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Tolerance of *Trichoderma, Gliocladium* and *Sclerotium* to Agrochemicals and biocontrol potency of *Trichoderma and Gliocladium*

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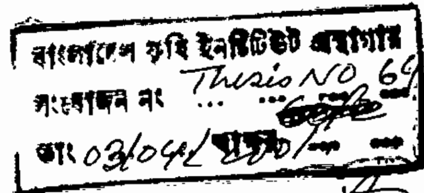

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Semester : July - December, 2000

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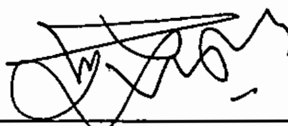

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December, 2000

ALLAH
the Benevolent
the most Merciful

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ABSTRACT

In-vitro test reveled that *Trichoderma harzianum* could tolerate Vitavax, Tilt 250EC, Ridomil and Thiovit at 1000µg/ml, while *Gliocladium viride* could grow better on PDA containing Ridomil and Thiovit. *Trichoderma harzianum* and *Gliocladium viride* could not tolerate even 100µg/ml of Bavistin. On the other hand, *Sclerotium rolfsii* could not tolerate Vitavax, Tilt 250EC and Ridomil even at 100µg/ml, but Bavistin and Thiovit reduced the growth of *Sclerotium rolfsii* by 86.18% and 75.64%, respectively at 1000µg/ml and 100µg/ml. Out of the nutrient elements tested, N, K, S and Mn reduced the growth of *Trichoderma harzianum* while slight induction in growth was recorded by P and Cu. Except P, the tested metal salts reduced the growth of *Gliocladium viride*, where higher concentration of metal salts in medium resulted higher inhibition of growth. Nutrients showed inhibition of growth of *Sclerotium rolfsii* at increasing the conc. in medium. The antagonists were found potential against *Sclerotium rolfsii*. Seed treatment with antagonists resulted upto 21.61%, 53.5%, 26.64% and 48.43% increase in germination in mungbean, blackgram, pigeon pea and tomato, respectively and showed good effect on seed borne mycoflora. Moreover, significant growth enhancement of mungbean, blackgram and tomato have been achieved by treating seeds with antagonists.

CHAPTER 1

INTRODUCTION

বাংলাদেশ কৃষি ইনস্টিটিউট গ্রন্থাগার
সংযোজন নং ৩৭৩৭৭
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1. INTRODUCTION

Pulses and vegetables constitute an important part of our daily diet next to cereals. Pulses are the cheap protein source in comparison with high cost animal protein. It is a direct source of protein of our daily life and also for animals (Satter *et al.*, 1996). Moreover, legumes have the remarkable quality of helping the symbiotic relationship with *Rhizobia* to fix atmospheric nitrogen and improving soil fertility. The important food legumes are Mungbean (*Vinga radiata*), Blackgram (*Vigna mungo*), Lentil (*Lens culinaris*), Chickpea (*Cicer arietinum*), Pigeon pea (*Cajanus cajan),* Pea (*Pisum sativum*), Kheshari (*Lathyrus sativus*), soyabean (*Glycine max*) and Groundnut (*Arachis hypogaea*). Among the vegetables, Tomato (*Lycopersicon esculentum*) is the most nutritive and lucrative one that supplies considerable amount of nutrition including vitamins and minerals.

In Bangladesh the yield of pulses is lower (0.8t/ha; BBS, 2000). This low yield may be due to various reasons, where. disease is one of the most vital factors that attribute significantly to lower the yield of pulses. Several phytopathogenic especially, soil borne mycoflora are responsible for

development of diseases in our economically important crops resulting in both quantitative and qualitative yield reductions.

Sclerotium rolfsii Sacc. is a soil borne pathogen with wide host range and almost have world-wide distribution. It attacks the plants in almost all growth stages (seedling to maturity) and is known to be more devastating in the seedling stage (Bag and Sinha, 1997). Foot and root rot of pulses caused by *Sclerotium rolfsii* is one of the cogent diseases that causes substantial yield losses (Dey et al., 1993). Germination failure, pre and post emergence death, wilting, seedling blight, southern blight of tomato are common damages caused by *Sclerotium rolfsii* (Singh, 1987; Borines and Ranchez, 1996).

The pathogen, *S. rolfsii* is difficult to combat by chemical means as it is soil borne (Bag and Sinha, 1997). In addition, unwise use of chemicals in agriculture causes environmental pollution, health hazards, destroying the natural balance and beneficial microflora of the soil.

Trichoderma harzianum has been found as an effective biocontrol agent of soil-borne plant pathogenic fungi such as *Fusarium, Sclerotium, Rhizoctonia* etc. (Chet and Inbar, 1994). The antagonism of *Trichoderma* to *S. rolfsii* and

suppression of growth by 63.6% has been reported by Iqbal *et al.* (1995), Begum and Hossain (1998). Biological seed treatment in tomato, potato, chickpea, lentil and peanut with *Trichoderma harzianum* and *Gliocladium virens* resulted in excellent protection against a wide range of pathogens including *Sclerotium rolfsii* and the treatments were consistently as effective as or better than fungicidal seed treatment (Mukhopadhyay, 1989). *Trichoderma* killed the sclerotia of *Sclerotium rolfsii* (Begum and Hossain, 1998; Suseelendra and Schlosser, 1999). Addition of *T. harzianum* to pathogen infested soil, it gave 45% control of foot and root rot disease of soybean and yield increase from 1.5g to 1.8g/plant (Deb and Dutt, 1991). However, in Bangladesh, least effort has been made to control foot and root rot of pulses by using antagonists like *Trichoderma* and *Gliocladium*. Research on using *Trichoderma* has been carried out at preliminary stage for controlling foot rot of pulses in Bangladesh (Begum and Hossain, 1998; Sultana, 1999). But no research has been carried out on the tolerance of biocontrol agents to agrochemicals. Papavizas (1985) proposed that biological approach can be successful only if antagonists are compatible with fungicides and biopesticides. So, it is an essential part of research in the discipline of biological control that there must be a study on compatibility of biocontrol agents with the agrochemicals used in agriculture. The successful

use of biocontrol agents completely depends on their fitness in nature where huge number of agrochemicals are being used now a days. Considering the above facts, the present study was, therefore, undertaken with the following objectives:

A. To asses the tolerance of *Trichoderma harzianum*, *Gliocladium viride* and *Sclerotium rolfsii* to different agrochemicals

B. To evaluate the antagonistic activity of *Trichoderma harzianum, Trichoderma hamatum, Trichoderma viride*, and *Gliocladium viride* against *Sclerotium rolfsii* and seed borne mycoflora of Pulses (Mungbean, Blackgram and Pigeon pea) and Tomato.

C. To determine the effect of *Trichoderma* species on germination and seedling vigor of Tomato.

CHAPTER 2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Bio-control agents or antagonists like *Trichoderma, Gliocladium* along with prudent use of different chemical fertilizers is of great interest to the researchers as an effective alternative to conventional chemical fungicide based disease management approach. Pulses and vegetables are subjected to be attacked by different soil-borne pathogens of which *Sclerotium rolfsii* is a devastating pathogen. Relevant literature of the present study has been compiled and presented below.

2.1 *Trichoderma* and *Gliocladium* as effective antagonist of *Sclerotium rolfsii*

Trichoderma harzianum has been isolated by Wells *et al.* (1972) and they found it as pathogenic to *S. rolfsii* in agar culture. They reported that *Trichoderma harzianum* effectively controlled *S. rolfsii* on peanuts, tomatoes and blue lupins under greenhouse condition. Moreover, *Trichoderma harzianum* has been found as highly effective in reducing *S. rolfsii* damage to tomato transplants under field condition when applied (1-3 times) over the plants on to the soil surfaces.

Agrawal *et al.* (1977) found *Trichoderma harzianum* as antagonistic against *Sclerotium rolfsii*. They reported that filtrates of *Trichoderma* inhibited the

growth of *S. rolfsii* on PDA. In pot trial the antagonist controlled seedling death. Culture was more effective when applied to seed rather than soil.

Arora and Dwivedi (1979) found that *Trichoderma harzianum* markedly depressed the growth of *S. rolfsii* causal organism of root disease of *Lens esculenta* Moench on agar.

Elad *et al.* (1980) reported that soil inoculation with *Trichoderma harzianum* reduced *S. rolfsii* and yield of bean was increased by 20%.

Elad *et al.* (1982) reported that *Trichoderma harzianum* excreted β-1, 3-glucanase and chitinase that showed high antagonistic activity against soil borne pathogen, especially *Sclerotium rolfsii.*

Henis *et al.* (1982) reported that *Trichoderma* produced volatile and non-volatile antibiotics that are active against *Sclerotium rolfsii* and also inhibited the sclerotial germination.

Elad *et al.* (1983) studied the parasitism of *Trichoderma harzianum* to the soil borne plant pathogen, *Sclerotium rolfsii.* They observed that hyphae of the parasites contact with their host, either producing appressorium like bodies or coiling around the hyphae, then enzymatically digest host cell walls. Extracellular fibrillar material deposited between the interacting cells.

Parasite organells (mitochondria, vesicles and dark osmiophilic inclusions) accumulate in the parasitizing cells. In response to invasion, the host produces a sheath matrix, which encapsulates the penetrating hyphase and the host cells become empty of cytoplasm.

Henis *et al.* (1983) conducted an experiment on the penetration of sclerotia of *Sclerotium rolfsii* by *Trichoderma.* They immersed sclerotia in an aqueous conidial suspension of *Trichoderma* spp. (1.5×10^7 conidia/ml) and incubated on water agar or on soil. *Trichoderma* hyphae penetrated the rind and cortex and lysed. Degraded sclerotia became dark, soft and empty, and disintegrated under slight pressure. The penetration of sclerotia of *S. rolfsii* by *Trichoderma* followed by lysis differed among the isolates of *Trichoderma.*

Upadhyay and Mukhopadhyay (1983) found that culture filtrate of three out of four isolates of *Trichoderma harzianum* significantly reduced the growth of *Sclerotium rolfsii*, when *S. rolfsii* was exposed to the vapour produced by the four isolates of which two were inhibitory and two stimulatory.

Ferrata and D'Ambra (1985) observed that *Trichoderma harzianum* isolate showed low ability in coiling round hyphae of *Sclerotium rolfsii*, but was very effective in penetrating or growing inside them. Hyphae of *Trichoderma harzianum* adversely affected them even without penetration.

Jacobs and Kamoen (1986) found that *Trichoderma harzianum* produced cell wall lysing enzymes which antagonism against plant pathogens and improve biological control.

Mutto *et al.* (1986) reported that hyphae of *Trichoderma harzianum* developed in the medulla of *Sclerotium rolfsii* sclerotia, growing on the inside of the cell walls and in the cell lumen, the cytoplasm of penetrated cells rapidly degenerated. The hyperparasite passed from cell by lytic perforation of the walls. Germ tubes of medullar cells were also parasitized, with wall lysis and digestion of cell cytoplasm.

Upadhyay and Mukhopadhyay (1986) reported that *Trichoderma harzianum* directly attacked and lysed the mycelium and sclerotia of *Sclerotium rolfsii* in dual culture. Hyphal coiling, entry through haustorial-like structures and direct entry into the hyphae and sclerotia of *S. rolfsii* were observed.

Harman *et al.* (1989) reported on combining effective strains of *Trichoderma harzianum* and solid matrix priming to improve biological seed treatments. They develop progeny strains (T12 and T95) by fusing two strains of *Trichoderma harzianum* and 2 of which were selected for further study. Seeds of cotton, cucumber, pea, snap bean (*phaseolus vulgaris*), maize and wheat were planted in soil infested with *Pythium ultimum*. Wheat seeds were also planted in soil containing *Fusarium graminearum*

[*Gibberella zeae*], cucumber and *P. vulgaris* seeds in soil containing *Sclerotium* [*Corticium*] *rolfsii*, and radish and cucumber seeds in soil infested with *Rhizoctonia solani.* In all crop pathogen combinations, seed treatment with parental and progeny *Trichoderma* strains with or wiothout solid matrix priming increased stands relative to the untrated control and were as effective as Vitavax 200 (carboxin+thiram) on wheat and thiram on other crops. In two field trials with strain 12T increased plant stand, reduced seedling mortality and increased plant growth relative to no treatment. The increased plant growth was evident for the entire duration (98 d) of the longest trial.

Ordentlich and Chet (1989) conducted an experiment in greenhouse and found that *Trichoderma harzianum* obtained from field soils were effective for control of diseases of various crop (caused by *Sclerotium* [*Corticum*] *rolfsii, Rhizoctonia solani*, *Pythium* and *Fusarium*) when grown in a semi-solid fermentation medium on wheat bran : peat.

Kumar and Khare (1990) found *Trichoderma harzianum* as antagonistic to *Sclerotium rolfsii* when soyabean seeds were treated and were sown.

Lim and Teh (1990) reported that isolates of *Trichoderma harzianum* inhibited the growth of *Sclerotium rolfsii* upto 67% in dual culture on malt agar and upto 100% using a cellophane overlay technique at 20 ± 1.5^{0}C.

Growth of the test organisms was inhibited by the production both of diffusable and volatile metabolites and various hyphal interactions were observed : hyphal coilings, appressoria and hooks were produced by the *Trichoderma* and host cells exhibited vacuolations, granulation, coagulation, disintegration and lysis.

Monaco *et al.* (1991) used *Trichoderma* for treating seeds as biocontrol agents of *Fusarium* and *Sclerotium*. They isolated *Trichoderma harzianum, Trichoderma koningii* and *Trichoderma aureoviride* from tomato fields in the horticultural area of La Plata, Argentina, naturally infected with *Fusarium* spp. and *Sclerotium* [*Corticium*] *rolfsii*. All 3 species of *Trichoderma* were effective against *Fusarium* spp. and *Croticum rolfsii,* in vitro. In subsequent field trials seedling emergence was significantly increased when *Trichoderma* spp. were applied to seeds sown in soil infected with the pathogens. They also reported that each *Trichoderma* sp. was effective against *F. oxysporum,* whilst *Trichoderma koningii* was not effective against *C. rolfsii..*

Singh (1991) reported that *Trichoderma harzianum* parasitized the mycelia and sclerotia of *Sclerotinia sclerotium* caused white mould of peas and destroyed sclerotia within 15 days.

Sugha *et al.* (1993) reported that conidial coating of the antagonists *Trichoderma harzianum* and *Trichoderma viride* on seeds significantly reduced seedling mortality (47-65%) infected by *Sclerotium rolfsii* compared with the untreated controls.

Xu *et al.* (1993) observed that both isolates of *Trichoderma* T82 or NF9 inhibited hyphal growth of *Sclerotium* (*Corticium*) *rolfsii.* Seed treatment with T82 or NF9 spore suspension (108 c.f.u./ml) increased emergence of cucumber seedlings by 14 and 20%, respectively, 11 d after inoculation with *C. rolfsii.*

Chet and Inbar (1994) found *Trichoderma harzianum* as effective biocontrol agents of soil-borne plant pathogenic fungi. Lectins were found to be involved in the recognition between *Trichoderma* and its host fungi, whereas Chitinase is involved in the degradation of the host wall.

Jagadeesh and Geeta (1994) observed that *Trichoderma harzianum* suppressed *Sclerotium rolfsii* (*C. rolfsii*) and increased seedlings emergence of groundnut. They recommended multiplication of *Trichoderma harzianum* in mixture of wheat bran and biogas manure for amending plots with it for continued survival of the control agent *Trichoderma harzianum.*

Kulkarni and Kulkarni (1994) reported that seed treatment with *Trichoderma harzianum* reduced seedling mortality effectively than other biocontrol agent. They observed soil drenching was more effective than seed treatment.

Tverdyukov *et al.* (1994) observed that *Trichoderma* produces Trichodermin which shows its antagonistic activity against various diseases.

Correa *et al.* (1995) reported that trichorzianins A & B obtained from *Trichoderma harzianum* possessed inhibitory activity on the mycelial growth of *Sclerotium rolfsii.*

Iqbal *et al.* (1995) showed that *Trichoderma harzianum,* significantly inhibited the mycelial growth of *Sclerotium rolfsii,* overlapped the pathogen and suppressed growth by upto 63.6%.

Mehta *et al.* (1995) has demonstrated the potential use of *Trichoderma harzianum* as biocontrol agent against various soil borne plant pathogens including *Sclerotium rolfsii.*

Mukherjee *et al.* (1995) observed that *Trichoderma harzianum* was effective in suppressing *Sclerotium rolfsii. Trichoderma harzianum* was found to be effective in destroying the sclerotia.

Benhamou and Chet (1996) studied the interaction between *Trichoderma harzianum* and sclerotia of the soilborne plant pathogen *S. rolfsii* (*Corticium rolfsii*). Hyphae of *Trichoderma harzianum* grew abundantly on the sclerotial surface forming a dense branched mycelium. Hyphae of the antagonist multiplied on the sclerotial surface and displayed the ability to penetrate the rind.

Muthamilan and Jeyarajan (1996) reported that *Trichoderma harzianum* reduced groundnut root rot caused by *Sclerotium rolfsii*. Maximum number of plant survived when the antagonist was applied as seed treatment.

Roberti *et al.* (1996) investigated the activity of *Trichoderma harzianum* 74 on bean (*Phaseolus vulgaris*) rot caused by *S. rolfsii* when applied to seeds. *Trichoderma* strains were active in bean root rot ensuring control of *S. rolfsii*. *Trichoderma harzianum* reduced the growth of *S. rolfsii* and parasitized *S. rolfsii* hyphae by direct contact, forming coils, short contact branches and hook-shaped hyphal tips.

Nakkeeran and Devi (1997) identified *Aspergillus flavus, A. niger, Penicillium sp., Cladosporium herbarum, Rhizopus stolonifer, Alternaria alternata, Macrophomina phaseolina and Fusarium udum* as seedborne fungi of pigeon pea. Sterilized and unsterilized pigeon pea seeds were treated with talc-based formulations *of Trichoderma viride, Trichoderma*

harzianum and Trichoderma longibrachiatum. Results indicated that all the seedborne fungi detected were most effectively reduced by seed pelleting with *Trichoderma harzianum*..

Ellil *et al.* (1998) reported that *Trichoderma harzianum* reduced the root rot infection 6.7-45.0% in bean. *Trichoderma* spp. obviously antagonized the effects caused by the pathogen, *Sclerotium rolfsii* and *Fusarium solani.*

Rajurkar *et al.* (1998) reported the antagonistic effect of *Trichoderma* on the wilt causing organism *Sclerotium rolfsii.*

Singh and Thapliyal (1998) investigated pre and post-emergence seedling rot. They effectively managed *Sclerotium rolfsii* and got good plant stand and number of normal seedlings by seed treatment with *Trichoderma harzianum*.

Sharma *et al.* (1999) reported that *Trichoderma harzianum* was most effective in inhibiting mycelial growth of *Sclerotinia sclerotium* (Lib) de Bary causing chickpea stem rot in dual cultures. They conducted the experiment in pot and field and reported that treatment of chickpea seeds with mycelial preparation of *Trichoderma harzianum* reduced seedling mortality in pot experiments and reduced the disease considerably in the

field. Pre- application of *Trichoderma harzianum* preferred as compared to the application at sowing time.

