STUDIES ON BIOLOGICAL CONTROL OF BLACK SCURF OF POTATO BY *TRICHODERMA* SPP.

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Dedicated to:

The *Shohada* (Martyrs), *Jaanbazan* (Injured and Disables) and all of the *Sarbazaan* (soldiers) of the eight year imposed war against my country, whose blood showed the right path, saved the integrity and sovereignty of Iran, and made it possible for all Iranian to live proudly in peace after the war.

Certificate –I

This is to certify that this dissertation entitled " **Studies on the biological control of potato black scurf by *Trichoderma* spp.**" submitted for the degree of Ph.D., in the subject of plant pathology of the CCS Haryana Agricultural University, is a bonafide research work carried out by **Mr. Eidi Bazgir** under my supervision and that no part of this dissertation has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE-II

This is to certify that this dissertion entitled " **Studies on the biological control of potato black scurf by *Trichoderma* spp.**" Submitted by **Mr. Eidi Bazgir** to the CCS Haryana Agricultural University in partial fulfilment of the requirements for the degree of Ph.D., in the subject of Plant Pathology has been approved by the student advisory committee after an oral examination on the same, in collaboration with an external examiner.

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Chapter 1

Inroduction

Potato (*Solanum tuberosum* L., Family *Solanaceae*) , with an annual production of nearly 300 million metric tons, is one of the major food crops grown in a variety of soils and climatic condition. Potato is one of the most important food crops in the world. In terms of production, it ranks as the fourth after wheat, maize and rice with respective production of 612, 585, 580 and 292 million tonnes in 1997. Potato production represent roughly half of the world's total annual output of 628 m tonnes from all root and tuber crops. Over one billion people consume potatoes world-wide and potatoes are part of the diet of half a billion people in the developing countries.

FAO statistics estimates world utilisation of current potato production as 45% for human food, 30% for animals, 15 %for seed, 2% for starch, and about 8% as waste.

Potato has been used at least 8000 years ago. The name "potato "is believed to be driven from the Inca name "papa". Potato has been introduced into Spain about 1570 from Sonth America and later to neighbouring European countries and in less than 100 years it was being grown extensively in many region of Europe. It was introduced into India about 1610, China 1770 and Japan1766. Scottish-Irish immigrants introduced the potato into the North America in the early 1700s.Out of 1500 *Solanum* species only 90 species are tuber producers and very few are cultivated. Potatoes are dicotyldonous short- lived perennials that are typically cultivated as annuals for their edible enlarged underground tubers (Rnbatzky and Yamaguchi, 1997).

Potato is an important cash crop of India and it is grown under wide range of climates, viz. temperate in the hills, sub-tropical in the north Indian plains, warmer plateau region and Nilgiri hills in the southern part. Amongst them the great north plains are very suitable for potato cultivation because of rich alluvial soil, availability of plenty of water and very congenial climate.

The region accounts for nearly 82% of potato area and about 85% of total potato production of the country.

Like other crops, potato is affected by a large number of diseases caused by fungi, bacteria, phytoplasma, viruses, viroids etc. Rhizoctonia or Black scurf caused by *Rhizoctonia solani* Kühn is one of the oldest and world wide distributed diseases of plants. It is world-wide in distribution and is called as Rhizoctonia or Rhizoctonia canker after the generic name of the imperfect stage of the causal fungus, rhizoctoniosis, stem canker, sprout canker, black scurf, or " the dirt that will not wash off " (O · Brien and Rich, 1976). The French call it *rizoctonie*.

Black scurf (*Rhizoctonia solani*) characterized by dark muddy encrustation on potato tubers is responsible for quantitative and qualitative reduction in yield besides causing root and stem cankers causing gaps in fields and also in certain cases leading to aerial tuber formation.

Banville (1978) reported that *R. solani* on seed pieces reduced yields of Norland and Green Mountain by 16-30% and 21-34%, respectively. It also affects size, shape, and appearance of potato tubers (Weinhold and Bowman, 1977). Lack of effective control measures adds to its importance.

Control of soil-borne plant pathogens is usually done by chemical, physical and cultural methods, and there are advantages and disadvantages in each method. There are no really good control measures for *Rhizoctonia* which are both practical and effective. Though soil disinfestation is an effective control measure for damping-off of seedlings, yet it is not practical for potatoes. Crop rotation may have some beneficial effect, but the fungus has such a wide host range and it is so easily reintroduced as sclerotia on seed potatoes that it is not very effective. Chemical treatment of seed potatoes with mercury compounds to kill the tuber-borne sclerotia was recommended and practised for many years. However, this did not control the soil-borne inoculum. The use of mercury fungicides is now prohibited in most countries. Soil-borne diseases are often very difficult to control with chemical pesticides because of physical and chemical characteristics of the soil. Complete eradication of pathogens from soil by steam –sterilisation and fumigation with chemicals could not be achieved mainly because of rapid recolonization of sterile soil by pathogens. The frequent failure of chemical control and frequent pathogen development in sterilised soil led to suggest that soil-borne diseases would be amenable to control by biological means. Host resistance

offers one of the best means of controlling the disease but commercially resistant cultivars are not available.Regardless of and may be in spite of , the complexity of environment surrounding the rhizosphere of plants, a number of biological control strategies aimed at reducing soil-borne plant pathgens and plant parasitic nemathodes have been developed as 1) release of naturally occurring antagonists, 2) release of genetically altered antagonists, or 3) enhancement of specific endemic antagonists through management (Vilich and Sikora 1997). Some of the widely used biocontrol agents in the world belong to the fungal genus *Trichoderma*. In particular, isolates of *Trichoderma harzianum, T. virens, and T. hamatum* are used against diseases in a wide variety of economically important crops for control of soil-borne, seed-borne and foliar diseases, and against storage rots. This wide range of application is due to the various antagonistic mechanisms found in different *Trichoderma* isolates, enabling them to function as potent biocontrol agent on many different crops, against a range of pathogens, and in several ecological situations (Papavizas 1985, Trosmo and Hjeljord 1997). Furthermore, there are several reports that *Trichoderma* isolates can stimulates plant growth even in the absence of pathogens (Windham *et al.*, 1986). . In this context, increasing number of researchers have convincingly shown the potential of some isolates of *Trichoderma spp.* as biocontrol agents of soil-borne plant pathogens(Cherif and Benhamou,1990).

Trichoderma spp. have many advatages as biocontrol agents. First, they have fast growth and a great arsenal of inducible polysacharid-degrading enzymes. Thus , the fungi may be propagated on a wide variety of carbon sources , so it is easy to finde a resonably cheap substrate for large scale production of the biocontrol agent (Papavizas 1985, Tronsmo and Hjeljord 1997). Another advantage is the wide range of environmental conditions tolerated by the various *Trichoderma* species and isolates, which means that it is possible to select isolates suited for most of the environmental conditions under which plant pathogens can cause disease. Isolates also vary in their tolerance to chemicals, and many show remarkable resistance to fungicides , either inherently or through mutation or adaptation. It is therefore possible to select fungicide tolerant or resistant biocontrol agents for use in integrated control(Tronsmo and Hjeljord,1997).

Biological control of plant diseases is an approach that is distinct from other control measures. Its technological base involves diverse scientific

disciplines that uniquely address the natural enemies and antagonists of the targeted species.

The growing concern regarding the use of hazardous fungicides for controlling plant diseases on one hand, and fascinating development of biotechnology on the other hand, have led to intensive research in agricultural biotechnology aimed at plant protection. The use of beneficial, naturally antagonistic fungi as biocontrol agents of plant disease has been studied for at least two decades. In some cases, biocontrol agents can probably serve as a suitable alternative to chemical fungicides, without harming the environment. However, more research is needed for biocontrol to became an integral component of modern agriculture. Biotechnology is defined by the European Federation of Biotechnology as "the integrated use of biochemistry, microbiology and engineering sciences in order to achieve technological (industrial) application of capabilities of microorganisms, cultured tissue cells and parts thereof." Biotechnology could make significant contribution to sustainable agriculture by producing improved means of disease control that are more compatible with environment. We are now at the beginning of a new era in which biological control should develop to increasingly advanced stages (Chet and Inbar, 1998).

Potato black Scurf caused by *Rhizoctonia solani* is an important and widely distributed disease of potato. Control of the disease by conventional methods is inadequate and not ecofriendly. Therefore biocontrol appears to be a good alternative either alone or in IPM system for management of black scurf. Not much work has been done in this direction, therefore the present investigation was undertaken with the following objectives.

- To find out effective isolates of *Trichoderma* spp. *in vitro*.
- To study the mechanism(s) of biocontrol of *R. solani* by *Trichoderma* spp.
- To test the performance of biocontrol agents under field condition.

CHAPTER 2

REVIEW OF LITERATURE

2-1 Rhizoctonioisis or Black scurf of potato

Black scurf is one of the oldest diseases of potato. The disease affects almost all parts of potato plant including foliage (Shekhawat *et al*,. 1993). It causes damping off of seedlings, root and stem cankers on growing plants, aerial tuberization and black scurf on potato tubers. It is world-wide in distribution and is referred to as Rhizoctonia canker after the generic name of the imperfect stage of the causal fungus, Rhizoctoniosis, Stem canker, Sprout canker, Black scurf, or "the dirt that will not wash off" in English. The French call it Rizoctonie (Dykstra, 1962 ; Pushkarnath, 1970 ; O' Brien and Rich, 1976 ; Rich, 1983).While Rhizoctoniosis, in general refers to disease symptom, black scurf refers to symptoms appeared on tubers alone in the form of black muddy encrustation (sclerotia) on them.

2-1-1. Importance

Rhizoctoniosis is one of the most serious diseases of potato and other crops. It reduces stands, yield, quality, and price of potato. *R. solani* on seed pieces reduced yields of Norland and Green Mountain varieties by 16-30% and 21-34%, respectively. It also affects size, shape, and appearance of potato tubers. Lack of effective control measures adds to its importance. It is widespread and probably occurs wherever potatoes are grown (Rich, 1983). It is one of the most Important diseases of potato in Iran (Zafari, 1999). Black scurf is one of the most common fungal diseases of potato in India (Pushkarnath, 1970 ; Dutt, 1976). Maheshwari and Srivastava (1991) while

investigating on tuber-borne diseases of potato, reported maximum disease incidence black scurf (10.1%) followed by soft rot (4.4 %), Charcoal rot 2.9 % , late blight (2.7 %), and common scab (1.9 %) from samples of four cold storage in Ambala region (Haryana), India. Planting infected seed led to almost 100% disease incidence on the progeny (Sahai 1987). R. *solani* exists as active mycelium in soil, in the absence of a host as dormant sclerotia for up to 6 years (Ghafar,1993).

2-1-2. Causal Agent

The imperfect (mycelial and sclerotial) stage of the fungus is usually cited as *Rhizoctonia solani* Kühn. The latest name for the teleomorph or perfect stage of the fungus appears to be *Thanatephorus cucumeris* (Frank) Donk . Old names or synonyms include *Pellicularia filamentosa* (Pat.) Rogers and *Corticium solani* (Prill.& Del.) Bourd. & Galz. (Rich, 1983). Occurrence of *Thanatephorus cucumeris* perfect stage of *R.solani* on potato plants in nature from India was reported by Singh *et al.* (1998) .

The most common anastomosis group of *R. solani* on potatoes is AG3 (Rich 1983; Mall and Suresh,1987; Kim and Kim, 1996 ; Zamani 1988). Of 25 isolates from field soils and infected part of potato, 21 belonged in AG3 and the remaining 4 in AG4. AG3 isolates obtained from stem , root , tubers and soil while AG4 was isolated from stem only (Suresh and Mall, 1982). Out of 24 isolates of *R.solani* pathogenic to potato 18 were AG3, four of AG4 and two isolates did not anastomose with any of the tester isolates (Mall and Suresh,1987). Anastomosis groups of *R.* solani of Iran were studied by Zamani, 1988, Rahimian, 1988, Bazgir,1991.

The sterile, septate mycelium is characterised by right- angle branching and a "pinched" appearance where it branches. It is hyaline when young but turns brown with age. In wet weather, the fungus grows as white crust along the base of the stem, such growth may extend and cover the petiole and leaflets of the basal leaves, but seldom cause any injury to the plant. This is the perfect stage of fungus (Pushkarnath, 1970). The basidial stage appears as a white to dirty grey, mat or mycelial felt at the base of the plant near ground level. Basidiospores, 7 - 12 × 1.5 - 3.5 µm, are produced on the basidia, which are borne on this mycelium. This stage occurs only under highly humid conditions (Rich 1983; Sneh, *et al.*,1991).

2-1-3. Symptomatology

Rhizoctonia frequently attacks the young sprouts or young shoots through epidermis and produces dark brown lesions thereby killing the sprout before emergence which result in gappy germination (Singh, 1965; Dutt, 1979). It causes brown cankers on the white stems below ground level. These cankers may girdle the stems , resulting in delayed emergence and uneven stands. Affected plants are weak if severely cankered or girdled. Elongated reddish brown lesions develop on stem at or below soil surface. The lesions enlarge and may girdle the stem. The affected plant lack vigour, when girdling is complete, the foliage curl and turn pinkish to purplish. Often aerial tubers are formed as a result of interference in starch translocation (Shekhawat *et al.*, 1993). Aerial tubers may develop in the axis of the leaves due to interference with starch translocation (Dikstra, 1962; Rich, 1883; Pushkarnath, 1970). Srivastava (1986) has reported wide spread occurrence of aerial tuber formation during 1985-1986 crop season in extensive potato growing belt of Haryana, India. Older plants may exhibit rolling of the leaves resembling leaf roll, psyllid yellows, or purple top wilt.

