANT PATHOLOGY** has been approved by the student's advisory committee after viva-voce examination in collaboration with the External Examiner.

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"There always are in the world few inspired men
whose acquaintance is beyond price"

- PLOTO -

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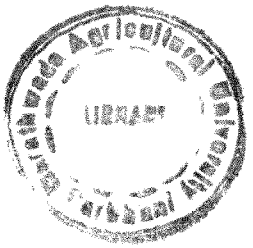


INTRODUCTION

Chapter 1

INTRODUCTION

The importance of vegetables in human diet is well known. Vegetables are rich and comparatively cheaper source of vitamins and minerals. They are important for the maintenance of good health and beneficial in protecting against some degenerative diseases. They play a key role in neutralizing the acids produced during digestion of proteinous and fatty foods.

Vegetable status in respect of area and production with special reference to India is far below the average. Unfortunately, no accurate data of area and production under vegetable crop in India is available. According to the rough estimate by different workers, vegetable crops occupy only 2.5 per cent of the total cultivated area of the country with the total annual production of about 45 million tonnes from a cropped area of 4 million hectares excluding potato and tubers (Singh, 1991).

Considering the present area and yield, the production of vegetables is inadequate to meet the needs of the country. As per information available, an average Indian consume 434 g cereals, 21 g leafy vegetables and 71 g other vegetables including tuber crops per day (Choudhary, 1979) whereas according to diaticians, each adult require 295 g vegetables per day for maintaining the proper health.

For increasing the availability of vegetables, its production needs to boosted up. Increase in area has limited scope and thus per unit productivity needs to be enhanced. Compared to other, in our country, the per hectare productivity of vegetable is quite low. Several factors accounts for this low productivity and diseases is one of the important factor in reducing the per hectare yield of vegetables. Several diseases like damping off, wilt, rots, seedling mortality caused by fungi belonging to *Fusarium, Pythium, Phytophthora, Rhizoctonia, Sclerotium* and *Macrophomina* are known to attack the vegetable crops. Management of these diseases needs to be taken up on priority for increasing the productivity of vegetables.

Developing resistant varieties is the most efficient and economic measure for the control of plant diseases. Looking into the nature of pathogens involved, there is little scope for developing resistant varieties of vegetables. Under this situation growers are left with no alternative but to go for fungicidal control. Fungicide though quite efficient in controlling diseases has several hazardous side effects. They, when applied to the soil, also kills the beneficial microflora of the soil disturbing the natural ecological balance. Their entry into food chain through vegetable consumption has already assumed an alarming proportion and peoples have already started rejecting the produce where heavy pesticides are applied.

These fungicides also enters the underground water body polluting the same. Here we must have an alternative to the fungicide use for disease control. Even the partial replacement of the fungicides with other methods will be most welcome situation to alleviate ill effects of pesticides Biological control is one such measure to reduce the fungicidal use with optimum disease control.

There are several examples of fungi that are able to control the plant pathogens (Barnet and Binder, 1973). Of these, only few have been studied to any extent with the aim of biological control. *Trichoderma* and *Gliocladium* probably have been studied to the greatest extent (Papavizas, 1985).

Biological control of soil borne plant pathogens by the addition of antagonistic micro-organisms to soil is a potential non-chemical mean for plant disease control. The species *Trichoderma* capable of hyperparasitizing pathogenic fungi are highly efficient antagonists (Barnet and Binder, 1973). Antagonists is a micro-organism that adversely effects another (e.g. target pathogen) growing in association with it (Baker and Cook, 1974).

The different species of *Trichoderma* used as antagonists are *Trichoderma viride*, *T. harzianum*, *T. hamatum*, *T. lignorum* and *T. koningii*. There have been many reports of successful uses of the biological control agents but no agent has been used

commercially. The mechanism of action of *Trichoderma* is chiefly by antibiosis and hyperparasitism.

For efficient use of a biocontrol agent, several aspects needs to be considered and the important ones are saprophytic ability of the agent, ecological situation of the area where it is to be applied, method of application, rate of bicontrol agent to be applied etc.

Considering these beneficial effects of *Trichoderma* spp., the present investigation was planned with following objectives.

1. To isolate the local *Trichoderma* spp.
2. Mass multiplication of five *Trichoderma* spp. viz. *T. viride*, *T. harzianum*, *T. hamatum*, *T. lignorum* and *T. koningii*.
3. To study the different carrier material for the preparation of *Trichoderma* inoculants.
4. To study the efficacy of *Trichoderma* spp. in controlling the seedling diseases.
5. To study the antagonism of *Trichoderma* against the pathogen in laboratory.

REVIEW OF LITERATURE

Chapter 2

REVIEW OF LITERATURE

2.1 Isolation of *Trichoderma* spp.

Trichoderma hamatum isolated from a *Rhizoctonia* suppressive soil (Harman *et al.*, 1980). *T. harzianum* (orignianlly described as *T. hamatum*) isolated from a soil suppressive to *Rhizoctonia* (Chet and Baker, 1981). *Trichoderma* spp. were isolated from soil on a *Trichoderma*-selective medium (TSM) (Elad *et al.*, 1981).

Elad and Chet (1983) reported improved selective medium for isolation of *Trichoderma* spp. The fungal antagonists like *T. harzianum*, *T. viride* and *Gliocladium virens* were isolated by a modified triple layer agar (TLA) technique from rhizospheric soil (Jee and Kim, 1987).

Askew and Laing (1993) reported modified *Trichoderma* selective medium (TSM) by replacing finaminosulf with propamocarb or metalaxyl for the quantitative isolation of *Trichoderma* spp.

2.2 Mass multiplication of *Trichoderma* spp.

The application of antagonistic fungi to the rhizosphere of crop plants to be protected from any soil borne disease requires mass production of the antagonist within a short period of time using cheap and easy technique.

Backman and Rodriquez-Kabana (1975) reported a diatomaceous earth granules impregnated with 10 per cent molasses solution was suitable for growth and delivary of *T. harzianum*. Wheat bran was used by several workers as substrate for multiplication of *Trichoderma* spp. (Henis *et al.*, 1978; Grienstein *et al.*, 1979).

Elad *et al.* (1980) reported wheat bran in combination with saw dust for multiplication of *Trichoderma*. A wheat bran + peat mixture (1:1 v/v) was the most efficient substrate for growing a new isolate of *T. harzianum* (T-315) (Sivan *et al.*, 1984).

Sawant and Sawant (1989) developed a simple method for achieving high CFU of *T. harzianum* on organic wastes for field application. The tapioca rind and well decomposed farm yard manure were good substrate for maximum number of colony forming units of *T. viride* (Kousalya and Jeyarajan, 1990).

The mass multiplication of *T. harzianum* on decomposed coffee husk was reported by Ganeshan *et al.* (1997).

Prasad *et al.* (1997) observed that potato dextrose broth (PDB) supported high amount of *T. harzianum* as compared to V-8 juice, molasses and brewer's yeast media. The combination of wheat straw, wheat bran and 2 per cent molasses as well as sugarcane straw and used tea leaves was most suitable for mass multiplication *T. viride* (Singh *et al.*, 1997).

Gaikwad and Sangale (1998) found that gobar gas slurry and dried cowdung were more superior for the growth and sporulation of *T. harzianum* and *T. viride* when it contains 50 per cent moisture and stored at 20 -35^{o}C for about 30 days incubation.

2.3 Carrier study

Wheat bran saw dust (WBSD) medium inoculated with *T. harzianum* was used as carrier of biocontrol agent into the soil (Upadhyay and Mukhopadhyay, 1986). A suitable carrier material needs to be inexpenssive and easily available (Gaind and Gaur, 1990).

Jeyarajan *et al.* (1994) reported the talc-based product of *T. viride* for seed treatment @ 4 gm/kg. The talc-based product of *T. viride* was also developed by Ramkrishnan *et al.* (1994).

Ranganathan *et al.* (1995) reported that gypsum is inexpensive and easily available, so it can be used for *Trichoderma* product.

Raguchander *et al.* (1995) evaluated a talc-based product of *T. viride* for the control of blackgram root rot caused by *Macrophomina phaseolina*.

Gandhikumar and Ranganathan (1997) carried out multiplication of *T. viride* and *T. harzianum* on sterilized substrate viz.,blackgram shell, shelled maize cob, coirpith, gypsum, peat soil and talk powder and reported

that the population increased from 17 x 10^6 CFU/g to 28 x 10^6 CFU/g on shelled maize cob and 26 x 10^6 CFU/g on blackgram shell in time interval of 30 days.

Nakkeeran *et al.* (1997). studied standardization of storage condition to increase the shelf life of *Trichoderma* formulations and reported that vermiculite bran acid fermentor biomass (VBAFB) of *T. viride* recorded highest mean population in milky white bags (205 x 10^6 CFU/g).

Prasad *et al.* (1997) developed eight types of *T. harzianum* formulation viz., talc, gypsum, vermiculite-wheat bran, three pesta granules combination (wheat flour, wheat flour-kaolin and wheat flour bentonite) and alginate prills and reported that the number of viable propagules were increased upto 30 days in vermiculite wheat bran and pesta granules, 45 days in gypsum, 60 days in alginate prills and talc.

Umamaheshwari and Ramakrishnan (1997) reported farm yard manure and tapioca thippi were the best substrate for rapid multiplication of *T. viride*.

2.4 Biological control

Biological control mainly consist of using a microorganism to control harmful microorganism (by biological destruction) causing plant disease without disturbing the ecological balance. The biological control

of root diseases of crop plants by introduction of antagonistic microorganisms has been suggested as an environmentally safer alternative to the use of fungitoxic chemicals (Baker and Cook, 1974).

D'Ercole and Nipoti (1986) reported control of *Fusarium* and *Verticillium* infections in tomato by using *T. viride, T.harzianum* and *T. koningii*.

Sivan *et al.* (1987) observed that *T. harzianum* effectively controlled *Fusarium* crown rot of tomato under field condition. The control of cucumber wilt pathogen, *Fusarium oxysporum f. sp. cucumerinum* by using *T. harzianum* and *G. virens* was reported by Cho *et al.* (1989).

Monaco *et al.* (1991) found *T. harzianum*, *T. koningii* and *T. aureoviride* were effectively controlled *Fusarium* spp. and *Sclerotium rolfsii*. The control of *Fusarium* crown and root rot of tomato by using *T. harzianum* was reported by Rattink (1993).

Shahida and Haque (1994) reported that *T. harzianum*, *T. koningii* and *G. virens* significantly controlled soil borne root infecting fungi in tomato and okra. The damping off disease caused by *Pythium ultimum* and *Rhizoctonia solani* was controlled by using *Trichoderma* spp. (Caliquet and Scheffer, 1996).

2.5 Biocontrol mechanism - antagonism

The microorganisms used in the biological control of plant diseases are termed as 'Antagonists' and the process is called as antagonism. The species of *Trichoderma* capable of hyperparasitising pathogenic fungi are highly efficient antagonists (Barnet and Binder, 1973).

The use of Tricoderma species as a biological control agents for various fungal diseases has been reported by Baker and Cook (1974).

Elad *et al.* (1980) studied the effectiveness of *T. harzianum*, as a biological control agent against *S. rolfsii* and *R. solani*.

Cipriano *et al.* (1989) observed the antagonistic activity of *T. koninigii*, *T. hamatum* and *G. virens* against *Fusarium oxysporum f. sp. radicis-lycopersici* by producing inhibition zones of 3 to 10 mm.

Xu *et al.* (1993) reported that *T. harzianum* T_{82} and *Trichoderma* sp. NF_9 inhibited the hyphal growth of *S. rolfsii*, *R. solani*, *Pythium aphanidermatum* and *F. oxysporum in-vitro*.

Xue Baodi *et al.* (1995) observed *Trichoderma* sp. isolate Tr-5 inhibitory to *R. solani*, *R. cerealis* and *F. oxysporum f. sp. niveum* at 10^6 - 10^7 CFU/ml; to *Sclerotinia sclerotiorum* and *Pythium kunmingense* at 10^4 - 10^5 CFU/ml and to *Phytophthora* at 10^5 - 10^6 CFU/ml.

- Selvarajan and Jeyarajan (1996) reported reduction in sporulation of *Fusarium solani* and sclerotial size, germination and germ tube number of *M. phaseolina* by using *Trichoderma* spp.

Padmodaya and Reddy (1996) reported *Trichoderma* spp. suppressed the growth of tomato wilt pathogen *F. oxysporum f. sp. lycopersici in-vitro.*

Dubey (1997) reported *T. harzianum, T. viride* and *G. virens* significantly inhibited the mycelial growth and sclerotial production of *Thanatephorus cucumeris* (=*Rhizoctonia solani*) *in-vitro.*

Jha and Singh (1997) studied the antagonistic effect of *T. viride* at different concentrations of spores against the growth and sporulation of *F. oxysporum f. sp. ciceri in-vitro* and concluded that 10^4 spore per ml concentration of antagonists completely inhibited the sporulation of pathogen.

2.5.1 Antibiosis

The antagonistic organism releases an antibiotics or other chemicals which are harmful to the pathogen and inhibits its growth. Production of antibiotics by *T. harzianum*, inhibitory to growth of *S. rolfsii* has been reported by Upadhyay and Mukhopadhyay (1983).

Jackson (1985) defined the antibiosis as antagonism mediated by specific or non-specific

metabolites of microbial origin or by lytic agents, enzymes, volatile componds or other toxic substances.

Mukhopadhyay and Kaur (1990) reported the production of antibiotics by *T. harzianum*, inhibitory to the growth of *R. solani* and *F. oxysporum*. The growth inhibition of the host mycelium is due to extracellular metabolites secreted by *Trichoderma* (Cherif and Benhamou, 1990).

Scarselletti and Faull (1994) reported the compound produced by *T. harzianum*, 6 pentyl-α-pyrone (6 pp) inhibited the growth of *R. solani* and *F. oxysporum f. sp. lycopersici*. The fungal isolates of *Trichoderma* sp. showed very strong antibiosis in both solid and liquid media against *S. rolfsii* (Bari *et al.*, 1997).

2.5.1.1 Role of volatile and non-volatile metabolites secreted by *Trichoderma*

Alkylpyrones, a volatile metabolite reported from *T. harzianum* (Claydon *et al.*, 1987) when added to peat soil mixture, suppressed *R. solani* induced damping off of lettuce. The metabolites produced by *Trichoderma* spp. are powerful inhibitors of several fungi *in-vitro* (Fravel, 1988).

Wilson *et al.* (1988) noticed *T. harzianum* isolate TRC-12 produced volatile and non-volatile antibiotics while TRC-33 produced only non-volatile one suppressing the

growth of *R. solani*. *T. harzianum* isolate T-42 strongly inhibited mycelial growth by means of non-volatile antibiotics (Cho *et al.*, 1989).

Calvet *et al.* (1990) found that non-volatile compounds released by *T. harzianum* isolate significantly inhibited the growth of *F. oxysporum* and *Verticillium dahliae in-vitro*.

Rathore (1992) reported volatile and non-volatile metabolites secreted by *T. viride* completely inhibited the growth of *Pythium myriotylum* and *F. solani* by 70 and 10 per cent, respectively.

Singh and Singh (1993) showed that *T. reesei* (*T. longibrachiatum*) produced volatile and non-volatile metabolites that inhibited the growth of *R. solani* by 71 per cent *in-vitro*.

2.5.2 Competition

The mechanism of control has been shown to be competition for iron between the biocontrol agent and plant pathogen.

Zhong *et al.* (1990) observed the hyperparasitism of *T. harzianum* on *R. solani* and reported that the hyphae of *Trichoderma* isolate grew parallel to and along the host hyphae, sometimes forming bulbous or hook like structures penetrating and growing within the host.

Sariah (1991) observed the hyphal interaction of *Trichoderma* spp. and *S. rolfsii* and concluded that the hyphae of *T. harzianum* grew towards and along the hypahae of *Corticium rolfsii*, forming several short branches and coils.