Suseelendra and Schlosser (1999) conducted a test with forty-four isolates of *Trichoderma* belonging to eight species groups for their ability to infect, macerate and kill the sclerotia of *Sclerotium rolfsii*, Sacc, while 14 isolates infected and killed sclerotia of *S. rolfsii.* They reported that eight isolates of *Trichoderma harzianum* could kill all the sclerotia inoculated. *Trichoderma harzianum,* biocontrol agent, could penetrate the sclerotial wall, establish and sporulate inside the sclerotium.

2.2 Sensitivity of *Trichoderma and Gliocladium* to fungicides

Wokocha (1990) studied the effect of simultaneous applications *of Trichoderma viride* with PCNB [quintozene], Captan or Aldrex T [thiram + aldrin] on the basal stem rot (*S. [Corticium] rolfsii*) of tomato in artificially inoculated field plots in Samaru (Northern Guinea Savanna Zone) during 1978-80. The results showed that a combination of *Trichoderma viride* with PCNB and *Trichoderma* viride alone gave good disease control during both dry and wet seasons. PCNB alone was less effective, giving a disease severity index (DSI) of 15.6%, whereas Captan and Aldrex T were ineffective, with DSI of 84.9% and 79.2% respectively, during wet-season trials when disease indices were generally high. Combined applications with

Trichoderma viride significantly enhanced the 3 fungicides in disease control, giving max. DSIs of 0.0% (*Trichoderma viride* + PCNB), 16.4% (*Trichoderma viride* + captan) and 19.0% (*Trichoderma viride* + Aldrex T).

Kaur and Mukhopadhyay (1992) reported chickpea wilt complex caused mainly by *Fusarium oxysporum* f.sp. *ciceris, Rhizoctonia solani* and *Sclerotium* (*Corticium*) *rolfsii* which effectively controlled by *Trichoderma harzianum* alone and in combination with fungicides. Soil application of *Trichoderma harzianum* gave 53.5 - 85.7% disease control in the glasshouse. In the field integrated use of *Trichoderma harzianum* with fungicidal seed treatments significantly reduced the incidence of chickpea wilt complex and increased crop yield. Seed treatment with Vitavax-200 (Carboxin + thiram) and Ziram resulted in 29.9% disease control. This control increasing to 63.3% when *Trichoderma harzianum* was also applied.

Lacicowa and Pieta (1994) observed that *Trichoderma* spp. gave the best disease control (*Ascochyta pisi, Fusarium culmorum, F. oxysporum* f.sp. *pisi, F. solani, Rhizoctonia solani* and *Sclerotinia sclerotium*) comparable with chemical control (carboxin and thiram).

Mondal et al. (1995) found that *Trichoderma koningii* proved to be compatible with commonly used fungicide carboxin against loose smut pathogen at 200 and 500 ppm. *Trichoderma harzianum and Trichoderma*

lignorum also grew in culture medium containing carboxin, but seeing the size of colony, *Trichoderma koningii* was more responsive to carboxin even at lower concentration. Contrary to this, the growth of *Trichoderma hamatum* and two isolates of *Trichoderma viride* was very poor indicating their incompatible response to carboxin. The mycelial growth of all the six *Trichoderma* spp. was arrested to a greater extent with the addition of 200 and 500 ppm carbendazim and tebuconazole in culture medium. These observations suggest that none of the antagonists may integrate with carbendazim or newly introduced tebuconazole.

Mukhopadhyay (1995) stated that two bioagents, viz., *Gliocladium virens* and *Trichoderma harzianum* were used for treating seeds of various crops, like chickpea, lentil, groundnut, tomato and cauliflower for protection against wide range of soil borne pathogens viz., *Rhizoctonia solani, Sclerotium rolfsii, Pythium spp. And Fusarium oxysporum*. Such biological treatment was also integrated with suitable fungicide in view of the insensitivity of the bioagents to some chemicals. The treatment was found highly effective and resulted in enhanced crop performance when compared with biological or chemical treatment alone.

Singh et al. (1995) studied selected isolates of *Trichoderma spp.* to screen against common fungicides like Captaf, Dithane M-45 and Thiram. All the isolates were grown on PDA medium containing variable concentrations of

test fungicides. The growth of *Trichoderma harzianum* were inhibited 94.5 and 85.0% at 500ppm of Captaf, 63.0 and 49.0% with 500 ppm of Dithane M-45, respectively after 3 days incubation. The 200 ppm concentration of Thiram completely inhibited the growth of local isolate, however, there was only 51% growth inhibition of Mtr-35 isolate of *Trichoderma harzianum*, respectively. Comparatively *Trichoderma viride and Trichoderma konigii* were found compatible for the all fungicides tested. The growth of these antagonists could not be inhibited completely at 1000ppm of Captaf and Dithane M-45. The 200 ppm of concentration of Thiram completely inhibited the growth of *Trichoderma viride*; however, its 1000 ppm concentration inhibited only 92% growth of *Trichoderma kongii.*

Mehta et al. (1995) stated that the potential use of *Trichoderma harzianum* as biocontrol agent demonstrated parasitic activity against various members of soil borne plant pathogens such as *Fusarium udum, Pythium aphanidermatum, Rhizoctonia solani and Sclerotium rolfsii. Trichoderma harzianum* controled plant pathogenic fungi when introduced as an alternate for, or in combination with sub-lethal dose of chemical fungicides like Bavistin, Dithane M-45 and Foltop.

De *et al.* (1996) conducted an experiment on comparative efficacy of biocontrol agents and fungicides in controlling chickepa wilt caused by *Fusarium oxysporum* f.sp. *ciceri.* Seed treatment with *Trichoderma*

harzianum significantly controlled *F. oxysporum* f.sp. *ciceris* wilt by 30-45%. Integration of biocontrol agents and carboxin significantly increased seed yield by 25.4-42.6%.

Karpagavalli (1997) investigated the effects of carbendazim (as Bavistin), thiram and copper oxychloride (as Fytolan) on the survival of *Trichoderma spp.* in soil. Carbendazim at 500 ppm decreased the initial populations of *Trichoderma* for upto 20 days in sterilized soil and for up to 10 in unsterilized soil. Population of *Trichoderma harzianum* and *Trichoderma viride* were reduced at all concentrations of thiram tested in all soils. Population of *Trichoderma* harzianum and *Trichoderma* viride increased with copper oxychloride treatment in both soil types.

Franke et al. (1998) determined the fungicide sensitivity of more than 450 isolates of *Sclerotium rolfsii* from 11 different peanut fields in Georgia based on percent inhibition of mycelial growth on agar amended with tebuconazole, flutolanil, or PCNB. Of the three fungicides tested, tebuconazole and flutolanil demonstrated the strongest positive correlation in 1994 and 1995.

2.3 Sensitivity of *Trichoderma and Gliocladium* to nutrient elements

Amendment of soil with sulphur increased the acidity and consequently the abundance of antagonistic species of *Trichoderma and Penicillium* has been increased (Liyange, 1983).

Sharma et al. (1995) studied the effect of different agrochemicals on the growth and spore germination of *Trichderma harzianum* Rifai *in vitro.* Among fertilizers urea was not only supportive but stimulatory (to growth and stimulation) followed by ammonium sulphate. Murate of potash was appreciably tolerated by the bioagents, whereas zinc sulphate was highly toxic.

Duggy et al. (1997) reported that disease suppression was not associated with the conduciveness of a soil to take-all, but rather to the supportiveness of a soil to biocontrol activity. Biocontrol activity was positively correlated with iron, nitrate-nitrogen, boron, copper, soluble magnesium, and percent clay, and negatively correlated with soil pH and available phosphorus. They identified a model that included nitrate-nitrogen, soil pH, copper, and soluble magnesium, and described the variance in take-all suppression by *Trichoderma koningii.* Potential applications of these results include amending soil or inoculants with beneficial factors that may be lacking in

the target soil and customizing biocontrol treatments for sites that have parameters predictive of a favorable environment for disease suppression.

2.4 Sensitivity of *S. rolfsii* to fungicide

Agnihotri et al. (1975) studied the role of fungitoxicants for controlling sclerotial root rot of sugerbeet. They reported from their in vitro tests that demosan, vitavax and PCNB [quintozene], in lower concs, were more effective in restricting growth of *S. rolfsii*, which causes extensive mature root rot of sugarbeet, than captan, thiram or benlate. Sclerotia were more resistant to fungicides than the mycelium.

Maiti and Chaudhuri (1975) studied efficacy of seven systemic fungicides on sclerotial germination and growth of *Sclerotium rolfsii*. They found that benodanil and carboxin were highly inhibitory to sclerotial germination and growth of *S. rolfsii*.

Diomande and Beute (1977) studied in soil plate method to evaluate 7 fungicides for control of *S. rolfsii*. Except for DCNA, effectiveness of chemicals in the tests was correlated with field performance. In all tests, carboxin at 0.56, 1.12 and 2.24 kg/ha and TPTH at 4.48 or 8.96 kg/ha were effective in preventing mycelial growth of *Sclerotium rolfsii*.

Yehia et al. (1979) tested fungicides in vitro against *Sclerotium rolfsii.* Seed treatment with Benlate [benomyl], Vitavax [carboxin]/thiram and Vitavax/captan, and soil treatment with Brassicol-75 [quintozene] reduced damping-off and root rot of groundnut in the field.

Peshney and Moghe(1980) conducted tests on 21 fungicides against an isolate of *Sclerotium rolfsii* from groundnut. They reported that the most effective inhibition of sclerotial germination and mycelial growth was given by Derosal [carbendazim], formaldehyde, Vitavax [carboxin] and Plantvax [oxycarboxin].

Fellman et al. (1983) stated that Carboxin, H-719, and especially oxycarboxin were potent inhibitors of sclerotium formation of *Sclerotium rolfsii* even though the fungus produced a heavy mycelial mat.

Motikhaye (1983) tested the effects of six fungicides (Bordeaux mixture, Dithane Z-78 [zineb], Vitavax [carboxin], Bavistin [carbendazim], BAS 70 F and aureofungin) on the germination and growth of *Sclerotium [Corticum] rolfsii.* The best results were given by aureofungin and carboxin.

Anilkumar and Gowda.(1984) evaluated eight fungicides against *S. rolfsii* on straw pieces in soil. They found that Bayleton [triadimefon], Sicarol

[pyracarbolid] and Vitavax [carboxin] were highly effective as dry-soil mixes and soil drenches.

Srikant et al. (1986) tested 19 fungicides against *Sclerotium rolfsii* in vitro and stated that Vitavax [carboxin] performed best by completely inhibiting growth at 50 ppm followed by Bayton (Baytan [triadimenol + fuberidazole]) and benodanil.

Fahim et al.(1986) studied the growth of *Sclerotium rolfsii* on solid and liquid media and found complete inhibition by Homai 80 (thiophanate-methyl + thiram) at 25 ppm, Orthocide 75 (captan) at 10 ppm., Vitavax (carboxin) + captan at 0.5 ppm and by carboxin + thiram at 1 ppm.

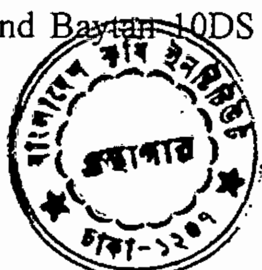
In vitro study of Palakshappa et al. (1987) with 12 fungicides showed that 100% inhibition of this *Sclerotium rolfsii* was given by carboxin, triadimenol, agallol, guazatine and quintozene at all concentrations tested.

Parcha et al. (1988) reported that the mycelial growth of *S. rolfsii* Sacc. on potato dextrose agar (PDA) after incubated for 24 hours with Vitavax, Sicarol and TPTA at 100 ppm; Baytan, Tilt, Thiram, Terrazole, NF-65 and Folpet at 150 ppm; Beam, Rovral and Saprol at 1,000 ppm were less effective. However, TPTA was the most effective fungicide after 72 hours of incubation.

Wokocha (1990) studied the effect of simultaneous applications *of Trichoderma viride* with PCNB [quintozene], Captan or Aldrex T [thiram + aldrin] on the basal stem rot (*S. [Corticium] rolfsii*) of tomato in artificially inoculated field plots in Samaru (Northern Guinea Savanna Zone) during 1978-80. The results showed that a combination of *Trichoderma viride* with PCNB and *Trichoderma* viride alone gave good disease control during both dry and wet seasons. PCNB alone was less effective, giving a disease severity index (DSI) of 15.6%, whereas Captan and Aldrex T were ineffective, with DSI of 84.9% and 79.2% respectively, during wet-season trials when disease indices were generally high. Combined applications with *Trichoderma viride* significantly enhanced the activity of three fungicides in disease control, giving max. DSIs of 0.0% (*Trichoderma* viride + PCNB), 16.4% (*Trichoderma* viride + Captan) and 19.0% (*Trichoderma* viride + Aldrex T).

Asghari and Mayee (1991) reported that application of *Trichoderma harzianum* and soil drenching with 0.2% carbendazim reduced stem rot by 44-60% and pod rot by 27-60% in groundnuts infected by *Sclerotium* [*Corticium*] *rolfsii*. Treatment increased pod yield by 17-47% and seed wt by 29-33%.

Raman et al. (1994) studied the effect of Vitavax-200 [carboxin], Apron-TZ [metalaxyl], Dithane M-45 [mancozeb], Thiram, Captan and Baytan-10DS



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[triadimenol] on foot and root rot disease (*Sclerotium rolfsii*) on cowpea (*Vigna unguiculata*). They have treated seeds of a susceptible variety before sowing. Vitavax-200 was the best fungicide with respect to controlling seedling mortality. Crop yield was also significantly increased due to seed treatment with fungicides. Highest seed yield was achieved after application of Vitavax-200.

Rondon et al. (1995) conducted in vitro and in the greenhouse experiments with tomato and sunflower using vinclozolin (Ronilan), iprodione (Rovral), metalaxyl (Ridomil), chlorothalonil (Daconil), PCNB (Terraclor [quintozene]), copper oxychloride (Cobrex), captan (Orthocide-50), benomyl (Benlate), carboxin + thiram (Vitavax-200), carbendazim (Derosal), kasugamycin (Kasumin) and thiabendazole (Tecto) at 5 concentrations. Measurements of mycelial growth and sclerotia production were taken for 21 d. The results after analysis showed that carboxin + thiram and quintozene were the most effective, both inhibiting mycelial growth and sclerotia formation at low concentration.

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De et al. (1996) observed that coating chickpea seeds with biocontrol agents *Bacillus subtilis, Gliocladium virens, Trichoderma harzianum* and *Trichoderma viride*, and carboxin (Vitavax) significantly controlled *Fusarium oxysporum* f.sp. *ciceris* wilt by 30-45.8% . Integration of biocontrol agents and carboxin significantly increased seed yield by 25.4-

42.6% as did carboxin treatment alone. Carbendazim (Bavistin WP) was more effective than carboxin in reducing wilt and increasing seed yield.

Borines and Ranchez (1996) studied the efficacy of selected fungicides against the basal stem rot or wilt of tomato caused by Sclerotium rolfsii Sacc. An in vitro assay of fungicides revealed that Mancozeb (Dithane M-45) was the most effective in suppressing the colony growth and sclerotia formation of *S. rolfsii*. Manganese ethylene bisdithiocarbamate (Maneb) also significantly reduced the colony growth of the pathogen but comparatively lesser effective by heat treatment. Captan (Captan) and thiophanate methyl (Fungitox) reduced growth to a lesser extent, while benomyl (Benlate) reduced colony growth only when not subjected to heat. Results of the field evaluation further proved the effectiveness of Dithane M-45 and Maneb in controlling sclerotium wilt in the field. These fungicides produced the lowest disease incidence and highest percent protection on the plants during the wet season and dry season trials. Fungitox was less effective than Dithane M-45 and Maneb, while Benlate was ineffective in controlling sclerotium wilt in the field.

Franke et al. (1998) determined the fungicide sensitivity of more than 450 isolates of *Sclerotium rolfsii* from 11 different peanut fields in Georgia based on percent inhibition of mycelial growth on agar amended with

tebuconazole, flutolanil, or PCNB. Of the three fungicides tested, tebuconazole and flutolanil demonstrated the strongest positive correlation in 1994 and 1995.

Srivastava and Tripathi (1998) studied the effectiveness of 5 combinations (2 fungicides in each combination) of 4 compatible fungicides, quintozene (Brassicol), thiram (TMTD), carboxin (Vitavax) and carbendazim (Bavistin) at the rate of 2.5 g/kg seed (mixed in equal proportion), incorporated in seed pellets of sugarbeet, was evaluated against a seedling disease complex caused by *Sclerotium rolfsii* [*Corticium rolfsii*]. Seed pelleted with all combinations of fungicides provided better disease control than steeping seeds in an aqueous suspension of a combination of fungicides. Of the combinations used, carbendazim + thiram was the most effective in reducing seedling mortality.

2.5 Sensitivity of *S. rolfsii* to nutrient elements

Thammasak et al. (1982) investigated pathogenicity of *Sclerotium rolfsii* on cotton. Observations showed that soil amendment with crop refuses, N-fertilizers, and lime decreased disease intensity. Seed dressing with five fungitoxicants showed that vitavax gave a complete protection when grown in infested soil.

Ortega et al. (1992) evaluated the effect of different combination levels of N-P-K, in the yield and other agronomic characteristics of the rice and in the severity of the disease. Natural infestation of "stem rot" caused a decrease in the growth and yield of rice. Potash fertilization increased the yield, dry matter production and K content of rice tissues and produced a drastic decrease in "stem rot" severity. N and P fertilization did not influence the disease severity, although high N content in the rice tissues, associated with high levels of N fertilization and K deficiency, could increase rice susceptibility to "stem rot". P fertilization increased the rice yield and dry matter production. The effect of N fertilization was observed only in presence of K fertilization. In soils infested with "stem rot", a balanced N-P-K fertilization will be the recommended practice.

Bag and Sinha (1997) tested six metal salts, selected out of 14 initially tested, viz. Barium sulphate, lithium sulphate, manganese sulphate, cupric chloride, ferric chloride, and zinc chloride, each at three concentrations for their in vitro fungitoxic action on the sclerotia germination of *Sclerotium rolfsii.* In plain water, sclerotia germinated heavily (84%). None of the salts, except cupric chloridce at 10^{-3}M and 10^{-4}M, and zinc chloride at 10^{-3}M, that allowed respectively 68, 72 and 74% germination, showed any appreciable inhibitory effect on sclerotia germination.

CHAPTER 3

MATERIALS AND METHODS

3. MATERIALS AND METHODS

Experiments were carried out both in the laboratory and in the field. The materials used and the methodology and procedures followed in the experiments have been described in this chapter.

3.1. Experimental site

In-vitro studies were done in Seed Pathology Laboratory (SPL) and Laboratory of the department of Plant Pathology, Bangladesh Agricultural University (BAU), Mymensingh. Field experiment was conducted in the field laboratory of Department of Plant Pathology, BAU, Mymensingh.

3.2. Experimental period

The experiments were carried out during September 1999 to November 2000.