Tubers are often rough, sometimes misshapen, and few in number, or numerous very small tubers may result. They often set near the surface, are exposed to light, and turn green. Stolons are frequently girdled resulting in a reduction in yield. Roots may also be attacked. The most conspicuous signs are the numerous small (1mm or less) to fairly large (more than 1cm) black sclerotia on the surface of infected potato tubers appearing as black muddy encrustation. They are more conspicuous when the tubers are wet, but they are not removed by washing- hence, the name "the dirt that won't wash off." has been given to the disease (Rich, 1983; O'Brien and Rich 1979). Beside, producing black scurf on tubers the pathogen also produces variety of symptoms. They include 'dry-core' symptoms where crator like depression is formed on the lenticels (Thirumalachar, 1953; CPRI, 1981), hard dry rot with browning of internal tissue (Thirumalachar, 1953), and seed-piece-decay (Chaudhary, 1981). The pathogen also reported to cause foliage blight of potato (Prasad and Agarwal, 1983).

2-1-4. Etiology

Rhizoctonia solani has a very wide host range. It can live on living plants, seeds, plant parts, or in the soil as active mycelium or in the sclerotial stage. It overwinters on potato tubers as black sclerotia of variable size.

Infected seed potato tubers are transported from one area to another and are planted in infested or noninfested soil. Thus it spreads readily from one place to another. The temperature range for growth of the fungus in culture is 8 and 35°C with an optimum of 25 to 35°C. Sclerotia germinate between 8 and 30°C. The optimum temperature for sclerotial germination is 23°C and for basidiospore germination is 21 to 25°C. The optimum temperature for development of stem lesions on potato is about 18°C. High summer temperatures are not conducive for production of sclerotia and their survival (Singh, 1964). However, they survive in the periods between autumn and spring crops. Therefore , *R. solani* has to over summer either as saprophyte or by infecting the crops grown during the summer period. Saprophytic survival of *R. solani* depends on many factors , the most important being soil fertility (Mall, 1977). Since survival of inoculum in soil is not granted, as far as plains are concerned, the seed tubers serve as the main source of the disease. Conversely in hills the fungus survives in the soil throughout the year and is potential source of the disease. Production of sclerotia on the tuber surface is governed by the soil temperature . At high temperature, the sclerotia are produced in abundance than at low temperature. This has been amply demonstrated by the data on the incidence of black scurf in produce harvested at different times. Late harvested crop showed more black scurf infection than early harvested crop (CPRI, 1963).

Apparently there are numerous pathogenic races of *R. solani* . Isolates from potato may or may not be pathogenic on other crops, such as sugar beet and cabbage, while isolates from these crops may or may not pathogenic on potato. Isolates from sclerotia on potato also vary widely in their Pathogenicity to potato. Soil-borne inoculum appears to be more important than tuber-borne inoculum in perpetuation of this disease (Rich, 1983).

R.solani produces a phytotoxin capable of causing disease symptoms (root necrosis, stolon pruning, leaf curling, stunting and chlorosis of leaf margins). Highly susceptible plants may be killed within a week. There is a technique to evaluate resistance to the root necrosis phase of the disease (Bertangnolli *et al.*, 1999).

Potato plants were more susceptible to stem-canker disease caused by *R. solani* in 7 to 15 days and less susceptible in 21 days after sowing. Endo polygalacturonase (endo-PG) is the main macerating enzyme and its activity is directly related to disease severity (Mall and Suresh , 1987). Physiological

and pathological aspects of some isolates of *R. solani* of Iran has been reported (Ghorbanii, 1976).

2-1-5. Management of potato black scurf:

2-1-5-1. Conventional method of control

There are no really good control measures for *Rhizoctonia* which are both practical and effective. Soil disinfestation is an effective control measure for damping-off of seedlings, but usually it is not pragmatic for potatoes. Crop rotation may have some beneficial effect, but the fungus has such a wide host range and it is so easily reintroduced as sclerotia on seed potatoes that it is not very effective. We often turn to resistant cultivars, but commercially resistant cultivars are not available. There is report on tolerance to disease (canker phase) in some cultivars, but there was no evidence of resistance to the black scurf stage (Rich, 1983).

The incidence of black scurf can be minimised if scurfed tubers are removed from seed lot to be used for sowing.

According to Lakra (1999) first week of October sown potato and harvested at 10 days curing period is quite helpful in minimising the disease (black scurf) incidence and intensity under Haryana conditions. Crop raised with disease free tuber exhibited necrotic stem (3.5%) and disease intensity (6.8%) indicating a big role of soil- borne inoculum in disease development (Lakra, 1999).

Effect of soil solarization coupled with application of *T. viride* (dipping the tubers in 10^6 spore/ml suspension) or 3% boric acid proved significantly effective in control of black scurf. Combination of soil solarization and seed treatment with *T. viride* was found best for control of black scurf in *R. solani* infested soil (Arora, 1999).

Singh and Jaswani (1983) reported that green sprouting or planting seed pieces with short, stubby, actively growing sprouts is beneficial. Shallow covering of seed pieces is one of the most important and most practical control measures. Tlus allows the fungus less opportunity to attack the susceptible sprouts. It is common practice among growers to level off the tops of the rows by dragging a plank, chain, or spike tooth harrow over them, thus permitting fairly deep planting and shallow covering. Soil is later worked in

around the base of the plants after emergence to control weeds and prevents greening of the new tubers . Crop rotation with cereals and grasses is beneficial in reducing *Rhizoctonia* and other soil-borne disease. Two years rotation of oats and potatoes has been suggested . Corn oats and soybean were poor substrates for *R.solani*, while buckwheat and sugar beets were good substrates. Black scurf (*Rhizoctonia solani)* infection was significantly reduced when maize or sunhemp was grown in long term potato rotation, while rotation with cowpea substantially increased the infection(Singh and Jaswani, 1983). Easton (1978) found that alfalfa and red clover favour the *Rhizoctonia* disease..

Organic amendments of soil with oil cakes or sawdust also significantly reduced the incidence of black scurf (Singh *et al.* 1972). Tuber treatment with Aretan-6 alone or in combination with green manures, FYM and organic wastes was best in reducing the black scurf disease of potato (Singh and Jaswani 1983). Best control of black scurf was provided by mustard cake followed in-order by peanut, margora and caster cakes (Singh, 1968). Soil amendment with rice husk failed to reduce the disease intensity/incidence (Jalali *et al.* 1981).

Thirteen plant species screened *in vitro* for the fungitoxicity against *R.. solani* the causal agent of sheath blight of paddy. Out of these, the maximum inhibition of mycelial growth was observed in cold water extract of *Prosopis juliflora* followed by *Thevetia peruviana.* On hot water extraction , *T. peruviana* stood first followed by *P. juliflora* extract. The complete inhibition of sclerotial production was recorded in the cold water extract of *Caesalpinia pulcherrima, Eucalyptus globosus* and *Lawsonia inermis* and hot water extracts of *Calotropis gigantea, Ocimum sanctum* and *P. juliflora* in all concentration tested. The fungitoxicity of *C. pulcherrima* and *E. globosus* was not lost even after air drying and autoclaving. All the plant extracts tested withstood their fungitoxicity to thermal treatment at 70°C for 10 min(Kurucheve, *et al.*, 1997).

Lemon grass (*Cymbopogon flexuosus* Stapf.) oil a by-product of distillation of lemon grass even at very low concentratiou (0.4 PPM) is effective in inhibiting the germination of sclerotia as well as arresting the mycelial growth of *R. solani (* Bhavani *et al.* 1982).

The control of tuber–borne diseases like common scab, black scurf and dry rot, etc. by dipping the tubers in 1% bleaching powder solution and then in 3% boric acid solution for half an hour were found economical and effective (Kumar and Sharma, 1999, Singh *et al.* 1999). Treatment of black scurf infected tubers with a mixture of Acetic acid (1%) and Zinc sulphate (0.05%) gave 100% mortality of the sclerotia. Treatment with Agallol-3 soil application of PCNB reduced the disease incidence by 73% as compared to control (Dutt and Gupta, 1978). Fungicides tested to control black scurf revealed that treatment of scurfed tubers with sulphuric acid (1.75%) for 20 minutes. provided the best control. Acetic acid (4%) , HCl (40%), formaldehyde (2%) for 30 min. also provided good control of the disease. (Dutt and Thaplyal 1978). A safe and economical method for control of black scurf of seed tubers has been reported by treating the seeds with a solution of acetic acid (1%) and zinc sulphate (0.05%) for 15 minutes(Dutt 1979). The efficacy of TBZ + 8 hydroxyquinoline at 1% concentration in killing all (100%) large size (3-4mm) sclerotia and its phytotoxic effect was similar to that of Emisan- 6 (0.5%) and acetic acid + ZnSO4 (0.05%). Dipping *Rhizoctonia solani* infected Kufri Chandramukhi tubers in 1% acetic acid +0.05% zinc sulphate for 15 min. controlled the disease as effectively as dipping them in organo- mercurial compound Emisan (0.5%) for 10-30 minutes. The chemicals were effective on tubers treated before or after cold storage. Seedling emergence and yield were unaffected by the treatments (Somani, 1986).

Chemical treatment of seed potatoes with mercury compounds to kill the tuber-borne sclerotia was recommended and practised for many years. However, this did not control the soil-borne inoculum and proved to be rather ineffective (Singh *et al.* 1999). The use of mercury fungicides is now prohibited in most countries.

Up to 40% reduction in black scurf infection on potato was achieved in experiment through the use of green manure crops (like Dhaincha and Sunhemp in rotation with potatoes) combined with seed treatment with an organomercurial fungicide and hot weather cultivation (Sikka *et al.* 1971).

Soil treatment with pentachloronitrobenzene is suggested for trial, Bolkan (1967) obtained beneficial results from seed treatment with benomyl, while Edgington and Busch (1967) observed that oxathiin reduced disease symptoms (Rich, 1983).

Control of black scurf was attempted with PCNB, Aretan, PCNB+ Aretan, mercuric chloride, potassium permangnate, copper sulphate and sulphur, in clay and sandy soil. All except Aaretan gave a consistent reduction in disease (Sharma and Sohi, 1965). Singh and Joshi (1969) found Rhizoctol alone or in combination with Ceredone effective against *R. solani*, *Pseudomonas solanacearum* and *Erwinia sp.*, and recommended it for field trials.

Best control of black scurf was provided by combined treatments of tubers with Agallol and soil with PCNB. Effect of seed dressing treatment with 6 fungicides (Sd 6334, 0.1%; captan 0.3%, Brassicol 0.4%, Duter 0.3%, thiram 0.5% and mercury chloride 0.1%) was studied on black scurf of potato. Duter treatment was the best. Mercury chloride treatment also proved effective in control of black scurf (Sidhu and Bansal, 1971). Combination of soil treatment with PCNB) and seed treatment with Aretan or Rhizoctol-combi gave better control of black scurf. Rhizoctol- combi proved better than Aretan because it consistently increased the yield (Singh *et al.*, 1972).

Scurf could be effectively controlled by Agallol-6 seed treatment for 10 min. followed by soil treatment with PCNB (25 kg/ha.) at the time of planting. Organic amendment of soils either with rice husk or saw dust reduced wilting and intensity of sclerotia on tubers. Combination of seed dips in Agallol or Aretan along with soil treatment either with PCNB or organic amendments gave most effective control (Singh *et al.*, 1978). Treatment of diseased potatoes for 10 min. with Daconil-2787 (2000ppm), Duter (0.3%), Rovral (0.45%), Tecto flowable (1%) and Emisan (0.5%) also provided satisfactory control of the disease (Dutt and Gupta, 1979). Mercurial fungicides and Benlate proved to be most effective in controlling tuber borne infection and increasing the yield (Dar and Dutta, 1980). Fungicidal seed dip treatment of scurfed seed potatoes indicated that both Bavistin and Agallol individually increased the number and weight of healthy marketable tubers with reduced tuber infection, but they did not increase the total yield. (Jalali *et al.*, 1981).

Wickes *et al.* (1996) indicated that the both soil and tuber-borne inoculum must be considered in any programme aimed at controlling *R. solani*. They reported that tuber treatment of either a 20 min. dip in 2% formaldehyde, spray with pencycuron (0.15 ml a.i. /10 kg seed or spore, iprodion (2ml a.i. /10 kg seed or a spore suspension of 10 ^{6}/ ml of

Verticillium biguttatum or a dust with tolclofos- methyl (4 g. a.i./ 10 kg seed were effective if planted in soil fumigated with 500 l / ha metam or soil with low levels of *R. solani*. A commercial formulation of Trichoderma *harzianum* and *T. koningii* applied as dust @ 1.3 g/ 10 kg seed was in the most cases ineffective when treated seeds were planted into either fumigated or unfumigated soil. The incidence of progeny tubers with sclerotia varied between sites and ranged from 85% in an unfumigated soil planted with infected tubers to 2% in fumigated soil planted with pencycuron- treated tubers. Neither seed treatment nor fumigation improved total or marketable yield (Wicks *et al.*, 1996).

Effect of nine systemic fungicides viz., carbendazim 50 WP, bitertanol 25 WP, benomyl 50 WP, metsulfovax 20WP, benalaxyl 35 SD, benomyl + mancozeb, benalaxyl + copper oxychloride, thiabendazole 60WP, and thiophanate-methyl 70 WP was tested on three isolates of *R. solani* AG-1, AG-2 and AG-3. The potato isolate AG-3 was least sensitive to the fungicides. However, it was more sensitive to carbendazim and bitertanol, moderately to benalaxyl and least to the remaining fungicides (Sugha, et al., 1989).