2.5.3 Mycoparasitism

Mycoparasitim is defined as parasitism of one fungus by another. The mechanism of hyperparasitism includs different kinds of interactions like coiling of hyphae around the pathogen, penetration, production of hausteria and lysis of hyphae, action of toxins and/ or enzymes.

Weindling (1934) reported the parasitism of *T. lignorum* (Tode) Harz. on *S. rolfsii* Sacc and *R. solani* Kuhn. *T. harzianum* directly attacked *R. solani* mycelinum (Hadar *et al.*, 1979).

Elad *et al.* (1983) observed the hyphal interaction between *T. harzianum* and *S. rolfsii* or *R. solani* by scanning electron microscopy (SEM).

Wokocha *et al.* (1986) reported *T. viride* and *T. harzianum* invaded colonies of *C. rolfsii* and inactivated pathogen *in-vitro*. *T. harzianum* isolate TR-5, was able to parasitize the hypahe of *R. solani* (Kim *et al.*, 1992).

Ghaffar (1997) reported the mycoparasitism of *Coniothyrium minitans* on *Sclerotium cepivorum* and *Sclerotinia sclerotiorum*; *T. hamatum* on *Sclerotium*

delphini; *T. viride* on *S. cepivorum*; *T. harzianum* on *R. solani* and antibiotic effects of *Penicillium nigricans*, *Penicillium expansum* and *Rhizobium* sp. on soil borne root infecting fungi.

Bazgir and Okhovvat (1997) observed the antagonistic activity of *Trichoderma* spp. against *R. solani* through hyphal coiling, hyphal penetration, hyphal lysis and production of volatile and non-volatile metabolites in laboratory experiment.

Singh and Singh (1997) reported a local isolate of *T. harzianum* (ITCC No. 5442) directly attacked and lysed the mycelium and sclerotia of *S. rolfsii* in dual culture technique.

Naik *et al.* (1997) reported that the interaction between *Fusarium* and *Trichoderma* showed mycoparasitism; the hyphae of both the fungi coiled all along resulting in formation of appresorium and further penetration by *Trichoderma* leads to death of hyphal cells of *F. oxysporum f. sp. capsici*.

2.5.3.1 Coiling of hyphae around the pathogen

Weindling (1932) reported coiling of hyphae of *Trichoderma* spp. around the hyphae of certain other fungi. The isoaltes of *T. harzianum* Rifai showed no appearent ability to produce antibiotics were capable of coiling around other hyphae (Dennis and Webster, 1971).

The group which produced non-volatile antibiotics are not able to coil around the hyphae of other fungi (Dennis and Webster, 1971). The hyphae of *Trichoderma* coiled around the hyphae of *S. rolfsii* or *R. solani* and then enzymatically digest cell walls (Elad *et al.*, 1983).

Wilson *et al.* (1988) reported *T. harzianum* parasitized *R. solani* by coiling around and lysing the host hyphae. The hyphae of *T. aureoviride* isolate grew and coiled around the hyphae of *F. oxysporum*. The hyphae of *Trichoderma* isolate coiled around host hyphae (Zhong *et al.*, 1990).

Dubey (1997) reported the principle mechanism of mycoparasitism of *Trichoderma* on *R. solani* was coiling of antagonistic hyphae around the host hyphae and lysis.

2.5.3.2 Penetration

Durell (1968) using more retained technique showed that penetration by *Trichoderma* hyphae is not limited to oomycetes, several members of mucorales and also *R. solani* to be penetrated by *Trichoderma* hyphae. The antibiotics are directly responsible for promoting penetration (Dennis and Webster (1971).

Dennis and Webster (1971) observed the penetration of *Pythium* hyphae by isolates of *T. harzianum* and *T. viride* . Direct penetration and utilization of protoplasmic contents of hyphae of *S. rolfsii* by *T. harzianum* was reported by Upadhyay and Mukhopadhyay (1986).

2.5.3.3 Production of hausteria and lysis of hyphae

Elad *et al.* (1983) studied on the parasitism of *Trichoderma* spp. and *S. rolfsii* or *R. solani* and showed that hyphae of parasite contact their host by producing appresorium like bodies and then enzymatically digest host cell walls.

Lederer *et al.* (1992) reported *Trichoderma* isolates were active against zoospores of *Phytophthora* causing lysis, formation of extracellular vesicles and hypertrophy of the water explusion vesicles resulting in death.

2.5.3.4 Action of enzymes

Elad *et al.* (1982) reported that *T. harzianum* excreted β-1-3 glucanase and chitinase into the medium when grown on laminarin and chitin respectively or on cell walls of the pathogen, *S. rolfsii* as a sole carbon source.

Cherif and Benhamou (1990) reported a chitinase, enzyme produced by *Trichoderma* spp. inhibited the growth of *F. oxysporum f. sp. radicis-lycopersici* in dual culture test.

Cruz *et al.* (1995) found that the enzyme β-1-6 glucanase, hydrolyses the filamentous cell wall, act cooperatively inhibiting the growth of fungi tested.

Barcelo (1997) reported cellulase from *T. viride* was capable of inducing disease resistance reaction in cultured grapevine (*Vitis venifera*).

Balsubramanian *et al.* (1997) purified and characterized *Trichoderma* chitinase effective against *R. Solani*, causing rice sheath blight.

2.6 Effect of *Trichoderma* spp. on method of application

2.6.1 Seed inoculation

Harman *et al.* (1980) reported seed treatment of *T. hamatum* (Bon) Bain protected seed and seedlings of radish and pea from the attack by *Pythium spp.* or *R. solani*. Seed treatment of tomato with *T. viride* and *Streptomyces griseus* reduced both pre-emergence and post-emergence damping off due to *Pythium debaryanum* and *F. oxysporum f. sp. lycopersici* (Yesh'a *et al.*, 1981).

Sivan *et al.* (1984) noted that the application of *T. harzianum* as seed coating was effective in sandy soil. Seed pelleting with various antagonists resulted in an increase in the rate of seed germination of cotton and a decrease in post-emergence mortality by infection with *R. solani* (Algarsamy *et al.*, 1987).

Monaco *et al.* (1991) observed that seed treatment with *Trichoderma* spp. significantly increases seedling emergence under field conditions. Seed treatment with *T. harzianum G. virens*, *Rhizobium meliloti* or *Streptomyces sp.* gave complete control of *F. oxysporum* causing root rot of tomato (Shahida and Ghaffar, 1991).

Xu *et al.* (1993) reported that seed treatment with *T. harzianum* T_{82} or NF_9 spore suspension (10^8 CFU/ml) increased the rate of seedling emergence of cucumber by 14 per cent and 20 per cent respectively.

Das *et al.* (1997) found that seed treatment of cabbage seed with spore suspension of *T. viride* , *T. harzianum* and *T. koningii* was more effective than soil application in reducing pre- and post-emergence damping off caused by *R. solani* and *T. harzianum* was more effective than *T. viride* and *T. koningii*.

Gandhikumar and Ranganathan (1997) reported seed treatment with *T. viride* + *Pseudomonas fluorescens* significantly decreased the wilt incidence caused by *F. oxysporum f. sp. corianderii*.

Mehta *et al.* (1997) noted seed inoculation with *Trichoderma* spp. viz. *T. harzianum* and *T. viride* increase the rate of seed germination as well as percentage of germination significantly in tomato and chilli.

Umamaheshwari and Ramkrishnan (1997) reported seed treatment was the best method of application than soil application for effective control of diseases under field conditions.

2.6.2 Soil inoculation

The control of damping off of tomato (*Pythium indicum*) by inoculating soil with *Trichoderma viride* , *T. harzianum* and *Laetiseria arvalis* was reported by Krishnamoorthy and Bhaskaran (1990).

Soil treatment with 0.6 per cent (w/w) *T. harzianum* isolate T_{82} bran culture (10^7 CFU/g) reduced the incidence of disease caused by *C. rolfsii*, *R. solani* and *P. aphanidermatum* by 46.5 per cent, 28.4 per cent and 81.2 per cent respectively (Xu *et al.*, 1993).

Gangopadhyay and Joshi (1997) reported soil application of *Trichoderma* formulation before sowing in addition to seed treatment provided better control of root rot of cotton and chickpea (*M. phaseolina*).

Lodha *et al.* (1997) reported soil application of resident biocontrol agents (BCA) *Trichoderma harzianum*, *T. aureoviride*, *G. virens*, *Pythium acanthophorum* and a non-resident isolate of *T. viride* suppressed ginger rhizome rot caused by *F. solani* or *P. myriotylum* or both.

Manoranjitham *et al.* (1999) reported soil application of antagonists like *T. viride* and *P. flourscens* effectively checked the pre-emergence and pos-emergence damping off of tomato caused by *P. aphanidermatum* under pot culture experiment.

2.7 Effect of *Trichoderma* spp. on seedling emergence

Windham *et al.* (1986) found that addition of *Trichoderma* spp. increased the rate of emergence of tomato and tobacco seedlings over that of control.

Manaco *et al.* (1991) reported that seed treatment with *Trichoderma* spp. increased seedling emergence significantly under field conditions.

Shahida and Ghaffar (1991) reported seed treatment with *T. harizanum*, *G. virens*, *R. meliloti* or *Streptomyces sp* resulted in higher seed germination.

Jacqmin *et al.* (1993) reported combining priming with *T. koningii* treatment either improved emergence rate or reduced post-emergence damping off caused by *Pythium sp* or *R. solani*.

Bari *et al.* (1997) found an isolate (TF-24) of *Trichoderma* spp. most effective in increasing seed germination, growth promotion and reducing foot and root rot of barley caused by *S. rolfsii*.

Singh *et al.* (1997) reported seed treatment with *T. viride* increased per cent seed germination and reduced seedling mortality due to wilt/root rot pathogen of chickpea.

2.8 Effect of *Trichoderma* spp. on growth parameters

Windham *et al.* (1986) found that application of *Trichoderma* spp. increased the root and shoot dry weight of tomato, radish and tobacco seedlings.

Shahida and Ghaffar (1991) reported seed treatment with *T. harzianum*, *G. virens*, *R. meliloti* or *Streptomyces sp.* increases fresh seedling weight, shoot and plant length over that of control.

Inbar *et al.* (1994) reported application of *T. harzianum* significantly increase 23.8 and 17.2 per cent

seedling height, 96.1 and 50.0 per cent leaf area and 24.7 and 28.6 per cent plant dry weight in cucumber and chilli seedlings, respectively as compared with control.

Trichoderma treated seedlings were more developed, grew more vigerously, contained higher level of chlorophyll and more resistant to damping off caused by *Pythium sp.* and *R. solani* (Inbar *et al.*, 1994).

Gandhikumar and Ranganathan (1997) reported application of *T. viride* + *P. fluorescens* increases seed germination, shoot and root length, dry matter production and yield.

Mehta *et al.* (1997) found application of *Trichoderma* spp. increases above ground biomass (AGB) and below ground biomass (BGB) nearly 150-200 per cent and 200-250 per cent, respectively in tomato and chilli over control.

Manoranjitham *et al.* (1999) reported talc-based formulation of *T. viride* and *P. fluorescens* significantly increased the shoot length, root length and dry matter production of tomato seedlings.

2.9 Efficacy of *Trichoderma* spp. in controlling seedling diseases of important vegetables

Elad *et al.* (1980) reported wheat bran preparation of *T. harzianum* significantly decreased diseases of tomato, bean or cotton, caused by *S. rolfsii* or *R. solani*. Mass

incorporation of *T. viride* in seedling plots protected approximately three lakh seedlings from root rot disease (Padmanabhan and Alexander, 1983).

Sivan *et al.* (1984) found that wheat bran/peat preparation of *T. harzianum* applied to either soil or rooting mixture efficiently controlled damping off induced by *P. aphanidermatum* in pea, cucumber, tomato and pepper. The application of *T. viride* and *T. koningii* reduced disease incidence significantly due to *Fusarium* and *Verticillium* infections in tomato (D'Ercole and Nipoti, 1986).

Siven and Chet (1986) observed that application of *T. harzianum* isolate effective against *F. oxysporum f. sp. radicis-lycopersici* on tomato and *F. oxysporum f. sp. niveum* on watermelon under field conditions. *T. harzianum* applied in the form of sorghum culture in *S. rolfsii* infested soil gave 76 and 88 per cent control of root rot of sugabeet in first and second growth cycle of seedlings, respectively (Upadhyay and Mukhopadhyay, 1986).

Mukhopadhyay (1987) reported the application of wheat bran saw dust (WBSD) preparation of *T. harzianum* or *T. koningii* brought about an excellent control of damping off of tomato and egg plant and wilt and root rot of lentil under field condition. *T. harzianum* isolate TRC-9 and 28 both reduced damping off of lettuce caused by *R. solani* (Wilson *et al.*, 1988).

Cho *et al.* (1989) effectively reduced the incidence of cucumber wilt (*F.oxysporum f. sp. cucumerinum*) by using *T. harzianum* (T_{42}) and *G. virens* (GC-27). The damping off of cauliflower was controlled by using *T. harzianum* alone or in combination with fungicide (Mukharjee *et al.*, 1989).

Georgieva (1992) reported the treatment of capsicum plant with trichodermin, a product of *T. koningii* N_{21} on barley seed, reduced *V. dahliae* wilt by 23-35 per cent compared with control.

Haque and Ghaffar (1992) reported *T. viride* *T. hamatum* and *R. meliloti* reduced *M. phaseolina* infection on fenugreek seedlings whereas *T. hamatum*, *T. harzianum*, *T. pseudokoningii* and *R. meliloti* completely control infection of *R. solani* and *T. harzianum*, *T. koningii* alone or *R. meliloti* with *T. viride* , *T. hamatum*, *T. koningii* or *T. pseudokoningii* compleltely controlled infection of *Fusarium spp.*

Khakimov and Abdullaev (1992) reported application of trichodermin to soil or tomato seed or dipping seedling roots in a trichodermin suspension gave good control of fusariose (*F. oxysporum f. sp. lycopersici*).

Flori and Roberti (1993) controlled *F. oxysporum f. sp. cepae* by treating the onion bulbs with *T. viride* 144, *T. harzianum* 312 and 68, *Trichoderma* spp. 13A under greenhouse conditions. *T. harzianum* was the most effective

antagonist which control *Fusarium* crown and root rot of tomato on a recirculation substrate system (Rattink, 1993).

Sivan and Chet (1993) controlled *Fusarium* crown and root rot of tomato with *T. harzianum* in combination with methyl bromide or soil solarization. The decomposed coirpit with *T. hamatum* and *T. viride* strain 2 gave 16.3 and 11.5 per cent post-emergence damping off of chilli (*P. aphanidermatum*) compared with 75 per cent in control under pot culture experiment (Mani and Marimuthu, 1994).

Shahida and Haque (1994) reported that *T. harzianum*, *T. koningii* and *G. virens* showed significant control of *M. phaseolina*, *F. oxysporum* and *F. solani* infections on tomato and okra.

Karpagavalli and Ramabadran (1995) noted that application of *Trichoderma* spp. reduced the incidence of damping off of tomato caused by *P. aphanidermatum* (Edson) Fitz in pot culture experiment.

Xue Baodi *et al.* (1995) reported isolate of *Trichoderma* spp. TR-5 significantly controlled damping off of cucumber caused by *P. aphanidermatum* and *Phytophthora drechsleri* by 88.2 and 94.5 per cent, respectively.

Caliquet and Scheffer (1996) reported four strains of *Trichoderma* spp. significantly controlled damping off caused by *P. ultimum* in cucumber and *R. solani* in radish.

Bazgir and Okhovvat (1997) noticed application of *T. harizanum*, *T. viride* and *G. virens* effectively control

bean damping off and seed rot caused by *R. solani*. *T. harzianum* (TH1, TH3) and *T. viride* (TV1) followed by *G. virens* (GV1) was the most effective in controlling lettuce drop caused by *S. sclerotiorum* + *S. minor* (Dhiman, 1997).