3.3. Isolation and preservation of *Sclerotium rolfsii* from the diseased Plant

Isolation of *Sclerotium rolfsii* from the diseased plants was accomplished by tissue planting method. Specimens having typical symptoms of foot and root rot were selected in the field of the farm of Bangladesh Agricultural

University (BAU), Mymensingh. Diseased plants were collected using the polyethylene bag and brought immediately to the laboratory of the Department of Plant Pathology, BAU, Mymensingh. The diseased samples were then initially washed in tap water to remove sand and soil particles. Thereafter, the specimens were cut into small pieces (1.0 cm) having healthy and dead tissues and surface sterilized with mercuric chloride solution (1 : 1000) for 1 minute. The plant pieces were then washed thoroughly with sterilized water thrice and placed on filter paper to remove excess water adhering with the pieces. Five pieces were plated in acidified (PDA) plates aseptically maintaining equal distance. The plates were incubated at room temperature for 7 days and observations were made regularly to see the growth of *Sclerotium rolfsii* from the plant pieces. The fungus that grew over PDA was isolated and purified by hyphal tip culture method (Fig. 1). The isolated fungus was identified following the key outlined by Booth (1971) and Singh (1982). The pure culture of *Sclerotium rolfsii* was preserved in PDA slant at 5 ± 1^0C in refrigerator as stock culture for future use.

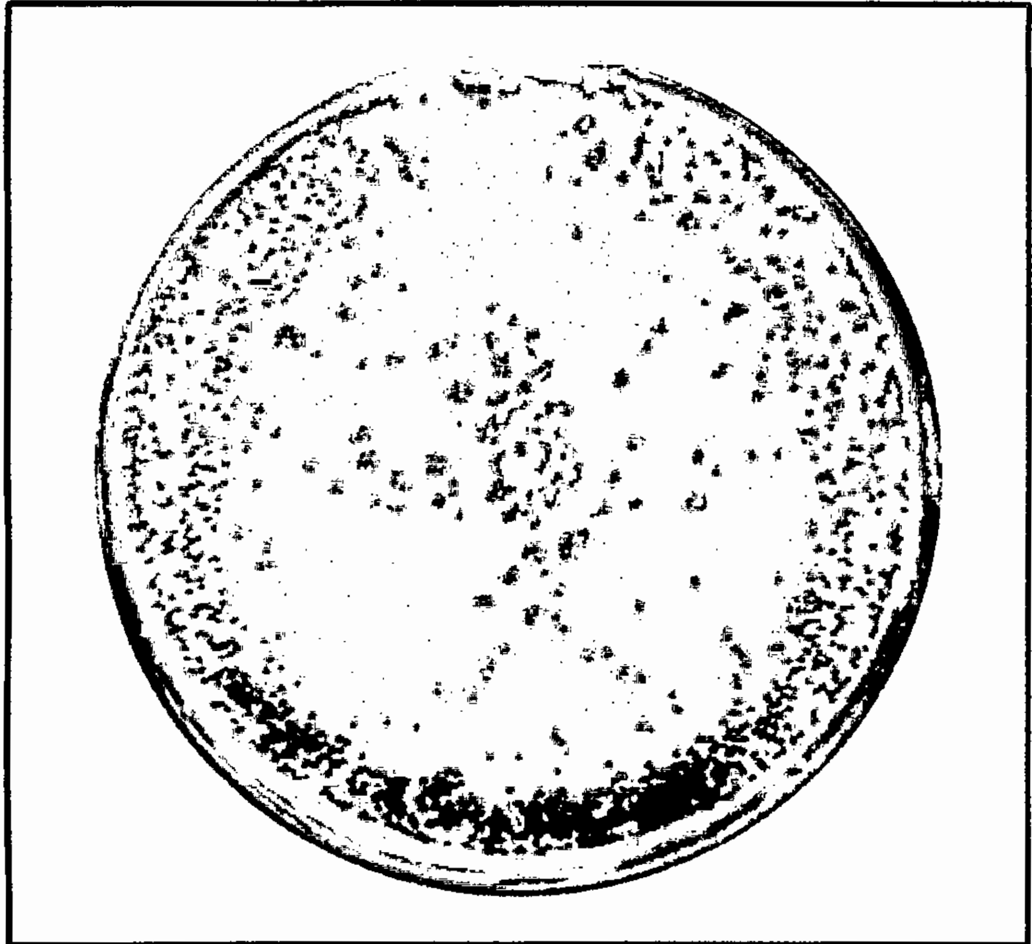


Fig. 1. Mycelia and sclerotia of *Sclerotium rolfsii*

3.4. Collection of *Trichoderma harzianum, Trichoderma hamatum, Trichoderma viride*, and *Gliocladium viride*

Trichoderma and *Gliocladium* were collected from the laboratory of Professor Dr. Ismail Hossain, Department of Plant Pathology, BAU, Mymensingh (Fig. 2a and 2b). The *Trichoderma* isolates and *Gliocladium viride* used in the present study are shown below:

Trichoderma harzianum (TL_1)

Trichoderma harzianum (TS_1)

Trichoderma harzianum (TBg_1)

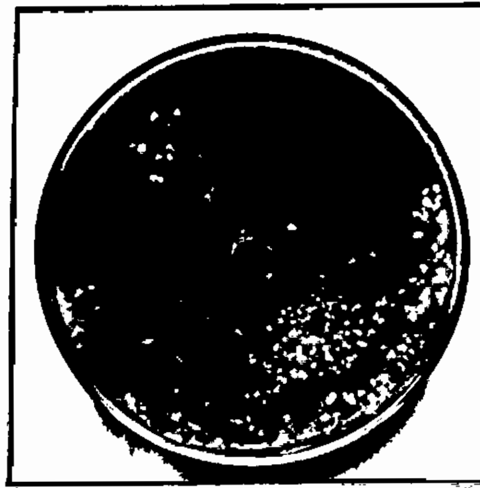
Trichoderma harzianum (TG_2)

Trichoderma harzianum (Tch_3)

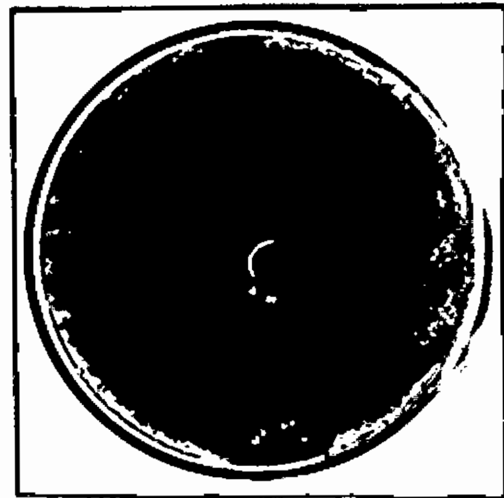
Trichoderma hamatum

Trichoderma viride

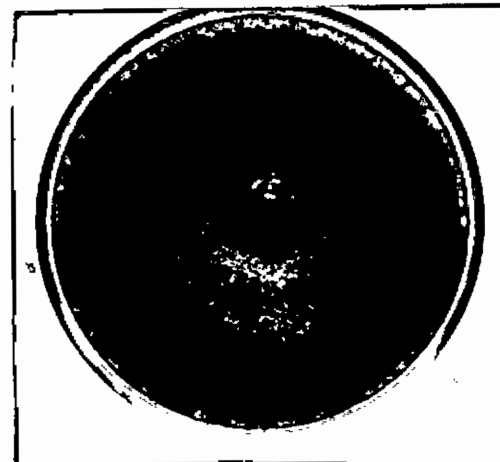
Gliocladium viride



Trichoderma viride

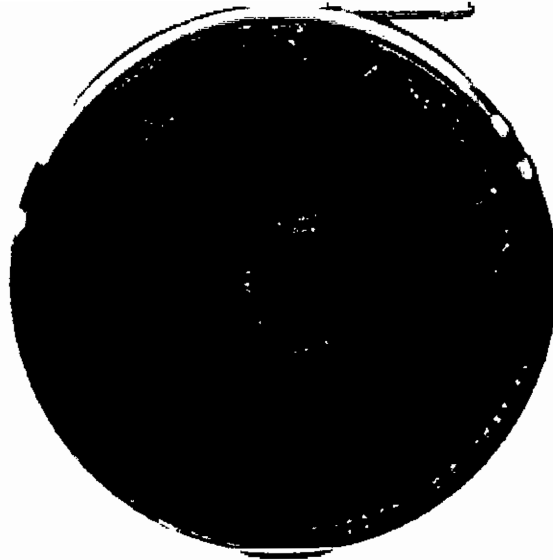


Trichoderma hamatum

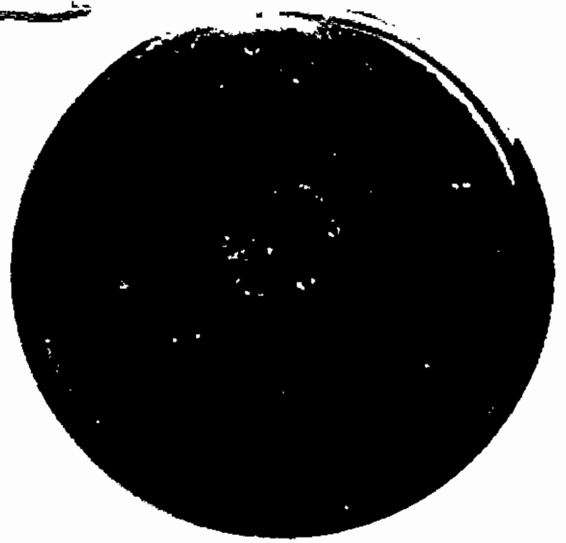


Gliocladium viride

Fig. 2a. Growth of *Trichoderma* and *Gliocladium* on PDA

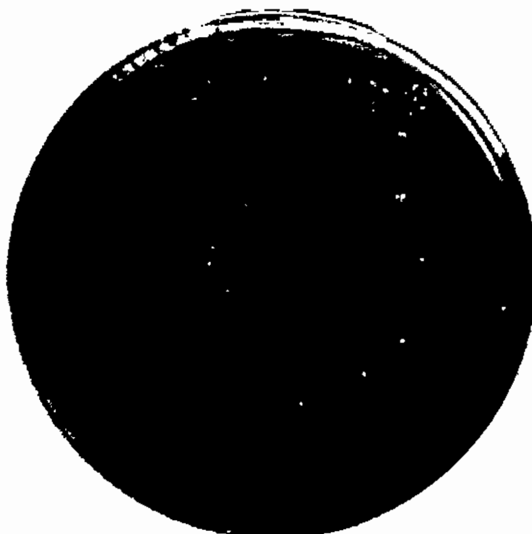


Trichoderma harzianum
TL-1

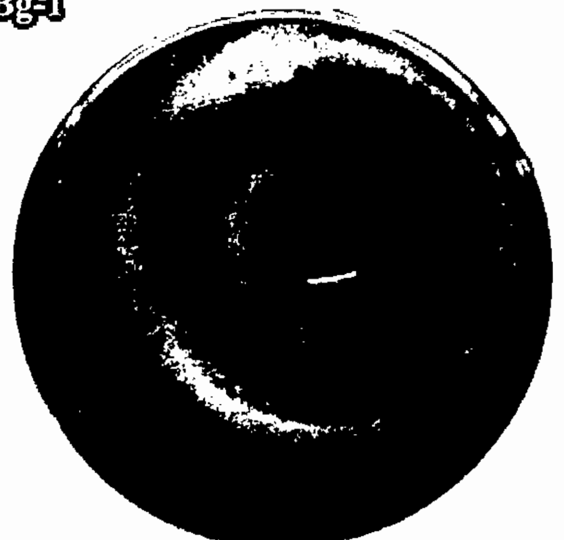


Trichoderma harzianum
TS-1

Trichoderma harzianum
TBg-1



Trichoderma harzianum
TG-2



Trichoderma harzianum
Tch-3

Fig. 2b. Growth of Different isolates of *Trichoderma harzianum* on PDA

3.5. Experiments conducted

Five sets of experiments were carried out during the study period for accomplishment of the research work in an well-orchestrated manner. These experiments have been entitled as shown below:

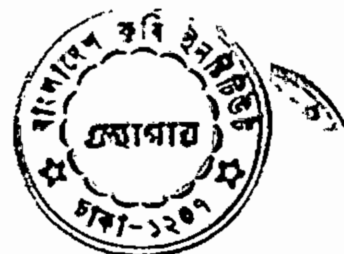
I. Studies on the tolerance of *Trichoderma harzianum; Gliocladium viride* and *Sclerotium rolfsii* to different fungicides and fertilizers

II. Antagonistic activity of *Trichoderma harzianum, Trichoderma hamatum, Trichoderma viride*, and *Gliocladium viride* against *Sclerotium rolfsii in vitro.*

III. Effect of *Trichoderma* and *Gliocladium* on germination of Pulses (Mungbean, Mashkalai, and Pigeon pea) and their efficacy of controlling seed borne mycoflora.

IV. Antagonistic effect of *Trichoderma* and *Gliocladium* on the seed borne mycoflora of Tomato *in-vitro*.

V. Effect of *Trichoderma harzianum* on germination and seedling vigor of Tomato.



3.5.1. Experiment I

Studies on the tolerance of *Trichoderma harzianum*, *Gliocladium viride* and *Sclerotium rolfsii* to different fungicides and fertilizers

3.5.1.1. Fungicides used

Commercial name	Active ingredient	Chemical name
Vitavax 200	Carboxin	2,3-Dihydro-6-6methyl-5-Phenuyl carbamoyl-1,4-oxathiin.
Bavistin	Carbendazim	Methyl benzimidazol-2-yl carbamate
Tilt 250 EC	Propiconazole	1-[2-(2,4-Dichlorophenyl-4-propyl-1,3-Dioxalen-2-ilmethyl) 1-H-L2, 4-Triazole
Ridomil MZ-72	Mancozeb	N-(2,6 dimethyl phenyl)-N-(methoxyacetyl)-alanine methyl ester ($C_{14}H_{21}$ NO_4
Thiovit	Sulphur	Wettable sulpher

3.5.1.1.1. Bio-assay of fungicides

The linear mycelial growth (mm) and percent growth inhibition of mycelium of *Trichoderma harzianum, Gliocladium viride* and *Sclerotium rolfsii* were observed *in-vitro* by poisoned food technique (Nene and Thapliyal, 1979). The test concentrations of the studied fungicides are shown below:

Name of Fungicides	Concentrations used (µg/ml)
Vitavax 200	0, 100, 250, 500 and 1000
Bavistin	0, 100, 250, 500 and 1000
Tilt 250EC	0, 100, 250, 500 and 1000
Ridomil MZ-72	0, 100, 250, 500 and 1000
Thiovit	0, 100, 250, 500 and 1000

3.5.1.1.2. Preparation of poisoned food

Fungicidal solution of each concentration was prepared and mixed thoroughly with melted PDA. PDA (100 ml) was taken in each conical flask and were labeled properly as 0, 100, 250, 500 and 1000µg/ml. The mouths of the conical flasks were then plugged with cotton and covered by brown paper and tied up with threads. The medium in flasks was then sterilized in autoclave at 121^0 C under 15 PSI for 15 minutes. After autoclaving, the

conical flasks were then allowed to cool at 50^0C. Then the specific amount of test chemical was added into the medium to get different concentrations of test chemicals viz. 100, 250, 500 and 1000 μg/ml. The flasks containing medium were shaked with the help of hand to mix the test chemical thoroughly. After that five drops of lactic acid per flasks was added and shaked well. Thus 100 ml poisoned PDA for each concentration was prepared. The poisoned food thus prepared was plated @ 20 ml in each five replicated plates which were previously marked as 0, 100, 250, 500 and 1000μg/ml as per treatment.

3.5.1.1.3. Inoculation of the test fungi (*Trichoderma harzianum*, *Gliocladium viride* and *Sclerotium rolfsii*) to poisoned food plates

Mycelial blocks of 5 mm diameter were cut from 7 days old culture of the test pathogens and were placed aseptically at the center of each plate. The plates were then incubated at 25+2 ^{0}C for a period of 3 – 4 days.

3.5.1.1.4. Data collection

Radial mycelial growth of the test fungi was determined at every 24 hr. until the whole plate of control treatment was covered by the mycelial mat of test fungi. Percent growth inhibition was also calculated.

3.5.1.2. Fertilizers Used

Commercial name of fertilizer	Chemical Formula	Available Nutrient	% Nutrient
Urea	$CO(NH)_2$	N	46
Triple Super Phosphate	$Ca(H_2PO_4)_2$	P	20
Murate of Potash	KCl	K	50
Gypsum	$CaSO_4.\ H_2O$	S	18
Managanese Sulphate	$MnSO_4.H_2O$	Mn	36
Copper Sulphate	$CuSO_4$	Cu	80

3.5.1.2.1. Bio-assay of fertilizers

The linear mycelial growth (mm) and percent growth inhibition of mycelium of *Trichoderma harzianum, Gliocladium viride* and *Sclerotium rolfsii* were observed *in-vitro* by poisoned food technique (Nene and Thapliyal, 1979). The test concentrations of the studied fertilizers are shown below:

Name of fertilizers	Concentrations used (μg/ml)
Urea	0, 100, 250, 500 and 1000
Triple Super Phosphate	0, 100, 250, 500 and 1000
Murate of Potash	0, 100, 250, 500 and 1000
Gypsum	0, 100, 250, 500 and 1000
Managanese Sulphate	0, 100, 250, 500 and 1000
Copper Sulphate	0, 100, 250, 500 and 1000

3.5.1.2.2. Preparation of poisoned food

This study was carried out following the same procedure as done in case of bio-assay of fungicides.

3.5.1.2.3. Inoculation of the test fungi *(Trichoderma harzianum, Gliocladium viride and Sclerotium rolfsii)*

This test has been done following the same procedure as done in case of inoculation test of fungi against fungicides.

3.5.1.2.4. Data collection

Radial mycelial growth of the test fungi was determined at every 24 hrs. until the whole plate of control treatment was covered by the mycelial mat of test fungi. Percent growth inhibition was also calculated.

3.5.2. Experiment II

Antagonistic activity of *Trichoderma harzianum, Trichoderma hamatum, Trichoderma viride* and *Gliocladium viride* against *Sclerotium rolfsii in-vitro*.

3.5.2.1 Growth study of *Trichoderma harzianum, Trichoderma hamatum, Trichoderma viride and Gliocladium viride*

Radial mycelial growth of *Trichoderma harzianum*, *Trichoderma hamatum, Trichoderma viride and Gliocladium viride* was studied. Mycelial block (5 mm) each of the isolate of each antagonist was aseptically placed at the centre of the petriplate containing PDA in five replications. Radial mycelial growth was measured after every 24hrs. upto four days until the plates under control treatments were filled with the mycelial mat of the test fungi.

3.5.2.2. Interaction of *Sclerotium rolfsii with Trichoderma harzianum, Trichoderma hamatum, Trichoderma viride* and *Gliocladium viride*

This study was carried out by using PDA following dual culture method as used by Sultana and Hossain (1999). In case of *Trichoderma harzianum,* a 5 mm block of pure culture was transferred to plate containing PDA at one side, another block of same size of *Sclerotium rolfsii* was placed on the other side of the plate in five replicates. The plates were then incubated at 25±1^{0}C for 7 days, and notes on selerotia formation, growth of *S. rolfsii* by *T. harzianum* and lysed zone formation were recorded. The same procedure

was followed in cases of *Trichoderma hamatum, Trichoderma viride* and *Gliocladium viride.* Another set of the same study was done for a period of 15 days and observation on interaction between antagonists *and S. rolfsii* was made and sclerotia formation by *S. rolfsii*, over growth of *S. rolfsii* by antagonists and formation of lysed zone between *S. rolfsii* and tested antagonists had also been noted. Moreover, mycelial mat along with culture from the lysed zone was transferred to freshly prepared PDA plates and incubated at room temperature for a week for observing the ability of *S. rolfsii* to grow further.