2-1-5-2.Biocontrol of potato black scurf

Soil-borne plant diseases are often very difficult to control with chemical pesticides because of physical and chemical characteristics of the soil. Therefore, until recently, the field of biological control of plant diseases has focused mainly on soil-borne diseases . Control of soil-borne plant pathogens is usually done by chemical, physical and cultural methods and there are advantages and disadvantages in each method. Complete eradication of pathogens from soil by steam –sterilization and fumigation with chemicals could not be achieved mainly because of rapid recolonization of sterile soil by pathogens. The frequent failure of chemical control and frequent pathogen development in sterilized soil led to suggest that soil-borne diseases would be amenable to control by biological means. In this context, an increasing number of researchers have convincingly shown the potential of some isolates of *Trichoderma spp.* as biocontrol agents of soil-borne plant pathogens (Cherif and Benhamou, 1990).

The rhizosphere provides the root's front line of defense against attack by soil- borne plant pathogenic fungi. Root colonization by introduced

biocontrol agents is, therefore assumed to be essential for biological control of soil-borne plant diseases (Weller, 1988).

The most commonly used fungal biocontrol agents are species of *Trichoderma*, *Gliocladium*, and *Coniothyrium* . For a biocontrol agent (such as *Trichoderma*) to become an integral component of plant-disease management, it must be effective, reliable, consistent and economical. To meet these criteria, a better and more basic understanding of the system must be reached. Superior strain must be developed and delivery systems must be improved to enhance its activity (Tronsmo and Hjeljord, 1997).

Antagonistic microorganisms have the potential to interfere with the growth and /or survival of plant pathogens, thereby contributing to biological control. Antagonistic interactions among microorganisms in nature include parasitism or lysis, antibiosis and competition . Baker and Griffin (1995) extended the scope of antibiosis to " inhibition or destruction of an organism by metabolic production of another. This definition includes small toxic molecules, volatile and lytic enzymes.They concluded that even in cases where antifungal metabolite production by an agent reduces disease, the impact of antibiosis in biological control is uncertain, because other mechanisms may also be operating. Exogenous nutrients are required for pathogens to germinate, penetrate and infect host tissue successfully (Baker and Griffin 1995).

Starvation is the most common cause of death of microorganisms. Therefore, competition for limiting nutritional factors. mainly carbon , nitrogen and iron, may result in biological control of plant pathogens (Garret, 1965).

Application of a single species or strain of microorganism, with proven biological activity, for control of a specific target pest or disease is presently the most popular strategy for increasing biological diversity in the soil on a commercial basis (Deacon, 1991). Regardless of, and may be in spite of , the complexity of environment surrounding the rhizosphere of plants, a number of biological control strategies aimed at reducing soil-borne plant pathgens and plant parasitic nematodes have been developed as 1) release of naturally occurring antagonists, 2) release of genetically altered antagonists, or 3) enhancement of specific endemic antagonists through management (Vilich and Sikora 1997).

Some of the widely used biocontrol agents in the world belong to the fungal genus *Trichoderma.* In particular, isolates of *Trichoderma harzianum, T. virens, and T. hamatum* are used against diseases in a wide variety of economically important crops for control of soil-borne, seed-borne and foliar diseases, and against storage rots. This wide range of application is due to the various antagonistic mechanisms found in different *Trichoderma* isolates, enabling them to function as potent biocontrol agent on many different crops, against a range of pathogens, and in several ecological situations (Papavizas 1985, Trosmo and Hjeljord 1997). Furthermore, there are several reports that *Trichderma* isolates can stimulates plant growth even in the absence of pathogens (Windham *et al.*,1986).

Trichoderma spp. have many advantages as biocontrol agents. First, they have fast growth and a great arsenal of inducible polysaccharide-degrading enzymes. Thus, the fungi may be propagated on a wide variety of carbon sources , so it is easy to finde a reasonably cheap substrate for large scale production of the biocontrol agent (Papavizas 1985, Tronsmo and Hjeljord 1997). Another advantage is the wide range of environmental conditions tolerated by various *Trichoderma* species and isolates, which means that it is possible to select isolates suited for most of the environmental conditions under which plant pathogens can cause disease. Isolates also vary in their tolerance to chemicals, and many show remarkable resistance to fungicides , either inherently or through mutation or adaptation. It is therefore possible to select fungicide tolerant or resistant biocontrol agents for use in integrated control (Tronsmo and Hjeljord 1997).

UV irradiation, chemical mutagens, protoplast fusion and gene coloning could be used for development of biotypes resistant to fungicides which are suitable for IPM application (Papavizas, 1985).

An antagonist insensitive to chemical(s) may be combined with it, extending the duration of effectiveness of control .An isolate of *T. harzianum* was developed by mutation, which was resistant to, and be combined to chemicals chlorothalonil, iprodion or benomyl (Papavizas *et al.*, 1982, Papavizas and Lewis, 1983). Under field and green house condition application of biotype Tu-203 (*T. harzianum).* Resistant to methyl bromide (MB) with reduced dose 200 kg/ h of MB controlled the *Rhizoctonia* disease of bean equal to recommended dose (500 kg/ h), and prevented reinfestation of soil by *R. solani* (Strashnow *et al.*, 1985).

Lo et al. (1997) by adding Triton X-100 at 1% to aqueous spray suspension of *Trichoderma harzianum* improved the effect of biocontrol on *R. solani*, and *Sclerotinia homoeocarpa.*

Prefoming an experiment with four isolates of *Trichoderma* as mycelial preparation and conidial preparation. It was reported that conidial preparation did not significantly prevent damping-off caused by *R.solani* but mycelial preparation of *Gliocladium. virense* (G21) and *T. hamatum* (TRI-4) significantly prevented damping- off on cotton , sugar beet and radish.(Lewis and Papavizas 1985).

Hadar *et al.* (1979), applied *Trichoderma* , in the form of wheat- bran culture to control *R. solani* in bean, tomato and egg plant seedling, under green house conditions. However, it was later found that during fungal growth, the pH of the preparation increased, possibly stimulating bacterial contamination. Therefore Sivan *et al.* (1984), in an attempt to overcome this problem, modified the preparation by mixing it with peat (1:1, v/v). In this modified preparation, the pH remained 5.5 during entire growth period. This preparation was a much better medium, with shelf life of one year at 25°C. Since then this formulation has proven succesful in many experiments in greenhouse and field (Chet and Inbar, 1997). *Trichoderma spp* were studied as biocontrol of *R. solani* on potato (Bari, 1988 and Beagle-Restanio and Papavizas, 1985), *Pythium aphanidermatum* on sugarbeet (Sawant and Mukhopadhyay, 1990) and ginger (Bhardwaj, et al.,1988), *Fuarium equiseti* on ginger (Bhardwaj, et al.,1988), *Macrophomina phaseolina* on soybean (Dubey and Dwivedi, 1988) , chickpea (Prakhar and Vishnav (1986), Mung (Kehri and Chandra, 1991) , mungbean (Raguchander *et al.*, 1997) and Sesamum (Sankar and Jeyarajan. 1996) and *Colletotrichun capsisi* on chilli (Jeyalakshmi, *et al.*, 1998) in India.

G. virens most effectively controlled damping-off of zinia, cotton and cabbage caused by *Pythium ultimum* or *R. solani* among 50 isolates of bacteria and fungi tested for biocontrol.(Lumsden, 1989). Damping-off diseases caused by *P. ultimum* and *R. solani* were supressed by *G. virens* in wheat bran-alginate and in two other comercial media containing peat moss as primary ingredient (Lumsden *et al.*, 1992). Seed treatment by *G. virens* reduced the preemergence damping -off of cotton in *R. solani* from 55 to 11. Introduction of *G. virens* into sand-corn meal culture containing mature

sclerotia of *R. solani* reduced sclerotial viability from 78 germinated in 100 mg of sclerptia in control to 8 germinated sclerotia(Howell, 1982).

Five substrates in different combinations with *T. hamatum*, *T.harzianum* or *G. virens* were tested in laboratory. Sugarcane bagasse and rice bran gave higher levels of cfu/g and took shorter time for full cover of the substrates . These were followed by wheat bran and crushed corn cobs (Amer and El-Desouky 1999). Studies on mass production for commercial application of *Trichoderma spp.* persuade and some formulation of *Trichoderma* has been developed for field application to control plant disease in Iran. (Zafari, personal communication).

Trichoderma viride, T. harzianum, T. koningii, Bacillus subtilis, and *Pseudomonas fluorescens* were tested for their potentially to suppress *R. solani* in dual culture as well as in screen house condition. All the microorganisms showed atagonistic activity to *R. solani* through hyphal coiling, penetration and lysis of hyphae of the pathogen. Coating potato tuber seeds with the antagonists protected seedlings from stem canker and black scurf in *R. solani* infested soil. *T. harzianum* and *P. fluorescence* were found to be more efficient in protecting the crop from the pathogen (Hazarika *et al.*, 1999).

Trichoderma spp. has been tested to control *R. solani* the causal agent of rice sheath blight (Zakii, 1975) and damping-off and seed rot of common bean (Bazgir, 1991), *Colletotrichum coccodes* , *Phytophthora erytroseptica, Fusarium solani* , *F. oxysporum and R. solani* on potato (Zafari, 1999), *Sclerotium rolfsii* on sunflower (Amirsadeqi, 1992), *Phytophthora dreschleri* and *Pythium aphanidermatum* on cucumber (Nazari, 1992) and other soil-borne pathogenic fungi in Iran.

Lignite stillage was used as carrier and substrate for application of *Trichoderma* @ 9.15 g/m of soil row to control *R, solani* (Jones, *et al.*, 1984). A method for encapsulation of biocontrol agents of plant pathogens was developed. Aqueus solution of 1% sodium alginate and 10% Pyrax contaminated with biocontrol spores or cells was dipped into solution of either 0.25 M $CaCl_2$ or calcium gluconate which cause aggregation of mixture and then they were dried and stored at room temperature (Fravel *et al.*, 1985).

Fermentor biomass (FB) preparations of *Trichoderma viride* (T-1-R9) and *G. Virens* (GI-21) applied as dusts to seed potatoes infested with sclerotia of *R. solani* before planting, reduced disease incidence in the field by 50 and 55%, respectively. Viability of sclerotia from seed pieces retrieved from the field was reduced 54- 89% by specific antagonists. In the greenhouse, up to 88% reduction in germination of sclerotia was obtained by treating sclerotia- infested tubers with FB of T-1-R9 before planting (Beagle-Restanio, Papavizas 1985).

Potato tubers treated with a spore suspension of *T. harzianum* and *T. viride* had much less stem and stolon canker and black scurf than untreated tubers. Biological control of *R.solani* black scurf disease of potato was observed when potato pieces were dipped in spore suspension of *T. pseudokoningii* or the *Bacillus sp.* (Ghaffar, 1993).

T. Harzianum, T. viride, and *G. viens* significantly inhibited the mycelial growth and sclerotia production of *Thanatephorus cucumeris* (= *R. solani)* causal agent of web blight of groundnut. *G. virens* was found the most effective principal mechanism of mycoparasitism was coiling of antagonistic hyphae around the host hyphae and lysis took place. Sclerotia , which were colonised by the antagonist, yielded only mycelium of antagonist. Hyphae of antagonist were also observed inside the sclerotia and finally sclerotia disintegrated (Dubey, 1997).

Aspergillus flavus was not effective in inhibiting mycelial growth of *Isariopsis griseola* and *R. solani*. Maximum growth inhibition (77.85%)of *R. solani* was obtained only by *T. viride* (Gupta et al., 1991). A standard preparation of *T. harzianum* and *T. konongii* in wheat bran- saw dust medium which can be very conveniently grown in autoclavable plastic bags of different size was developed. The preparation is less expensive and showed high potential for commercial use in the biological control of soil-borne plant diseases in sugarbeet, tobacco, tomato, brinjal, lentil and chickpea (Mukhopadhyay, 1987).

Epicoccum purpurascens was found to be strongly antagonistic to *R. solani* causing black scurf of potato. Fifty per cent culture filtrate of antagonist inhibited 84% mycelial growth and did not allow the sclerotia formation. Further 71.8 and 80.25 inhibition of black scurf was observed in unsterilized and sterilised soil respectively in the presence of *Epicoccum purpurascens*. However, the inhibition of disease was only 7.9 and 17.48%

in unsterilised and sterilised soil , respectively in second year trial. Antagonist supressed the population of *R. solani* up to 5 months. Population of pathogen increases in soil after elimination of antagonist due to high summer temperature (Singh and Sekhon 1984).

A conidial suspension of *Verticillium bigutattum* applied to potato seed tubers either as dip or a spray has been used on field-scale for control of collar rot and black scurf (*Rhizoctonia solani*). The antagonist was able to spread from the coated tubers to sprouts and stolons, protecting them from infection by *R. solani* and providing 60% reduction in sclerotium production by the pathogen(Jager and Velvis, 1986).

R.solani was successfully controlled by *Trichoderma* spp. in bean, tomato, peanut (Hadar *et al.*, 1979) lentil and chickpea (Mukhopadhyay, 1987).

T. longibrachiatum and *G. virens* were strongly antagonistic to rice sheath blight pathogen *R. solani* (Manibhushanrao *et al.*,1989).

Conidial and mycelial suspensions of *T. harzianum* and *G. deliquescens* when applied as seed treatment to control damping-off of chickpea caused by *R. solani* gave very less plant stand when compared to soil application of mycelial preparation (on wheat bran) of the above bioagents. Seed treatment with mycelial suspensions was found to be more effective than conidial treatment. When mycelial preparations of bioagents were applied in pathogen infested soil and incubated, gave more plant stand than treatment without incubation and was comparable to or even better than the fungicide mancozeb after three weeks duration (Prasad and Rangeshwaran, 1997).