Joseph and Sivaprasad (1997) reported *T. viride* and *Aspergillus fumigatus* significantly reduced rhizome rot of ginger caused by *P. aphanidermatum* (Edson) Fitz. The reduction in sclerotium pod rot of groundnut in pot culture experiment by *T. harzianum* was reported by Mayee and Asghari (1997).

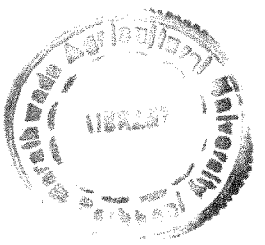
Mehta *et al.* (1997) found that *T. harzianum* and *T. viride* were most promising for controlling root rot and wilt of tomato caused by *Fusarium spp.* The fungal isolate TF-24 of *Trichoderma* spp. and SF-17 of sterile fungus were effecient in controlling pre- and post-emergence damping off of cauliflower seedlings caused by *S. rolfsii* or *R. solani*, respectively (Mondal *et al.* (1997).

Mukhopadhyay (1997) reported *Trichoderma* and *Gliocladium* highly effective biocontrol agents because of their broadspectrum activity against several plant pathogens and seed treatment of these agents resulted in management of a large number of soil borne diseases in cereals, pulses, vegetables, oilseeds and flowering plants.

Nallathambi (1997) reported some isolates of *Trichoderma* and *P. fluorescens* were effective bicontrol agents against *Pythium graminicola* causing seedling rot of sugarcane under *in-vitro* condition.

Phookan and Chaliha (1997) noted thatcollor rot and wilt of brinjal caused by *S. sclerotiorum* were effectively controlled by using antagonists like *T. viride*, *Bacillus subtilis* and *G. virens*.

Robert and Saha (1997) reported *T. harzianum*, Rifai and *T. viride* Pers as effective biocontrol agents against *S. rolfsii* Sacc, the collor rot pathogen in pigeonpea.



MATERIALS AND METHODS

Chapter 3

MATERIALS AND METHODS

3.1 General considerations and precautions

During the investigation on "Studies on the efficacy of *Trichoderma* in controlling the seedling diseases of important vegetables", a series of experments were carried out in the field and laboratory. *In-vitro* experiments were done to study the antagonistic activity of *Trichoderma* spp. against the pathogen.

Isoaltion, inoculation and sub-culturing were done under aseptic condition on Laminar Air Flow Bench. The glasswares used in the present investigation were sterilized in hot air oven. The media in test tubes and in petriplates were sterilized in autoclave at 15 lbs psi for 20 minutes. The soil used for raising the plants under glasshouse condition was sterilized in autoclave at 30 lbs psi for 30 minutes in two successive cycles at the interval of 24 hours.

During isolation 0.1 per cent mercuric chloride ($HgCl_2$) solution was used for surface sterilization of infected root pieces with three subsequent washings in sterilized water. The mercuric chloride solution was used for disinfecting the Laminar Air Flow Bench.

Clean seeds of tomato, chilli and brinjal were procured from Vegetable Growing Scheme, Department of Horticulture, Marathwada Agricultural University, Parbhani and in vivo trials were conducted at the same place under standard conditions of raising vegetable seedlings.

3.2 Isolation of *Trichoderma* spp.

Isolates of five *Trichoderma* spp. viz., *T. viride*, *T. harzianum*, *T. hamatum*, *T. lignorum* and *T. koningii* were available at Department of Plant Pathology, College of Agriculture, Parbhani.

These isolates were maintained on potato dextrose agar (PDA) slant. The cultures were transferred to fresh media periodically and stored at 28 $\pm$ 2^{o}C temperature.

3.3 Mass multiplication of *Trichoderma* spp.

Mass multiplication of five *Trichoderma* spp. viz., *T. viride*, *T. harzianum*, *T. hamatum*, *T. lignorum* and *T. koningii* was done on potato dextrose broth (PDB).

Mass multiplication was done by inoculating 5 mm disc of 5 days old *Trichoderma* culture grown on potato dextrose agar (PDA) into 250 ml conical flask containing 150 ml potato dextrose broth (PDB). The inoculated flasks were kept at room temperature (28 $\pm$ 2^{o}C).

3.4 Assessment of seedling diseases

The field experiment on effect of *Trichoderma* spp. on seedling diseases of tomato, chilli and brinjal was conducted using Factorial experiment layed in Randomised Block Design. The details of treatments were as under:

Factor I

M_1 - Seed inoculation

M_2 - Soil inoculation

Factor II

I_0 - No inoculation

I_1 - *Trichoderma viride*

I_2 - *T. harzianum*

I_3 - *T. hamatum*

I_4 - *T. lignorum*

I_5 - *T. koningii*

The treatment combinations were as under:

M_1I_0	M_2I_0
M_1I_1	M_2I_1
M_1I_2	M_2I_2
M_1I_3	M_2I_3
M_1I_4	M_2I_4
M_1I_5	M_2I_5

The above treatment combinations were layed down in Randomised Block Design with three replications. The plot size of individual treatment was 1.8 x 1.0 m.

3.4.1 Inoculation techniques

3.4.1.1 Seed and soil inoculation

The field was prepared by ploughing and harrowing. Raised beds of 1.8 x 1.0 m size were prepared. Each bed had 10 lines and each line was sown with 0.5 g seeds of tomato (215 seeds), chilli (127 seeds) and brinjal (142 seeds) i.e. 5 g seeds/treatment.

Seed inoculation was carried out by rolling the seeds in young culture of *Trichoderma* spp., air dried and sown.

In soil inoculation, the inoculum was mixed in the soil prior to sowing.

3.4.2 Plan and layout

The plan and layout of the present investigation on tomato, chilli and brinjal under field conditions is appended.

3.5 Effect of *Trichoderma* spp. on seedlings

3.5.1 Effect of *Trichoderma* spp. on seedling emergence

The observations on seedling emergence of tomato, chilli and brinjal was recorded for each treatment and replication on 10-15 DAS.

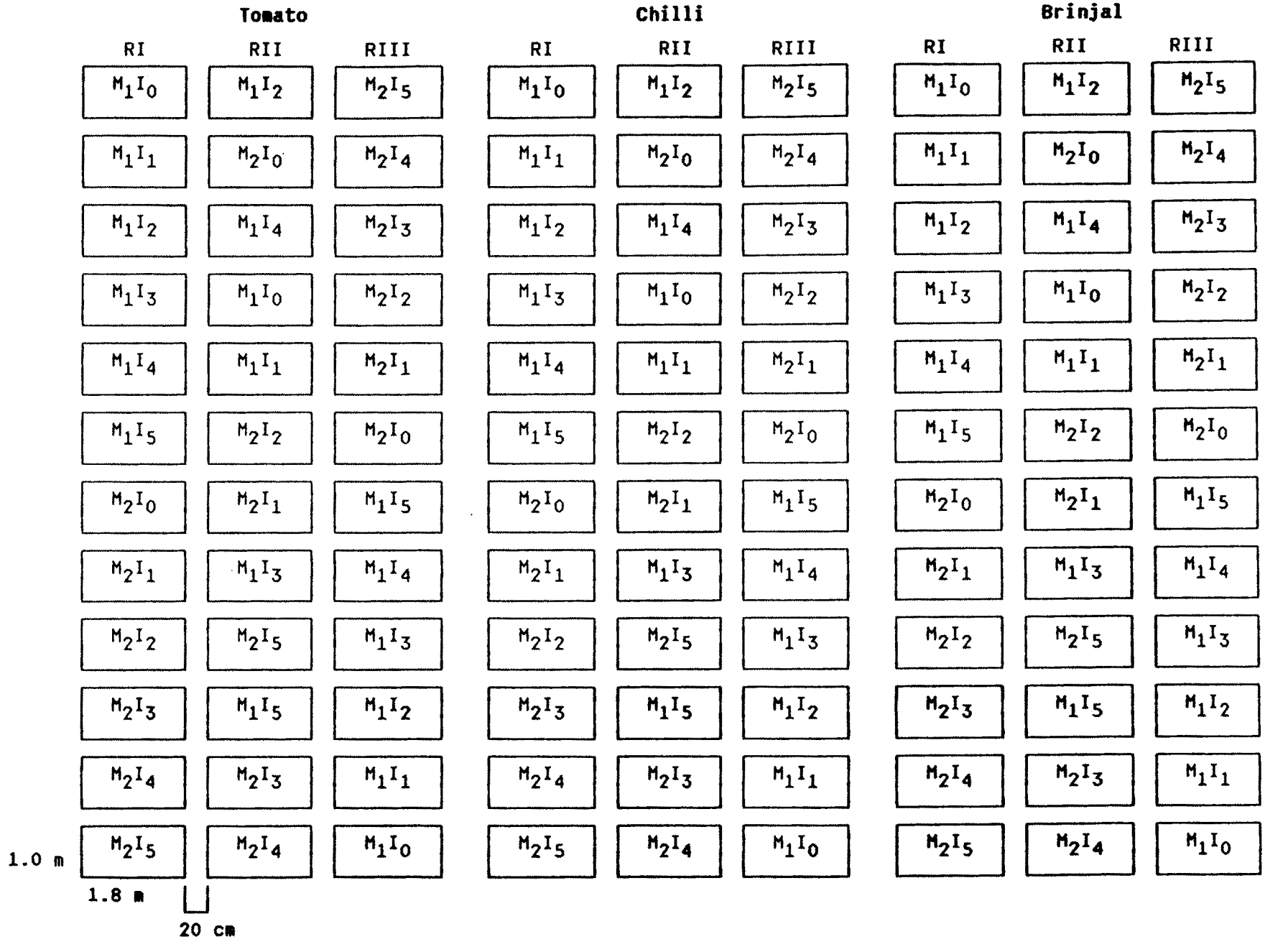


Fig. 1. Plan of layout

3.5.2 Effect of *Trichoderma* spp. on seedling mortality

The observations on seedling mortality of tomato, chilli and brinjal was recorded on 20th, 25th, 30th, 35th, 40th and 45th DAS.

3.5.3 Effect of *Trichoderma* spp. on growth parameter

The observations on root length, shoot length, number of leaves, root fresh and dry weight and shoot fresh and dry weight of tomato, chilli and brinjal seedlings were recorded for each treatment and replication on 35th DAS based on five seedlings.

3.5.4 Effect on chlorophyll content

The chlorophyll content of tomato, chilli and brinjal seedlings was estimated using standard procedure as given below.

The fresh leaves (200 mg) were cut into small pieces and homoginized in mortor and pestle with pure acetone. The supernatant was filtered through Whatman No. 42 filter paper. The sufficient quantitiy of 80 per cent acetone was added on filter paper until colourless and 15 ml volume was made. Then 1 ml extract was transferred into 10 ml volumetric flask and diluted by

making the volume with 80 per cent acetone. Optical density (OD) at 663 nm and 645 nm was measured for determination of chlorophyll-a and chlorophyll-b respectively.

The chlorophyll content was calculated by using following formulae.

$$\text{Chlorophyll-a (mg/g)} = (12.7 \times D663) - (2.69 \times D645) \times \frac{\text{T.E.}}{1000} \times \text{Dil. used} \times \frac{1}{\text{Wt. of sample}}$$

$$\text{Chlorophyll-b (mg/g)} = (22.9 \times D645) - (4.65 \times D663) \times \frac{\text{T.E.}}{1000} \times \text{Dil. used} \times \frac{1}{\text{Wt. of sample}}$$

$$\text{Total chlorophyll (mg/g)} = (20.2 \times D645) + (8.02 \times D663) \times \frac{\text{T.E.}}{1000} \times \text{Dil. used} \times \frac{1}{\text{Wt. of sample}}$$

T.E. - Total extract.

3.6 Isolation of pathogen

The affected portion of root and stem were cut into small pieces. These pieces were surface sterilized in 0.1 per cent mercuric chloride ($HgCl_2$) solution followed by three changes in sterile water. Then these pieces were planted on sterilized potato dextrose agar (PDA) in petriplates. The petriplates were incubated at 28 $\pm$ 2^{o}C temperature in inverted position.

After three days sub-culturing was done on potato dextrose agar slant by transferring the young mycelial bit with the help of sterile inoculating needle. The slants were kept at 28 ± 2°C temperature. Sub-culturing was done at regular intervals of 15-20 days, on PDA slants. The pathogen was identified as *Fusarium* according to characters given by Booth (1971).

Pathogenecity of *Fusarium* was tested by incorporating the inoculum in soil prior to sowing. The culture obtained by re-isolation compared with original culture and found it identical with original culture. The re-isolated culture was maintained on PDA slant for further studies.

3.7 *In-vitro* inhibition of pathogen by *Trichoderma* spp.

The antagonistic activity of *T. viride*, *T. harzianum*, *T. hamatum*, *T. lignorum* and *T. koningii* on *Fusarium* were studied *in-vitro*.

The 5 mm mycelial disc of *Fusarium* was inoculated at one side of sterile petriplate containing potato dextrose agar and other 5 mm disc of *Trichoderma* spp. was inoculated at other side of the same petriplate. Petriplate containing potato dextrose agar with *Fusarium* alone served as control. Petriplates were incubated at 28 ± 2°C temperature in inverted position.

After 72 hours, the size of inhibition zones were measured for the difference between the growth of the pathogen and antagonist.

3.8 Statistical analysis

The replicated data was subjected to the computer for statistical analysis using Factorial Randomized Block Design.

RESULTS

Chapter 4

RESULTS

The beneficial effects of inoculation of different *Trichoderma* spp. on various growth parameters and seedling mortality of tomato, chilli and brinjal were studied in the field experiment. The five species of *Trichoderma* were inoculated by two methods with a uninoculated control The results so obtained are presented as below.

4.1 Effect of *Trichoderma* spp. on seedling emergence of tomato, chilli and brinjal

The effect of five species of *Trichoderma* inoculated by two methods, on per cent seedling emergence are recorded and presented in Table 1.

The data presented in Table 1 clearly revealed that the method of inoculation did not have significant impact on emergence of tomato, chilli and brinjal seedlings.

With regard to the different species of *Trichoderma*, significant differences were observed in tomato and brinjal, however, it was non-significant in chilli.

In tomato, highest seedling emergence (82.86 %) was observed in the plots where *T. harzianum* was inoculated which was statistically superior over uninoculated control

Table 1: Effect of *Trichoderma* spp. on seedling emergence of tomato, chilli and brinjal

Treatments	per cent seedling emergence		
	Tomato	Chilli	Brinjal
Method of inoculation			
M_1 - Seed inoculation	80.89 (64.21)	80.35 (63.76)	79.76 (63.38)
M_2 - Soil inoculation	80.48 (63.90)	80.28 (63.77)	77.85 (61.91)
S.E. ±	0.68	0.81	0.60
C.D. at 5 %	N.S.	N.S.	N.S.
Inoculation			
I_0 - No inoculation	75.34 (60.28)	76.37 (60.95)	72.88 (58.69)
I_1 - *T. viride*	82.32 (65.21)	82.02 (64.94)	80.51 (63.83)
I_2 - *T. harzianum*	82.86 (65.69)	82.01 (65.09)	80.60 (63.91)
I_3 - *T. hamatum*	81.00 (64.20)	80.70 (64.12)	80.04 (63.32)
I_4 - *T. lignorum*	80.93 (64.13)	80.18 (63.66)	79.69 (63.25)
I_5 - *T. koningii*	81.70 (64.83)	80.60 (63.84)	79.10 (62.88)
S.E. ±	1.18	1.40	1.03
C.D. at 5 %	3.45	N.S.	3.04
Interaction (M x I)			
S.E. ±	1.67	1.98	1.46
C.D. at 5 %	N S	N S	N S

(Figures in the parentheses indicates arcsin values)
N S - Non significant

(75.34 %). However, this was statistically similar with the emergence (82.32 %) obtained with the inoculation of *T. viride* and other species i.e. *T. koningii* (81.70 %), *T. hamatum* (81.00 %), *T. lignorum* (80.93 %).