3.5.2.3. Treatment of Sclerotia of *S. rolfsii* with antagonists

Sclerotia of *Sclerotium rolfsii* were treated with the conidia of the *Trichoderma and Gliocladium* and then placed on PDA plate (3 sclerotia/plate) at equal distance maintaining three replications in each study following the method of Begum et al. (1998). In each case control was maintained by placing untreated sclerotia on PDA plate in triplicates. . Moreover, the sclerotia which were over grown by the *Trichoderma* and *Gliocladium* were transferred to PDA plates and their germinability was also studied following the same procedure as of mycelial growth study from the lysed zone.

3.5.3. Experiement III

Effect of *Trichoderma* and *Gliocladium* on germination of Pulses (Mungbean, Blackgram and Pigeon pea) and their efficacy in controlling seed borne mycoflora.

3.5.3.1. Collection of seeds

Seed samples of pulses viz. Mungbean, Blackgram, and Pigeon pea were collected from local market of Mymensingh. Seeds after collection were taken in brown paper packets and stored in refrigerator at 5-7 ^{0}C until use for subsequent studies.

3.5.3.2. Health status of the collected seed samples

Health status of the collected seed samples were tested following the blotter method (ISTA, 1996). Three hundred seeds were randomly taken from each seed sample. Twenty-five seeds without surface sterilization were placed on three layers of moist blotter paper contained in each 9 cm-diameter glass petridish. Then the petriplates were incubated under near UV light (12/12) for a period upto 10-12 days and germination of the seeds was recorded following the method of ISTA (1993). Incubated seeds were examined under stereobionocular microscope for the detection of fungi that grew on the

seeds. To resolve confusion for identification of fungi, temporary slides were prepared and observed under microscope and the fungi were identified.

3.5.3.3. Effect of *Trichoderma* and *Gliocladium* in controlling seed borne mycoflora

3.5.3.3.1. Seed treatment with *Trichoderma* and *Gliocladium*

Seeds of test crops were treated following the method of Begum et al.(1998). Three hundred seeds of each crop were treated for each isolate of the antagonists. One hundred seeds were taken in a beaker and 2 drops of water were added and moistened the seed surface uniformly to allow maximum adherence of conidia on the whole surface of seeds. Seeds were then transferred to 7 days old culture of bioagents (antagonists) and stirred the plate gently by hand until the whole surface of seeds coated with the conidia of the specific test antagonist (*Trichoderma/Gliocladium)*. The treated seeds were then subjected for testing the effect of bioagents in controlling seed borne mycoflora. The bioagents were used as treatments where control (without bioagent) was maintained. The treatments were as follows:

T_1=Control

T_2=*Trichoderma harzianum*

T_3=*Trichoderma hamatum*

T_4=*Trichoderma viride*

T_5=*Gliocladium viride*

3.5.3.3.2. Collection of Data

Data were collected on percent seed germination and percent mean incidence of seed borne mycoflora.

3.5.3.4. Effect of *Trichoderma* and *Gliocladium* on seedling vigor of Mungbean and Blackgram under laboratory condition

This experiment was done following the Standard blotter method (ISTA, 1996). Three hundred seeds were randomly taken from each seed sample. Seeds were then treated with antagonist following the same procedure as of Begum et al. (1998) and described in section 3.5.3.3.1. Then the petriplates were incubated under defused sunlight for 10-12 days. After incubation shoot length of the germinated seeds was considered for calculating vigor index.

3.5.3.4.1. Design of Experiment

The experiments were carried out following completely randomized design with three replications having 100 seed per replication.

3.5.4. Experiment IV

Antagonistic effect of *Trichoderma* and *Gliocladium* on the seed borne mycoflora of Tomato *in vitro.*

3.5.4.1. Collection of seeds

Seed sample of tomato c.v. Ratan were collected from local market of Mymensingh. After collection of seeds, the seed sample was kept in brown paper packets and stored in refrigerator at 5 ^{0}C until use for subsequent studies.

3.5.4.2. Laboratory Experiment

3.5.4.2.1. Health status of the collected seed samples

This test has been done following the same procedure as done in case of Experiment III section 3.5.3.2.

3.5.4.2.2. Seed treatment with *Trichoderma* and *Gliocladium*

Seeds of tomato were treated with conidia of *Trichoderma* and *Gliocladium* following the method as described in the Experiment III section3.5.3.3.1. The seeds were treated with the conidia of antagonist and each isolate of *Trichoderma* was considered as treatment. The treatments used for this study are shown below:

T_1= Control

T_2= *Trichoderma harzianum* (Tch_3)

T_3= *Trichoderma harzianum* (TL_1)

T_4= *Trichoderma harzianum* (TS_1)

T_5= *Trichoderma harzianum* (TBg_1)

T_6= *Trichoderma harzianum* (TG_2)

T_7= *Trichoderma. hamatum*

T_8= *Trichoderma viride*

T_9= *Gliocladium viride*

3.5.4.2.3. Collection of Data

Data were collected on percent seed germination and percent mean incidence of seed borne pathogen .

3.5.4.2.4. Design of Experiment

The experiments were carried out following completely randomized design with three replications having 100 seeds per replication.

3.5.5. Experiment V

Effect of *Trichoderma harzianum* on germination and seedling vigor of Tomato

3.5.5.1. Experimental site

The experiment was conducted in the field laboratory of the Department of Plant Pathology, Bangladesh Agricultural University (BAU) Mymensingh.

3.5.5.2. Soil condition

The soil of the experimental site belongs to the non-calcareous Grey flood plain under the Agro-Ecological Zone of Old Brahmaputra Alluvial Soil. The land was a medium high land and soil texture was sandy loam with pH value from 6.5-6.8 (Raman and Ali, 1970)

3.5.5.3. Experimental period

The experiments were conducted during October1999 to February 2000.

3.5.5.4. Materials

Seed sample of tomato c.v. Ratan were collected from local market of Mymensingh. After collection of seeds, the seed sample was kept in brown paper packets and stored in refrigerator at 5 ^{0}C until use for subsequent studies.

3.5.5.5. Land preparation

The soil of experimental plot were opened with power tiller. Later, four ploughings and cross ploughings followed by three laderings were done during the course of land preparation. Weeds and other rubishes were removed. Then the unit plot was prepared. Chemical fertilizers were not used in the experimental plot.

3.5.5.6. Manuring

Cowdung was applied to the soil at the rate of 0.75 cft per unit plot (1m X 1m) at the time of land preparation.

3.5.5.7. Experimental design and layout

The field experiment was conducted in Randomized Complete Block Design (RCBD) with three replications. The size of the individual plot was 1 m x 1 m with spacing 50 cm between plots and 1m between blocks.

3.5.5.8. Treatments used

T_1= Control	T_4= *Trichoderma harzianum* (TS_1)
T_2= *Trichoderma harzianum* (Tch_3)	T_5= *Trichoderma harzianum* (TBg_1)
T_3= *Trichoderma harzianum* (TL_1)	T_6= *Trichoderma harzianum* (TG_2)

3.5.5.9. Tomato seed treatment with *Trichoderma harzianum* as per treatment

Seeds of tomato were treated with the conidia of different isolates of *Trichoderma harzianum* following the same procedure as mentioned in the Experiment III section 3.5.3.3.1.

3.5.5.10. Sowing of treated seeds in the field (Seed bed)

The treated seeds were sown in lines about 2.0 cm depth and immediately the seeds were covered with soil. The line to line and plant to plant distances were maintained 20 cm and 5 cm, respectively. The seeds were sown in the field in the afternoon on November 19, 1999.

3.5.5.11. Data collection

Data on germination and growth of seedlings were recorded. After six weeks, twenty seedlings from each plot were randomly selected and washed in water. Then root length, shoot length, root weight and shoot weight were taken.

3.5.5.12. Data analysis

The data were analyzed statistically and treatment effect were compared to each other by employing Duncun's New Multiple Range Test (DMRT). Vigour index was calculated by using the formula (Abdul Baki and Anderson, 1973) as shown below:

Vigour Index (VI) = (mean shoot length + mean root length) X percent germination.

CHAPTER 4

RESULTS

4. RESULTS

4.1. Experiment I

4.1.1. Tolerance of *Trichoderma harzianum* to fungicides

Tolerance of *Trichoderma harzianum* was tested against five fungicides, namely Vitavax 200, Bavistin, Tilt 250EC, Ridomil MZ-72 and Thiovit. It has been observed that Vitavax 200 showed significant effect on inhibition of radial mycelial growth of *Trichoderma harzianum* over untreated control by 89.25% at 1000 µg/ml followed by 88.57%, 82.59% and 12.59% at 500, 250 and 100 µg/ml respectively after 96 hrs of incubation (Table 1). 500 µg/ml and 1000 µg/ml resulted statistically similar effect on radial mycelial growth but differed significantly from other treatments. Tolerance study clearly showed that *Trichoderma harzianum* could not tolerate even 100µg Bavistin/ml i.e, Bavistin totally inhibited the mycelial growth of *Trichoderma harzianum* at 100µg/ml after 96 hr incubation (Table 2). Tilt 250EC showed significant effect in arresting the mycelial growth of *Trichoderma harzianum* (Table 3). It was observed that Tilt 250EC reduced the mycelial growth of *Trichoderma harzianum* by 52.59%, 79.25% and 87.03% at 100, 250 and 500 µg/ml, respectively. *Trichoderma harzianum* could not tolerate Tilt 250EC at 1000 µg/ml upto 94 hrs incubation period.

Table 1. Tolerance of *Trichoderma harzianum* against Vitavax

Concentration (µg/ml)	Radial mycelial growth (mm)				% growth inhibition
	24hrs.	48hrs.	72hrs.	96hrs.	
Control	2.93a	42.66a	68.50	90.00a	
100	2.46b	33.50b	55.50ab	78.66b	12.59
250	0.00c	8.50c	9.50c	15.66c	82.59
500	0.00c	8.50c	8.83c	10.33d	88.57
1000	0.00c	2.16d	7.50c	9.66d	89.25
LSD (p=0.05)	0.1603	5.086	4.710	4.600	

Data represents mean of five replications

Table 2. Tolerance of *Trichoderma harzianum* against Bavistin

Concentration (µg/ml)	Radial mycelial growth (mm)				% growth inhibition
	24hrs.	48hrs.	72hrs.	96hrs.	
Control	2.93a	42.66a	68.50a	90.00a	
100	0.00	0.00	0.00	0.00	100.00
250	0.00	0.00	0.00	0.00	100.00
500	0.00	0.00	0.00	0.00	100.00
1000	0.00	0.00	0.00	0.00	100.00
LSD (p=0.05)					

Data represents mean of five replications

Table 3. Tolerance of *Trichoderma harzianum* against Tilt

Concentration (μg/ml)	Radial mycelial growth (mm)				% growth inhibition
	24hrs.	48hrs.	72hrs.	96hrs.	
Control	2.93a	42.66a	68.50a	90.00a	
100	0.00b	14.83b	24.83b	42.66b	52.59
250	0.00b	6.83c	11.83c	18.66c	79.25
500	0.00b	0.00d	0.00d	11.66d	87.03
1000	0.00b	0.00d	0.00d	0.00e	100.00
LSD (p=0.05)	0.1603	2.028	2.941	0.7169	

Data represents mean of five replications

But *Trichoderma harzianum* could grow later on PDA. The growth of *Trichoderma harzianum* 10 days after incubation at 1000µg Tilt 250EC/ml was found to some extent as shown in Fig. 3. Ridomil did not show any fungicidal effect upto 500µg/ml rather it increased the growth of *Trichoderma harzianum* significantly over untreated control. It induces *Trichoderma harzianum* growth upto 22.36% at 500 µg/ml, while the highest mycelial growth was obtained at 100 µg/ml. It has clearly been observed that *Trichoderma harzianum* could not tolerate higher concentrations of Ridomil. Its mycelial growth was totally arrested and no growth was found at 1000 µg/ml of Ridomil. (Table 4). The mycelial growth of *Trichoderma harzianum* has been found to be increased significantly by 31.36% upto 1000 µg/ml of Thiovit though the conc. of Thiovit from 100 to 1000 µg/ml did not show any significant variation in respect of mycelial growth after 72 hrs of incubation (Table 5)

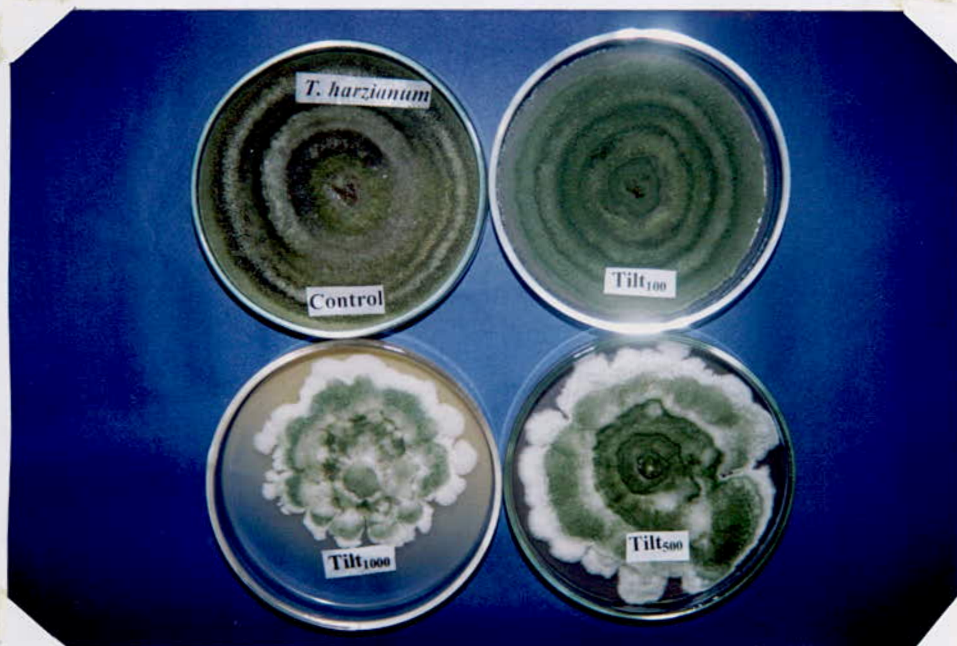


Fig. 3. Comparative tolerance of *Trichoderma harzianum* to Tilt 250 EC

Table 4. Tolerance of *Trichoderma harzianum* against Ridomil

Concentration (μg/ml)	Radial mycelial growth (mm)			% growth induction (+) or inhibition (-)
	24hrs.	48hrs.	72hrs.	
Control	2.93ab	42.66b	68.50c	
100	3.00a	51.33a	90.00a	+31.36
250	2.73b	43.33b	81.16b	+18.47
500	2.60b	44.50b	83.83b	+22.36
1000	0.00	0.0000	0.00	-100.00
LSD (p=0.05)	0.4118	2.682	3.349	

Data represents mean of five replications

Table 5. Tolerance of *Trichoderma harzianum* against Thiovit

Concentration (μg/ml)	Radial mycelial growth (mm)			% growth induction
	24hrs.	48hrs.	72hrs.	
Control	2.93d	42.66c	68.50b	
100	24.33c	74.66b	90.00a	31.36
250	24.66c	74.66b	90.00a	31.36
500	27.33b	74.50b	90.00a	31.36
1000	28.66a	83.50a	90.00a	31.36
LSD (p=0.05)	1.180	5.933	2.634	

Data represents mean of five replications

4.1. 2. Tolerance of *Gliocladium viride* to fungicides

It has been observed that Vitavax 200 showed significant effect on inhibition of radial mycelial growth of *Gliocladium viride* over untreated control by 85.70% at 250 μg/ml followed by 22.08% at 100 μg/ml after 96 hr. of incubation (Table 6). The radial mycelial growth of *Gliocladium viride* was totally arrested at 500 μg/ml. Tolerance study of *Gliocladium viride* against Bavistin showed that *Gliocladium viride* could not tolerate even 100μg Bavistin/ml i.e, Bavistin totally inhibited the mycelial growth of *Gliocladium viride* even at 100μg/ml after 96 hrs incubation (Table 7). Tilt 250EC showed significant effect in arresting the mycelial growth of *Gliocladium viride* (Table 8). It was observed that Tilt 250EC reduced the mycelial growth of *Gliocladium viride* by 52.40% and 84.43% at 100 and 250 μg/ml, respectively. *Gliocladium viride* could not tolerate at 500 and 1000 μg Tilt 250EC /ml. Ridomil did not show any fungicidal effect upto 100μg/ml rather it increases the growth of *Gliocladium viride* significantly over untreated control. It induces *Gliocladium viride* growth upto 23.15% at 100 μg/ml while the mycelial growth was similar to untreated control at 250 μg/ml. Growth inhibition 1.29% was observed in *Gliocladium viride* at 500 μg/ml followed by 17.20% at 1000 μg/ml after 96 hour of incubation (Table 9). Thiovit has increased significantly the mycelial growth of *Gliocladium*

Table 8. Tolerance of *Gliocladium viride* against Tilt

Concentration (μg/ml)	Radial mycelial growth (mm)				% growth inhibition
	24hrs.	48hrs.	72hrs.	96hrs.	
Control	2.33	24.01	45.16	64.16	
100	0.00	0.00	13.2	31.00	52.40
250	0.00	0.00	2.10	10.00	84.43
500	0.00	0.00	0.00	0.00	100.00
1000	0.00	0.00	0.00	0.00	100.00
LSD (p=0.05)					

Data represents mean of five replications

Table 9. Tolerance of *Gliocladium viride* against Ridomil

Concentration (μg/ml)	Radial mycelial growth (mm)				% growth induction (+) or inhibition (-)
	24hrs.	48hrs.	72hrs.	96hrs.	
Control	2.33a	24.01a	45.16b	64.16b	
100	2.26a	26.66a	54.33a	79.00a	+23.15
250	1.76b	23.00a	42.66b	64.16b	0.00
500	0.00c	17.00b	39.00b	60.00b	-1.29
1000	0.00c	0.00c	30.00c	49.83c	-17.2
LSD (p=0.05)	0.2028	5.816	8.111	8.111	

Data represents mean of five replications

viride by 82.18% and 92.88% at 100 and 250 μg/ml, respectively over control. At higher concentration of 500 and 1000 μg/ml the mycelial growth was increased by 99.54% after 72hr of incubation (Table 10).

Table 10. Tolerance of *Gliocladium viride* against Thiovit

Concentration (μg/ml)	Radial mycelial growth (mm)			% growth induction
	24hrs.	48hrs.	72hrs.	
Control	2.33 e	24.00 e	45.16 d	
100	16.58 d	47.10 d	82.22 c	82.18
250	18.35 c	52.16 c	86.33 b	92.88
500	20.76 b	60.30 b	90.00 a	99.54
1000	24.56 a	66.00 a	90.00 a	99.54
LSD (p=0.05)	1.53	2.026	1.396	

Data represents mean of five replications

4.1.3. Tolerance of *Sclerotium rolfsii* to fungicides

Tolerance study clearly showed that *Sclerotium rolfsii* could not tolerate even 100µg Vitavax/ml i.e, Vitavax totally inhibited the mycelial growth of *Sclerotium rolfsii* even at 100µg/ml after 96 hr incubation (Table 11). It has been observed that Bavistin showed significant effect on inhibition of radial mycelial growth of *Sclerotium rolfsii* over untreated control by 86.18% at 1000 µg/ml followed by 61.59% at 250µg/ml, respectively after 96 hrs of incubation (Table 12). The treatment 100, 250, and 500 µg/ml resulted statistically similar effect on radial mycelial growth but they differ significantly from other treatments. *Sclerotium rolfsii* could not tolerate even 100µg Tilt 250EC and Ridomil i.e, both of them totally inhibited the mycelial growth of *Sclerotium rolfsii* at 100µg/ml after 96 hrs incubation (Table 13 &14). Thiovit showed significant effect on inhibition of radial mycelial growth of *Sclerotium rolfsii* over untreated control by 74.64% at 100 µg/ml followed by 69.22% at 500 µg/ml after 72 hours of incubation (Table 15).