Antagonistic activity of four isolates of *G. virens* and a single isolate of *Streptoverticillium sp.* was demonstrated towards phytopathogenic fungi, viz., *Aspergillus flavus, Colletotrichum gloesporioides, Fusarium solani* and *Rhizoctonia solani* . Spore germination and radial growth of test fungi were inhibited by cell-free culture filtrate of all bio-agents. Moreover, the disease symptoms were restricted on pre-treated plants groundnut, brinjal and paddy , which were artificially inoculated by pathogenic fungi , respectively (Mishra and Narain, 1994).

2-2. *Trichoderma*

Colonies often growing rapidly, but variable among species; mycelium mostly submerged, some strains eventually with matted, floccose, wooly or arachnoid aerial mycelium. Reverse uncoloured or in different species variously buff, yellow,amber, reddish or yellow-green. Conidiation effuse or tufted forming compact pustules; white at first, remaining so or turning green, grey or brown. Chlamydospores usually present, often abundant in submerged mycelium; intercalary, or terminal on short lateral branches of vegetative hyphae; globose to ellipsoid, colourless to pale yellowish or greenish, smooth- and sometimes thick-walled (to 4µm thick). Conidiophores in most species with a broad, stright or flexous main axis; primary branches usually arising at regular intervals and producing smaller secondary branches that also may branch, and so on, with successive branches at higher levels apically and distally usually progressively shorter and narrower ; branches more or less divergent, solitary, paired or in verticils ; in some species repetitive verticillate branching results in highly ramified pyramidal structure; in other species branching is less regular with branches solitary or paired and not extensively rebranched. The conidiophore main axis and primary branches in some species are terminaated by sterile conidiophore elongations which may be simple or branched, straight, flexuous, undulate, hamate or coiled. Adjacent conidiophores frequently anastomose in some species, but not in others. In many species, phialides and fertile branches also arise from otherwise undifferntiated aerial hyphae in areas of effuse conidiation. Conidiogenous cells phialides, usually disposed in divergent verticils terminally on branches of the conidiophore, less often solitary or in whorls directly beneath septa along the conidiophore and branches; cylindrical, subulate, lageniform, ampulliform or subglobose; usually attenuate to narrow, short- cylindrical, conidium-bearing neck. Conidia 1-celled, colourless, greyish, green or brown; smooth- walled to distinctly roughened, or a few species with sinuate, bullate or winglike projections from the outer wall; subglobose, obovoid, ellipsoide, oblong or short cylindrical; accumulating in apparent gloeoid heads, or enclosed in a sac-like sheat in two species examined for this stucture (Meyer, Plakowitz 1989). Teleomorph, where known, is *Hypocrea.(* Bissett 1991).

Lectotype species: *Trichoderma viride* Pers. , Fr. (Fide Hughes 1985). The genus *Trichoderma* is divided into five sections,namely Pachybasium, Trichoderma, Saturnisporum, Longibrachiatum and Hypocreanum. Section

Pachybasium contains four species (*T. hamatum, T. polysporum* and *T. piluliferum*), section Trichoderma contains (*T. koningii, T. aureoviride* , *T. viride, T. atroviride, T. harzianum*), section Longibrachiatum contains : (*T. pseudokoningii,* and *T. Longibrachiatum*), in section Saturnisporum contain *T. saturnisporum* , and section Hypocreanum contains *Hypocrea* anamorphs.

The five sections of Trichoderma may be identified according the Bissett 1991 key as follow:

Key to the sections of Trichoderma

1a. Conidiation effuse;conidiophores with few or no lateral branches; phialides born in simple terminal verticils, cylindrical to lageniform ..... Section Hypocreanum

1b. Conidiation effuse or fasciculate to pustulate; conidiophores with frequent lateral branches; phialides mostly lageniform to ampulliform 2

2a. Conidiophores main axes long with short secondary branches, not extensively rebranching; branches and phialides frequently arising singly .....section Longibrachiatum

2b. Conidiophores repeatedly rebranching; branches and phialides paired or verticillate..... 3

3a. Phialides ampulliform to broadly lageniform, mostly in verticls of 2 or 3; conidia with conspicuous, sinuate, wing like or bullate ornamentation.....section Saturnisporum

3b. Phialides lageniform to subulate, or if ampulliform, then in verticils of up to 7 ; conidia smooth or verrucose..... 4

4a. Conidiophores and branches relatively broad (main axis to 10µm wide , phialides in verticils of 2-7 , ampulliform to lageniform

.....section Pachybasium.

4b. Conidiophores and branches narrow and flexuous (main axis to 6µm wide) ; phialides mostly in verticils of 2 or 3, lageniform to subulate.....section Trichoderma

Species of the genus *Trichoderma* can be identified according to Rifai 1969 and Domsch 1980 using the following key:

Key for species of the genus *Trichoderma*:

- **1a.**-Conidiophores long and thick, often with sterile hyphal elongations; side branches mostly short and thick, bearing crowded, short and plump phialides; colonies white or whitish green to green, generally with compact tufts of conidiophores (**2**)
- **1b.**- Conidiophores and their side branches long and slender, without sterile hyphal elongations ; phialides not crowded, rather slender; colonies yellowish, bright, dull to dark green, floccose or with compact conidiophore tufts (**5**).
- **2a.**-Sterlile hyphal elongations absent; conidia globose, hyaline (***T. piluliferum***).
- **2b.**-Sterile hyphal elongations present or modified or rarely absent; conidia not globose (**3**).
- **3a.**-Conidia green, short ellipsoid, surrounded by a wide irregular veil (***T. saturnisporum*****).**
- **3b.**-Conidia smooth-walled or finely punctuated (**4**)
- **4a.**-Conidia hyaline, small , 2.4-3.8×1.8-2.2µm (***T. polysporum***).
- **4b.**-Conidia green, small to large, 3.8-6.0 × 2.2-2.8µm (***T. hamatum***).
- **5a**.-Conidia roughened, 3.6- 4.8× 3.5-4.5µm (***T. viride***).

- **5.b**-Conidia smooth-walled (**6**).

- **6a.**-Conidiophores with complicated dendroid branching system; phialides± regularly disposed in numbers of 3 or more (**7**).

- **6b**.-Conidiophores with simpler branching system; phialides irregularly laterally disposed, often arising singly (**9**).

- **7a.**-Conidia ellipsoid or oblong , often appearing angular, 3.0-4.8 ×1.9-2.8µm (***T. koningii***).

- **7b.**-Conidia shorter with a length : width ratio of lessthan 1.5 (**8**).

- **8a.**-Conidia obovoid, with truncate base, 3.0-4.8×2.0-3.0µm ;reverse of colonies often discoloured; colonies reaching 3 cm diameter in 5 days at 20°c on OA(***T.aureoviride***).

- **8b**.-Conidia globose, subglobose or short-obovoid, with length: width ratio of less than 1.25, 2.8-3.2×2.5-2.8µm ; colony reverse uncoloured; ; colonies reaching>9 cm in 5 days at 20°c on OA (***T.harzianum***).

- **9a**.-conidia subglobose to ovoide, 3.8-4.5×2.5- 3.0µm(***T.T. resei***).

- **9b**.-Conidia ellipspoidal (**10**).

- **10a-Phi**alides usually only slightly attenuate at the base; conidia large, partly dark green, to 7µm long, mostly elipsoidal (***T. longibrachiatum***).

- **10b**-Phialides usually distinctly atteuate at the base; conidia smaller, pale green 2.8-4.8µm long, mostly oblong ellipsoidal (***T. pseudokoningii***).

The genus *Trichoderma* was introduced by Persoon (1794) for four macroscopically similar fungi, which were described by Persoon as appearing like a mealy powder enclosed by hairy covering, now those species are known to be unrelated to each other. (Bissett 1991, Gams and Bissett, 1998), and consists of anamorphic fungi isolated primarily from soil and decomposing organic matter. Isolates of *Trichoderma* are ubiquitous and are relatively easy to isolate and culture. In addition , isolates grow quickly on many substrates, produce metabolites with demonstrable antibiotic activity, and may be mycoparasitic against a wide range of pathogens.

Most species of the genus grow rapidly in artificial culture and produce large number of small green or white conidia from conidiogenus cells situated at the ends of widely branched conidiophores. This characteristic allows a relatively easy identification of *Trichoderma* as a genus, but species concepts are difficult to interpret and there is considerable confusion over the application of specific names. Rifai (1969) divided *Trichoderma* into nine species aggregates on the basis of morphological features. Bisset (1991) revised the genus and also included some *Hypocrea* anamorphs in the genus, resulting in the establishment of five new sections. Species concepts within *Trichoderma* are very wide, and this has resulted in the establishment of many specific and subspecific taxa (Samuels 1996). Systematic of *Trichoderma* of Iran has been studied (Khodakramian, 1998) and application of molecular biology for classification of *Trichoderma* of Iran is under study (Zafari, personal communication).

Trichoderma harzianum Rifai is a species aggregate which includes a plethora of strains that can be used as biological control agents of plant pathogenic fungi and viral vectors (Grondona *et al* ,. 1997).

Papavizas *et al.* (1982) developed benomyl resistant *T. harzianum* by exposing spore suspension of *Trichoderma* to UV radiation , and reported that 19 colonies tolerated 100-500mg/ml of benomyl. Carbendazim at 5 ppm completely inhibited *T. viride* a common weed mould in oyster mushroom (*Pleurotus sajor-caju*) cultivation. (Rai and Vijay, 1992).

The ecological preferences of *Trichoderma* are discused in comprehensive reviews of Danilson & Davey(1973 a-c). When dry condition in soil are maintained for long periods of time, the population of *Trichoderma* and *Gliocladium* as a group decrease. Danilson and Davey also concluded that certain strains of *T. hamatum and T. pseudokoningi* are adapted to condition of excessive soil moisture. *T. viride* and *T. polysporum* are restricted to areas where low temperature prevail, whereas *T. harzianum* is commonly found in warm climatic regions and *T. hamatum* and *T. kononingi* are widely distributed in areas of diverse climatic conditions (Papavizas (1985).

Through the work of several scientists, *Trichoderma* spp. have proven their potential as superior biocontrol agents. The time has come to move from small-scale greenhouse and field experiment to commercial application of *Trichoderma.* Large-scale production of *T.harzianum* as a biocontrol

agent is now under way in several countries, and commercial preparations are available as Trichodex in Israel, BINAB-T, F. Stop, Gliogard and T-22 in USA, Trichoject in New Zealand , Supresivit in the Czech Republic , Trichokill and Gliostar in India (Barry 1993, Deacon 1997, Mishra, 1997).

It would be unrealistic to expect that biological control agents like *Trichoderma* can completely replace chemical fungicides in disease control. There are however, areas in which biological agents are superior to chemical agents, and future research should be directed toward exploiting such niches. For example, rhizosphere competent *Trichoderma* isolates can colonise the entire root zone and afford localised protection unattainable through chemical control. Furthermore, successful colonisation by *Trichoderma* isolates adapted to specific applications will provide persistent protection only achievable by repeated doses of chemicals. Biological agents achieve their effect through a variety of mechanisms and, thus avoid the development of resistance that so often renders fungicides obsolete.

Risk evaluation is another aspect of *Trichoderma* biocontrol needing clarification, in order to gain public acceptance of these biological protectants used on food crops.

Finally, though *Trichoderma spp.* have proven their worth as biocontrol agents in the field, yet we know very little about the actual mechanisms underlying their antagonism. Interactions between *Trichoderma spp* and pathogenic fungi are currently being analysed and monitored on a molecular biological level in laboratories around the world. Further research should give us insight necessary to improve the agents themselves as well as the appropriate delivery systems to achieve consistent, effective and environmentally sound biological control in a wide range of applications. (Tronsmo and Hjeljord, 1997).

High yields (approx. 10^8 /ml) of protoplasts of high purity (99%) can be obtained from young thalli of strains T12 and T95 of *Trichoderma harzianum* by digestion of cell walls with NovoZym234. Protoplasts derived from mycelium or immature conidia contained 2-12 nuclei and they regenerate readily on basal osmotically stabilised medium (Staz et al 1998).

All *Trichoderma* thalli that have been examined are polynucleate and it seems reasonable that wild strain may be heterokaryotic. The sexual stage of *Trichoderma* and *Gliocladium* is rare, and may be entirely lacking for some

biocontrol strains and sexual recombination may not be possible. Protoplast fusion is a method to efficiently induce heterokaryosis. Of several enzymes and mixture of enzymes examined for release of viable protoplast NovoZyme 234 at 13 mg/ml gives the good results.(Harman and Hayes,1993). To increase recovery selective methods have been devised by [Askew and Laing, 1993 ; Davet, 1979 ; Papavizas and Lumsden, 1982; Elad and Chet, 1983; Elad *et al.*, 1981 ; Johnson *et al.*, 1987 ; and Smith *et al.*,(1990)] (Gams and Bissett 1998).

2-3. Mechanism(s) of biocontrol:

2-3-1. Antibiosis and competition:

Antagonistic microorganisms have the potential to interfere with the growth and /or survival of plant pathogens, thereby contributing to biological control. Antagonistic interactions among microorganisms in nature include parasitism or lysis, antibiosis and competition (Baker and Grifinn 1995). Diverse mechanisms of action of biocontrol are antibiosis, repellents, toxins, and induced resistance (Cook 1993).Under nutrient- rich conditions, many *Trichoderma* isolates posses three antagonistic mechanisms : competition, antibiosis, and mycoparasitsm (Dennis and Webster 1971 a-c).