In chilli, the inoculation of different species of *Trichoderma* improved the seedling emergence, however, it failed to achieve the level of significance. Highest seedling emergence (82.02 %) was obtained with the inoculation of *T. viride* and *T. harzianum* (82.01 %) followed by *T. hamatum* (80.70 %) and *T. koningii* (80.60 %). *T. lignorum* showed minimum (80.18 %) amongst *Trichoderma* species.

In brinjal, *Trichoderma* inoculations significantly improved the seedling emergence as compared to uninoculated plots. Highest seedling emergence (80.60 %) was noticed with the inoculation of *T. harzianum*. This was followed by the emergence count obtained with *T. viride* (80.51 %), *T. hamatum* (80.04 %) and *T. lignorum* (79.69 %) with least in *T. koningii* (79.10 %).

The interaction effects due to *Trichoderma* spp. with the methods of inoculation were absent indicating no preference of *Trichoderma* spp. to the method of inoculation.

4.2 Effect of *Trichoderma* spp. on per cent seedling mortality of tomato

The effects of different species of *Trichoderma* on percent seedling mortality of tomato were studied and the relevant data so obtained are presented in Table 2.

The data presented in Table 2 clearly indicated that the method of inoculation failed to show any significant influence on seedling mortality of tomato which was monitored from 20 to 45 DAS at a interval of 5 days.

The inoculation of *Trichoderma* spp. brought about significant beneficial effects in seedling mortality of tomato. Significant reduction in seedling mortality was observed with the inoculation of *Trichoderma* spp. compared to uninoculated plots on observations recorded at different intervals except the first two recorded on 20 and 25 DAS. The superiority of *T. harzianum* can clearly be noticed since it has recorded lowest mortality on all the observations recorded at different intervals. Lowest seedling mortality was recorded by *T. harzianum* on 20 DAS (2.08 %), 25 DAS (3.21 %), 30 DAS (4.03 %), 35 DAS (5.26 %), 40 DAS (6.16 %) and 45 DAS (6.84 %). *T. viride* was next to *T. harzianum* in reducing the seedling mortality, however, this too was statistically similar with the mortality observed in the plots inoculated with *T. harzianum*. *T. hamatum* was next to *T. harzianum* and *T. viride* in reducing the seedling mortality.

Table 2: Effect of *Trichoderma* spp. on percent seedling mortality of tomato

Treatments	percent seedling mortality (Days after sowing)					
	20 DAS	25 DAS	30 DAS	35 DAS	40 DAS	45 DAS
Method of inoculation						
M_1 - Seed inoculation	2.46 (7.74)	4.26 (10.79)	5.69 (12.59)	7.09 (14.94)	8.50 (16.47)	9.26 (17.24)
M_2 - Soil inoculation	3.13 (9.98)	4.97 (12.45)	6.55 (14.23)	8.17 (16.12)	9.19 (17.14)	9.93 (17.90)
S.E. ±	0.95	1.02	0.90	0.93	0.88	0.79
C.D. at 5 %	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Inoculation						
I_0 - No inoculation	4.48 (11.99)	8.07 (16.32)	12.05 (20.05)	14.51 (22.11)	16.77 (23.95)	18.52 (25.32)
I_1 - *T. viride*	2.13 (7.58)	3.24 (9.15)	4.28 (11.54)	5.49 (13.22)	6.44 (14.45)	7.11 (15.23)
I_2 - *T. harzianum*	2.08 (7.49)	3.21 (9.17)	4.03 (11.33)	5.26 (13.07)	6.16 (14.17)	6.84 (15.00)
I_3 - *T. hamatum*	2.60 (8.32)	4.14 (11.28)	5.01 (12.55)	6.64 (14.64)	7.57 (15.75)	8.17 (16.37)
I_4 - *T. lignorum*	2.86 (9.38)	4.80 (12.46)	5.85 (13.55)	7.19 (15.31)	8.35 (16.62)	8.62 (16.93)
I_5 - *T. koningii*	2.62 (8.40)	4.25 (11.34)	5.50 (11.43)	6.70 (14.83)	7.78 (15.89)	8.34 (16.55)
S.E. ±	1.64	1.77	1.56	1.62	1.52	1.37
C.D. at 5 %	N.S.	N.S.	4.59	4.76	4.47	4.01
Interaction (M x I)						
S.E. ±	2.33	2.50	2.21	2.30	2.15	1.93
C.D. at 5 %	N S	N S	N S	N S	N S	N S

(Figures in the parentheses indicates arcsin values)
N S - Non significant

T. koningii was least effective among different species of *Trichoderma* in controlling the seedling mortality of tomato.

The interaction effects between method of inoculation and *Trichoderma* spp. were non-significant indicating no preference of *Trichoderma* spp. to the method of inoculation.

4.3 Effect of *Trichoderma* spp. on per cent seedling mortality of chilli

The seedling mortality of chilli was monitored from 20 to 45 DAS at interval of 5 days in plots uninoculated and inoculated with different *Trichoderma* spp. The data so obtained are presented in Table 3.

The data (table 3) on seedling mortality of chilli clearly indicated that the method of inoculation has no effect in influencing the mortality of seedling.

The inoculation of *Trichoderma* spp. however brought about the significant reduction in seedling mortality on 30, 35, 40 and 45 DAS compared to uninoculated control. The superiority of *T. harzianum* can clearly be noticed since it showed lowest mortality at four intervals out of six. *T. viride* showed lowest mortality on 25 and 30 DAS. However, this too was statistically similar with the mortality observed in the plots

Table 3: Effect of *Trichoderma* spp. on per cent seedling mortality of chilli

Treatments	per- cent seedling mortality (Days after sowing)					
	20 DAS	25 DAS	30 DAS	35 DAS	40 DAS	45 DAS
Method of inoculation						
M_1 - Seed inoculation	2.23 (7.12)	3.90 (9.51)	6.34 (13.39)	9.20 (16.79)	11.40 (19.00)	12.45 (19.87)
M_2 - Soil inoculation	2.75 (8.12)	5.22 (12.44)	8.49 (16.49)	11.12 (19.00)	12.67 (20.37)	13.94 (21.45)
S.E. ±	1.21	1.24	1.06	1.03	1.03	1.07
C.D. at 5 %	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Inoculation						
I_0 - No inoculation	4.62 (12.08)	8.76 (16.83)	14.26 (21.75)	19.21 (25.67)	22.68 (28.09)	25.75 (30.11)
I_1 - *T. viride*	1.59 (5.39)	2.71 (8.23)	4.77 (11.98)	7.00 (14.64)	8.44 (16.27)	9.08 (16.97)
I_2 - *T. harzianum*	1.57 (5.86)	3.06 (8.06)	4.89 (12.45)	6.87 (14.77)	8.31 (16.39)	8.64 (16.81)
I_3 - *T. hamatum*	2.22 (6.98)	3.84 (9.95)	6.36 (14.43)	8.83 (17.12)	10.45 (18.75)	11.41 (19.65)
I_4 - *T. lignorum*	2.52 (7.39)	4.47 (11.73)	7.43 (15.59)	9.99 (18.13)	11.65 (19.82)	12.60 (20.74)
I_5 - *T. koningii*	2.43 (8.01)	4.54 (11.05)	6.78 (13.44)	9.08 (17.06)	10.70 (18.82)	11.68 (19.67)
S.E. ±	2.09	2.15	1.84	1.79	1.80	1.85
C.D. at 5 %	N.S.	N.S.	5.40	5.24	5.27	5.44
Interaction (M x I)						
S.E. ±	2.96	3.05	2.60	2.53	2.54	2.62
C.D. at 5 %	N S	N S	N S	N S	N S	N S

(Figures in the parentheses indicates arcsin values)
N S - Non significant

inoculated with *T. harzianum*. Lowest seedling mortality was recorded by *T. harzianum* on 20 DAS (1.57 %), 35 DAS (6.87 %), 40 DAS (8.31 %) and 45 DAS (8.64 %). *T. viride* showed lowest seedling mortality on 25 DAS (2.71 %) and 30 DAS (4.77 %). *T. hamatum* was next to *T. harzianum* and *T. viride* in reducing the seedling mortality. *T. koningii* was least effective amongst different species of *Trichoderma* in controlling the seedling mortality of chilli.

The interaction effects were non-significant indicating no preference of *Trichoderma* spp. to the method of inoculation.

4.4 Effect of *Trichoderma* spp. on per cent seedling mortality of brinjal

The seedling mortality of brinjal was monitored from 20 to 45 DAS at interval of 5 days in plots uninoculated and inoculated with different *Trichoderma* spp. The data so obtained are presented in Table 4.

The data (Table 4) on seedling mortality of brinjal clearly indicated that the method of inoculation had no effect in influencing the mortality of seedlings.

The inoculation of *Trichoderma* spp. however brought about the significant reduction in seedling mortality compared to uninoculated control. The superiority of *T. harzianum* can clearly be noticed since it showed lowest mortality at five intervals out of six.

Table 4: Effect of *Trichoderma* spp. on percent seedling mortality of brinjal

Treatments	Percent seedling mortality (Days after sowing)					
	20 DAS	25 DAS	30 DAS	35 DAS	40 DAS	45 DAS
Method of inoculation						
M_1 - Seed inoculation	1.25 (4.23)	2.26 (6.33)	4.27 (10.92)	6.21 (13.53)	8.37 (16.04)	9.83 (17.57)
M_2 - Soil inoculation	1.92 (5.65)	2.90 (8.57)	4.88 (11.77)	6.88 (14.05)	8.97 (16.78)	10.14 (17.82)
S.E. ±	1.19	1.16	0.94	1.20	1.13	1.13
C.D. at 5 %	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Inoculation						
I_0 - No inoculation	3.27 (9.34)	5.38 (13.08)	9.64 (17.75)	13.75 (21.36)	17.18 (24.09)	19.59 (25.92)
I_1 - *T. viride*	1.04 (4.77)	1.49 (6.05)	2.61 (9.20)	3.80 (11.00)	5.69 (13.47)	6.86 (14.89)
I_2 - *T. harzianum*	0.91 (3.46)	1.49 (5.35)	2.33 (6.54)	3.78 (9.50)	5.86 (13.69)	6.27 (13.88)
I_3 - *T. hamatum*	1.21 (3.18)	2.28 (5.69)	3.74 (10.71)	5.41 (12.84)	6.58 (14.33)	8.41 (16.48)
I_4 - *T. lignorum*	1.62 (5.57)	2.64 (9.15)	5.00 (12.76)	6.77 (14.88)	8.69 (16.83)	9.74 (17.86)
I_5 - *T. koningii*	1.47 (3.34)	2.21 (5.35)	4.13 (11.12)	5.78 (13.17)	8.03 (16.03)	9.04 (17.13)
S.E. ±	2.06	2.01	1.63	2.08	1.97	1.97
C.D. at 5 %	N.S.	N.S.	4.78	6.10	5.76	5.77
Interaction (M x I)						
S.E. ±	2.92	2.84	2.31	2.94	2.78	2.78
C.D. at 5 %	N S	N S	N S	N S	N S	N S

(Figures in the parentheses indicates arcsin values)
N S - Non significant

T. viride showed lowest mortality on 20 and 40 DAS (1.49% and 5.69% respectively), however this too was statistically similar with the mortality observed in the plots inoculated with *T. harzianum*. Lowest seedling mortality was recorded by *T. harzianum* on 20 DAS (0.91 %), 25 DAS (1.49 %), 30 DAS (2.33 %), 35 DAS (3.78 %) and 45 DAS (6.27 %). *T. viride* showed lowest seedling mortality on 25 DAS (1.49 %) and 40 DAS (5.69 %). *T. hamatum* was next to *T. harzianum* and *T. viride* in reducing the seedling mortality. *T. koningii* was least effective amongst different species of *Trichoderma* in controlling the seedling mortality of brinjal.

The interaction effects were non-significant indicating no preference of *Trichoderma* spp. to the method of inoculation.

4.5 Effect of *Trichoderma* spp. on growth of tomato

The effect of inoculation of five species of *Trichoderma* on different growth parameters of tomato was studied in a field experiment. The observations were recorded on 35 DAS and the data so obtained are presented in Table 5.

The data presented in table 5 clearly revealed that the method of inoculation in general do not have any significant impact on different growth parameters except for shoot length and shoot fresh weight, where soil

Table 5: Effect of *Trichoderma* spp. on growth parameter of tomato

Treatments	Root length (cm)	Shoot length (cm)	No.of leaves	Root Wt. (g)		Shoot Wt. (g)	
				Fresh	Dry	Fresh	Dry
Method of inoculation							
M_1 - Seed inoculation	6.88	14.35	21.55	0.254	0.033	2.92	0.32
M_2 - Soil inoculation	6.96	15.29	21.47	0.259	0.035	3.27	0.31
S.E. ±	0.16	0.27	0.38	0.016	0.002	0.11	0.01
C.D. at 5 %	N S.	0.81	N S.	N S	N S	0.34	N S.
Inoculation							
I_0 - No inoculation	6.49	13.50	20.54	0.188	0.024	2.21	0.27
I_1 - *T. viride*	7.24	14.98	22.60	0.264	0.046	3.69	0.36
I_2 - *T. harzianum*	7.25	15.36	22.94	0.334	0.043	3.97	0.36
I_3 - *T. hamatum*	6.69	15.15	21.33	0.250	0.032	3.00	0.32
I_4 - *T. lignorum*	6.86	14.92	20.55	0.252	0.027	2.80	0.29
I_5 - *T. koningii*	6.99	15.03	20.71	0.254	0.030	2.91	0.30
S.E. ±	0.29	0.47	0.67	0.028	0.004	0.20	0.02
C.D. at 5 %	N S	N.S.	N.S.	0.082	0.012	0.59	N S
Interaction (M x I)							
S.E. ±	0.41	0.67	0.95	0.039	0.005	0.28	0.03
C.D. at 5 %	N.S.	N S	N S	N S.	N.S	N S.	N S

N S - Non significant

application of *Trichoderma* has significantly improved the shoot length (15.29 cm) and fresh weight of shoot (3.27 g) compared to the shoot length and fresh weight recorded in the plots where seeds were treated with *Trichoderma*.

With regard to the different species of *Trichoderma*, significant differences occured in root weight and shoot weight of tomato. Root/shoot length, number of leaves and shoot dry weight remain unchanged with different species of *Trichoderma*. Significantly highest (22.94) number of leaves were recorded with *T. harzianum* followed by *T. viride* (22.60), *T. hamatum* (21.33), *T. koningii* (20.71) and *T. lignorum* (20.55).

Significantly highest root fresh weight was observed with *T. harzianum* (0.334 g) and lowest amongst the species, in *T. hamatum* (0.250 g).

The root dry weight of tomato (Table 5) was significantly highest in plots inoculated with *T. viride* (0.046 g) which was at par with the dry weight recorded with *T. harzianum* (0.043 g) and *T. hamatum* (0.032 g). *T. lignorum* recorded lowest (0.027 g) root dry weight amongst the species.

Shoot fresh weight was also influenced significantly by *Trichoderma* spp. *T. harzianum* recorded the highest (3.97 g) and *T. lignorum* the lowest (2.80 g).

All the growth parameters showed the lowest value in uninoculated control irrespective of significant

or non-significant differences. The interaction effects due to *Trichoderma* spp. and their method of inoculation were non-significant.

4.6 Effect of *Trichoderma* spp. on growth of chilli

The effect of *Trichoderma* inoculations on growth of chilli was studied. The observations were recorded at 30 DAS on different growth parameters and data obtained are presented in Table 6.

The data presented in table 6 clearly indicated that the method of inoculation of *Trichoderma* had no influence on different growth parameters of chilli.