Table 11. Tolerance of *Sclerotium rolfsii* against Vitavax

Concentration (µg/ml)	Radial mycelial growth (mm)				% growth inhibition
	24hrs.	48hrs.	72hrs.	96hrs.	
Control	4.00	38.00	80.83	90.00	
100	0.00	0.00	0.00	0.00	100.0
250	0.00	0.00	0.00	0.00	100.00
500	0.00	0.00	0.00	0.00	100.00
1000	0.00	0.00	0.00	0.00	100.00
LSD (p=0.05)					

Data represents mean of five replications

Table 12. Tolerance of *Sclerotium rolfsii* against Bavistin

Concentration (µg/ml)	Radial mycelial growth (mm)			% growth inhibition
	24hrs.	48hrs.	72hrs.	
Control	4.00a	38.00a	81.83a	
100	0.00b	3.56b	35.50b	52.17
250	0.00b	3.33bc	31.16b	61.59
500	0.00b	2.36c	29.66b	56.26
1000	0.00b	0.00d	11.33c	86.18
LSD (p=0.05)	0.5069	1.024	5.941	

Data represents mean of five replications

Table 13. Tolerance of *Sclerotium rolfsii* against Tilt

Concentration (µg/ml)	Radial mycelial growth (mm)			% growth inhibition
	24hrs.	48hrs.	72hrs.	
Control	4.00	38.00	81.83	
100	0.00	0.00	0.00	100.00
250	0.00	0.00	0.00	100.00
500	0.00	0.00	0.00	100.00
1000	0.00	0.00	0.00	100.00
LSD (p=0.05)				

Data represents mean of five replications

Table 14. Tolerance of *Sclerotium rolfsii* against Ridomil

Concentration (µg/ml)	Radial mycelial growth (mm)			% growth inhibition
	24hrs.	48hrs.	72hrs.	
Control	4.00	38.00	81.83	-
100	0.00	0.00	0.00	100.00
250	0.00	0.00	0.00	100.00
500	0.00	0.00	0.00	100.00
1000	0.00	0.00	0.00	100.00
LSD (p=0.05)				

Data represents mean of five replications

Table 15. Tolerance of *Sclerotium rolfsii* against Thiovit

Concentration (µg/ml)	Radial mycelial growth (mm)			% growth inhibition
	24hrs.	48hrs.	72hrs.	
Control	4.00a	38.00a	80.83a	
100	0.00b	1.83b	19.83c	75.64
250	0.00b	2.23b	27.83b	65.72
500	0.00b	2.53b	25.00bc	69.22
1000	0.00b	2.43b	25.33bc	68.82
LSD (p=0.05)	0.506	0.964	5.675	

Data represents mean of five replications

4.1.4. Tolerance of *Trichoderma harzianum* to fertilizers

Six fertilizers namely, Urea, Triple Super Phosphate, Murate of Potash, Gypsum, Manganese Sulphate and Copper Sulphate containing essential nutrients viz. N, P, K, S, Mn. and Cu were tested for their effect on the radial mycelial growth of *Trichoderma harzianum*. Addition of nitrogen to the culture medium resulted inhibition of mycelial growth upto 11.66% (Table 16). The highest inhibition of mycelial growth *of Trichoderma harzianum* has been noticed at 1000µg/ml. The mycelial growth of *Trichoderma harzianum* has been observed to be induced by the application of P in culture medium (Table 17). P at 100µm/ml showed maximum induction (7.2%) while the minimum (0.39%) was observed at 1000µm/ml. Addition of K to the culture medium resulted inhibition of mycelial growth of *Trichoderma harzianum* and the highest inhibition (7.54%) of mycelial growth was recorded at 1000µgK/ml and the lowest inhibition (0.24%) was observed in 250µgK/ml (Table 18). In case of S the mycelial growth of *Trichoderma harzianum* was not inhibited significantly though variation was found among the treatments (Table 19). Addition of Cu to the culture medium resulted an ameliorating effect on the radial mycelial growth of *Trichoderma harzianum* (Table 20). A gradual inductive effect with increase in Cu was observed and the highest (19.80%) induction was found at 1000µg/ml over

Table 16. Effect of N on radial mycelial growth of *Trichoderma harzianum*

Concentration (µg/ml)	Radial mycelial growth (mm)				% growth inhibition
	24hrs.	48hrs.	72hrs.	96hrs.	
Control	2.93ab	42.66	68.50ab	90.00a	
100	2.40b	41.33	69.83a	84.16b	6.48
250	3.00ab	39.66	61.50bc	81.16b	9.82
500	2.33a	41.16	68.50ab	90.00a	0.00
1000	3.00ab	32.33	58.50c	79.50b	11.66
LSD (p=0.05)	0.473	3.232	7.317	5.545	

Data represents mean of five replications

Table 17. Effect of P on radial mycelial growth of *Trichoderma harzianum*

Concentration (µg/ml)	Radial mycelial growth (mm)			% growth induction
	24hrs.	48hrs.	72hrs.	
Control	2.93b	42.66b	83.33b	
100	3.33b	53.16a	89.33a	7.20
250	4.46a	48.16ab	83.83b	0.65
500	4.53a	51.00a	87.66a	5.19
1000	4.43a	49.50a	83.66b	0.39
LSD (p=0.05)	0.6125	5.622	5.325	

Data represents mean of five replications

Table 18. Effect of K on radial mycelial growth of *Trichoderma harzianum*

Concentration (µg/ml)	Radial mycelial growth (mm)			% growth induction (+) or inhibition (-)
	24hrs.	48hrs.	72hrs.	
Control	2.93a	42.66ab	68.50ab	
100	2.83a	46.66a	73.33a	+7.04
250	2.00a	38.16b	68.33ab	-0.24
500	2.66a	39.33b	65.00b	-5.10
1000	2.66a	39.16b	63.33b	-7.54
LSD (p=0.05)	1.351	6.661	5.268	

Data represents mean of five replications

Table 19. Effect of S on radial mycelial growth of *Trichoderma harzianum*

Concentration (µg/ml)	Radial mycelial growth (mm)			% growth inhibition
	24hrs.	48hrs.	72hrs.	
Control	2.93 bc	42.66 a	68.50 a	
100	2.70 c	40.50 a	66.50 ab	2.91
250	3.13 ab	41.50 a	64.50 b	5.83
500	3.16 ab	40.16 a	66.00 ab	3.64
1000	3.33 a	41.83 a	66.16 ab	3.41
LSD (p=0.05)	0.2776	2.999	3.905	

Data represents mean of five replications

the untreated control. Contrary to that a gradual inhibition in the radial mycelial growth was observed while the medium was supplemented with Mn (Table 21). The highest inhibition (11.37%) was observed at 1000 µgMn/ml and the lowest at 100 µgMn/ml in comparison to untreated control.

Table 20. Effect of Cu on radial mycelial growth of *Trichoderma harzianum*

Concentration (ppm)	Radial mycelial growth (mm)			% growth induction
	24hrs.	48hrs.	72hrs.	
Control	2.93	42.66b	68.50c	
100	3.10	44.66ab	71.50b	4.35
250	2.93	44.00b	73.16b	6.75
500	2.76	45.00a	76.66ab	11.83
1000	3.13	47.50ab	82.16a	19.8
LSD	NS	3.22	3.026	

Data represents mean of five replications.
NS= Not significant

Table 21. Effect of Mn on radial mycelial growth of *Trichoderma harzianum*

Concentration (μg/ml)	Radial mycelial growth (mm)			% growth inhibition
	24hrs.	48hrs.	72hrs.	
Control	2.93	42.66a	68.50a	
100	3.06	43.00a	67.00a	2.18
250	3.03	41.00a	66.33a	3.15
500	3.03	39.00ab	65.66a	4.12
1000	2.80	34.66b	60.66b	11.37
LSD (p=0.05)	NS	4.58	4.97	

Data represents mean of five replications.

4.1.5. Tolerance of *Gliocladium viride* to fertilizers

Six fertilizers namely, Urea, Triple Super Phosphate, Murate of Potash, Gypsum, Manganese Sulphate and Copper Sulphate containing essential nutrients viz. N, P, K, S, Mn. and Cu were tested for their effect on the radial mycelial growth of *Trichoderma harzianum*. Addition of nitrogen to the culture medium resulted inhibition of mycelial growth upto 75.29% (Table 22). The highest inhibition of mycelial growth *of Gliocladium viride* has been noticed at 500µgN/ml. The mycelial growth of *Gliocladium viride* has been observed to be induced by the application of P in culture medium (Table 23 and Fig. 4). P at 1000µm/ml showed a maximum induction (13.24%), while the minimum (2.53%) was observed at 100µm/ml. In the study of adding K to the culture medium, the mycelial growth of *Gliocladium viride* was inhibited and the highest inhibition (75.81%) was found at 250 µg/ml and the lowest inhibition (67.49%) was observed at 1000 µg/ml (Table 24). S inhibited the mycelial growth of *Gliocladium viride* significantly and the highest inhibition (74.91%) was observed at 100µg/ml, while the lowest (67.14%) was found at 1000µg/ml (Table 25). Cu resulted an ameliorating effect on the radial mycelial growth of *Gliocladium viride* upto 11.62% at 100µg/ml (Table 26 and Fig. 5), but the

Table 22. Effect of N on radial mycelial growth of *Gliocladium viride*

Concentration (µg/ml)	Radial mycelial growth (mm)				% growth inhibition
	24hrs.	48hrs.	72hrs.	96hrs.	
Control	2.33a	17.63a	45.16a	64.16a	
100	0.00b	0.16b	5.217c	19.83b	69.06
250	0.00b	0.20b	6.33bc	16.00b	75.03
500	0.00b	0.20b	5.00c	15.83b	75.29
1000	0.00b	0.13b	8.66b	18.16b	71.66
LSD (p=0.05)	0.1242	13.38	2.923	9.310	

Data represents mean of five replications

Table 23. Effect of P on radial mycelial growth of *Gliocladium viride*

Concentration (µg/ml)	Radial mycelial growth (mm)				% growth induction
	24hrs.	48hrs.	72hrs.	96hrs.	
Control	2.33c	24.00c	45.16b	64.16b	
100	2.16c	25.16bc	43.66b	66.50c	2.53
250	2.56b	28.00ab	47.33b	66.00b	2.86
500	2.83a	28.33a	51.83a	71.33a	11.17
1000	2.90a	29.33a	53.16a	72.66a	13.24
LSD (p=0.05)	0.2151	2.868	4.321	4.505	

Data represents mean of five replications

Fig. 4. Comparative tolerance of *Gliocladium viride* to Phosphorus (P)

Table 24. Effect of K on radial mycelial growth of *Gliocladium viride*

Concentration (μg/ml)	Radial mycelial growth (mm)				% growth inhibition
	24hrs.	48hrs.	72hrs.	96hrs.	
Control	2.33a	24.00a	45.16a	64.16a	
100	0.00b	8.33b	10.83b	15.83b	75.29
250	0.00b	8.33b	11.33b	15.50b	75.81
500	0.00b	10.00b	13.16b	17.00b	73.47
1000	0.00b	10.66b	11.00b	20.84b	67.49
LSD (p=0.05)	0.1242	3.512	3.298	7.027	

Data represents mean of five replications

Table 25. Effect of S on radial mycelial growth of *Gliocladium viride*

Concentration (μg/ml)	Radial mycelial growth (mm)				% growth inhibition
	24hrs.	48hrs.	72hrs.	96hrs.	
Control	2.33a	24.00a	45.16a	64.16a	
100	0.00b	7.00b	13.33bc	18.50cd	74.91
250	0.00b	7.66b	10.66c	14.83d	75.42
500	0.00b	6.66b	14.50b	24.16b	73.09
1000	0.00b	6.66b	14.16bc	22.00bc	67.14
LSD (p=0.05)	0.1242	2.822	3.702	5.316	

Data represents mean of five replications

Table 26. Effect of Cu on radial mycelial growth of *Gliocladium viride*

Concentration (μg/ml)	Radial mycelial growth (mm)				% growth induction (+) or inhibition (-)
	24hrs.	48hrs.	72hrs.	96hrs.	
Control	2.33a	24.00a	45.16a	64.16a	
100	2.16ab	25.00a	48.33a	71.66a	+11.62
250	2.00b	25.33a	48.50a	68.33a	+6.480
500	1.90b	18.83b	32.16b	43.33b	-34.26
1000	1.66c	16.00b	27.50b	38.50b	-39.99
LSD (p=0.05)	0.2682	4.524	5.650	8.116	

Data represents mean of five replications

Fig. 5. Comparative tolerance of *Gliocladium viride* to Copper (Cu)

radial mycelial growth of *Gliocladium viride* was inhibited at 500µg/ml and the highest inhibition (39.99%) was found at 1000 µg/ml. It was observed that Mn inhibited the radial mycelial growth of *Gliocladium viride* when the medium was supplemented with Mn (Table 27). The highest (57.40%) inhibition was observed at 1000 µg/ml and the lowest (52.54%) at 500 µg/ml.

Table 27. Effect of Mn on radial mycelial growth of *Gliocladium viride*

Concentration (µg/ml)	Radial mycelial growth (mm)				%growth inhibition
	24hrs.	48hrs.	72hrs.	96hrs.	
Control	2.33a	24.00a	45.16a	64.16a	
100	0.00b	7.00b	10.50b	14.83b	54.62
250	0.00b	6.33b	11.33b	14.83b	53.32
500	0.00b	6.66b	11.00b	17.33b	52.54
1000	0.00b	6.33b	9.83b	13.83b	57.40
LSD (p=0.05)	0.1242	2.822	4.149	14.43	

Data represents mean of five replications

4.1.2.3. Tolerance of *Sclerotium rolfsii* to fertilizers

Addition of nitrogen to the culture medium resulted inhibition of mycelial growth of *Sclerotium rolfsii* upto 28.82% at 1000µgN/ml (Table 28). The mycelial growth of *Sclerotium rolfsii* has also been observed to be inhibited by the application of P in culture medium. P at 1000µm/ml showed maximum inhibition (100.00%), while the minimum (42.55%) was observed at 100µm/ml after 72 hrs of incubation (Table 29). Addition of K to the culture medium exerted inhibition of the mycelial growth of *Sclerotium rolfsii* and the highest inhibition (69.63%) of mycelial growth was found at 1000 µg/ml and the lowest inhibition (25.45%) was observed at 100 µg/ml (Table 30). In case of S the mycelial growth of *Sclerotium rolfsii* was inhibited significantly, while the highest count (44.67%) was made at 1000µg/ml and the lowest (24.83%) at 100µg/ml (Table 31). Cu resulted a gradual inhibition of radial mycelial growth of *Sclerotium rolfsii*. The highest growth inhibition (43.77%) of *Sclerotium rolfsii* was observed at 1000µgCu/ml, while the lowest (4.52%) was recorded at 100µgCu/ml (Table 32). The highest (42.35%) mycelial growth inhibition of *Sclerotium rolfsii* was observed at 1000 µgMn/ml and the lowest (24.38%) at 100 µgMn/ml in comparison to untreated control (Table 33).

Table 28. Effect of N on radial mycelial growth of *Sclerotium rolfsii*

Concentration (µg/ml)	Radial mycelial growth			% growth inhibition
	24hrs.	48hrs.	72hrs.	
Control	4.20a	37.30a	80.10 a	
100	3.10c	30.50b	58.70 b	26.70
250	3.26bc	28.60b	57.30 b	28.45
500	3.24bc	30.30b	59.30 b	25.95
1000	3.28b	28.80b	57.00 b	28.82
LSD (p=0.05)	0.584	2.622	3.579	

Data represents mean of five replications

Table 29. Effect of P on radial mycelial growth of *Sclerotium rolfsii*

Concentration (µg/ml)	Radial mycelial growth (mm)			% growth inhibition
	24hrs.	48hrs.	72hrs.	
Control	4.20a	37.30a	80.10a	
100	3.10b	23.00b	46.00b	42.55
250	0.00c	25.10b	43.60b	45.55
500	0.00c	3.20c	8.54c	89.35
1000	0.00c	0.00c	0.00c	99.96
LSD (p=0.05)	1.756	5.176	11.31	

Data represents mean of five replications

Table 30. Effect of K on radial mycelial growth of *Sclerotium rolfsii*

Concentration (µg/ml)	Radial mycelial growth (mm)			% growth inhibition
	24hrs.	48hrs.	72hrs.	
Control	4.20a	37.30a	80.10a	
100	3.00b	30.70b	59.70b	25.45
250	2.90bc	27.60c	54.40c	32.07
500	2.40c	24.30d	48.50d	39.43
1000	1.30d	11.10e	24.30e	69.63
LSD (p=0.05)	0.585	2.752	4.629	

Data represents mean of five replications

Table 31. Effect of S on radial mycelial growth of *Sclerotium rolfsii*

Concentration (µg/ml)	Radial mycelial growth (mm)			% growth inhibition
	24hrs.	48hrs.	72hrs.	
Control	4.20a	37.30a	80.10a	
100	1.22b	30.90b	60.20b	24.83
250	0.00c	27.90c	52.30c	34.69
500	0.00c	28.30c	51.90c	35.19
1000	0.00c	28.00c	44.30d	44.67
LSD (p=0.05)	0.4613	2.290	4.161	

Data represents mean of five replications

Table 32. Effect of Cu on radial mycelial growth of *Sclerotium rolfsii*

Concentration (μg/ml)	Radial mycelial growth (mm)			% growth inhibition
	24hrs.	48hrs.	72hrs.	
Control	4.00a	37.83ab	80.66a	
100	3.06b	35.83ab	77.00a	4.52
250	2.66b	34.83ab	64.50ab	20.02
500	2.56c	33.06b	54.33ab	32.62
1000	1.96c	26.80b	45.33b	43.77
LSD (p=0.05)	0.629	3.26	7.11	

Data represents mean of five replications

Table 33. Effect of Mn on radial mycelial growth of *Sclerotium rolfsii*

Concentration (μg/ml)	Radial mycelial growth (mm)			% growth inhibition
	24hrs.	48hrs.	72hrs.	
Control	4.00a	37.83ab	80.83a	
100	3.06b	37.00ab	60.20b	24.83
250	2.86b	35.44b	54.40c	32.07
500	2.59bc	33.05b	52.33c	34.53
1000	2.46c	26.60b	45.33d	42.35
LSD (p=0.05)	0.6291	11.96	5.204	

Data represents mean of five replications

4.2. Experiment II

4.2.1. Growth study of *Trichoderma harzianum, Trichoderma hamatum, Trichoderma viride* and *Gliocladium viride*

Radial mycelial growth of *Trichoderma harzianum, Trichoderma harzianum Trichoderma hamatum, Trichoderma viride and Gliocladium viride* were studied on PDA. At first the colony colour was whitish and later it turn bright green with floccose texture. The radial mycelial growth of the isolates varied significantly from each other after 24 hrs, 48 hrs, 72hrs and 96 hrs of growth (Table 34). After 24 hrs the maximum radial mycelial growth was found in *Trichoderma harzianum* (2.93 mm) followed by *Gliocladium viride* (2.60 mm), *Trichoderma hamatum* (2.33 mm) and *Trichoderma viride* (2.00 mm). This trend was observed until 72hrs of incubation. After 96 hrs of incubation, the highest mycelial growth was recorded in *Trichoderma harzianum* (90.00 mm) followed by *Gliocladium viride* (78.00 mm) and *Trichoderma viride* (56.50 mm). The lowest radial mycelial growth (54.16 mm) was recorded in case of *Trichoderma hamatum*.