Dennis and Webster (1971 a-b) reported the inhibitory effect of volatile and non volatile metabolites of *Trichodermaon* against other fungi.

Baker and Griffin (1985) extended the scope of antibiosis to " inhibition or destruction of an organism by metabolic production of another. This definition includes small toxic molecules, volatile and lytic enzymes.They concluded that even in cases where antifungal metabolite production by an agent reduces disease, the impact of antibiosis in biological control is uncertain, because other mechanisms may also be operating (Lumsden *et al.*,1992).

Species of *Trichoderma* are antagonistic to other fungi, and have shown primacies as biocontrol agents of several soil-borne diseases (Papavizas ,1985 ; Jensen & Wolffhechl,1995). *G. virens* produces secondary non volatile metabolites (gliotoxin, gliovirin, gliocladic acid, heptalidic acid, viridin and viridol), that have biological activity in vitro against plant pathogens. Gliotoxin adversely affects membrane of *Pythium* and *Rhizoctonia* and may be a factor of leakage of metabolites from hyphae of *Rhizoctonia*

treated with extracts from cultures of *G. virens*. Anastomosis groups (AGs) of *R. solani* were variable when treated with gliotoxin, Ag1, AG4 and AG5 required much more gliotoxin for inhibition (Lumsden *et al.*, 1992).

Trichodermin, suzukacillin and alamaticin are among the non-voatile metabolites produced by *Trichoderma spp.* produces 6- pentyl- α- pyron (6PAP) which is toxic to plant pathogens. PAP inducing isolates of *T. virens* are more effective in biocontrol. Gliovirin producing strains inhibit the growth of *Pythium ultimum* but not *R. solani* in vitro, conversely gliotoxin is more effective against *Rhizoctonia* than Pythium (Deacon, 1997).

Trichoderma hamatum was isolated from a soil sample suppressive to *R. solani* which was able to attack *R. solani* mycelium in dual culture and produced cell wall degrading enzymes β- 1,3 glucanase and chitinase. Addition of *T. hamatum* to conducive soil @ 10^6spore/ g soil made it suppressive to *R. solani* (Chet and Baker, 1981). *G. virens* caused cytoplasmic leakage of *R. solani*, mycelium, and prevented secondary branching of hyphae and occasionally coiled around *Rhizoctonia* hyphae (Harris and Lumsden, 1997).

Exogenous nutrients are required for pathogens to germinate, penetrate and infect host tissue successfully (Baker and Grifin 1995). Garret (1965) concluded that starvation is the most common cause of death microorganisms. Therefore, competition for limiting nutritional factors. mainly carbon , nitrogen and iron, may result in biological control of plant pathogens.

The direct mycoparasitic activity of *Trichoderma spp.*is one of the major mechanisms proposed to explain their antagonistic activity against soil-borne plant pathogenic fungi (Dennis &Webster,1971; Elad *et al.* 1982;Ridout *et al.* 1986).

2-3-2. Mycoparasitism

Barnett and Binder (1973) divided mycoparasitism (direct attack on fungal thallus, followed by nutrient utilization by the parasite), as:

- Necrotrophic (destructive) parasitism, in which the relationship results in death and destruction of one or more components of the host thallus.

- Biotrophic (balanced) parasitism; in which the development of the parasite is favored by a living host stucture.

Biotrophic mycoparisites tend to have restricted host ranges and produce specialized structures to absorb nutrients from their host In contrast, necrotrophic mycoparasites (e.g. *Trichoderma spp.*) tend to be more aggressive, have a broader host range extending to a wide variety of taxonomic groups, and are relatively unspecialized in their mode of parasitism The antagonistic activity of necrotrophic mycoparasites is attributed to the production of antibiotics toxins or hydrolytic enzymes in proportion that cause the death and destruction of their host (Manocha , 1990).

Due to their nature (being more common, saprophytic in nature and less spcialized in their mode of action), the majority of mycoparasites used as biocontrol agents to date have been necrotrophs (Chet and Inbar, 1997).

Mycoparasitic activity and antibiotic production were first demonstrated in *Trichoderma* by Weindling in 1932 and 1934, and many modern biotechnological applications of these fungi as biocontrol agents are derived from these early works (Grondona et al 1997).

Mycoparasitism by *Trichoderma* is a complex process, initiated by *Trichodermas* directed growth toward the host hyphae, probably by chemotropism, and followed , at contact with the host, by its coiling around the host hyphae and penetrating the cell wall by secreting lytic enzymes, mainly chitinase and ß-glucanase. Mycoparasitism relies on the production of lytic enzymes by the mycoparasite for degradation of cell walls of the host fungus .Biological control by mycoparasite involves the following steps:1) chemotropic growth of mycoparasite towards its host, 2) recognition and attachment of host by the mycoparasite, 3) secretion of lytic enzymes, chitinases and other hydrolyses, 4) penetration of host by mycoparasite, and 5) lysis and killing of host by mycoparasite. The success of mycoparasitism may be impeded or hindered at any one of these points (Manocha and Govindsamy 1997).

Recently , using gold cytochemistry, *T. harzianum* hyphae were shown to coil around and penetrate cells of *R. solani* , causing extensive damage such as cell wall alteration, plasma-membrane retraction and cytoplasmal aggregation (Benhamou and chet, 1993). Inbar and Chet (1992) established a system based on the binding of lectin onto a surface of nylon

fibers , which mimicked live hyphae and could induce changes in the behavior and morphogenesis of filamentous fungi (e.g. *T.harzianum*).These results provided the first evidence of the role of lectins in mycoparasitism.

Studies on parasitism of *Sclerotium rolfsii* and *Rhizoctonia solani* by *Trichoderma hamatum* and *T. harzianum* demonstrated additional phenomena at the molecular level (e.g. exteracellular fibrillar material deposited between the interacting hyphae, the accumulation of parasite organelles in the parasitizing cells, the production of sheat matrix that encapsulates the penetrating hyphae (Elad *et al.* 1983).

Parasitism of *R. solani sasakii* by *T. harzianum* was observed in two ways. Hyphae of the latter grew appressed that the former and small protuberances developed at certain points , which continued to grow coiling around the hyphae of *R. solani sasakii.* In another method , the hyphea of *T. harzianum* itself coiled around that of *R. solani sasakii* (Roy and Sayre, 1984). A few fungi , particularly *Penicillium ehrlichii, Fusarium solani* and *Pseudourotium multisporum* were found to have antagonistic effect against *R. solani sasakii.* (Roy 1985).

Two isolates of *T. harzianum* (Th1 and Th2) exhibited strongly hyperparasitic activity against *R. solani (* causal agent of paddy sheath blight). Culture filtrate of both isolates inhibited sclerotial germination of *R. solani* . Soaking sclerotia in culture filtrate of antagonists reduced their viability by 45 and 50 % , respectively and reduced disease severity and disease incidence in green house tests.(Kumaresan and Manibhushanrao, 1991).

Lectins sugar-binding proteins or glycoproteins have also been found to be produced by some soil-born plant pathogens such as *R. solani* and *S .rolfsii*, researchers suggested that lectins play a role in the recognition and specificity between *Trichoderma* and pathogenic fungi (Barak and Chet, 1990 ; Chet and Inbar 1997). The peptide toxin mastoparan and the activator fluoroaluminate (AlF_4^-) increased coiling more than two-fold in comparision with control whereas aluminum ions alone were ineffective.cAMP increased coiling about three-fold when the chemicals were used in a biomimetic system consisting of nylon fibers (Herrera-Estrella et al 1999).

Five substrates in different combinations with *T. hamatum, T.harzianum* or *G. virens* were tested in laboratory. Sugarcane bagasse and rice bran gave higher levels of cfu/g and took shorter time for full cover of the

substrates . These were followed by wheat bran and crushed corn cobs (Amer and El-Desouky 1999).

Application of a single species or strain of a micro-organism, with proven biological activity, for control of a specific target pest or disease is presently the most popular strategy for increasing biological diversity in the soil on a commercial basis (Deacon 1991).

G.virens utilised the sclerotia of *R.solani* as food base in natural soil in green house at 30±5°C. Surface sterilised sclerotia colonised by *G. virens* yielded *G. virens* on PDA indicating internal; parasitization of sclerotia by mycoparasite (Mukherjee and Mukhopadhyay , 1995). The resting structures like sclerotia of *S. Sclerotiorum, S. rolfsii,* and *R. solani* are directly invaded and degraded by *Trichoderma spp (* Tu *.et al.*, 1980; Elad *et al.*, 1984; Mukhopadhyay and Mukherjee 1996; Benhammou and chet 1996). Parasitism of sclerotia is suggested as the principal mechanism of biological control of *S. rolfsii* and *R. solani* by *G. virens (* Mukherjee *et al.*, 1995).

Weindling & Emerson (1936) working with *Trichoderma lignorm* (? *G. virens*) isolated gliotoxin in crystalline form which was highly toxic to *R. solani* and Brain & Mc Gowan (1945) described viridin produced *T. viride.* Dennis & Webester (1971) showed that *Trichoderma spp.* produce other toxic antibiotics such as trichodermin.

2-3-3. Production of lytic enzymes:

2-3-3-1. Chitinase

Chitin, a homopolymer of β-1,4 -linked N- acetyl –D-glucosamine (GlcNAc) , is one of the most abundant polymers in the nature, second to cellulose . It is a major structural component in a wide variety of organisms; it is found in the exoskeletons of many invertebrates and in cell walls of most fungi and algae. Vessey and Pegg (1973) extracted Chitin from lobster exoskeleton .

The cell walls of basidiomycetes and ascomycetes contain chitin and laminarin (β-1,3 glucan) but no cellulose. Oomycetes contain β-1,3 glucans and cellulose, and relatively small amount of chitin (<1%, as in *Pythium*) or no chitin (as in *Phytophthora*). The chitin in fungal cell walls occurs as a primary microfibrilar layer or as an inner complex layer with glucans and proteins connected via peptide bridges. Cell wall of *Rhizoctonia solani* is

composed of β-1,3 glucan and chitin. Chitin content of cell wall of *R. solani* is 8% of cell wall dry weight (Chet and Inbar , 1997). In fungal cell wall chitin is cross-linked covalently to other wall components notably β-1,3 glucan (Goody, 1994).

Chitin degradation is therefore, important to a wide variety of applications, ranging from the seafood industry and environmental cleanup of chitinous wastes, plant defence system (Graham & Sticken, 1994) and biological control (Chet & Inbar, 1997).

Chitinases, the chitin degrading enzymes, are produced by several groups of organisms and have been shown to have various physiological functions (Vasseur et al., 1990).

Chitin an insoluble polymer, induces the chitinolytic system of *T. harzianum* , which made up of two N-acetylglucosaminidases and four endochitinases (Schickler et al 1998). One of the best studied chitinolytic system is that of the soil-borne *Trichoderma harzianum*, an effective biocontrol agent of several economically important plant-pathogenic fungi. Chitinase activity in *T.harzianum* grown in liquid cultures is induced by chitin, fungal cell walls and to a certain extent by GlcNAc (Ulhoa & Peberdy, 1991). The chitinolytic system of *T. harzianum* has been found to be composed of six enzymes: two N-acetylglucoseaminidases and four endochitinases.Chitin induces the expression of various components of this system (Haran *et al.*, 1996).

The capacity to produce cell wall- degrading enzyme is common among saprophytic fungi. More than 10% of 160 fungi examined produced β-1,3 glucanase (Chesters and Bull 1963). In mycoparasitism, the host may first be softened simultaneously by toxic metabolic products, including enzymes, before disorganization and dead occure (Papavizas, 1985).

Efficacy in biocontrol of plant pathogenic fungi may be increased by combining different organisms, metabolites, or genes Biocontrol strains from the genera of *Enterobacter* and *Pseudomonas* and two chitinolytic enzymes from *Trichoderma harzianum* were combined and tested for antifungal activity in bioassay. Inhibitory effects on spore germination and germ tube elongation of pathogenic fungi (*Botrytis. cinerea, Fusarium. solani, Uncinula necatore*) were synergistically increased by mixing fungal enzymes and cells of *Entrobacter clocae* but not of *Pseudomonas spp.* However , the

combination of bacterial culture filtrate with fungal chitinolytic enzymes generated only an additive response, indicating that the presence of bacterial cell was required for synergistic effect (Lorito *et al.* 1993).

Six different enzymes exhibiting chitinolytic activity were found to be secreted when *T. harzianum* was grown on synthetic medium with chitin as sole carbon source; two were *β*-1-4N-acetylglucosaminidases [CHIT 102 and CHIT 73] and four were endochitinases [CHIT 52, CHIT42, CHIT33 andCHIT31] (Haran *et al.*, 1995; Lorito *et al.*, 1993).

As early as 12 h after contact between *T. harzianum* and *S. rolfsii* , activity of the costitutive *β*- 1,4- N acetylglucoseaminidase (CHIT 102) was to increase greatly. Twenty four hours after contact between *T. harziznum* and *S. rolfsii* , CHIT102 began to disappear and concomitantly, CHIT73 was activated , from then on, untill the end of the incubation period (120 h). CHIT 73 was the major active chitinolytic enzyme. Tlus phenomenon could be eliminated by autoclaving *S. rolfsii* mycelium perior to its incubation with *T. harzianum* in this case CHIT102 remained active up to 48 h after contact between *T. harzianum* and the dead *S. rolfsii* mycelium. These results suggest that heat- libale factors in the live mycelium of the host are responcible, for at least in part, for this phenomenon a very interesting finding from this work was that induction of chitinolytic enzymes in *Trichoderma* during mycoparasitism is a very early event which is triggered only by the recognition signal.