With regard to the species of *Trichoderma* their inoculations brought about the enhancement in all the growth parameters however, this increase could not achieve the level of statistical significance except for the root length. All the parameters were better in the plots inoculated with *T. harzianum*. The root length due to *T. harzianum* (5.89 cm) was statistically significant over uninoculated control (4.81 cm) and *T. lignorum* (4.83 cm). It was however, at par with *T. viride* (5.85 cm), *T. hamatum* (5.29 cm) and *T. koningii* (5.09 cm).

The interaction effects due to method of inoculation and *Trichoderma* spp. were non-significant.

Table 6: Effect of *Trichoderma* spp. on growth parameter of chilli

Treatments	Root length (cm)	Shoot length (cm)	No.of leaves	Root Wt. (g)		Shoot Wt. (g)	
				Fresh	Dry	Fresh	Dry
Method of inoculation							
M_1 - Seed inoculation	5.24	16.15	8.27	0.135	0.053	0.670	0.215
M_2 - Soil inoculation	5.35	15.89	8.34	0.129	0.043	0.630	0.205
S.E. ±	0.14	0.30	0.13	0.007	0.004	0.035	0.012
C.D. at 5 %	N.S	N.S	N S.	N S	N.S.	N.S.	N.S.
Inoculation							
I_0 - No inoculation	4.81	15.21	8.16	0.115	0.037	0.572	0.185
I_1 - *T. viride*	5.85	16.49	8.21	0.133	0.052	0.728	0.240
I_2 - *T. harzianum*	5.89	16.66	8.49	0.149	0.056	0.769	0.248
I_3 - *T. hamatum*	5.29	16.53	8.27	0.130	0.046	0.607	0.200
I_4 - *T. lignorum*	4.83	16.16	8.38	0.133	0.050	0.596	0.191
I_5 - *T. koningii*	5.09	15.09	8.32	0.132	0.045	0.630	0.197
S.E. ±	0.25	0.53	0.23	0.012	0.007	0.060	0.021
C.D. at 5 %	0.73	N.S	N.S	N S.	N S	N S	N S
Interaction (M x I)							
S.E. ±	0.35	0.75	0.33	0.018	0.010	0.086	0.031
C.D. at 5 %	N.S.	N.S.	N.S	N S	N.S	N S	N.S.

N.S - Non significant

4.7 Effect of *Trichoderma* spp. on growth of brinjal

The effects of inoculation of different *Trichoderma* spp. on growth of brinjal were studied in a field trial. The observations were recorded 35 DAS on different growth parameters and are presented in Table 7.

The data from Table 7 clearly indicated that the methods of inoculation of *Trichoderma* had no significant difference in helping the plant to grow better. All the parameters showed statistically similar values.

With regard to the species of *Trichoderma* the inoculation brought about improvement in all the growth parameters of brinjal, however, it was not statistically significant except for the shoot length. Amongst the different species of *Trichoderma*, *T. harzianum* proved to be most effective in enhancing the growth of brinjal. It showed highest improvement in all the growth parameters of brinjal. The shoot length was significantly improved by the inoculation of *Trichoderma* spp. *T. harzianum* showed significantly more shoot length (8.87 cm) and was at par with the shoot length observed with *T. viride* (8.27 cm) and *T. hamatum* (8.16 cm), lowest being in uninoculated control (7.20 cm).

The interaction effects due to inoculation methods and *Trichoderma* spp. were non-significant.

Table 7: Effect of *Trichoderma* spp. on growth parameter of brinjal

Treatments	Root length (cm)	Shoot length (cm)	No.of leaves	Root Wt. (g)		Shoot Wt. (g)	
				Fresh	Dry	Fresh	Dry
Method of inoculation							
M_1 - Seed inoculation	5.07	8.11	4.57	0.083	0.027	0.792	0.100
M_2 - Soil inoculation	4.96	8.13	4.57	0.070	0.028	0.778	0.099
S.E. ±	0.12	0.14	0.14	0.005	0.004	0.047	0.008
C.D. at 5 %	N S	N.S	N S	N S	N S.	N S.	N S.
Inoculation							
I_0 - No inoculation	4.65	7.20	4.44	0.070	0.019	0.688	0.087
I_1 - *T. viride*	5.37	8.27	4.66	0.080	0.032	0.845	0.095
I_2 - *T. harzianum*	5.40	8.87	4.72	0.100	0.040	0.856	0.134
I_3 - *T. hamatum*	5.04	8.16	4.61	0.068	0.033	0.783	0.097
I_4 - *T. lignorum*	4.84	8.05	4.49	0.077	0.020	0.786	0.090
I_5 - *T. koningii*	4.79	8.17	4.49	0.062	0.022	0.753	0.094
S.E. ±	0.21	0.25	0.24	0.010	0.007	0.081	0.014
C.D. at 5 %	N S	0.75	N S.	N S	N S.	N S.	N S
Interaction (M x I)							
S.E. ±	0.30	0.36	0.35	0.014	0.010	0.115	0.020
C.D. at 5 %	N S	N S	N S	N S	N S	N S	N S

N S - Non significant

4.8 Effect of *Trichoderma* spp. on chlorophyll content of tomato

The chlorophyll content of tomato seedlings was estimated on 40 DAS in plots uninoculated and inoculated with *Trichoderma* spp. The data obtained are presented in Table 8.

The data from table 8 clearly reveal that method of inoculation of *Trichoderma* has no significant effect on chlorophyll content of tomato seedlings. The estimates of chlorophyll-a, chlorophyll-b and total chlorophyll d not differ significantly with the method of inoculation.

With regard to different species *Trichoderma*, the differences in chlorophyll content were non-significant. However, the inoculations increase the chlorophyll content. *T. viride* showed maximum chlorophyll-a (10.11 mg/g) and total chlorophyll (18.48 mg/g). The maximum chlorophyll-b content (7.95 mg/g) was observed in the seedlings inoculated with *T. hamatum*.

The interaction effects due to method of inoculation and *Trichoderma* spp. were nonsignificant.

Table 8: Effect of *Trichoderma* spp. on chlorophyll content of tomato seedlings

Treatments	Chlorophyll-a (mg/g)	Chlorophyll-b (mg/g)	Total chlorophyll (mg/g)
Method of inoculation			
M_1 - Seed inoculation	8.01	8.82	16.57
M_2 - Soil inoculation	9.40	7.10	16.47
S.E. ±	0.49	0.71	0.60
C.D. at 5 %	N S	N S	N S
Inoculation			
I_0 - No inoculation	6.96	6.08	14.10
I_1 - *T. viride*	10.11	7.51	18.48
I_2 - *T. harzianum*	8.71	6.85	16.33
I_3 - *T. hamatum*	8.44	7.95	16.96
I_4 - *T. lignorum*	8.36	7.28	15.84
I_5 - *T. koningii*	9.64	6.93	17.43
S.E. ±	0.85	1.24	1.05
C.D. at 5 %	N S	N S	N S
Interaction (M x I)			
S.E. ±	1.20	1.76	1.48
C.D. at 5 %	N S	N S	N S

N S - Non significant

4.9 Effect of *Trichoderma* spp. on chlorophyll content of chilli

The effect of *Trichoderma* inoculations on chlorophyll content of chilli seedlings was estimated at 40 DAS. The data on chlorophyll-a, chlorophyll-b and total chlorophyll content are presented in Table 9.

The data (table 9) clearly indicated that method of inoculation did not show any significant effect on chlorophyll content.

The *Trichoderma* spp. don't have significant effect on chlorophyll-a and chlorophyll-b content of chilli seedlings. It however improved the total chlorophyll content significantly. The seedlings inoculated with *T. viride* showed significantly more chlorophyll content (18.75 mg/g) compared to uninoculated control (14.47 mg/g). This was statistically similar with the chlorophyll content obtained with *T. harzianum* (18.35 mg/g), *T. koningii* (17.88 mg/g) and *T. hamatum* (17.10 mg/g). *T. lignorum* showed least effect on improving the chlorophyll content (15.69 mg/g).

The interaction effects between species of *Trichoderma* and method of inoculation were non-significant.

Table 9: Effect of *Trichoderma* spp. on chlorophyll content of chilli seedlings

Treatments	Chlorophyll-a (mg/g)	Chlorophyll-b (mg/g)	Total chlorophyll (mg/g)
Method of inoculation			
M_1 - Seed inoculation	8.35	8.19	16.70
M_2 - Soil inoculation	9.13	8.42	17.38
S.E. ±	0.48	0.56	0.53
C.D. at 5 %	N.S.	N S	N S
Inoculation			
I_0 - No inoculation	7.46	7.03	14.47
I_1 - *T. viride*	8.25	10.96	18.75
I_2 - *T. harzianum*	9.80	7.47	18.35
I_3 - *T. hamatum*	8.60	9.08	17.10
I_4 - *T. lignorum*	8.23	7.48	15.69
I_5 - *T. koningii*	10.10	7.80	17.88
S.E. ±	0.83	0.97	0.92
C.D. at 5 %	N S	N S	2.71
Interaction (M × I)			
S.E. ±	1.18	1.37	1.30
C.D. at 5 %	N S	N S	N S

N S - Non significant

4.10 Effect of *Trichoderma* spp. on chlorophyll content of brinjal

The effect of *Trichoderma* inoculations on chlorophyll content of brinjal was studied in field experiment. The observations were recorded on 40 DAS in respect of chlorophyll-a, chlorophyll-b and total chlorophyll content. The data so obtained are presented in Table 10.

The data from table 10 revealed that the method of inoculation did not have any significant influence on these parameters. The chlorophyll content in both these treatments was statistically similar.

With regard to different species of *Trichoderma*, the differences were non-significant for all the three chlorophyll content. Little improvement in chlorophyll content was observed due to inoculation of *Trichoderma* spp. Maximum chlorophyll-a content was obtained (8.44 mg/g) with the inoculation of *T. hamatum* and *T. koningii* and total chlorophyll (14.25 mg/g) was obtained with the inoculation of *T. hamatum*. With regard to chlorophyll-b content, it was maximum in seedlings inoculated with *T. harzianum* (5.89 mg/g).

The interaction effects due to *Trichoderma* spp. and method of inoculation were non-significant.

Table 10: Effect of *Trichoderma* spp. on chlorophyll content of brinjal seedlings

Treatments	Chlorophyll-a (mg/g)	Chlorophyll-b (mg/g)	Total chlorophyll (mg/g)
Method of inoculation			
M_1 - Seed inoculation	7.83	5.59	13.46
M_2 - Soil inoculation	7.93	5.35	13.25
S.E. ±	0.33	0.45	0.33
C.D. at 5 %	N.S.	N.S.	N.S.
Inoculation			
I_0 - No inoculation	7.22	4.95	12.15
I_1 - *T. viride*	7.96	5.54	13.48
I_2 - *T. harzianum*	7.75	5.89	13.91
I_3 - *T. hamatum*	8.44	5.84	14.25
I_4 - *T. lignorum*	7.47	5.21	12.56
I_5 - *T. koningii*	8.44	5.39	13.79
S.E. ±	0.58	0.79	0.57
C.D. at 5 %	N.S.	N.S	N.S
Interaction (M x I)			
S.E. ±	0.82	1.12	0.81
C.D. at 5 %	N.S.	N.S	N.S

N.S - Non significant

4.11 *In-vitro* inhibition of pathogen by *Trichoderma* spp.

To evaluate the efficacy of *Trichoderma* spp. in inhibiting the growth of *Fusarium*, a laboratory testing was done. The pathogen and the antagonists were inoculated on agar plates at a distance of 45.00 mm apart. The laboratory testing clearly indicated that all the species inhibited the growth of *Fusarium* (Plate 1, 2, 3, 4 and 5).

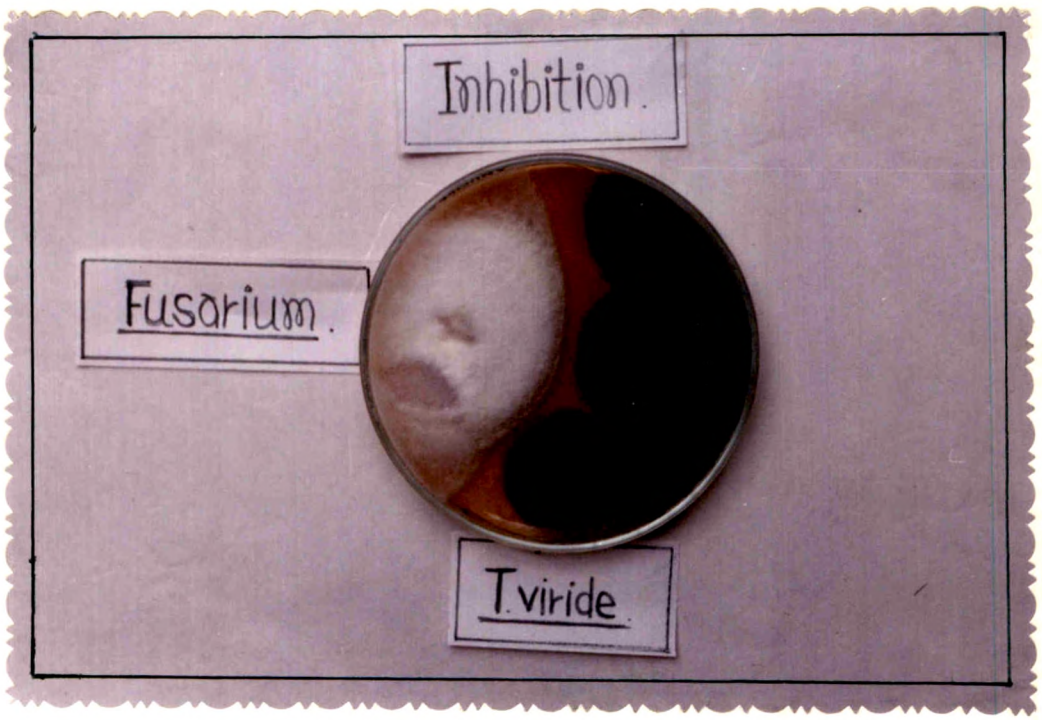
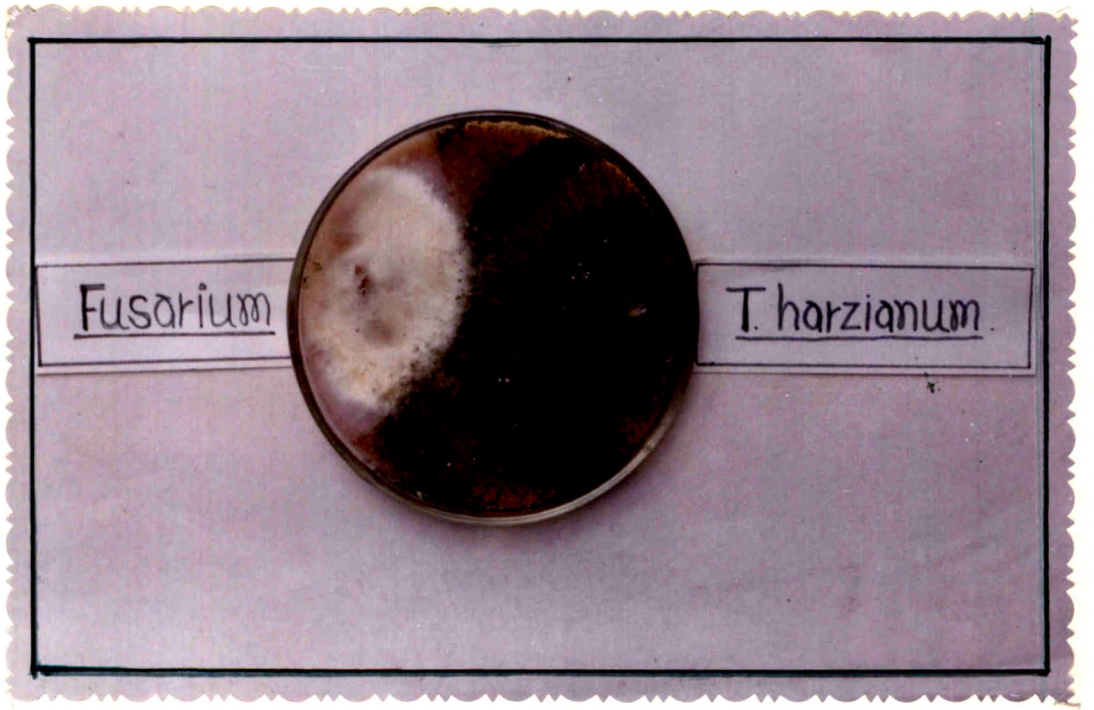