Table 34. Radial mycelial growth of *Trichoderma* and *Gliocladium* (PDA medium)

Trichoderma/Gliocladium	Radial mycelial growth (mm) after			
	24 h	48 h	72h	96h
Trichoderma harzianum	2.93 a	42.66 a	68.50 a	90.00 a
Trichoderma hamatum	2.33bc	24.00 c	45.16 c	54.16 c
Trichoderma viride	2.00 c	23.16 c	37.66 d	56.50 d
Gliocladium viride	2.60ab	29.66 b	57.16 b	78.00 b
LSD (p=0.05)	0.445	3.73	5.66	4.76

Data represents mean of five replications

4.2.2. Interaction study

4.2.2.1. Interaction of *Trichoderma* and *Gliocladium* with *Sclerotium rolfsii* (7 days of incubation period)

Trichoderma and *Gliocladium* inhibited the growth of *Sclerotium rolfsii* (Table 35). In case of control (only *S. rolfsii*), the fungus grew well and produced luxuriant mycelia with sclerotia.

4.2.2.2. Interaction of *Trichoderma* and *Gliocladium* with *Sclerotium rolfsii* (15 days of incubation)

It was observed that in all the cases *Trichoderma* and *Gliocladium* grew over the *Sclerotium rolfsii* and lysed zone was formed at the point of meeting though Sclerotia of *Sclerotium rolfsii* were formed but the *Trichoderma* and *Gliocladium* grew over Sclerotia (Table 35). In case of control, luxuriant mycelial growth along with huge Sclerotia of *Sclerotium rolfsii* were observed.

Table 35. Interaction of *Trichoderma* and *Gliocladium* with *Sclerotium rolfsii* (dual culture method)

Trichoderma/Gliocladium	Interaction after	
	7 days	15 days
Trichoderma harzianum	TIS	TGS + Lysed
Trichoderma hamatum	TIS	TGS + Lysed
Trichoderma viride	TIS	TGS + Lysed
Gliocladium viride	GIS	GGS + Lysed

TIS = *Trichoderma* inhibited growth of *Sclerotium rolfsii*
GIS = *Gliocladium* inhibited growth of *Sclerotium rolfsii*
TGS = *Trichoderma* grew over *Sclerotium rolfsii.*
GGS = *Gliocladium* grew over *Sclerotium rolfsii*

4.2.2.3. Test of Sclerotia of *Sclerotium rolfsii* which were over grown by *Trichoderma* and *Gliocladium*

The Sclerotia which were over grown by antagonists, *Trichoderma* and *Gliocladium*, were tested by growing on PDA. It was observed that the sclerotia of *Sclerotium rolfsii* could not grow on PDA. On the other hand, *Trichoderma* and *Gliocaldium* were observed that they grew luxuriantly and produced huge number of conidia and mycelium. Moreover, lysed mycelial blocks of *S. rolfsii* along with the culture (PDA) were transferred to PDA. The growth of *Trichoderma* and *Gliocladium* have been observed but no growth of *Sclerotium rolfsii* was noticed (Fig. 6).

4.2.2.4. Treatment of Sclerotia of *Sclerotium rolfsii* with the conidia of antagonists, *Trichoderma* and *Gliocladium*

The sclerotia that were treated with the conidia of *Trichoderma* and *Gliocladium* separately were tested by growing on PDA. It was observed that *Trichoderma* and *Gliocaldium* were grown luxuriantly and produced huge number of conidia and mycelium, but the treated sclerotia of *S. rolfsii* could not germinate on PDA (Fig. 7). In case of control, sclerotia of *Sclerotium rolfsii* were easily germinated and showed luxuriant mycelial growth.

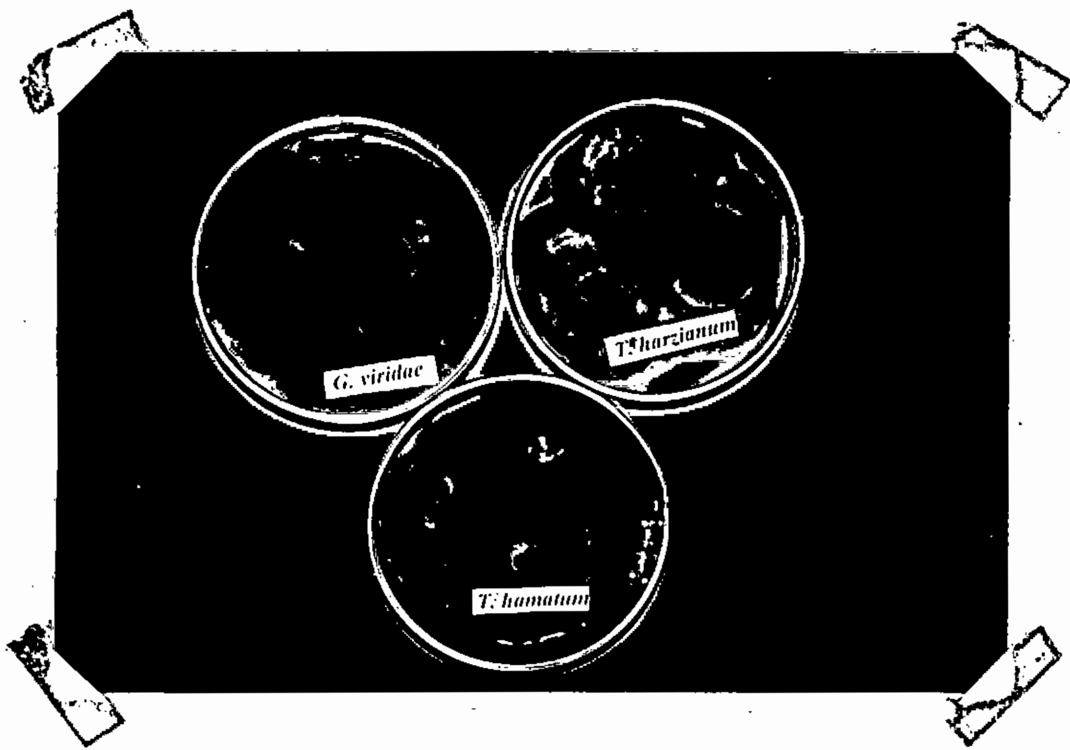


Fig. 6. Growth of *Trichoderma harzianum, Trichoderma hamatum* and *Gliocladium viride* from lysed zone

4.3. Experiment III

4.3.1 Effect of *Trichoderma* and *Gliocladium* on germination of seeds of Pulses

Germination of the seeds of some important pulses (Mungbean, Blackgram and Pigeon pea) has been found to differ significantly among the various treatments (Table 36 and Figs. 8 & 9). In case of mungbean the maximum germination was 87.66% obtained by using T_2 (*Trichoderma harzianum*) and minimum was recorded (72%) in case of T_1 (control). Treatments T_3, T_4 and T_5 yielded 82.00, 81.00 and 80.33% germination resulting 13.8, 12.42 and 11.49% increase in germination, respectively over T_1 (control). The treatments T_3, T_4 and T_5 showed statistically similar effect on per cent germination while T_2 differed significantly from other treatments proving its highest performance (21.61% increase in germination) over untreated control.

In case of Blackgram, antagonists showed significantly different effect in case of germination. Highest germination (65.00%) was found in case of T_3 (*Trichoderma hamatum*) followed by T_2 (56.66%), T_4 (52.66%), and T_5 (48.00%) resulting 53.50%, 33.81%, 24.37% and 13.38% increase in germination, respectively. The lowest germination (42.33%) was recorded in case of T_1 (Control). Treatment T_2 and T_4 showed statistically similar effect

Table 36. Effect of *Trichoderma/Gliocladium* on germination of some pulses (standard blotter method)

Trichoderma/ Gliocladium	Germination (%)		
	Mungbean	Blackgram	Pigeon pea
T_1=Control	72.00 c	42.33 d	45.00 c
T_2=*Trichodermaharzianum*	87.66 a (21.61)	56.66 b (33.81)	57.00 a (26.64)
T_3=*Trichoderma hamatum*	82.00 b (13.8)	65.00 a (53.50)	53.66 ab (19.22)
T_4=*Trichoderma viride*	81.00 b (12.42)	52.66 bc (24.37)	51.00 b (13.32)
T_5=*Gliocladium viride*	80.33 b (11.49)	48.00 c (13.38)	52.66 ab (17.00)
LSD (p=0.05)	4.26	5.169	4.83

100 seeds per treatment were treated in three replications

( ) data in parentheses indicate per cent increase over control

Fig. 8. Germination of Blackgram seed

a. Untreated (control) seeds
b. *Gliocladium viride* treated seeds

Fig. 9. Germination of Blackgram seed

a. Untreated (control) seeds
b. *Trichoderma harzianum* treated seeds

on germination as of T_4 and T_5, but all the treatments differed significantly from T_1(control).

In case of Pigeon pea the highest (57%) germination was found in case of T_2 (*Trichoderma harzianum*) that resulted 26.26% increase in germination followed by 19.22, 13.32 and 17% increase by T_3 (*Trichoderma hamatum*), T_4 (*Trichoderma viride*), and T_5 (*Gliocladium viride*) respectively, over untreated control. The treatments T_3, T_4 and T_5 shows statistically similar effect on per cent germination of seeds of Pigeon pea while T_2 (*Trichoderma harzianum*) differed significantly from other treatments.

4.3.2. Effect of *Trichoderma* and *Gliocladium* on incidence of seed borne mycoflora of pulses

It had been observed that *Trichoderma* and *Gliocladium* showed significant effect on seed borne mycoflora in Mungbean (Table 37). Significant variation in reduction of seed borne mycoflora (*Aspergillus, Pnenicillium, Rhizopus and Fusarium*) were observed among the tested antagonists. All the antagonists used in the study were found highly effective in controlling seed borne *Fusarium*. Similar significant antagonistic effect was found in case of Black gram (Table 38) and Pigeon pea (Table 39 and Fig. 10).

Table 37. Effect of *Trichoderma* and *Gliocladium* on incidence of Seed borne pathogens in Mungbean (Standard Blotter Method)

Trichoderma/ Gliocladium	% Mean incidence of			
	Aspergillus	*Penicillium*	*Rhizopus*	*Fusarium*
T_1=Control	13.66 a	8.50 a	12.66 a	2.33 a
T_2=*Trichoderma harzianum*	3.00 b	2.73 b	3.5b cb	0.00 b
T_3=*Trichoderma hamatum*	4.00 b	1.66 bc	1.93 c	0.00 b
T_4=*Trichoderma viride*	3.33 b	0.00 c	4.66 bb	0.00 b
T_5=*Gliocladium viride*	3.66 b	1.66 b	4.00 bc	0.00 b
LSD (p=0.05)	2.48	1.98	5.53	1.55

100 seeds per treatment were treated in three replications

Table 38. Effect of *Trichoderma* and *Gliocladium* on the incidence of Seed borne pathogens in Blackgram (Standard Blotter Method)

Trichoderma/ Gliocladium	% Mean incidence of			
	Aspergillus	*Penicillium*	*Rhizopus*	*Fusarium*
T_1=Control	7.66a	28.66a	12.33a	4.66 a
T_2=*Trichoderma harzianum*	2.00c	6.00bc	4.00b	0.00 c
T_3=*Trichoderma hamatum*	2.66bc	3.66c	4.33b	1.66 b
T_4=*Trichoderma viride*	3.66b	8.00b	4.00b	0.00 c
T_5=*Gliocladium viride*	2.33c	5.66bc	4.00b	0.00 c
LSD (p=0.05)	1.17	2.48	2.41	0.82

100 seeds per treatment were treated in three replications

Table 39. Effect *Trichoderma* and *Gliocladium* on the incidence of Seed borne pathogens in Pigeon pea (standard blotter method)

Trichoderma/Gliocladium	% Mean incidence of			
	Aspergillus	*Penicillium*	*Rhizopus*	*Fusarium*
T_1=Control	16.33 a	46.66 a	4.33 a	2.66a
T_2=*Trichoderma harzianum*	11.33 b	14.00 b	0.00	1.33b
T_3=*Trichoderma hamatum*	6.33 c	13.00 b	0.00	0.00
T_4=*Trichoderma viride*	6.66 c	12.00 b	0.00	0.00
T_5=*Gliocladium viride*	11.00 b	8.33 c	0.00	0.00
LSD (p=0.05)	2.689	2.190	1.171	0.826

100 seeds per treatment were treated in three replications.

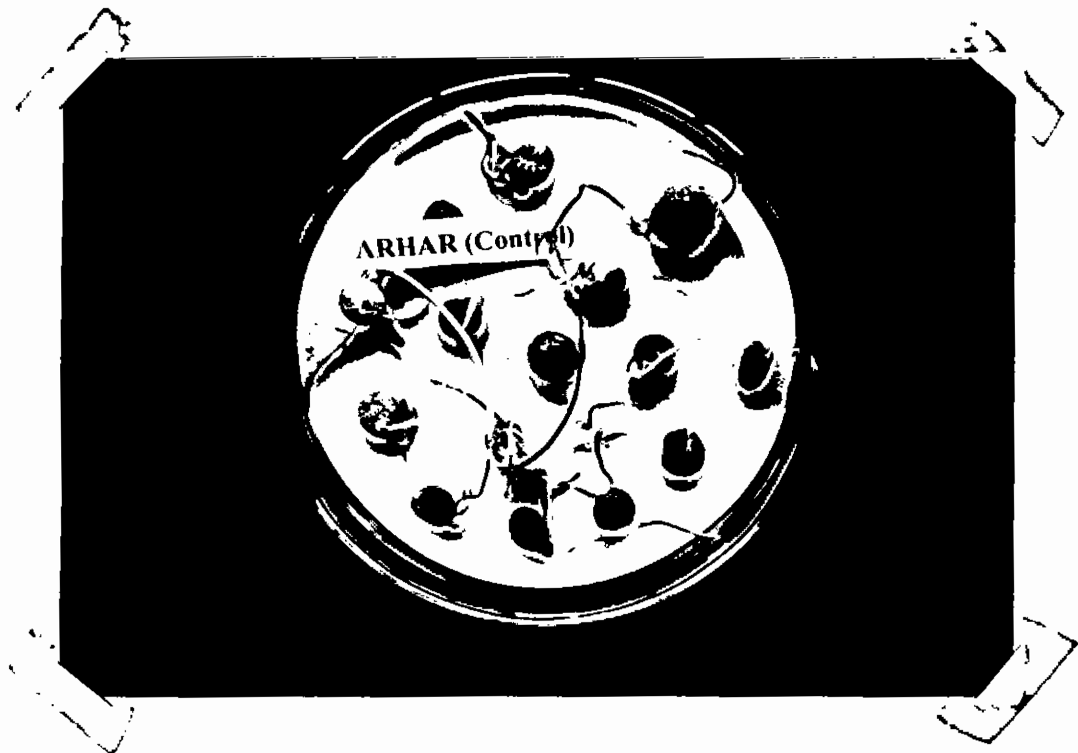


Fig. 10. Growth of different seed-borne mycoflora from Pigeon pea seeds

4.3.3. Effect of *Trichoderma* and *Gliocladium* on shoot length of Mungbean and Blackgram (TP method)

The seed treating biocontrol agents, *Trichoderma* and *Gliocladium* showed excellent inducing effect on shoot length of mungbean and blackgram (Table 40). In case of mungbean the shoot length ranged from 60.63mm to 91.06 mm, while in blackgram form 61.33 to 86.95mm. The effect of bioagents on shoot length of mungbean and blackgram differed significantly over the untreated control and also among themselves. In all the cases, the lowest shoot length was recorded in seedlings under control. *Trichoderma harzianum* and *Trichoderma hamatum* exerted highest effect by showing maximum increase in shoot length by 91.06% and 86.95%, respectively over control.

Table 40. Effect of *Trichoderma* and *Gliocladium* on shoot length of Mungbean and Blackgram (Standard Blotter Method)

Trichoderma/ Gliocladium	Shoot length (mm)	
	Mungbean	Blackgram
T_1=Control	60.63d	61.33e
T_2=*Trichoderma harzianum*	91.06a	75.60d
T_3=*Trichoderma hamatum*	88.66ab	86.95a
T_4=*Trichoderma viride*	83.20c	83.93b
T_5=*Gliocladium viride*	87.33b	80.38c
LSD (p=0.05)	3.697	2.622

100 seeds per treatment were treated in three replications.

4.4. Experiment No IV

4.4.1. Effect of *Trichoderma* and *Gliocladium* on germination of Tomato (Standard blotter method)

Significant increase in germination of tomato seeds over the control was obtained when seeds were treated with *Trichoderma* and *Gliocladium* (Table 41 and Fig. 11). The germination of Tomato seeds ranged from 53.56% to 79.6%, and the highest germination was observed in case of seed treated *with Trichoderma harzianum* (TG-2) that resulted 48.43% increase in germination over untreated control. Treatments T_4 (*Trichoderma harzianum* TS-1) and T_9 (*Gliocladium viride*) gave 77.93% and 77.67% germination resulting 45.32% and 44.84% higher germination over control and showed statistically similar effect as of T_6 (*Trichoderma harzianum* TG-2).

4.4.2. Effect of *Trichoderma* and *Gliocladium* on the incidence of seed borne pathogen of Tomato (Standard blotter method)

It was observed that *Trichoderma* and *Gliocladium* showed significant effect on seed borne pathogen of Tomato (Table 42). Significant variations in reduction of seed borne mycoflora (*Aspergillus, Pnenicillium, Rhizopus and Fusarium*) were recorded among the tested antagonists. All the antagonists used in the present study were found to be highly effective in controlling seed borne pathogens of Tomato.