Increased activity of CHIT 102 was detected when *T. harzianum* was grown on nylon fibers coated with the purified lectin of *S. rolfsii* in the biomimetic system , in which no chitin was present. (Chet and Inbar, 1997).

Trichoderma harzianum excreted *β*-1,3 glucanase and chitinase into the medium (Chet *et al.*, 1967; Kitamoto and Kono, 1987). The production of chitinases has been described in several fungal species, but most typically by those which are antagonistic to other fungi for example *T. harzianum* and those pathogenic to insects such as *Metarhizum anisopliae* .

Filamentous fungi in particular *Aspergillus spp.* and *Trichoderma reesei* have been used as hosts for the synthesis of many important proteins. *Aspergillus oryzae* is presently the most commonly used fungal host for the production of recombinant industrial enzymes derived from other fungi including amylases, lipases, proteases, cellulases, xylanases and peroxidases.

Trichoderma reesei shows similar potential to the Aspergilli as a host for recombinant protein production and it has been successfully transformed to constitutively produce chitnase ,. The transformed *T. reesei* expressed an exteracellular enzyme at a specific activity 6.5 fold higher than the interacellular level at peak production, and enzyme had a calculated molecular mass of 52 kDa , the *T. harzianum* endochitinase gene (ThWn-42)also has been expressed in *T. reesei*. These results show that *T. reesei* can be used as an alternative host *to Aspergillus sp.* for the production of commercially important proteins (Deane *et al.*, 1999).

Recently chitinases have taken the center stage in the investigations on host-parasite interactions and biological control of fungal pathogens. The degradation of chitin occurs in two steps. In the first step, endo- or exochitinase, or both cleave chitin microfibrils. Exochitinase cleaves chitin in stepwise fashion resulting in the progressive release of chitobiose. No other monomers or oligomers are formed. Endocitinase, in the otherhand, cleaves chitin randomly resulting in soluble low molecular weight GlcNAc oligomers such as chitotetraose and chitotriose with dimer, chitobiose, being predominant. In the second step, chitobiose as well as chitotriose and chitotetraose are hydrolysed to GlcNAc monomers in an exotype fashion by ß-1,4 N acetyglucoseaminidase

Chitinases with their molecular masses are differing consideably (MW 27-130 kDa) in various fungi are generally active at slightly acidic pH, have high temperature optima, and high degrees of stability. They are inhibited by divalent cations, specially copper and mercury, and are competitively inhibited by chitobionocton oxime and allosamidin (Manocha and Govindsamy , 1997).

Three isoforms of chitinases were induced by MNT7 (a mutant *T. viride*) when the exteracellular protein fractions were probed with tobacco chitinase antiserum. Three proteins resembling chitinases were purified from the concentrated culture filtrate of MNT7 by protein precipitation with ammonium sulphate, gel filtration and finally chitin chromatography with molecular weights 30 kDa. 41kDa and 64kDa, The antifungal activity of 41kDa chitinase against the *R. solani* was proved using inhibition zone technique (Balasubramanian *et al., 1997*).

As molasses and brewer's yeast medium is cheap it could be used for mass production of *T. harzianum*.

An amendment of chitin to soil leads to an increase in the population of chitinolytic microbes and decrease in the population of soil-borne fungal plant pathogens (Boller 1986).

Reissige *et al.* (1955) developed a modified colorimetric method for the estimation of N- acetyl amino sugars using concentrated borate buffer.

Two *T. harzianum* strains which were highly mycoparasitic to *R. solani* and *Pythium aphanidermatum* when grown in liquid culture containing laminarin, chitin or fungal cell walls as sole carbon sources, released ß- 1,3 glucanase and chitinase into the medium (Sivan and Chet 1989).

Lorito *et al.* (1994) purified 1,3 - ß-glucanase (EC.3.2.1.58) and N – acetyl glucoseaminidase(Ec 3.2.1.30) from *T.harzianum* and detected synergistic inhibitory effect on both spore germination and germ tube elongation of *Botrytis cinerea* when the enzymes were applied together. When used alone at concentration of 150µg ml^{-1} the endochitinase inhibited spore germination of the test fungus and caused cell wall damage, resulting in bursting of hyphal tips.

Combining the endochitinase and chitobiosidase for their antifungal activity against nine different fungal species resulted in a synergistic increase of antifungal activity. and the degree of inhibition of spore germination or cell replication was proportional to the level of chitin in the cell wall of the target fungi (Lorito *et al.*, 1993).

When gliotoxin and endochitinase were applied together a synergistic inhibitory effect was observed. Addition of 25 or 50 µg ml^{-1} endochitinase reduced the ED_{50} of gliotoxin from 1.25µg ml^{-1} to 0.75 and 0.5µg ml^{-1}, respectively (Di Pietro *et al.*, 1993).

Chitinase gene from *Aphanocladium album* fused in frame to the glyceraldehyde -3-phosphate dehydrogenase promoter of *Aspergillus nidulance* has been successfully transferred to *Trichoderma reesei* and up to 70% of the stable transformants exhibited exteracellular chitinase activity (Deane *et al.*, 1999).

Infection of plants by potentially pathogenic micro-organisms has shown to result in accumulation of novel class of proteins termed "

pathogenesis related proteins" or PR-proteins like chitinase and ß-1,3 glucanase (Van Loon, 1985).

The fungus *T. harzianum* was able to grow on *R. solani* cell wall as a sole carbon source (Hadar *et al.*, 1979). Isolates of *T. harzianum* produced exteracellular β-1,3 glucanase and chitinase when grown on cell walls of *R. solani* (Chet *et al.* ,1979; Hadar *et al.*, 1979).

The chitinase gene Chi A, encoding one of the chitinases from *Serratia marcescens*, a well- known biocontrol agent , was isolated and cloned into *E. coli* (Shipra *et al.*, 1989). To improve the biocontrol activity of *T. harzianum* , the Chi A gene from *Serratia* was introduced into the fungus via protoplast transformation using plasmid DNA, the plasmid used for transformation carried the Chi A gene , pSL3ChiAII, controlled by a viral promoter which allowed constitutive expression of the gene, and a selectable marker, the amdS gene carried on the plasmid p3SR2, which enabled the transformed fungi to grow on acetamide or acrylamide as the sole nitrogen source. Two transformants showed increased chitinase activity (specific activity, 11 and 5 times more than the recipient) and excreted a protein of about 58 kDa, the expected size of the *S. marcescens* chitinase , when grown on synthetic medium without chitin. Antagonistic activity of the transformants was significantly.higher than that of wild type *T. harzianum*, as evaluated by their ability to overgrow the plant pathogen *S. rolfsii* in dual culture (Haran *et.al.*, 1993).

The major advantage of such genetic manipulation is the ability to isolate genes from one strain and to introduce them into other varieties of fungi or bacteria. This can be used to enhance the potency of biocontrol agents and make a single strain effective, stable and consistent against more than one plant pathogenic fungus., without the hazardous effects of chemical pesticides.

Geremia *et al.*, (1993) identified a basic proteinase of *T.harzianum* (Prbl) which is induced in the presence of either autoclaved mycelia or cell-wall preparation of phytopathogenic fungi.

2-3-3-2. β-1,3 glucanase

β-1,3-glucans are known to be present in several plant structures including the wall of pollen tubes, endosperm cell walls , and the wall of seive elements (Broglie et al 1993). β-1,3 glucan (laminarin) are found in microorganisms and higher plants as structural entities of cell walls, as cytoplasmic and vascular reserve materials, and exteracellular substances of uncertain significance. Laminarin is a vascular content of marine algae. *Laminaria* and *Alaria* species , the best source of laminarin, consitituting 22-34% of dry weight of these algae. Laminarin functions as storage product and is usually accumulated during summer months in Northern hemisphere. Extraction methods for laminarin have been developed by Black *et al.* (1951). These methodes involve the precipitation of the polysaccharide from aqueous solutions with ethanol or other organic solvents thereby yielding a cold water- soluble product ("soluble laminarin"), or the spontaneus precipitation from acidic solutions (pH 2.4) to give a cold water-insoluble product which will dissolve in warm water ("insoluble laminarin"). Present evidence allows us to conclude that laminarin consists of linear mannitol- or glucose-terminated chains of β-1,3- linked glucose residues joined by β-1,6 interresidue bonds. B-1,3 glucans have been reported in a number of fungal cell walls.

Enzymes which hydrolyse β-1,3 glucans have been known as laminarinases (= laminarases) and more suitably β-1,3 glucanases. These are ubiquitous enzymes and appear to be implicated in the intercellular mobilization of food reserves in fungi, algae, and higher plants; wall plasticity in budding and dividing fungal cells; extracellular depolymerization of plant debries by microorganisms; and digestive metabolism of invertebrates.

Fungi genrally have powerful laminarinases. It appears that endohydrolases, including laminarinase are primary attackers of the cell walls, which produce substrates for exohydrolysis. Being a constitutive enzyme in fungi, laminarinase has been prepared using a variety of carbon sources for growth. In contrast bacterial laminarinase appears to be inducible. The enzyme is conveniently extracted from cold cell free filterates at pH 5-6 by precipitation with acetone, ethanol,or ammonium sulfate. Amylase and nucleic acids are removed from fungal preparation by adsorption chromatography on carboxymethyl cellulose and protamine sulfate precipitation respectively. Both exo- and endo- β-1,3 glucanases are

stimulated by Fe^{3+}, Mn^{2+} and Ca^{2+} ions at 1mM concentration, but heavy metals ions such as Cu^{2+}, Ag^{+}, and Hg^{2+} were highly inhibitory to the enzymes.

Trichoderma viride STR produced 92 and 8% exo- and endo- β-1,3 glucanase ,respectively. Soil treatment with chitin and /or laminarin resulted in a decline in disease severity caused by pathogenic *Fusaria*, but *Pythium debaryanum* and *Agrobacterium tumefaciens*, which do not contain these polsaccharides in their cell walls were not suppressed. Natural sources of these polysaccharides, ground lobster shells and *Laminaria* fronds, were equally effective (Bull and Chesters, 1955).

A strain of *T. harzianum* isolated from Shiitake logs produces remarkable amount of β-1,3- glucanase and chitinase when it is cultured in a medium containing the powder of fruit-bodies of basidiomycetes as carbon source(Kitamoto *et al* . 1987).

T.harzianum isolate Th008 secreted trichodermin (MW=292) and a small peptide (Mw=876) in culturte, which were antagonistic to *R.solani* mycelial growth.. When 100 mg of dried autoclaved mycelium of *R.solani* was added to 200 ml of liquid cultures of *T. harzianum*, the quantity of antimycotic compounds increased 3.5 times geater than that of antagonist alone. *R. solani* secreted cumarin (MW=313) in liquid culture, which inhibited the mycelial groth of *T.harzianum*, however, the inhibition of the growth of the antagonist required a greater concentration than that for the antimycotic compound produced by the antagonist against the pathogen. The inclusion of 100 mg of dried autoclaved mycelial mat of *T. harzianum* in 200 ml liquid culture of *R.solani* did not effect the quantity of the antimycotic compound produced by the pathogen (Bertagnolli *et al*., 1999).

β-1,3 glucanase is a semiconstitutive enzyme which may be induced by several inducers such as laminarin , starch, xylose, mannitol and glycerol. However, in the presence of laminarin the excretion of this enzyme increases (Sivan and Chet , 1989). It has been suggested that the lytic activity of several starains of *T. harzianum* on cell walls of *Sclerotium rolfsii*, *Rhizoctonia solani* and *Pythium aphanidermatum* can be correlated with the degree of biocontrol of those pathogens *in vivo* (Elad *et al*., 1982). When grown in liquid cultures contaning laminarin, chitin or fungal cell walls as sole carbon

sources, both the two strains of *T. harzianum* released β-1,3 glucanase and chitinase into the medium (Sivan and Chet, 1989).

2-3-3-3. Cellulase

β- glucosidase is common among plants, fungi and bacteria. The enzyme system for the conversion of cellulose to glucose comprises endo-1-4- *β*-glucanase (EC 3.2.1.4) cellobiohydrolase (Ec3.2.1.91), and *β*-glucosidase(*β*-D-glucosidic glucohydrolase (EC 3.2.1.21). The role of the *β*-glucosidase in the saccharification of cellulose is to degrade cellobiose, an inhibitor of depolymerization, and cellooligosaccharides to glucose.(Oh *et al.*, 1998).

The exteracellular enzyme system produced by *Trichoderma reesei* is composed of three major enzyme components: endoglucanases, exocellobiohydrolases and *β*-glucosidases. These enzymes are usually glycosylated, occur in multiple formes and have distinct specific activities. In general, the random action of endoglucanases appears to gradully inerease the availability of cellulose chain ends, therefore increasing the specific area of substrate for exoglucanase catalysis. More recently modified celluase preparations were obtained from selected *Trichodema reesei* strains after they were subjected to high frequency gene replacement. As a result several enzyme preparations were produced in which at least one of the four main components of the cellulase system, CBHsI and II and EGsI and II, was missing. Deletion of the gene encoding for CBH I from the genome *of T. reesei* decreased the filter paper activity of culture filterate by 70 per cent and endoglucanases were over-expressed when both genes encoding for CBHsI and II were deleted. Altough CBHs are the main components of cellulase system, the relevance of endoglucanase-rich preparations should not be underestimated. Endoglucanases have very important applications in the hydolysis of amorphous and/or soluble cellulose derivatives and due to their properties, these enzymes are rather useful in sevral industrial processes including biopolishing of textile fibers and fiber modification in pulp and paper manufacturing (Ramos et al., 1999).