Plate 1: *In-vitro* inhibition of *Fusarium* by *T. viride*



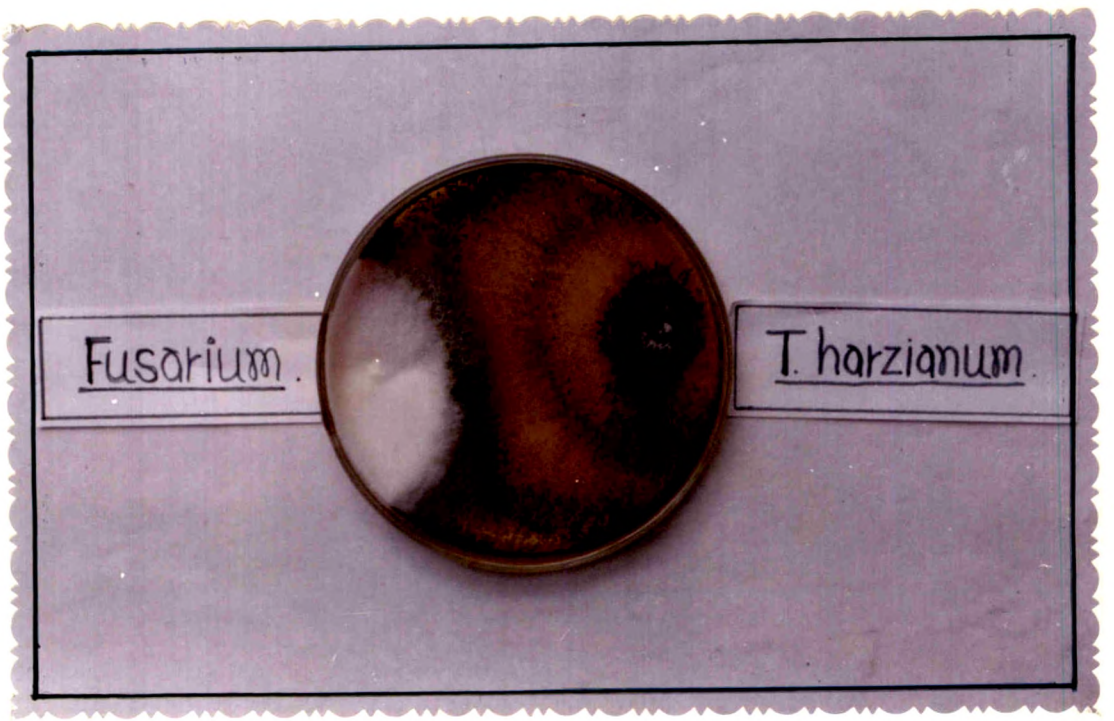
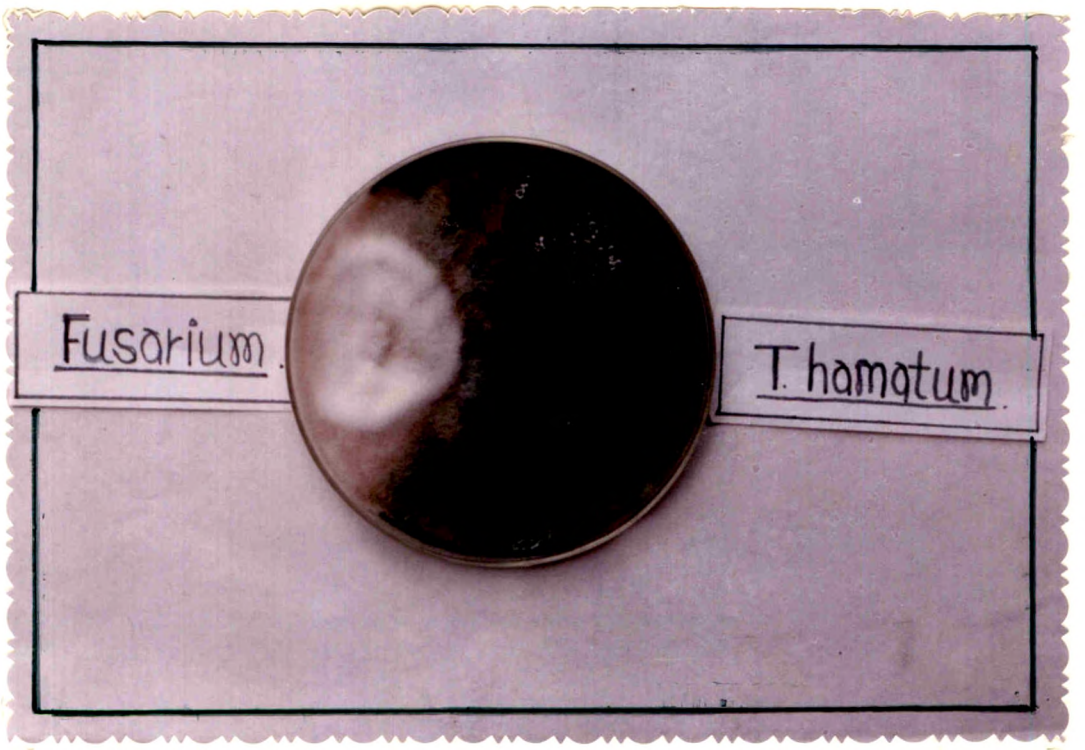


Plate 2: Progresssive overrunning of *Fusarium* colony by *T. harzianum* at a interval of five days



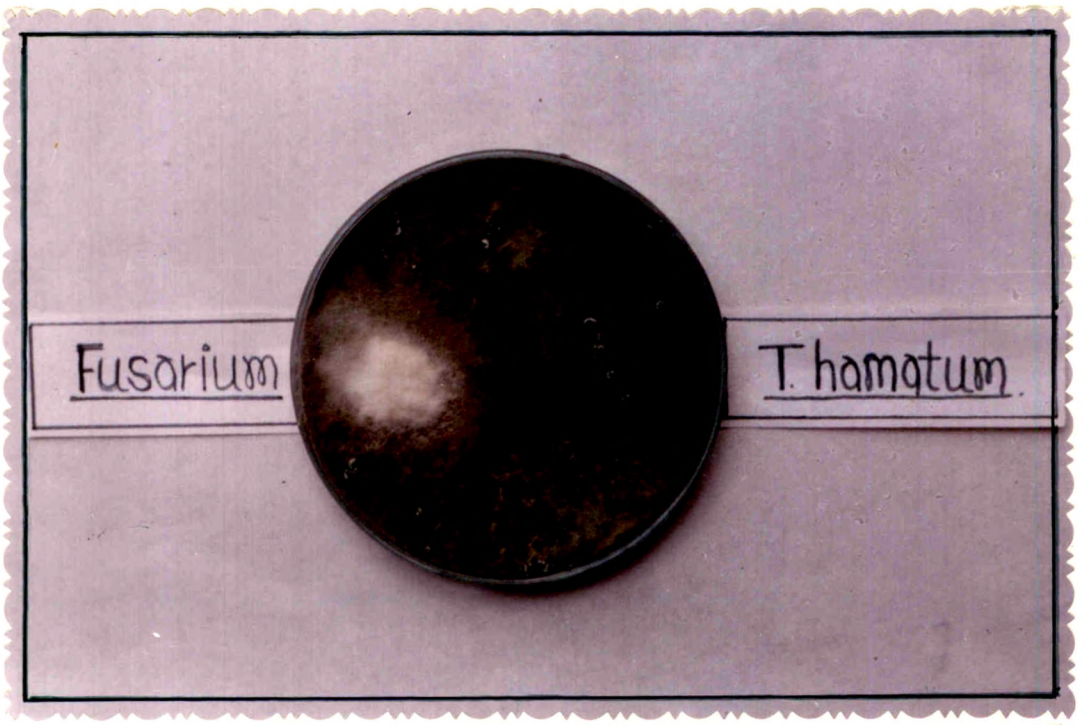


Plate 3: Progresssive overrunning of *Fusarium* colony by *T. hamatum* at a interval of five days

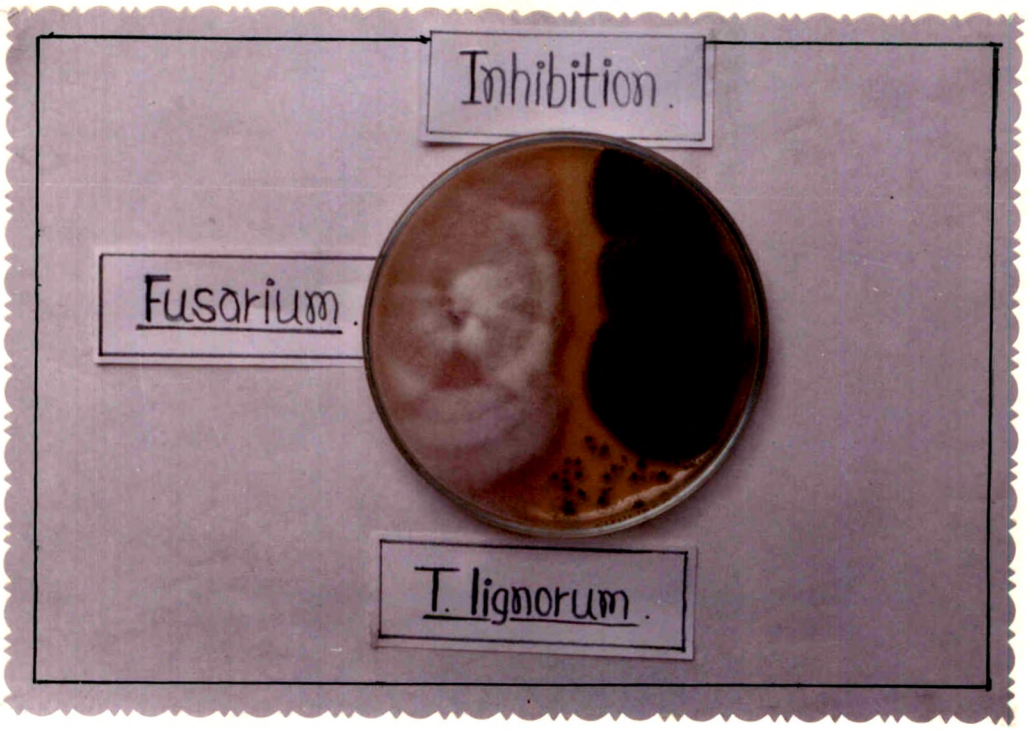


Plate 4: *In-vitro* inhibition of *Fusarium* by *T. lignorum*

Plate 5: *In-vitro* inhibition of *Fusarium* by *T. koningii*

DISCUSSION

Chapter 5

DISCUSSION

The beneficial effects of inoculation of *Trichoderma* spp. on seedling emergence and growth parameter were evaluated in field experiment conducted at Vegetables Research Unit, Marathwada Agricultural University, Parbhani. The *Trichoderma* spp. were inoculated by seed inoculation and soil application. The results obtained were elaborated in previous chapter.

5.1 Effect on seedling emergence

The results obtained with regard to seedling emergence have clearly indicated no significant difference when the inoculations were done either by seed treatment or by soil application. However the critical perusal of the results showed that the emergence was better when *Trichoderma* inoculant was used as seed dresser though it was not supported by statistical analysis.

Sivan *et al.* (1984) reported similar beneficial effects of seed inoculation with *T. harzianum*. He reported an increase in seed germination of cotton with reduction in post-emergence mortality. Monaco *et al.* (1991) reported significant increase in seedling emergence when *Trichoderma* was used for seed treatment. Similar beneficial effects

due to seed treatment of *Trichoderma* spp. were reported by different workers in the past (Xu *et al.*, 1993; Das *et al.*, 1997 and Mehta *et al.*, 1997). Umamaheshwari and Ramkrishnan (1997) reported that seed treatment was the best method of *Trichoderma* application than soil application for effective control of diseases in the field.

The five different species of *Trichoderma* used in the present experimentation has proved to be quite effective in improving the seedling emergence. The results obtained were statistically significant with regard to tomato and brinjal. Though it was not statistically significant in case of chilli, the inoculation has brought about increment in the seedling emergence. In all the three crops, maximum seedling emergence was obtained with the inoculation of *T. harzianum*. It was immediately followed by *T. viride* indicating the superiority of these two species in improving the seedling emergence.

Monaco *et al.* (1991) reported an increase in the seedling emergence with inoculation of *Trichoderma* species. Windham *et al.* (1996) reported similar increase in emergence of tomato and tobacco seedling due to inoculation of *Trichoderma* species. Similar beneficial effects on seed germination and seedling emergence with inoculation of *Trichoderma* spp. has been reported in the past (Shahida and Ghaffar, 1991; Jacqmin *et al.*, 1993; Bari *et al.*, 1997 and Singh *et al.*, 1997).

The results obtained with regard to the seedling emergence when inoculated with *Trichoderma* spp. are in full agreement with those reported in the past. The absence of interaction between the methods of inoculation and *Trichoderma* spp. has clearly indicated that a particular species of *Trichoderma* do not behave differently with the change in method of inoculation.

5.2 Effect on seedling mortality

The seedling mortality of tomato, brinjal and chilli was observed from 20 to 45 DAS in a field experiments. The results clearly indicated that the method of inoculation does not have any impact in reducing the seedling mortality on these three vegetables. The data indicated somewhat better effects of soil inoculations though the differences were not significant.

Krishnamoorthy and Bhaskaran (1990) reported an efficient control of damping off of tomato with the soil application of *Trichoderma* spp. Xu *et al.* (1993) noted t reduction in seedling mortality with the application of *T. harzianum* @ 0.6 per cent (w/w). Gangopadhyay and Joshi (1997) reported the better control of root rot of cotton and chickpea with soil application of *Trichoderma* formulations before sowing. Similar beneficial effects on seedling mortality has been reported by Lodha *et al.* (1997) and Manoranjitham *et al.* (1999) where they reported the

reduction in pre- and post-emergence damping off of tomato and ginger.

The observations of present investigation are fully in agreement with those reported in the past indicating the beneficial effects of *Trichoderma* inoculations on seedling mortality. Nonsignificant interaction effects indicated that *Trichoderma* spp. were equally effective in both the methods of application.

5.3 Effect on growth

The effect of *Trichoderma* inoculations on the growth of tomato, chilli and brinjal seedlings were evaluated in field experiment. The results with regard to the different growth parameters of tomato, chilli and brinjal as influenced by the method of inoculation has clearly indicated that the inoculations done either by seed treatment or by soil application has no influence in promoting the growth of these vegetables. The non-significant results obtained in the present experimentation has aptly cleared this view except for two parameters in tomato which can be taken up as exception. This is probably because of the fact that the inoculum is reaching to the rhizosphere area in both the methods of application where there is maximum microbial activity.

The beneficial effects reported in the past due to seed inoculation (Harman *et al.*, 1980; Sivan *et al.*, 1984; Algarsamy *et al.*, 1987; Xu *et al.*, 1993 and Mehta *et al.*, 1997) and soil inoculation (Krishnmoorthy and Bhaskaran, 1990; Gangopadhyay and Joshi, 1997; Lodha *et al.*, 1997 and Monoranjitham *et al.*, 1999) has made it quite clear that *Trichoderma* spp. were quite effective in controlling the disease and improving the growth parameter in both the methods of application.