Table 41. Effect of *Trichoderma* and *Gliocladium* on germination of Tomato (standard blotter method)

Trichoderma/Gliocladium	Germination (%)	% increase over control
T_1= Control	53.56 g	
T_2= *Trichoderma harzianum* (Tch_3)	73.40 cd	36.90
T_3= *Trichoderma harzianum* (TL_1)	68.66 e	28.08
T_4= *Trichoderma harzianum* (TS_1)	77.93 ab	45.32
T_5= *Trichoderma harzianum* (TBg_1)	62.00 f	15.56
T_6= *Trichoderma harzianum* (TG_2)	79.60 a	48.43
T_7= *Trichoderma hamatum*	75.40 bc	40.62
T_8= *Trichoderma viride*	70.66 d	31.80
T_9= *Gliocladium viride*	77.67 ab	44.84
LSD (p=0.05)	2.90	

200 seeds were tested for each isolate or species of *Trichoderma/ Gliocladium*

Table 42. Effect of *Trichoderma* and *Gliocladium* on the incidence of seed borne pathogen in Tomato (standard blotter method)

Trichoderma/Gliocladium	% Mean incidence of			
	Fusarium	*Penicillium*	*Rhizopus*	*Aspergillus*
T_1= Control	17.66a	21.66a	13.33a	14.66a
T_2= *Trichoderma harzianum* (Tch_3)	3.00bc	0.00c	1.1c	0.66c
T_3= *Trichoderma harzianum* (TL_1)	2.66c	3.33b	3.0b	2.33bc
T_4= *Trichoderma harzianum* (TS_1)	4.66b	2.66b	0.93c	1.33bc
T_5= *Trichoderma harzianum* (TBg_1)	3.00bc	3.00b	1.73bc	1.33bc
T_6= *Trichoderma harzianum* (TG_2)	2.66c	2.33b	1.33c	3.00bc
T_7= *Trichoderma. Hamatum*	3.33bc	2.66b	1.67bc	1.33bc
T_8= *Trichoderma viride*	3.66bc	2.66b	1.33c	1.66bc
T_9= *Gliocladium viride*	3.00bc	0.00c	0.66c	1.66bc
LSD (p=0.05)	1.883	1.404	1.171	2.321

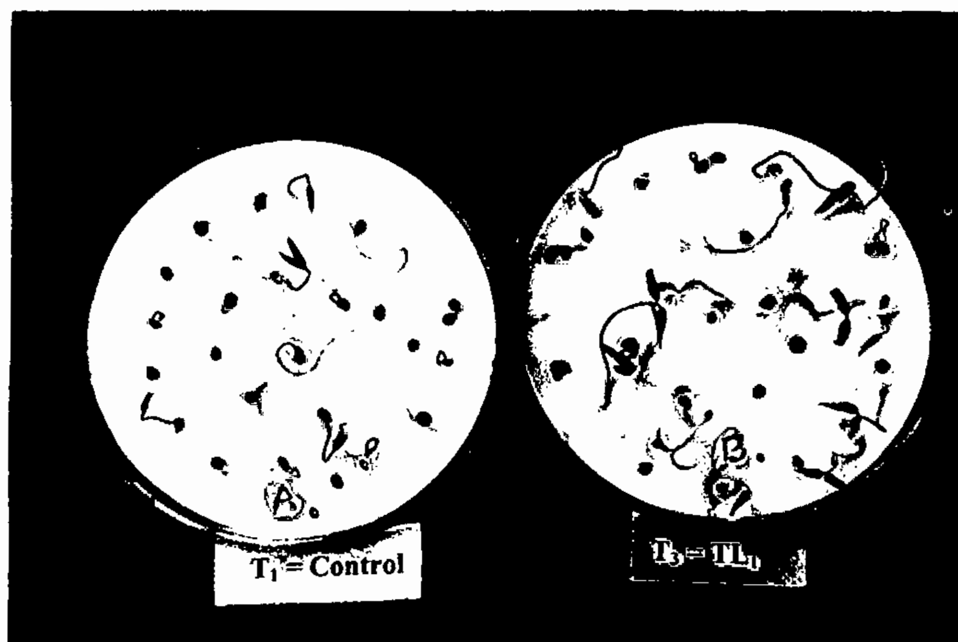


Fig. 11. Germination of seeds of Tomato c.v Ratan

A. Untreated (control) seed

B. *Trichoderma harzianum* (TL-1) treated seed

4.5. Experiment V

4.5.1. Effect of different isolates of *Trichoderma harzianum* on germination and seedling vigor of Tomato under field condition.

4.5.1.1.Germination

Trichoderma harzianum showed significant effect on germination of tomato seeds (Table-43). The lowest germination (60.12%) was found in case of untreated control and the highest germination (71.58%) was recorded when seeds were treated with the conidia of *Trichoderma harzianum* isolate TCh3. The isolates TG-2, TL-1 and Tch-3 resulted statistically similar effect on germination. Again the isolates TBg-1 and TS-1 showed statistically similar effect on germination.

4.5.1.2. Shoot length

Shoot length under different treatments ranged from 30.03 to 37.92 cm, while the highest and the lowest counts were made in *Trichoderma harzianum*(TS-1) and in untreated control, respectively (Table 43 and Fig. 12). It was observed that the isolates of *Trichoderma harzianum* showed significantly higher shoot length over control.

4.5.1.3. Root length

Root length under different treatments ranged from 11.40 to 14.89cm, while the highest and the lowest results were obtained in seedlings that received *Trichoderma harzianum*(TS-1) and in untreated control, respectively (Table 43 and Fig. 13).

4.5.1.4.Shoot weight

It has been observed that *Trichoderma harzianum* induced significantly increased the shoot weight of Tomato (Table 43). The highest shoot weight (198.16g) was induced by applying *Trichoderma harzianum* (TCh-3) and it differed significantly from all other treatments and the lowest shoot weight (178.96g) was recorded when *Trichoderma harzianum* (TBg-1) was used.

4.5.1.5. Root weight

The effect of isolates differed significantly in case of root weight. Isolates TBg-1, TG-2, TL-1, Tch3 and control showed similar effect on root weight. The highest root weight (24.92g) was found by using isolate TS-1 and it differed significantly from the rests.

4.5.1.6. Vigor Index

The vigor index varied from 2490.77 to 3434.36. The highest and lowest vigor index (VI) were determined in case of application of *Trichoderma harzianum* (TCh-3) and control, respectively (Table 43).

Table 43. Effect of different isolates of *Trichoderma harzianum* on germination and seedling vigor under field conditions

Trichoderma isolates	Germination (%)	MRL (cm)	MSL (cm)	MRW (g)	MSW(g)	VI
Control	60.12c	11.40ab	30.03c	21.18ab	185.40a	2490.77
TBg-1	64.51b	12.50b	34.34b	19.61b	178.96ab	3021.64
TG-2	71.08a	13.69ab	33.23b	18.74b	189.93abc	3335.07
TL-1	70.50a	13.72ab	34.20b	19.56b	190.43abc	3378.36
TS-1	65.03b	14.89a	37.92a	24.92a	193.00bc	3434.36
TCh-3	71.58a	13.69ab	33.20b	22.91ab	198.16c	3356.38
LSD (p=0.05)	1.777	2.38	2.99	5.06	18.95	

Value are the means of three replications of 20 plants each

MRL = Mean Root Length
MSL = Mean Shoot Length
MSW = Mean Shoot Weight
MRW = Mean Root Weight
VI = Vigor Index

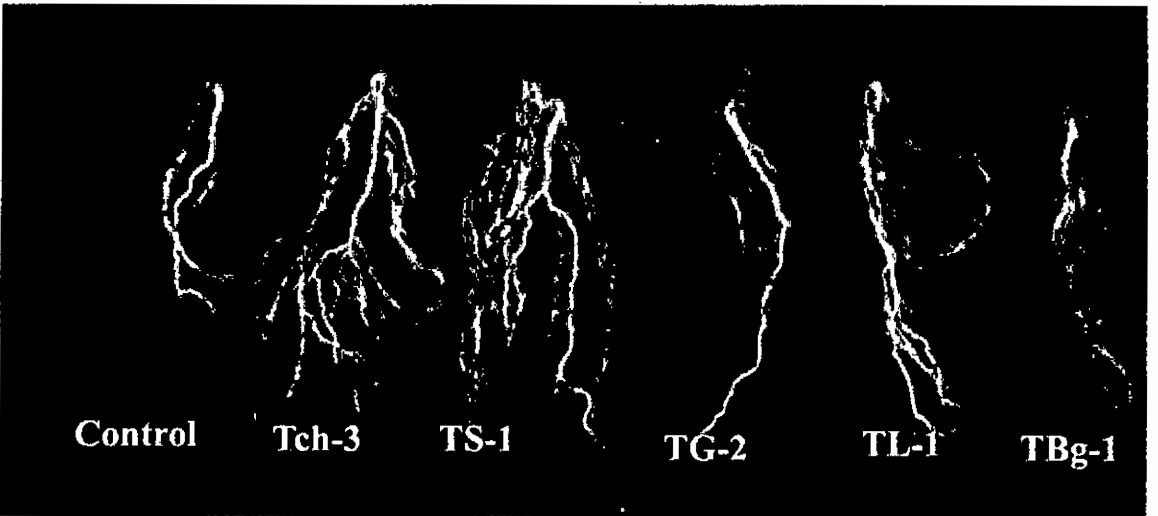


Fig. 13. Variation in root length of Tomato cv. Ratan due to seed treatment with different isolates of *Trichoderma harzianum*

DISCUSSION

5. DISCUSSION

Investigations were carried out to find out the fitness of *Trichoderma* and *Gliocladium* for controlling soil borne *Sclerotium rolfsii* and seed borne mycoflora of pulses and tomato in traditional agricultural practices where use of agrochemicals is a common phenomena. Now a days huge number of agrochemicals viz. fungicides, pesticides and metal salts are being used. The use of aforesaid chemicals in soil environment exerting heavy pressure on microbial populations. Thus, making the soil environment imbalance and disturbing the beneficial microorganisms resulting environmental hazards. Keeping these points in mind, the present studies were carried out in different steps. The tolerance of *Trichoderma* and *Gliocladium* to commonly used fungicides (Vitavax, Bavistin, Tilt 250EC, Ridomil and Thiovit) is imperative for their application as biocontrol agents in plant disease control program. *Trichoderma* could tolerate Vitavax, Tilt 250EC, Ridomil and Thiovit at 1000µg/ml, where *Gliocladium* could grow on PDA containing Ridomil and Thiovit. These findings are supported by Kaur and Mukhopodhayay (1992), Lacicowa and Pieta (1994), Mondal et al. (1995) and Karpagalli (1997). Wokocha (1990) applied *Trichoderma viride* with Thiram simultaneously for controlling *Sclerotium rolfsii* and obtained 19.0%

DSI. In the present study *Trichoderma* and *Gliocladium* showed sensitive reaction to Bavistin. This is in accordance with the findings of Mondal et al. (1995), Mehta et al.(1995) and Karpagavalli (1997). The effect of the tested fungicides on the growth of *Sclerotium rolfsii* is profound. *S. rolfsii* could not tolerate Vitavax, Tilt 250EC and Ridomil even at 100μg/ml , where Bavistin and Thiovit reduced the growth of *Sclerotium rolfsii* by 86.2% and 75.7%, respectively at 1000μg/ml and 100μg/ml. The findings of the present study are supported by other researchers (Agnihotri et al. 1975; Yahia et al. 1979; Peshney and Moghe, 1980; Motikhaye, 1983; Anilkumar and Gowda, 1984; Srikant et al. 1986; Fahim et al. 1986; Palashshappa et al. 1987; Ashgari and Mayee, 1991; Raman, 1994 and Rondon et al. 1995). But Parcha et al. (1988) reported that Vitavax at 100μg/ml and Ridomil at 1000μg/ml were less effective in controlling *Sclerotium rolfsii.*

Application of N, K, S and Mn to the culture medium exhibited inhibition and P and Cu resulting slight increase in growth of *Trichoderma*, but except P, the applied metals reduced the growth of *Gliocladium.* Application of metal salts in higher concentration in medium resulted higher reduction of growth of *Gliocladium*. The increase/decrease in growth of *Trichoderma* due to agrochemicals has been supported by Liyange(1983), Sharma et al. (1995)

and Duggy et al. (1997). Inclusion of metal salts to medium profoundly decrease the growth of *Sclerotium rolfsii*. Ortega et al. (1992) reported increased yield and dry matter production of rice fertilization with N, P and K for controlling stem rot disease.

The radial mycelial growth of different species of *Trichoderma* and *Gliocladium* differed significantly from one to another. This variation may be due to difference in genetic make up of the antagonists. The antagonist, *T. harzianum* was grown on PDA and its conidial productivity has been studied. Use of PDA medium has been supported by Cliquent and Scheffer (1997) and they mentioned that PDA is the best substrate and it enhanced the viability of *T. harzianum*. Interaction study of *Trichoderma* and *Gliocladium* with *Sclerotium rolfsii* resulted that *Trichoderma* and *Gliocladium* inhibited the growth of *S. rolfsii*. They grew over *S. rolfsii* and lysed mycelia and slcerotia of *S. rolfsii*. This is in accordance with the findings of Begum et al. (1998) and Sultana (1999) and Sultana and Hossain (2000). The antagonistic effect of *T. harzianum* of the present study has also been supported by other researchers. Elad *et al.* (1982) obtained high degree of antagonistic activity of *T. harzianum* against *S. rolfsii* as *T. harzianum* excreted β-1, 3-glucanase and chitinase. Jacobs and Kamoen (1986) mentioned cell wall lysing enzymes produced by *T. harzianum* responsible for antagonistic activity against plant pathogens. Henis *et al.* (1982) reported that *Trichoderma* produced volatile and non-volatile antibiotics which were responsible for

inhibition of *S. rolfsii* and suppression of sclerotia germination. But Ferrata and D'Ambra (1985) obtained high antagonistic effect of *Trichoderma harzianum* against *S. rolfsii* because of penetration and growing of *T. harzianum* inside *S. rolfsii.* This is also supported by Mutto *et al.* (1986), while Upadhyay and Mukhopadhyay (1986) reported direct attack and lysis of sclerotia and mycelia of *S. rolfsii* by *T. harzianum.*

Treatment of seeds of pulses (mungbean, blackgram and pigeon pea) and tomato with conidia of *Trichoderma* and *Gliocladium* were used. It was observed that the antagonists did not produce any phytotoxic effect on germination rather induce germination. This has been supported directly by Begum et al. (1998), Sultana (1999), Sultana and Hossain (2000). Sumitha and Gaikwad (1995) observed no adverse effect of *T. harzianum* on pigeon pea seed germination. The increase in germination of the treated seeds of pulses may be due to induction or stimulation by *Trichoderma/Gliocladium* or suppression of growth of seed borne fungi. In the study seed treatment with *Trichoderma* and *Gliocladium* significantly decreased the seed borne mycoflora. This result is strongly supported by Begum et al. (1998) and Sultana (1999). It has been observed that treatment with *Trichoderma* and *Gliocladium* significantly increased seedling growth of pulses (mungbean and blackgram) and tomato. According to Sumitha and Gaikwad (1995). Seeds coated with *T. harzianum* induced germination better than untreated seeds and produced longer roots and shoots. Application of *Trichoderma* and *Gliocladium* as seed treating biocontrol agent resulted longer shoot and root

length over control. That may be due to stimulation incited by antagonists. This is in accordance with the findings of Sumitha and Gaikwad (1995). They coated seeds with *T. harzianum* and obtained longer shoots and roots when sown in either infested or sterilized soil. Moreover, Khan *et al.* (1997) proved from their investigation that *T. harzianum* application showed stronger inhibition of *Fusarium solani* and resulted 35% incrase in plant growth. As *T. harzianum* increased shoot and root length of lentil cv. Barimasur-1, there was also higher weight of plants (either dry weight or weight of plants just after harvest). This has been achieved by treating seeds with *T: harzianum* isolates (Sultana, 1999). Monaco et al. (1991) treated seeds with *Trichoderma* and obtained significantly higher seedling emergence by suppressing *Fusarium* and *Sclerotium*. Seedling vigor induction of the present study has been supported by Krishnamoorthy and Bhaskaran (1990).

The findings of the present investigations clearly pointed out the fitness of *Trichoderma* and *Gliocladium* to commonly used fungicides and metal salts by showing their tolerance and exerting high potentiality against seed borne mycoflora of pulses (mungbean, blackgram and pigeon pea) along with tomato and soil borne *Sclerotium rolfsii*, in vitro and micro plot trials. The use of *Trichoderma* and *Gliocladium* as potential biocontrol agents need to be further investigated in elaborate form under field condition.

CHAPTER 6

SUMMARY

6. SUMMARY

Experiments were carried out to explore the possibility of finding out the fitness of biocontrol agents with conventional chemical control strategies for management of soil borne *Sclerotium rolfsii* and seed borne mycoflora of pulses and tomato. The conducted experiments were:

1. Tolerance of *Trichoderma harzianum*, *Gliocladium viride* and *Sclerotium rolfsii* to five different fungicides (viz. Vitavax 200, Bavistin, Tilt 250EC, Ridomil MZ-72, Thiovit) and six fertilizers (viz. Urea, Triple Super Phosphate, Murate of Potash, Gypsum, Manganese Sulphate and Copper Sulphate)
2. Antagonistic activity of *Trichoderma harzianum, Trichoderma hamatum, Trichoderma viride*, and *Gliocladium viride* against *Sclerotium rolfsii in vitro*.
3. Effect of *Trichoderma* and *Gliocladium* on germination of Pulses (Mungbean, blackgram, and Pigeon pea) and their efficacy of controlling seed borne mycoflora.
4. Antagonistic effect of *Trichoderma* and *Gliocladium* on the seed borne mycoflora of Tomato *invitro*.
5. Effect of *Trichoderma harzianum* on germination and seedling vigor of Tomato.

Tolerance of *Trichoderma harzianum, Gliocladium viride* and *Sclerotium rolfsii* were tested at 0, 100, 250, 500 and 1000 µg/ml concentrations. In vitro

test reveled that *Trichoderma harzianum* could tolerate Vitavax, Tilt 250EC, Ridomil and Thiovit at 1000µg/ml, where *Gliocladium viride* could grow on PDA containing Ridomil and Thiovit. *Trichoderma harzianum* and *Gliocladium viride* could not tolerate even 100µg/ml of Bavistin. On the other hand *Sclerotium rolfsii* could not tolerate Vitavax, Tilt and Ridomil event at 100µg/ml, but Bavistin and Thiovit reduced the growth of *Sclerotium rolfsii* by 86.18% and 75.64%, respectively at 1000µg/ml and 100µg/ml. Out of the nutrient elements tested, N, K, S and Mn showed reduction in growth of *Trichoderma harzianum* but slight induction of growth was recorded by P and Cu. Except P, the tested metals reduced the growth of *Gliocladium viride*, where higher the concentration of metals in medium resulted higher inhibition of growth. Nutrients showed inhibition of growth of *Sclerotium rolfsii* by increasing the conc. in medium.

Maximum mycelial growth was recorded in *Trichoderma harzianum* followed by *Gliocladium viride*. Both the antagonists were found potential against *Sclerotium rolfsii*. Seed treatment with antagonists resulted upto 21.61%, 53.5%, 26.64% and 48.43% increase in germination in mungbean, blackgram, pigeon pea and tomato, respectively and showed good effect on seed borne mycoflora. Moreover, significant growth enhancement of mungbean, blackgram and tomato have been achieved by treating seeds with antagonists.

The findings of the present study clearly pointed out the ample scope of using *Trichoderma* and *Gliocladium* for controlling soil borne *Sclerotium rolfsii* and seed borne mycoflora where there is a usual practice of application of agrochemicals. But detail study in this regard needed to be carried out.

REFERENCES

REFERENCES

Abdul Baki, A. A. and J. D. Anderson. 1973. Vigor determination in soybean by multiple criteria. Crop Sci. 13, 630-633.

Agnihotri, V. P., S. M. Srivastava and C. Sen.. 1975. Role of fungitoxicants in the control of Sclerotium root rot of sugarbeet, *Beta vulgaris* L. Indian J. Experimental Biology 13 (1): 89-91.