A simple cultural test for relative cellulolytic activity of fungi was developed. In this method the organisms were screened on china blue aurin cellulose medium having : K_2HPO_4 1.0g ; $MgSO_4$ $7H_2O$ 1.0g; Na_2CO_3 1.0g ; $(NH_4)_2SO_4$,1.0 g; Distilled water 1000 ml amended with different carbon

sources viz., CMC, cellobiose or cellulose powder. *T. reesei* QM 9414 and *Pleurotus sajor-caju* produced the higher amount of cellulase. (Kanotra and Mathur, 1995).

Goyal *et al.*, (1986) reported that the optimum pH and temprature for cellulase assay were 4.8 and 55°C, respectively, and sodium nitrate supported higher cellulase production . Cellulase powder at 2% was optimum and local *Aspergillus terreus* showed higher ß-glucosidase activity than *T. reesei* QM 9414 and its mutants.Production of cellulase by *Trichoderma reesei* has been reported by Anand (1979) and Chaudhary (1981).

Chapter 3

Materials and Methods

3-1. Isolation of *Trichoderma* from soil

Soil samples from 0-25cm dept of potato fields of Ambala ,Kurukshetra, Karnal and Hisar districts of Haryana were colected in plasic bags (5 samples of 250 g weight from each district) and carried to lab. Davet (1979) selective media was used for isolation of *Trichderma* from soil. The media consisted of the following ingredients :

$Ca(NO_3)_2$	1g
$CaCl_2.2H_2O$	1g
Sucrose	2g
KNO_3	250mg
$MgSO_4,.7H_2O$	250mg
KH_2PO_4	125mg
Citric acid	50mg
Agar-agar	25g
Distilled water	1000ml
Allyl alcohol	0.5ml
Vinchlozoline	2.5mg
Streptomycin	30mg

Allyl alcohol, streptomycin and vinchlozoline were added to sterlized medium when it was cooled to about 60 °C.

Two grams of each soil sample was added to 20 ml of sterile distilled water and 2 ml of the suspension was added to 18 ml sterilized melted Water Agar (WA), mixed and it was added to a petri plate. After solidifaction of WA disks of 1 cm diameter was removed form it using corck borer. The disks were inoculated to the center of plates having 20 ml of Davet media and

incubated at 28°C for 7 days. The mycelia which emerged from the disks were tranfered to PDA for purification and further use.PDA medium composition was:

Extract of 200 g boiled potato in water
Dextrose 20g
Agar 20g
Volume was adjusted to 1000 ml by adding distilled water.

The isolates were identified using publications of Rifai (1969), Domsch and Aderson (1980), Bisset (1984 and 1991a-c) and Gams and Bissett (1998).

3-2 Isolation of *Rhizoctonia solani*

R. solani was isolated from sclerotia of black scurf infected potato tubers using Streptomycin sulfate (30 mg/ L) amended PDA and they were subcultred on PDA for purification and further use . The isolates of *R. solani* were identified according to Sneh *et al.*, (1991).

3-3 Dual Culture of *Trichoderma* vs. *R. solani*

Disks of 5 mm diameter from the margin of 4 days old culture of *R. solani* grown on PDA at 28°C were inoculated at one side of petri plate containing PDA, and on the oposite side the plate was inoculated with a disk of 5 mm diameter of 4 days old culture of an isolate of *Trichoderma* grown on the same situation as *R.solani.* Plates were incubated at 28°C and they were checked daily and the radial growth of *R. solani* measured and recorded. In control only *R.solani* was inoculated on the plates, each treatment repeated in 3 petriplates and experiment was repeated three times. Growth inhibition of *R. solani* by *Trichoderma* was calculated using the following formula:

$$\frac{100 \times (\text{Growth of } \textit{R. solani} \text{ in control} - \text{Growth of } \textit{R. solani} \text{ in treatment})}{\text{Growth of } \textit{R. solani} \text{ in control}}$$

Cultures were checked for colonization of *R.solani* mycelium by *Trichoderma* isolates. Hyphal interaction *R.solani* and *Trichoderma* were studied using Light microscope and Scanning Electron Microscope (SEM). For light microscopic studies slids of samples from the area of contact of *Trichoderma* and. *R.solani* were prepared and they were examined under 100, 400 and1000X magnification for hyphal coiling, hyphal penetration and other hyphal interacions and abnormalities in *R. solani* hyphae.

Scanning Electron Microscopy sudies of hyphal interaction

Samples from the area of contact of *Trichoderma* and. *R.solani* were removed and fixed in 2.5% Gluteraldehyde for 48 hrs, then they were washed 3 times (30 min each) in 0.1M phosphate bufer pH 7.0 and dehydrated with graded series of ethanol (30, 50, 70 % and absolute) 15 minutes each and the samples were chemically dried using 1,1,1,3,3,3-Hexamethyl Di Silizone (HMDS) for 15 minutes.

The specimens were mounted on stubs using double sided sticky tape and they were gold coated and examined under a LEO 435 VP (Clifton Road, Cambridge CBI 3QH England) Scanning Electron Microscope (using the facilities of All India Institute of Medical Scinces , New Delhi) for hyphal interactions of *Trichoderma* and *R. solani* and abnormalities in *R. solani* hyphae.

3-4. Effect of Culture filtrate of *Trichoderma* on *R.solani* mycelial growth

Disks of 5 mm diameter of 4 days old culture of *Trichoderma* isolates were added to 100 ml conical flasks containg 50 ml PDB (8 flasks for each isolate) and they were incubated in a rotary shaker incubator at 28°C and 140 shake/ min. for 7 days. The cultures were centrifuged at 15000 rpm at 4°C for 30 min. and were filtered through Watman No.1 filter paper.

The culture filterates was added to PDA at about 60°C @ 10%(v/v) and petriplates were filled with 20 ml of the mixture in control only filtered PDB was used and in 2 treatments suspension 15 ppm a.i. of MEMC (

Emisan 6% Hg Formulation from Excel Industries Limited) and 125 ppm a.i.carbendazim (Bavistin 50% WP , from BASF) added to cooling PDA.

Plates were inoculated with 5 mm diameter disks from the margin of 4 days old culture of *R. solani* grown on PDA at 28°C and incubated at 28°C for 4 days in this experiment 3 plates were used for each treatment and the experiment replicated twice.The radial growth (mm) of *R.solani* in petriplates was recorded after 48, 72 and 96 hrs.The data of 96 hrs. were subjected to statistical analysis and they were compared using Duncan multiple range test 5%.

3-5. Effect of Culture filtrate on dry weight of *R.solani*

Culture filtrate was added to PDB @ 10% in 100 ml conical flasks containing 20 ml sterilised PDB and fungicide treatments MEMC 15 ppm(Emisan 6% Hg Formulation from Excel industries Limited) and 125 ppm carbendazim (Bavistin50%, from BASF) were used, in control only PDB filtrate was added @10% . Flasks were inoculated with 5mm disks of 4 days old culture of *R.solani* grown on PDA at 28°C and they were incubated in a shaker incubator at 140 rpm at 28°C for 4 days. Each treatment consisted of 3 flasks and the experiment was repeated twice. The mycelial mat of *R.solani* was collected *on* Watman No. 1 filter paper and washed 3 times with sterile distilled water and dried at 80°C for 48 hrs. The dry weight of *R.solani* mycelia (mg) was recorded for each treatment and the data were subjected to statistical analysis and compared using Duncan multiple range test (5%).

3-6. Effect of volatile metabolites of *Trichoderma* on *R. solani*

Disks of 5 mm diameter of 4 days old PDA culture of *Trichoderma* and *R. solani* were inoculated on the centre of separate petriplates having solidified PDA. Removing the lids of the plates the *R. solani* plates were reversed on the *Trichoderma* plates and the two plates were airtighted using adhesive tape to prevent gas exit from the plates. In control only a disk of PDA was used instead of *Trichoderma* to inoculate the plates and the *R. solani* plates were reversed on these plates. Each treatment included 3 petriplates, and the experiment was repeated twice. The radial growth of *R. solani* recorded at 48, 72 hrs and data of 72 hrs incubation were statistically analysed and means compared using Duncan multiple range test (5%).

3-7. Effect of Fungicides on *R. solani*

Fungicides used in this experiment were tebuconazole (Folicur^{R} 250 EC, Bayer) carbendazim (Bavistin 50 % WP. from BASF) ,MEMC (Emisan, 6% Hg), , chlorothalonil (75% WP.). The fungicides were mixed with PDA @ 1, 10,, 100, 200, 500 ppm of active ingredient . Fungicides stock solutions or suspensions were prepared on sterile distilled water and added to autoclaved PDA while it was cooled to about 60 °C. Disks of 5 mm diameter of *R. solani* grown on PDA at 28°C for 4 days were removed by cork borer and they were inoculated in the centre of fungicide amended PDA, and plates were incubated at 28°C for 4 days. In control disks of *R. solani* inoculated on PDA only. Radial growth of *R. solani* was recorded for each treatment at 48 , 72 and 96 hrs. after inoculation and data were statistically analysed and the means compared using Duncan multiple range test (5%).

3-8. Effect of Fungicides on *Trichoderma* spp.

The fungicides used in this experiment were: tebuconazole (Folicur^{R} 250EW , Bayer) @100,200 and 300 , carbendazim (Bavistin50% WP, from BASF) @ 50, 100, 250 and 500 , MEMC (Emisan 6% Hg Formulation, Excel industries Limited)@ 5, 10, 20 and 50 ppm and chlorothalonil @ 100 and 200 ppm of active ingredients. The fungicides were added to cooling sterilised PDA at desired concentrations.

Disks of 5 mm diameter of 4 days old culture of *Trichoderma* isolates grown on PDA at 28°C were inoculated on fungicide amended PDA plates and they were incubated at 28 °C for 4 days. Radial growth of *Trichoderma* isolates recorded at 48, 72 and 96 hrs. Each treatment consisted of 3 petri plates and the experiment was repeated twice. In control only PDA was used. Data were subjected to statistical analysis and compared using Duncan multiple range test (5%).

3-9. Mutation of *Trichoderma* for fungicides resistace

This experiment was conducted to obtain fungicides resistant / tolerant mutants for use in combination with fugicides . The methods of [Papavizas *et al*., (1982) and Papavizas and Lewis (1983)] for UV induced mutation and (Vashisht, 1973 and Yadav, 1991) for chemical mutation were adopted with slight modifications.

3-9-1. UV Mutation in *Trichoderma* :

Two wild types (WT) isolates of *T. longibrachiatum* viz. WT_5 and WT_4 were grown on PDA at 28 °C for 7 days in petriplates. Then 5 ml distilled water was added to each plate and spores of *Trichoderma* were gently rubbed in water by sterile cotton tipped needle. The spore content of the suspension was adjusted to 10^7 spore/ml by addition of sterile distilled water. Ten ml of each spore suspensions (10^7 spore/ml) transferred to sterile petri plates, and the plates were exposed to Ultra Violet (UV) [germicidal tube] irradiation from 20 cm distance for different periods of time i.e. 0, 5, 10, 20,40, 60, 80, and 100 minutes. The treated suspension was diluted with sterile distilled water to give 10^3spore/ ml. One ml of UV treated suspension spreaded on sterile PDA (5 plates for each treatment) and they were incubated at 28 °C for 4 days.

Growing colonies were monitored and the number of colonies developed in each plate was recorded and based on these data the suitable time for UV treatment (95- 99 % mortality) was selected. It was found that 60 min exposure to UV is suitable for mutation. It gives more than 95% mortality, so the experiment was conducted by exposing the spore suspension to UV at 20 cm distance for 60 minutes. 1 ml of UV treated suspension was spreaded on fungicide amended PDA and the plates were incubated at 28 °C for 4 days. The survived colonies were transferred to PDA and incubated for 7 days for complete sporulation and spores were collected in distilled water as before and exposed to UV for 60 min. followed by incubation on fungicide

amended PDA for 4 days as before and transferring to PDA for sporulation. This process repeated for the third time for producing stable mutants.

3-9-2. EMS mutation

In this experiment (Yadav, 1991 and Vashishat, 1973) method was used . Ethylmethane sulphonate (EMS) was used as chemical mutagen. Suspension of 10^6 spore/ml of T_5 and T_4 isolates of T. *longibrachiatum* was prepared in 0.1 M phosphate buffer pH 7.0 in 50 ml conical flasks (9.8 ml/ flask). To that added 0.2 ml of EMS and flasks were incubated for 0, 5, 15, 30, 40, 50 and 60 min with constant shaking (50 shake / min) after each period of time the corresponding flasks were removed from the shaker incubator and EMS was inactivated. For EMS inactivation 4 ml of 6% sodium thiosulphate solution was added to 1 ml of EMS treated spore suspension . After inactivation of EMS for 10 min, the suspension was diluted with sterile distilled water to give 1000 spore / ml. And 1 ml of diluted suspension was spreaded on each petri plate having PDA (5 Plate for each treatment) and plates were incubated at 28 °C for 48 hrs. Growing colonies were monitored and the number of colonies developed in each plate was recorded and based on these data the suitable time for EMS treatment (giving more 95- 99 % mortality) was selected .

It was found that 40 minutes is the suitable period time for mutation. In the next stage the suspension of conidia were treated with EMS for 40 minutes. After inactivation of EMS, 1 ml of diluted suspension 10^3 spore / ml was plated on fungicide amended PDA, and the plates were incubated at 28 °C for 4 days aud number of germinated spores and their colony criteria were recorded. The germinated spores were transferred to PDA and incubated at 28 °C for 7 days for complete sporulation. The processes of EMS treatment for 40 minutes was repeated on the spore suspension prepared from the fungicide tolerant colonics. They were cultured on fungicide amended PDA again. The pure culture of fungicide tolerant was maintained on slant PDA and preserved in refrigerator.