The different species of *Trichoderma* behaved differentially to enhance the growth parameters of tomato, chilli and brinjal seedlings. The critical perusal of the results obtained clearly indicated that *T. harzianum* was most effective in improving the growth parameters of these vegetables followed by *T. viride*. On many occasions, this improvement was not able to cross the critical difference to make it significant statistically, however, the improvement was evident. This aptly clear the superiority of *T. harzianum* in enhancing the growth parameter over other species. *T. viride* reported to be quite efficient species in biocontrol was not found better under Marathwada situation where the soil pH ranges between 7.5 to 8.0 possibly unsuitable to these species. This may probably because of the high potency and diversified mechanism of action of these species. Similar differential effects have been reported in the past.

Dennis and Webster (1971) reported the better penetration of *Pythium* by *T. harzianum* than *T. viride*. D'Ercole and Nipoti (1986) reported differential control of *Fusarium* and *Verticillium* infections in tomato by *T. viride*, *T. harzianum* and *T. koningii*. Xu *et al.* (1993) reported differential seedling emergence of cucumber with TH_2 and NF_9 isolates of *T. harzianum*. Flori and Roberti (1993) reported the most effective control of *F. oxysporum* in onion with *T. harzianum* compared to *T. viride*. Mani and Marimuthu (1994) reported that *T. viride* was better in controlling the damping off in chilli as compared to *T. hamatum*.

Dhiman (1997) observed that *T. harzianum* was the most effective in controlling the lettuce drop compared to *T. viride*. Similar differential effect were also observed by Mehta *et al.* (1997), Mondal *et al.* (1997), Mukhopadhyay (1997) and Robert and Saha (1997) in the past.

5.4 Effect on chlorophyll content

Chlorophyll is the most important component involved in the growth and productivity of any crop. The results of present experimentation on chlorophyll content influenced due to *Trichoderma* inoculations as made it aptly clear that the method of inoculation does not have any effect on chlorophyll content of the seedlings of tomato, chilli and brinjal.

The species of *Trichoderma* also do not have any significant effect on influencing the chlorophyll content of the seedling. In all the three crops, the chlorophyll content was enhanced due to the inoculation of *Trichoderma* spp. The superiority of *T. viride* and *T. harzianum* was evidenced in increasing the chlorophyll content of the seedlings. The increased chlorophyll content can be attributed to the increased productivity. Such increased productivity due to different species of *Trichoderma* has been reported in the past (D'Ercole and Nipoti, 1986; Xu *et al.*, 1993; Flori and Roberti, 1993; Dhiman, 1997; Mehta *et al.*, 1997; Mondal *et al.*, 1997; Mukhopadhyay, 1997 and Robert and Saha, 1997).

With the foregoing discussion following conclusions can be drawn.

1. The method of application should ensure that the *Trichoderma* inoculum should reach to the rhizosphere.
2. The increment in growth parameters is because of better root health besides managing the pathogen.
3. *T. harzianum* and *T. viride* used in the present investigation are the best species in managing the soil borne diseases.
4. The species of *Trichoderma* needs to be identified looking into the ecological situation of the area.

SUMMARY

Chapter 6

SUMMARY

The beneficial effects of inoculation of biocontrol agents are well established from the point of view of disease control and increased root health. *Trichoderma* spp. has been established as the most important bicontrol agents for soil borne pathogens. In present study, the beneficial effects of *Trichoderma* inoculation on soil borne diseases has been studied under field conditions in three vegetable crops.

The results of present investigation has clearly proved that inoculated plots gave better seedling emergence compared to uninoculated plots. The two methods of application of *Trichoderma* has indicated that both the methods are equally efficient in improving the seedling emergence. Of the five different species of *Trichoderma* used, *T. harzianum* has been observed to be most effective in promoting the seedling emergence. *T. viride*, another species has also proved to be equally effective in promoting the seedling emergence of all the three vegetables.

The seedling mortality monitored from 20 to 45 DAS was significantly reduced where *Trichoderma* inoculations were followed. With regard to the method of application,

seed treatment or soil application were equally effective in reducing the seedling mortality of tomato, chilli and brinjal. Of the five species of *Trichoderma* use for inoculation, *T. harzianum* was most effective in reducing the seedling mortality of tomato, chilli and brinjal monitored from 20 to 45 DAS. *T. viride* here too was found equally effective in reducing the seedling mortality.

Different growth parameters, as observed on 35th DAS were significantly influenced by the inoculation of the biocontrol agent. Both the methods of application were equally efficient in bringing about the increment in the growth parameters of tomato, chilli and brinjal. Of the five different species of *Trichoderma*, it was *T. harzianum* which brought about the significant increment in different growth parameters of the seedlings. *T. viride* was equally efficient in enhancing the growth of seedlings. *T. lignorum* was least effective in promoting the growth parameters of tomato, chilli and brinjal seedlings.

The results of present investigation made it aptly clear that *T. harzianum* and *T. viride* available in Department of Plant Pathology are quite efficient in managing the soil borne diseases of vegetables in Parbhani situation and thus can be promoted for commercial exploitation of these species for biocontrol of vegetable diseases.

LITERATURE CITED

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Algarsamy, G., Mohan, S. and Jeyarajan, R. (1987). Effect of seed pelleting with antagonists in the management of seedling diseases of cotton. J. Biol. Control. **1**: 66-67.

Askew, D. J. and Laing, M.D. (1993). An adapted selective medium for the quantitative isolation of *Trichoderma* spp. Plant Pathology **42**(5): 686-690.

Backman, P.A. and Rodriguez - Kabana, R. (1975). A system for growth and delivery of biological control agents to the soil. Phytopathology **65**: 819-821.

Baker, K.F. and Cook, R.J. (1974). Biological control of Plant Pathogens. San. Franscisco: Freeman, pp. 433.

Balsubramanian, P., Samiyappan, R., Stephen Jebakumar, R., Krishnamoorthy, J., Nakkeeran, S. and Vidhyasekaran, P. (1997). Purification and characterization of *Trichoderma* chitinase effective agaist the rice sheath blight pathogen, *R. solani*. Abstr. Ind. Phytopathol. Soc. Int. Conf. on Integrated Pl. Disease Management for Sustainable Agriculture Nov., 10-15, New Delhi. pp. 209.

Barcelo, A. Ros (1997). Cellulase from *Trichoderma viride*; an elicitor of disease resistance reactions in grapevines. Abstr. Ind. Phytopathol. Soc. Int. Conf. on Integrated Pl. Disease Management for Sustainable Agriculture Nov., 10-15, New Delhi. pp. 134.

Bari, M.A., Mondal, S.N., Rahman, M.Z. and Ahmad, K.M. (1997). Control of foot and root rot of barley (*Hordeum vulgare* L.) using fungial antagonist. Abstr. Ind. Phytopathol. Soc. Int. Conf. on Integrated Pl. Disease Management for Sustainable Agriculture Nov., 10-15, New Delhi. pp. 31.

Barnet, H.L. and Binder, F.L. (1973). The fungal host-parasite relationship. Annu. Rev. Phytopathol. **11**: 273-292.

Bazgir, E. and Okhovvat, M. (1997). Biological control of *R. solani* by *Trichoderma* spp. and *G. virens*. Abstr. Ind. Phytopathol. Soc. Int. Conf. on Integrated Pl. Disease Management for Sustainable Agriculture Nov., 10-15, New Delhi. pp. 196.

Booth, C. (1971). The genus *Fusarium*. Commonwealth Mycol. Inst. Kew Surrey, U.K. pp. 237.

Caliquet, S. and Scheffer, R.J. (1996). Biological control of damping off caused by *P. ultimum* and *R. solani* using *Trichoderma* spp. applied as industrial film coating on seeds. European J. Pl. Pathol. **102**(3): 247-255.

Calvet, C., Pera, J. and Barea, J.M. (1990). Interaction of *Trichoderma* spp. with *Glomus mosseae* and two wilt pathogenic fungi. Agriculture, Ecosystem and environment **29**(4): 59-65.

Cherif, M. and Benhamou, N. (1990). Cytochemical aspect of chitin breakdown during the parasitic action of a *Trichoderma* spp. on *Fusarium oxysporum f. sp. radicis-lycopersici* Phytopathology **80**(12): 1406-1414.

Chet, I and Baker, R. (1981). Isolation and biocontrol potential of *T. hamatum* from soil naturally suppressive to *R. solani*, Phytopathology **71**: 286-290.

Cho, C.T., Moon, B.J. and Ha, S.Y. (1989). Biological control of *F. oxysporum f. sp. cucumerinum* causing cucumber wilt by *G. virens* and *T. harzianum* Korean J. Pl. Pathol. **5**(3): 239-249.

Choudhury, B. (1979). Role of vegetable crops in meeting food requirements. Commerce Research Bureau, Commerce (Annual Number), 139:73-91.

Cipriano, T. Cirivilleri, G. and Cartia, G. (1989). In-vitro activity of antagonistic microorganism against *F. oxysporumf. sp. radicis - locopersici*, the causal agent of tomato crown root rot. *Attivita in vitro di microogranism antagonist di Fusarium oxysporum f. sp. radicis-lycopersici agente dale merciume basale der Pomodoro Informatore fitopathologica*. **39**(5): 46-48.

Claydon, N, Allan, M., Hanson, J.R. and Avent, A.G. (1987). Antifungal alkyl pyrones of *T. harzianum* Trans. Br. Mycol. Soci. **88**: 503-513.

Cruz, J., De L.A., Pintor-Toro, J.A., Benitez, T. and Llobell, A. (1995). Purification and characterization of an endo-β-1-6-glucanase from *T. harzianum* that is related to its mycoparasitism. J. Bacteriology. **177**(7): 1864-1871.

Das, B.C., Roy, S. and Bora, L.C. (1997). Biological seed treatment with *Trichoderma* spp. for management of damping off of cabbage caused by *R. solani* Kuhn, Abstr. Ind. Phytopathol. Soc. Int. Conf. on Integrated Pl. Disease Management for Sustainable Agriculture Nov., 10-15, New Delhi. pp. 258.

Dennis, C. and Webster, J. (1971). Antagonistic properties of species - groups of *Trichoderma*. Trans. Br. Mycol. Soc. **57**(3): 363-369.

D'Ercole, N. and Nipoti, P. (1986). Biological control of *Fusarium* and *Verticillium* infections in tomatoes under protected cultivation. *Colture Protette*. **15**(3): 55-59.

Dhiman, J.S. (1997). Management of lettuce drop using antoganistic fungi Abstr. Ind. Phytopathol. Soc. Int. Conf. on Integrated Pl. Disease Management for Sustainable Agriculture Nov., 10-15, New Delhi. pp. 201.

Dubey, S.C. (1997). Biological control of web blight of groundnut caused by *Thanetophorus cucumeris*. Abstr. Ind. Phytopathol. Soc. Int. Conf. on Integrated Pl. Disease Management for Sustainable Agriculture Nov., 10-15, New Delhi. pp. 194.

Durrell, L.W. (1968). Hyphal invasion by *T. viride*: Mycopathol. et Mycol. Appl. **35**: 138-144.

Elad, Y., Barak, R., Chet, I and Henis, Y. (1983). Ultrastructural studies of the interaction between *Trichoderma* spp. and Plant Pathogenic fungi. *Phytopathologische, Zeitschrift.* **107**(2): 168-175.

Elad, Y., Chet, I. and Henis, Y. (1981). A selective medium for improving quantitative isolation of *Trichoderma* spp. from soil. Phytoparasitica **9**: 59-67.

Elad, Y. and Chet, I. (1983). Improved selective media for isoltion of *Trichoderma* spp. and *Fusarium* spp. Phytoparasitica. **11**: 55-58.

Elad, Y., Chet, I. and Katan, J. (1980). *Trichoderma harzianum*: A biological agent effective against *S. rolfsii* and *R. solani*. Phytopathology**70**: 119-121.

Elad, Y., Kalfon, A. and Chet, I. (1982). Control of *R. solani* cotton by seed coating with *Trichoderma* spp. spores. Plant Soil. **66**: 279-281.

Elad, Y., Chet, I. and Henis, Y. (1980). A selective medium for isolation and counting of *Trichoderma* sp. from soil. Phytoparsitica. **8**: (In press).

Flori, P. and Roberti, R. (1993). Treatment of onion bulbs with antagonistic fungi for the control of *F. oxysporum f. sp. cepae. Difesa delle piante.* **16**(4): 5-12.

Fravel, D.R. (1988). Role of antibiosis in the biocontrol of plant diseases. Ann. Rev. Phytopathol. **26**: 75-91.

Gaikwad, S.J. and Sangale, U.R. (1998). Use of cheaper available substrate for mass multiplication of bioagents viz. *Trichoderma* spp. Abstr. Ind. Phytopathol. Soc., Fiftieth Ann. Meeting, National Symp. on present scenario in diseases of oilseeds and pulses, Feb., 17-19, held at Dr, B.A.M.U., Aurangabad.

Gaind, S. and Gaur, A.C. (1990). Shelf life of phosphate solubilizing inoculants as influenced by type of carrier, high temperature and low moisture. Can. J. Microbiol. 36: 846-849.

Gandhikumar, N. and Ranganathan, K. (1997a) Biocontrol of *Fusarium* wilt of coriander. Abstr. Ind. Phytopathol. Soc. Int. Conf. on Integrated Pl. Disease Management for Sustainable Agriculture Nov., 10-15, New Delhi. pp. 197.

Gandhikumar, N. and Ranganathan, K. (1997 b) Evaluation of different substrates on the survival of *T. viride* and *T. harzianum*. Abstr. Ind. Phytopathol. Soc. Int. Conf. on Integrated Pl. Disease Management for Sustainable Agriculture Nov., 10-15, New Delhi. pp. 239.

Ganeshan, G., Ravishankar, H. and Bhargava, B.S. (1997). Mass multiplication of *T. harzianum* at ches, chethalli, Coorg. Abstr. Ind. Phytopathol. Soc. Int. Conf. on Integrated Pl. Disease Management for Sustainable Agriculture Nov., 10-15, New Delhi. pp. 203.

Gangopadhyay, S. and Joshi, R.K. (1997). Efficacy of *Trichoderma* in controlling root rot of cotton and chickpea. Abstr. Ind. Phytopathol. Soc. Int. Conf. on Integrated Pl. Disease Management for Sustainable Agriculture Nov., 10-15, New Delhi. pp. 198.

Georgieva, O. (1992). Antagonistic characteristics of *T. koningii* towards *V. dahliae* on pepper. Bulletin. OILB/SROP. 15(1): 18-20.

Ghaffar, A. (1997). Prospects in the biological control of sclerotial fungi. Abstr. Ind. Phytopathol. Soc. Int. Conf. on Integrated Pl. Disease Management for Sustainable Agriculture Nov., 10-15, New Delhi. pp. 49.

Greinstein, A. Elad, Y., Katan, J. and Chet, I. (1979). Control of *S. rolfsii* by means of a herbicide and *T. harzianum*. Pl. Dis. Reptr. 63: 823-826.

Hadar, Y., Chet, I. and Henis, Y. (1979). Biological control of *R. solani* damping off with wheat bran culture of *T. harzianum*. Phytopathology **69**: 64-68.

Harman, G.E., Chet, I. and Baker, R. (1980). *Trichoderma hamatum* effects on seed and seedling diseases induced in radish and pea by *Pythium* spp. or *Rhizoctonia solani*. Phytopathology **70**: 1167-1172.

Haque, S.E. and Ghaffar, A. (1992). Efficacy of *Trichoderma spp.* and *Rhizobium meliloti* in the control of root rot of fenugreek. Pakistan J. Botany. **24**(2): 217-221.

Henis, Y., Ghaffar, A., Baker, R. and Gillespie, S.L. (1978). A new soil sampler and its use for the study of population dynamics of *R. solani* in soil. Phytopathology **68**: 371-376.