Agrawal, S. C., M. N. Khare and P. S. Agrawal. 1977. Biological control of *Sclerotium rolfsii* causing collar rot of lentill. 30(2): 176-179.

Anilkumar, T. B. and K. T. P. Gowda. 1984. Effect of pesticides and fungicides on the saprophytic survival of *Sclerotium rolfsii*. Indian Phytophology 18: (1): 43- 46.

Arora, D. K. and R.S.. Dwivedi. 1979. Rhizosphere fungi of *Lens esculenta* Moench antagonistic to *Sclerotium rolfsii* Sacc. Soil Biology and Biochemistry 11(6): 563-566.

Asghari, M. R. and C. D. Mayee. 1991. Comparative efficiency of management practices on stem and pod rots of groundnut. Indian Phytopathology 44(3): 328-332.

Bag, T. K. and A. K. Sinha. 1997. Control of stem rot of soybean incited by *Sclerotium rolfsii* through metal salts. Indian Phytopathology 50 (3): 350-357.

BBS, 2000.Year Book of Agricultural Statistics, Bangladesh., 1998. Bangladesh Bureau of Statistics, Statistics Division, Ministry of Planning, Govt. of the People's Republic of Bangladesh. p.61.

Begum, M. M., I. Hossain and M. S. Hoque.1998. Biocontrol of seed borne *Fusarium oxysporum* with *Trichoderma harzianum*. Bangladesh J. Enviorn. Sci. Vol. 4, 128-133.

Benhamou, N. and I. Chet. 1996. Parasitism of Sclerotia of *Sclerotium rolfsii* by *Trichoderma harzianum* : Ultrastructural and cytochemical aspects of the interaction. Phytopathology 86(4): 405-416.

Booth, C. 1971. The genus *Fusarium*. Commonwealth Mycol. Inst. Kew, Surrey, England. 236 pp.

Borines, L. M. and C. V. Ranchez. 1996. Efficacy of selected fungicides against basal stem rot or wilt of tomato caused by *Sclerotium rolfsii*. Annals of Tropical Research (Philipines), 18 : p 3 – 11.

Chet, I. and J. Inbar. 1994. Biological control of fungal pathogens. Applied Biochem. and Biotech. 48 (1): 37-43.

Correa, A., S. Rebuffal, B. Bodo, M.F. Roquebert, J. Dupont and L. Bettucci. 1995. In vitro inhibitory activity of trichorzianines on *Sclerotium rolfsii* Sacc. Cryptogamie, Mycologie 16(3): 185-190.

De, R. K., R. G. Chaudhary and S. Naimuddin. 1996. Comparative efficacy of biocontrol agents and fungicides of controlling Chickpea wilt caused by *Fusarium oxysporum* f.sp. *ciceri*. Indian J. Agril. Sci. 66(6): 370-373.

Deb, P.R. and B.K. Dutta. 1991. Studies on biological control of foot rot disease of soyaben caused by *Sclerotium rolfsii* Sacc. Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz 98(5): 539-546.

Dey, T.K., A. Aub, H.U. Ahmed and M.H. Rahman. 1993. Screening of chickpea germplasms against *Fusarium* wilt. Bangladesh J. Plant Path. 9(1 & 2): 29-30.

Diomande, M and M. K. Beute. 1977. Comparison of soil plate fungicide screening and field efficacy in control of *Sclerotium rolfsii* on peanuts. Plant Disease Reporter 61 (5): 408-412.

Duggy, B. K., B. H. Ownley and D. M. Weller.1997. Soil chemical and physical properties associated with suppression of take-all of wheat by *Trichoderma koningii.* Phytopathology. 87(11): 1118-1124.

Elad, Y., I. Chet and J. Katan. 1980. *Trichoderma harzianum* : a biocontrol agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. Phytopathology 70(2): 119-121.

Elad, Y., I. Chet and Y. Henis. 1982. Degradation of plant pathogenic fungi by *Trichoderma harzianum*. Canadian J. Microbiol. 28(7): 719-725.

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

Ellil, A.H.A.A., N.G.H. Awad and S.T.A. El-Haleem. 1998. Biocontrol of vegetable root rot disease by *Trichoderma harzianum* and *T. viride* role of sugars, protein and amino acides in host resistance. African J. Mycology and Biotechnology 6(2): 25-41.

Fahim, M. M., M. A. Kararah, E. I. Gharabawi and K. A. M. Abada. 1986. Chemical control of damping-off of sugar beet (Beta vulgaris) caused by *Sclerotium rolfsii*. Egyptian J. Phytopathology. 16 (1-2): 35-42.

Fellman, J. K., D. Tourneau and D. L. Stiers. 1983.. Effects of some carboxamide fungicides on growth and sclerotium formation of *Sclerotium rolfsii*. Transactions British Mycological Society. 80(1): 170-172.

Ferrata, M. and V. D'Amba. 1985. Morphological aspects of the parasitism of *Trichoderma harzianum* Rifai on hyphae and sclerotia of *Sclerotium rolfsii* Sacc. Rivista di Patologia Vegetal 21(2): 53-60.

Franke, M. D. and T. B. Brenneman. 1998. Sensitivity of isolates *of Sclerotium rolfsii* from peanut in Georgia to selected fungicides. Plant disease. 82(5): 578-583.

Harman, G.E., A.G. Taylor and T.E. Stasz. 1989. Combining effective strains of *Trichoderma harzianum* and solid matrix priming to improve biological seed treatments. Plant Diseases 73(8): 631-637.

Henis, Y. and G.C. Papavizas. 1982. Factors affecting susceptibility of *Sclerotium rolfsii* Sclerotia to *Trichoderma harzianum* in natural soil. Abstr. Phytopathology. 72: 1010.

Henis, Y., P.B. Adams, J.A. Lewis and G.C. Papavizas. 1983. Penetration of sclerotia of *Sclerotium rolfsii* by *Trichoderma* spp. Phytopathology 73(7): 1043-1046.

Iqbal, S.M., A. Bakhsh, S. Hussain and B.A. Malik. 1995. Microbial antagonism against *Sclerotium rolfsii*, the cause of collar rot of lentil. Lens Newsletter 22(1/2): 48-49.

ISTA. 1993. International Rules for seed Testing. Int. Seed Testing Assoc. Seed Sci. and Technol. 21(Supplement): 25-29 and 141-160.

Jacobs, D. and O. Kamoen. 1986. Roll of cell wall lysing enzymes of *Trichoderma harzianum*. Med. Fac. Landbouw. Rijks. Gent. 56(26): 751-758.

Jagadeesh, K. S. and G. S. Geeta. 1994. Effect of *Trichoderma harzianum* grown of different food bases on the biological control of *Sclerotium rolfsii* Sacc. in groundnut. Environment and Ecology 12(2): 471-473.

Karpagavalli, S. 1997. Effect of different fungicides on the survival of *Trichoderma* spp. Plant Disease Research. 12 (1): 79-82.

Kaur, N. P. and A. N. Mukhopadhyay. 1992. Integrated control of Chickpea wilt complex by *Trichoderma* and chemical methods in India. Tropical Pest Management 38(4): 372-375.

Khan, T. A., S. T. Khan, M. Fazal and Z. A. Siddiqui. 1997. Biological control of *Meloidogyne incognita* and *Fusarium solani* disease complex in papaya using *Paecilomyces lilacinus* and *Trichoderma harzianum*. International J. Nematology 7(2): 127-132.

Krishnamoorthy, A. S. and A. Bhaskaran. 1990. Biological control of damping – off disease of tomato caused by *Pythium indicum* Balakrishnan. Journal of Biological control. 4 (1): 52-54.

Kulkarni, S. A. and S. Kulkarni. 1994. Biological control of *Sclerotium rolfsii* Sacc. a causal agent of stem rot of groundnut. Karnataka J. Agril. Sci. 7(3): 365-367.

Kumar, S.M. and M.N. Khare. 1990. Studies on the antagonistic relationship of soyaben spermosphere microflora with *Rhizoctonia bataticola* and *Sclerotium rolfsii*. J. Biological control 4(1): 72-74.

Lacicowa, B. and D. Pieta. 1994. Protective effect of microbiological dressing of pea seeds (*Pisum sativum* L.) against pathogenic fungi living in soil. Annales-Universitatis-Mariae, Curie Sklodowska, Sectio. EEE. Horticultura 2: 165-171.

Lim, T. K. and B. K. Teh. 1990. Antagonism in vitro of *Trichoderma* spp. against several basidiomycetous soil-borne pathogens and *Sclerotium rolfsii*. Z. PflKrank und. PflSchutz. 97(1): 33-41.

Liyange, A. D. S. 1983. Review of the Plant Pathology Department. Annual review, Rubber Research Institute, Srilanka, 53-68.

Maiti, S and S. Chaudhuri 1975. Effect of some systemic fungicides on sclerotial germination and growth of *Sclerotium rolfsii* in vitro. Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz. 82 (4): 233-235.

Mehta, R. D., K. A. Patel K. K. Roy and M.H. Mehta. 1995. Biological control of soil borne plant pathogens with *Trichoderma harzianum*. Indian J. Mycol. and Plant Path. 25(1 & 2): 128.

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

Mondal, G., K. D. Srivastava and R. Aggarwal. 1995. Antagonistic effect of *Trichoderm spp. on Ustilago segetum var. tritici* and their compatibility with fungicides and biocides. Indian Phytopathology 48 (4): 466-470.

Motikhaye, S. G. 1983. Studies on the efficacy of some fungicides against five phytopathogenic fungi in the laboratory. Hindustan Antibiotics Bulletin 25 (1-2): 46-48.

Mukherjee, P.K., A.N. Mukhopadhyay, D.K. Sarmah and S.M. Shrestha. 1995. Comparative antagonism properties of *Gliocladium virens* and *Trichoderma harzianum* on *Sclerotium rolfsii* and *Rhizoctonia solani* - its relevans to understanding the mechanisms of biocontrol. J. Phytopathology 143(5): 275-179.

Mukhopadhyay, A. N. 1989. National Seminar and VII workshop of AICRP on Biological Control. Lucknow. Oct. 23-25.1989.

Mukhopadhyay, A.N. 1995. Exploitation of *Gliocladium virens* and *Trichoderma harzianum* for biological seed treatment against seed borne disease. Indian J. Mycol. Pl. Path. 25(1 & 2): 124.

Muthamilan, M. and R. Jeyarajan. 1996. Integrated management of *Sclerotium* root rot of groundnut involving *Trichoderma harzianum*, *Rhizobium* and Carbengazim. Indian J. Mycol. and Plant Path. 26(2): 204-209.

Mutto, S., V. D'Ambra and M. Ferrata. 1986. Ultrastructual aspects of the parasitism of *Trichoderma harzianum* on sclerotia of *Sclerotium rolfsii*. Phytopathologia Mediterranea 25(1-3): 10-18.

Ordentlich, A. and D. Chet. 1989. Biological control of seed borne plant pathogenic fungi by *Trichoderma*. Israel Agresearch 3(1-2): 137-152.

Ortega, B. R. A. A. Roberto and B. N. Ciro. 1992. Effect of N P K fertilization on severity of stem rot (*Sclerotium hydrophilum* Sacc.) in rice. Agricultura Tecnica, 52(2): 162-169.

Palakshappa. M. G., K. Srikant, R. K. Hegde and S. Kulkarni.1987. Bioassay of fungicides against *Sclerotium rolfsii* Sacc.: a casual agent of foot rot of betelvine. Current Research University Agricultural Sciences. 16 (10): 140-142.

Papavizas, G.C. 1985. *Trichoderma* and *Gliocladium*: biology, ecology and potential for biocontrol. Annual Review of Phytopathology 23: 23-54.

Peshney, N. L and P. G. Moghe. 1980. In vitro evaluation of some fungicides against *Sclerotium rolfsii* Sacc. Tropical Grain Legume Bulletin. No. 21: 24-27.

Pracha, L. C. Chana and S. Sangchote. 1988. Seedling blight of barley caused by *Sclerotium rolfsii* Sacc. Bangkok (Thailand).p 1-20

Rajurkar, R.B., R.M. Gade, A.N. Paslawar and R.P. Chauke. 1998. Management of betelvine wilt through cultural and biological methods. J. of soils and crops 8(2): 176-178.

Raman, M. L., M. S. Haque, A. Muqit, K. B. Alam and S. Ali.1994. Response of *Sclerotium rolfsii* to different fungicides. Bangladesh J. P. Pathol. 10 (1-2): 35-36.

Roberti, R., P. Flori and A. Pisi. 1996. Biological control of soil borne *Sclerotium rolfsii* infection by treatment of bean seeds with species of *Trichoderma.* petria 6(2): 105-116.

Rondon, A., Y. Flores, E. Soto and Y. Mujica. 1995. Chemical control in vitro and in the greenhouse of the fungus causing white rot. : Revista de la Facultad de Agronomia, Universidad del Zulia. 12(1): 1-13.

Sattar, M.A., A.R. Podder, M.C. Chanda and M. Rahman. 1996. Rhizobial biofertilizers: the most promising BNF technology for increased of grain legume production in Bangladesh. Biological nitrogen fixation associated with rice production. Based on selected papers presented in the International Symposium, Dhaka, Bangldesh, 28 Nov. - 2 Dec. 1994. pp. 15-20.

Sharma, S. K., B.R. Verma and B.K. Sharma. 1999. Biocontrol of *Sclerotinia sclerotium* causing stem rot of chickpea. Indian Phytopath. 52(1): 44-46.

Singh, D. 1991. Biocontrol of *Sclerotinia sclerotium* (Lib.) de Bary by *Trichoderma harzianum*. Tropical Pest Management 37(4): 374-378.

Singh, D. 1995. Biocontrol of *Sclerotinia sclerotiorum* (Lib.) de Bary by *Trichoderma harzianum*. Tropical-Pest-Management. 37(4): 374-378.

Singh, R. S. 1987. Diseases of vegetable crops. Oxford & IBH Publishing Co. Pvt. Ltd. New Delhi.

Singh, U. and P.N. Thapliyal. 1998. Effect of inoculum density, host cultivars and seed treatment on the seed and seedling rot of soyabean caused by *Sclerotium rolfsii*. Indian Phytopathology 51(3): 244-246.

Srikant, K, S. N. Chattannavar, R. K. Hegde and S Kulkarni.1986. Laboratory evaluation of fungicides against foot rot of wheat caused by *Sclerotium rolfsii* Sacc. Pesticides. 20(9): 27 - 31.

Srivastava, S. N .and R. C. Tripathi. 1998. Management of sugarbeet seedling disease complex by combination of fungicides. Indian Phytopathology. 51 (1): 75-77.

Sattar, M.A., A.R. Podder, M.C. Chanda and M. Rahman. 1996. Rhizobial biofertilizers: the most promising BNF technology for increased of grain legume production in Bangladesh. Biological nitrogen fixation associated with rice production. Based on selected papers presented in the International Symposium, Dhaka, Bangldesh, 28 Nov. - 2 Dec. 1994. pp. 15-20.

Sharma, S. K., B.R. Verma and B.K. Sharma. 1999. Biocontrol of *Sclerotinia sclerotium* causing stem rot of chickpea. Indian Phytopath. 52(1): 44-46.

Singh, D. 1991. Biocontrol of *Sclerotinia sclerotium* (Lib.) de Bary by *Trichoderma harzianum*. Tropical Pest Management 37(4): 374-378.

Singh, D. 1995. Biocontrol of *Sclerotinia sclerotiorum* (Lib.) de Bary by *Trichoderma harzianum*. Tropical-Pest-Management. 37(4): 374-378.

Singh, R. S. 1987. Diseases of vegetable crops. Oxford & IBH Publishing Co. Pvt. Ltd. New Delhi.

Singh, U. and P.N. Thapliyal. 1998. Effect of inoculum density, host cultivars and seed treatment on the seed and seedling rot of soyabean caused by *Sclerotium rolfsii*. Indian Phytopathology 51(3): 244-246.

Srikant, K, S. N. Chattannavar, R. K. Hegde and S Kulkarni.1986. Laboratory evaluation of fungicides against foot rot of wheat caused by *Sclerotium rolfsii* Sacc. Pesticides. 20(9): 27 - 31.

Srivastava, S. N .and R. C. Tripathi. 1998. Management of sugarbeet seedling disease complex by combination of fungicides. Indian Phytopathology. 51 (1): 75-77.

Sugha, S.K., B.K. Sharma and P.D. Tyagi. 1993. Factors affecting development of collar rot of gram (*Cicer arietinum*) caused by *Sclerotium rolfsii*. Indian J. Agril. Sci. 63(6): 382-385.

Sultana, N. 1999. Biological control of Foot and Root Rot of Lentil with *Trichoderma harzianum*. M.S. Thesis. Dept. of Plant Pathology, BAU, Mymensingh.

Sultana, N. and I. Hossain. 2000. Biological control of Foot and root rot of lentil with *Trichoderma harzianum*. 17 th Annual Conference of Bangladesh Society of Microbiology, held on July 27, 2000. p 26.

Sumitha, R. and S. J. Gaikwad. 1995. Cheecking *Fusarium* wilt of pigeon pea by Biological Means. J. Soils and Crops 5(2): 163-165.

Suseelendra D. and E. Schlosser. 1999. Parasitism of *Sclerotium rolfsii* by *Trichoderma*. Indian Phytopath. 52(1): 47-50.

Thammasak, S., W. Chaliphom and K. Chunnahawong. 1982. Studies on damping-off of cotton caused by *Sclerotium rolfsii* Sacc. Bangkok (Thailand). pp33.

Tverdyukov, A. P., P. V. Nikonov and N. P. Yushchenko. 1994. Trichodermin. Rev. Plant Pathology 73(4): 237.

Upadhyay, J.P. and A.N. Mukhopadhyay. 1983. Effect of non-volatile and volatile antibiotics of *Trichoderma harzianum* on the growth of *Sclerotium rolfsii*. Indian J. Mycol. and Plant Path. 13(2): 232-233.

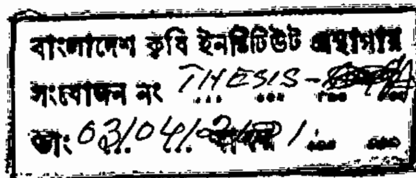
Upadhyay, J.P. and A.N. Mukhopadhyay. 1986. Biological control of *Sclerotium rolfsii* by *Trichoderma harzianum* in sugarbeet. Tropical pest Management 32(3): 215-220.

Wells, H.D., D.K. Bell and C.A. Jaworski. 1972. Efficacy of *Trichoderma harzianum* as a biological control for *Sclerotium rolfsii*. Phytopathology 62(4): 442-447.

Wokocha, R. C. 1990. Integrated control of *Sclerotium rolfsii* infection of tomato in the Nigerian Savanna: effect of *Trichoderma viride* and some fungicides. Crop-Protection. 9(3): 231-234..

Xu, T., J. P. Zhong and D.B. Li. 1993. Antagonism of *Trichoderma harzianum* T82 and *Trichoderma* sp. NF9 against seed borne fungal pathogens. Acta. Phytopathologica Sinica. 23(1): 63-67.

Yahia, A. H., E. Said and A. A. Deep. 1979. Fungicidal control of damping-off and root rot diseases of peanuts in Egypt. Agricultural Research Review. 57(2): 95-102.



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