Inbar, J., Abramsky, M., Cohen, D. and Chet, I. (1994). Plant growth enhancement and disease control by *T. harzianum* in vegetable seedlings grown under commerical condition. European J. Pl. Pathol. **100**(5): 337-346.

Jackson, R.M. (1985). Antibiosis and fungistasis of soil microorganism. In ecology of soil borne plant pathogen ed. K.F. Baker, W.C. Snyder, pp. 363-369. Berkeley Univ. Calif. Press. pp. 571.

Jacqmin, B., Cotes, A.M., Lepoivre, P. and Semal, J. (1993). Effect of the combination of seed priming and *Trichoderma* treatment on incidence of damping off agents. *Mededelingen Van de facultiet Landbouwwetenschappen Universiteit Gent.* **58**(3b): 1321-1328.

Jee, H.J.and Kim, H.K. (1987). Isolation, identification and antagonism of rhizospheric antagonists to cucumber wilt pathogen, *F. oxysporum f. sp. cucumerinum* Owen. Korean J. Pl. Pathol. **3**(3): 187-197.

Jeyarajan, R., Ramkrishnan, G., Dinkaran, D. and Srider, R. (1994). Development of product of *T. viride* and *B. subtilis* for root rot diseases of pulses and oilseeds. In "Biotechnology in India" (B.K. Dwivedi and G. Pandey Eds.) by Bioved Res. Soc. Allahabad.

Jha, D.K. and Singh, D.K. (1997). Biological control of chickpea wilt. Abstr. Ind. Phytopathol. Soc. Int. Conf. on Integrated Pl. Disease Management for Sustainable Agriculture Nov., 10-15, New Delhi. pp. 194.

Joseph, P.J. and Sivaprasad, P. (1997). Development of antagonistic fungi against rhizome rot pathogen (*P. aphanidermatum* (Edson) Fits) of ginger. Abstr. Ind. Phytopathol. Soc. Int. Conf. on Integrated Pl. Disease Management for Sustainable Agriculture Nov., 10-15, New Delhi. pp. 205.

Karpagavalli, S. and Ramabadran, R. (1995). Effect of *Trichoderma* spp. on damping off disease of tomato caused by *P. aphanidermatum* (Edson) Fitz. J. Biol. Control. 9(1): 59-60.

Khakimov, A.Kh., Abdullaev, B.Ya. (1992). Trichodermin against fusariose of tomato. *Zashchita Rastenil* (Moskva) 8(25): 79-81.

Kim, S.I., Shim, J.O, Shin, H.S., Choi, H.J. and Lee, M.W. (1992). Suppressive mechanism of soil borne disease development and its practical application. Isolation, identification of species of *Trichoderma* antagonstic to soil diseases and its activities in the rhizosphere. Korean J. Mycology. 20(4): 337-346.

Kousalya, G. and Jeyarajan, R. (1990). Mass multiplication of *Trichoderma* spp. J. Biol. Control. 4(1): 70-71.

Krishnamoorthy, A.S. and Bhaskaran, R. (1990). Biological control of damping off disease of tomato caused by *P. indicum*. Balkrishan. J. Biol. control. 4(1): 52-54.

Lederer, W., Lorenz, K.H., Seemuller, E. (1992). Studies on antagonistic effects of *Trichoderma* isoaltes against *Phytophthora cactorum* J. Phytopathol. **136**(2): 154-164.

Lodha, B.C., Mathur, K. and Ram. B. (1997). Integrated management of rhizome rot of ginger. Abstr. Ind. Phytopathol. Soc. Int. Conf. on Integrated Pl. Disease Management for Sustainable Agriculture Nov., 10-15, New Delhi. pp. 29.

Mani, M.T. and Marimuthu, T. (1994). Effect of decomposed coconut coirpith, fungicide and biocontrol agent on damping off of chillies. Indian J. Mycol. Plant Path. **24**(1): 20-23.

Manoranjitham, S.K., Prakasam, V., Rajappan, K. and Amutha, G. (1999). Effect of antagonist on damping off disease of tomato (Unpublished).

Mayee, C.D. and Asghari, M.R. (1997). Biological control of *S. rolfsii* Sacc by *T. harzianum* Rifai in groundnut. Abstr. Ind. Phytopathol. Soc. Int. Conf. on Integrated Pl. Disease Management for Sustainable Agriculture Nov., 10-15, New Delhi. pp. 258.

Mehta, R.D., Patel, S.J., Johnson, V., Patel, K.A., Varshney, A.K., Shah, J.C. and Mehta, M.H. (1997). Plant growth promotion by *T. harzianum*. Abstr. Ind. Phytopathol. Soc. Int. Conf. on Integrated Pl. Disease Management for Sustainable Agriculture Nov., 10-15, New Delhi. pp. 249.

Mehta, R.D., Patel, S.J., Johnson, V., Patel, K.A., Varshney, A.K., Shah, J.C. and Mehta, M.H. (1997). Biological control of *Fusarium* wilt by *T. harzianum* and *T. viride*. Abstr. Ind. Phytopathol. Soc. Int. Conf. on Integrated Pl. Disease Management for Sustainable Agriculture Nov., 10-15, New Delhi. pp. 249.

Monaco, C., Perello, A., Alippi, H.E. and Pasquare, A.O. (1991). *Trichoderma* spp: a biocontrol agent of *Fusarium* spp. and *Sclerotium rolfsii* by seed treatment. Advance in Hort. Sci. 5(3): 92-95.

Mondal, S.N., Ali, M.S., Yasmin, K. and Ahmed, K.M. (1997). Biological control of damping off of cauliflower seedling. Abstr. Ind. Phytopathol. Soc. Int. Conf. on Integrated Pl. Disease Management for Sustainable Agriculture Nov., 10-15, New Delhi. pp. 196.

Mukherjee, P.K., Upadhyay, J.P. and Mukhopadhyay, A.N. (1989). Biological control of *Pythium* damping off of Cauliflower by *T. harzianum*. J. Biol. Control. 3(2): 119-124.

Mukhopadhyay, A.N. (1987). Biological control of soil borne plant pathogens by *Trichoderma* spp. Indian J. Mycol.Plant Path . 17: 1-10.

Mukhopadhyay, A.N. (1997). Biological managemnet of soil borne plant diseases- A reality or Myth. Abstr. Ind. Phytopathol. Soc. Int. Conf. on Integrated Pl. Disease Management for Sustainable Agriculture Nov., 10-15, New Delhi. pp. 46.

Naik, M.K., Singh, S.J. and Sinha, P. (1997). Mechanism of biological control of wilt of cilli caused by *F. oxysporum f. sp capsici*. Abstr. Ind. Phytopathol. Soc. Int. Conf. on Integrated Pl. Disease Management for Sustainable Agriculture Nov., 10-15, New Delhi. pp. 253.

Nakkeeren, S.P., Shankar and Jayrajan, R. (1997). Standardization of storage conditions to increase the shelf life of *Trichoderma* formulations. J. Mycol.Plant Path . 27(1): 60-63.

Nallathambi, P., Mohanraj, D. and Rajendra Prasad, S. (1997). Biological control of seedling rot of sugarcane by using *Trichoderma* and *P. flourescens*. Abstr. Ind. Phytopathol. Soc. Int. Conf. on Integrated Pl. Disease Management for Sustainable Agriculture Nov., 10-15, New Delhi. pp. 193.

Padmanabhan, P. and Alexander, K.C. (1983). Seedling root rot of sugarcane. Extn. Publ. 5(1983). Sugarcane Breeding Institute, Coimbtore.

Padmodaya, B. and Reddy, H.R. (1996). Screening of *Trichoderma* spp. against *F. oxysporum f. sp. lycopersici* causing wilt in tomato. Ind. J. Mycol. Plant Path. **26**(3): 266-270.

Papavizas, G.C., Dunn, M.T., Lewis, J.A. and Beagle-Ristaino, J. (1984). Liquid fermantation technology for experimental production of biocontrol fungi. Phytopathol. **74**: 1174-1175.

Papavizas, G.C. (1985). *Trichoderma* and *Gliocladium*: Biology, Ecology and Potential for biocontrol. Ann. Rev. Phytopathol. **23**: 23-54.

Phookan, A.K. and Chaliha, K. (1997). Biological control of collor rot of brinjal caused by *S. sclerotiorum*. Abstr. Ind. Phytopathol. Soc. Int. Conf. on Integrated Pl. Disease Management for Sustainable Agriculture Nov., 10-15, New Delhi. pp. 259.

Prasad, R.D., Rangeshwaran, R. and Sreeramakumar, P. (1997). Mass production of *T. harizanum* and its shelf life in different formulation. Abstr. Ind. Phytopathol. Soc. Int. Conf. on Integrated Pl. Disease Management for Sustainable Agriculture Nov., 10-15, New Delhi. pp. 212.

Raguchander, T., Rajappan, K. and Prabaker, K. (1995). Evulation of talc based product *T. viride* for the control of black gram root rot. J. Biol. Control **9**(1): 63-64.

Ramakrishnan, G., Jeyaranaj, R. and Dinkaran, D. (1994). Talc based formulation of *T. viride* for biocontrol of *M. phaseolina* J. Biol. Control **8**: 44.

Ranganathan, K., Sridar, R. and Jeyarajan, R. (1995). Evaluation of gypsum as carrier in the formulation of *T. viride* J. Biol. Control **9**(1): 61-62.

Rathore, V.R.S., Mathur, K. and Lodha, B.C. (1992). Activity of volatile and non-volatile substances produced by *T. viride* on ginger rhizome rot pathogen. Indian Phytopath . **45**:(2): 253-254.

Rattink, H. (1993). Biological control of *Fusarium* crown and root rot of tomato on a reciculation substrate system. *Mededelingen Van de faculteit Landbouwwetenscheappen Universiteit Gent.* **58**(3b): 1329-1336.

Robert, C.B. and Saha, L.R. (1997). Biological control of collor rot in pigeonpea with *Trichoderma*. Abstr. Ind. Phytopathol. Soc. Int. Conf. on Integrated Pl. Disease Management for Sustainable Agriculture Nov., 10-15, New Delhi. pp. 211.

Sariah M. (1991). Hyphal interaction of *Trichoderma* spp. and *S. rolfsii*, the causal pathogen of foot rot of chilli. *Pertanika* **14**(2): 167-169.

Sawant, I.S. and Sawant, S.D. (1989). Coffee fruit skin and cherry husk as substrats for mass multiplication of *T. harzianum* as antagonist of citrus *phytophthora*. Indian Phytopath . **42**: 336.

Scarselletti, R. and Faull, J.L. (1994). In vitro activity of 6-pentyl-α-pyrone, a metabolite and *T. harzianum* in the inhibition of *R. solani* and *F. oxysporum f.sp. lycopersici*. Mycolgical Research. **98**(10): 1207-1209.

Selvarajan, R. and Jeyrajan, R. (1996). Inhibition of chickpea root rot pathogens, *F. solani* and *M. phaseolina* by antagonists. Indian J. Mycol. Plant Path . **26**(3): 248-251.

Shahida, P. and Ghaffar, A. (1991). Effect of microbial antaganists in the control of root rot of tomato. Pakistan J. Botany **23**(2): 179-182.

Shahida, P. and Haque, E.S. (1994). Biological control of soil borne root infecting fungi in tomato and okra. Pakistan J. Botany **26**(1): 181-186.

Singh, B., Mane, S.S. and Pal, M. (1997). Management of chickpea wilt. Abstr. Ind. Phytopathol. Soc. Int. Conf. on Integrated Pl. Disease Management for Sustainable Agriculture Nov., 10-15, New Delhi. pp. 283.

Singh, D. and Singh, A. (1997). Biocontrol of *Sclerotium rolfsii* by *Trichoderma* spp. in brinjal. Abstr. Ind. Phytopathol. Soc. Int. Conf. on Integrated Pl. Disease Management for Sustainable Agriculture Nov., 10-15, New Delhi. pp. 195.

Singh, J. and Singh, R.S. (1993). Effect of *T. reesei* on mycelial and sclerotial characters of *R. solani* causing black scurf of potato. Bioved **4**(1): 69-72.

Singh, K. (1991). Survey in Indian Agriculture, The Hindu. pp. 192.

Singh, R.S., Singh, S.V. and Singh, Y. (1997). Efficacy of *Trichoderma* spp. against *R. solani* and selection of substrate for their mass production. Abstr. Ind. Phytopathol. Soc. Int. Conf. on Integrated Pl. Disease Management for Sustainable Agriculture Nov., 10-15, New Delhi. pp. 247.

Sivan, A. and Chet, I. (1986). Possible machanism for control of *Fusarium* spp. by *T. harzianum*. British Crop Protection Conference Pest and Diseases **2**: 865-872.

Sivan, A. and Chet, I. (1993). Integrated control of *Fusarium* crown and root rot of tomato with *T. harzianum* in combination with methyl bromide or soil solarization. Crop Protection **12**(5): 380-386.

Sivan, A., Elad, Y. and Chet, I. (1984). Biological control effects of a new isolate of *T. harzianum* on *P. aphanidermatum*. Phytopathology **74**(4): 498-501.

Sivan, A., Ucko, O. and Chet, I. (1987). Biological control of *Fusarium* crown rot of tomato by *T. harzianum* under field conditions. Plant Dis . **71**(7): 587-592.

Umamaheshwari, C. and Ramakrishnan, G. (1997). Factors fevouring the growth and multiplication of *T. viride*. Abstr. Ind. Phytopathol. Soc. Int. Conf. on Integrated Pl. Disease Management for Sustainable Agriculture Nov., 10-15, New Delhi. pp. 238.

Upadhyay, J.P. and Mukhopadhyay, A.N. (1983). Effect of non-volatile and volatile antibiotics of *T. harzianum* of the growth of *S. rolfsii*. Indian J. Mycol. Plant Path . 13: 232-233.

Upadhyay, J.P. and Mukhopadhyay, A.N. (1986). Biological control of *S. rolfsii* by *T. harzianum*. Tropical Pest Management **32**: 215-220.

Weindling, R. (1932). *Trichoderma lignorum* on a parasite of other soil fungi. Phytopathol. **22**(10): 837-845.

Weindling, R. (1934). Studies on lethal principle effective in the parasitic action of *T. lignorum* on *R. solani* and other soil fungi. Phytopathology **24**: 1153-1179.

Wilson, M., Crawford, E.K. and Campbell, R. (1988). Biological control by *T. harzianum* of damping off of lettuce caused by *R. solani*. Bulletin OEPP. **18**(1): 83-89.

Windham, M.T., Elad, Y. and Baker, R. (1986). A mechanism for increased plant growth induced by *Trichoderma* spp. Phytopathology **76**(5): 518-521.

Wokocha, R.C., Ebenebe, A.C. and Erinle, I.D. (1986). Biological control of the basal stem rot disease of tomato caused by *Corticium rolfssi* (Sacc) Curzi in Northern Nigeria. Tropical Pest Management **32**(1): 35-39.

Xu, T., Zhong, J.P. and Li, D.B. (1993). Antagonism of *Trichoderma harzianum* T_{82} and *Trichoderma* sp. NF_9 against soil borne fungual pathogens. *Acta Phytopathologica Sinica* **23**(1): 63-67.

Xue Baodi, Li J. and Chen Y. (1995). Studies on antagonism of *Trichoderma* spp. against six pathogenic fungi and biological control. J. Nanjing Agril. Univ. **18**(1): 31-36.

Yesh'a, A.H., El-Hassan, S.A. and Ismall, F.K. (1981). Studies on damping off disease of tomato seedlings and its biological control. Mesopot. J. Agric. **11**: 115-124.

Zhong, J.P., Chen, J.H., Xu, T. and Li, D.B. (1990). Hyperparasitism of *Trichoderma* sp. on *R. solani*. *Acta Agriculturae Universitatis Zhejiangensis* **16**(suppl 2): 73-77.