3-10. Effect of fungicides on mycelial growth of mutants

The mutants and wild types were grown on PDA at 28 °C for 4 days. Disks of 5 mm diameter of the mutants and wild types cultures (WT) were inoculated on the fungicide amended PDA, each treatment included 3 petri plates and the experiment was repeated 3 times. Plates were incubated at 28 °C for 4 days and radial growth of the fungi were recorded. The fungicides used in this experiment were MEMC (10,50,100 ppm), carbendazim (100, 200 ppm), tebuconazole (50, 100 and 200 ppm) and chlorothalonil (100 and 200 ppm).

3-11. Production of lytic enzymes by *Trichoderma*

3-11-1. Preparation of Fungal Cell-walls Materials (FCW) of *R. solani*

For preparation of cell wall material of *R. solani* the method of Mitchell and Alexander (1963) was adopted. *R. solani* was grown on PDB in shaker incubator at 28 °C and 120 shake/minute for 7 days . The culture broth was filtered through Watman No. 1 filter paper and mycelial mat was collected, washed frequently with distilled water to remove the media from the mycelial mat and then boiled for 30 min in 500 ml of 2% sodium dodcyl sulphate (SDS). This was then filtered and repeatedly washed with distilled sterile water to remove SDS. Finally it was extracted twice with 50ml ethyl ether at 4°C and dried at 50°C for 24 hours. The cell wall material was powdered in a grinder at the highest speed and used as the carbon source for enzyme assay.

3-11-2. Chitinase assay

3-11-2-1. Preparation of colloidal chitin:

Unbleached chitin was used for the preparation of colloidal chitin by the method of Lingappa and Lockwood (1962). Crude chitin (20g) was washed alternatively for 24 h with 1 N NaOH and 1 N HCl [5-6 times] followed by washing with 95% ethanol [3-4times]. The cleaned chitin was mixed with acetone and dissolved in 100 ml of cold concentrated HCl by stirring for 20 minutes in an ice bath. The thick syrupy solution was then filtered by suction through a thin glass wool pad on a Buchner funnel into a 2L of stirred ice cold distilled water for precipitation the chitin as a fine colloidal suspension. The colloidal chitin was sedimented by allowing standing and washing in 5 litres of distilled water 4-5 times and storing in a refrigerator until used. This suspension was used as substrate for assay of chitinase The dry weight of colloidal chitin was determined by placing 1 ml of colloidal suspension on pre-weighted filter paper and drying it at 60°C for 24 hours followed by calculation of differences of loaded and unloaded filter paper. Selected *Trichoderma* isolates were grown on SMCS medium with the following ingredients :

SMCS medium composition (Di Pietro ET al., 1993):

KH_2PO_4	680 mg
K_2HPO_4	870 mg
KC	200 mg
$CaCl_2$	200 mg
$MgSO_4.7H_2O$	200 mg
$FeSO_4$	2 mg
$ZnSO_4$	2 mg
$MnSO_4$	2 mg
Sucrose	5 g
NH_4NO_3	1 g

containing 1% chitin for 7 days in shaking ineubator at 27 °C and 140 shake/minute. The broth was centrifuged at 10000 rpm for 30 minutes and supernatant was used for chitinase assay. One ml of colloidal chitin (10mg/ml) was mixed with 3 ml of citrate buffer (pH 5.05) and was incubated

with 1ml of cell free supernatant of *Trichoderma* culture for 60 minutes in a water bath at 37 °C. After this 1ml sample was transferred to a screw cap tube containing 1 ml of distilled water and the reaction was stooped by placing the tube in a boiling water bath for 15 minutes and then cooled to room temperature. The mixture was centrifuged and the amount of N-acetyl glucose amine (NAG) was determined in 1ml of the supernatant by method of Ressieg *et al.* (1955). For this, 0.5 ml of sample, was mixed with 0.1 ml of potassium tetraborate and this mixture was boiled in a water bath for 3 minutes and cooled. Three ml of p-dimethyl amino benzaldehyde (DMAB) reagent was added and the mixture was incubated at 36-38 °C in a water bath for 20 minutes and cooled. The absorbance was read at 585 ηm using a Spectronic-20 spectrophotometer. The amount of NAG released by 1 ml of supernatant was determined from the standard curve and specific activity is expressed as μmoles of N- acetyl glucoseamine per mg chitin per hour.

3-11-2-2. Release of N- acetyl glucose amine from fungal cell wall

2 ml of supernatant of *Trichoderma* isolates as in the case of chitinase assay was transferred to a test tube having 1.6 mg of FCW of *R. solani* and incubated for 24 hours at 35 °C. In control only distilled water was added to FCW. The release of N- acetyl glucoseamine was measured according to Ressiege *et al.*, (1955) and compared with standard curve prepared from grading dilution of N- acetyl glucose amine . Enzyme activity was expressed as μmoles of N- acetyl glucoseamine per milligram of CFW per hour.

3-11-3. β-1,3- glucanase activity

Trichoderma isolates were grown on SMCS containing 1% FCW of *R. solani* as carbon source in shaker incubator at 27 °C and 120 shake/minutes for 7 days. The broth was centrifuged at 10000 rpm at 4 °C for 5 minutes and dialysed against distilled water till there was no trace of glucose. The dialysed supernatant was used for β-1,3- glucanase assay.

To 1.5 ml of citrate buffer 0.1 M pH 5.1 was added 1.6 mg of laminarin (Sigma, USA) and mixed in a test tube. Then 0.5 ml of dialysed supernatant was added to the mixture and tubes were incubated at 40 °C for one hour in a water bath. The reaction stopped by boiling the mixture for ten minutes. Amount of glucose released was measured according to Nelson (1944).

1 ml of sample was added to 1 ml of freshly prepared copper reagent in a test tube and it was boiled in a water bath for 20 minutes . The tubes were cooled to room temperature. One ml arsenomolybdate reagent was added to each tube and volume adjusted to 10 ml by addition of sterile distilled water. Absorbance was read at 520 nm using a spectronic 20 spectrophotometer, and the amount of glucose released in 1 ml of sample was determined from the standard curve developed using graded dilution of glucose. Specific activity is expressed in μ mole of glucose released / mg laminarin/ hour.

Citrate buffer (pH 4.8)

prepared according to Gomori, (1995) by preparing stock solutions A [0.1 M solution of citric acid (21.01 g/1000 ml distilled water)] and B [0.1 M solution of sodium citrate (29.41 g/ 1000 ml distilled water)]. Citrate buffer pH 4.8 was prepared by mixing, 23 ml of stock A with 27 ml of Stock B.

Copper reagent A:

It was prepared by dissolving 25g of anhydrous Na_2CO_3, 25g of Rochelle salt (potassium sodium tartarate), 20 g of $NaHCO_3$ and 200g of anhydrous Na_2SO_4 in about 800 ml of distilled water and then the volume was made to 1 litre by addition of distilled water.

Copper reagent B:

Fifteen grams of $CuSO_4.5H_2O$ was dissolved in 100 ml of distilled water containing 1-2 drops of concentrated H_2SO_4. Copper reagent A and B were mixed in ratio of 25:1,before use.

Arsenomolybdate reagent:

Twenty five grams of ammonium molybdate was dissolved in 450 ml of distilled water, and 21ml of concentrated H_2SO_4 was added to it with stirring. 3 g of $Na_2HAsO_4.7H_20$ dissolved in 25 ml of distilled water was

added with mixing and the solution was kept in incubator at 37 °C for 24 hours. This reagent was stored in a glass-stoppered brown bottle.

3-11-3-1. Release of glucose from fungal cell wall

2 ml of supernatant of *Trichoderma* isolates as in the case of chitinase assay was transferred to a test tube having 1.6 mg of FCW of *R. solani* and incubated for 24 hours. at 35 °C . In control only distilled water was added to FCW. The release of glucose was measured according to Nelson (1944) and compared with standard curve prepared from grading dilution of glucose. Enzyme activity was expressed as μm moles of glucose per miligram of CFW per hour.

3-11-4. Cellulase assay

Cellulase activity in *Trichoderma* culture filtrates was determined by growing the disk of 5 mm diameter of *Trichoderma* isolates in 100 ml flask containing Mandels and Sternberg (1976) medium as adopted by Anand (1979), which consisted of:

$(NH_4)_2SO_4$	1.4 g
KH_2PO_4	2.0 g
Urea	0.3 g
$CaCl_2$	0.3 g
$MgSO_4.7H_2O$	0.3 g
$FeSO_4.7H_2O$	5.0 mg
$MnSO_4.H_2O$	1.6 mg
$ZnSO_4.7H_2O$	1.4 mg
$CoCl_2$	2.0 mg
Distilled water	1000ml
pH	5.5

containing 1% cellulose powder. The flasks were incubated at 28 °C and 140 shake /minutes for 7 days in CIS 24 Orbital Shaking Incubator (REMI Instruments). The broth were centrifuged at 10000 rpm for 5 min at

4°C and the supernatant was dialysed against distilled water till the time there was no trace of glucose in the supernatant. It was used for cellulose activity according to Mandels *et al.*, (1976) method with modification and reducing sugars were estimated by Nelson method (1944), as described for β-1,3-glucanase.

Preparation of 1% CMC solution:

One gram CMC (carboxy methyl cellulose) was dissolved in 80 ml hot distilled water (80-90 °C) by adding the dry powder slowly with continuous agitation. To it 10 ml of 0.5 M citrate buffer (pH 4.8) was added. The volume was made to 100 ml by distilled water.

3-11-4-1. Exoglucanase (C1) activity:

One ml of 1% CMC solution and one ml of culture filtrate were taken in a test tube and mixed well. The mixture was incubated for 30 min at 50 °C in a water bath with frequent shaking. The amount of reducing sugars estimated according to Nelson (1944) as in case of β-1,3 glucanase. Enzyme specific activity is expressed in μ mole of glucose released / mg protein/ hour.

3-11-4-2. Endoglucanase (Cx) activity:

Fifty mg of cellulose powder was taken in each test tube and to this was added 1 ml of culture filtrate and 1 ml citrate buffer (pH4.8). The test tubes were shaken well and were incubated for 60 min at 50 °C in water bath shaker. The amount of reducing sugars estimated according to Nelson (1944) as in case of β-1,3 glucanase. Enzyme specific activity is expressed in μ mole of glucose released / mg protein/ hour.

3-12. Field application of *Trichoderma* to control black scurf of potato

3-12-1. Mass production of biocontrol agent inoculum

Selected isolates of *Trichoderma* were grown on autoclaved wheat bran in polypropylene (PP) bags at room temperature. For this to 500 g dry

wheat bran 500 ml of tap water was added and mixed thoroughly in PP bags and after closing the bags with rubber band the bags were sterilised in an autoclave for 3 successive hours at 120 °C . The bags allowed to be cooled for 24 hours to reach the room temperature. Then the spore suspension of *Trichoderma* isolates was added to make 10^7spore/g wheat bran. And the bags were incubated at room temperature for 20 days. The wheat bran culture of *Trichoderma* isolates were used in field experiments @ 83 g. wet weight/m^2 of field as soil application of biocontrol agent's treatments. In chemical and control infested treatments only sterile uninoculated wheat bran was added @ 83 g wet weight/m^2 .

3-12-2. Preparation of *R.solani* inoculum

R. solani was prepared by addition of mixture of 6 isolates of the fungus (2 petri plates from each 4 days old culture on PDA) to wheat bran prepared as before and the bags were incubated at room temperature for 20 days. The wheat bran culture was used as inoculum of *R. solani* @ 75 g wet weight/m^2 of field in all treatments except check not inoculated treatment in which only not inoculated sterilised wheat bran was used at the same rate.

3-12-3. Treatment of Tubers with spores of *Trichoderma*

Potato tubers were dipped in 10^8-spore/ml suspension of *T. harzianum* (WT_8), *T. longibrachiatum* (WT_5), *T. longibrachiatum* (WT_4) in plastic bags for 1 hour.

3-12-4. Fungicides Tuber dipping

The fungicides used in this experiment were: tebuconazole (Folicur R 250EW , Bayer) @. 2 ml/l, carbendazim (Bavistin 50% WP, from BASF India Ltd.) @ 2.5 g/ l , MEMC (Emisan 6% Hg , from Excel Industries Limited) @ 2.5 g formulation / l water. Calculated amount of fungicides was added to 20 litres of water in buckets. Potato tubers were dipped for 20 minutes in the buckets. These treated tubers were used for chemical and integrated (chemical + biological) treatments.

3-12-5. Exprimental design

Completely Randomised Design (RBD) was adopted with 16 and 32 treatments in the first (1998-99) and second (1999-2000) years of experiment, respectively in 3 replications. Each treatment consisted of 3 rows of 2 meters length in each row 10 potato seed were seeded at 20 cm distance from each other and inter row space was 60 cm. The experiment was conducted in the field of Department of Plant Pathology, CCS Haryana Agricultural University, and Hisar, India.

Treatments in the first year of experiment were:

(T1) Check not infested either with pathogen or biocontrol.
(T2) Seed treatment with spores of *T. longibrachiatum* (WT_4)
(T3) Seed treatment with spores of *T. longibrachiatum* (WT_5).
(T4) Seed treatment with spores of *T. harzianum* (WT_8).
(T5) Soil application of wheat bran culture of WT_4.
(T6) Soil application of wheat bran culture of WT_5.
(T7) Soil application of wheat bran culture of WT_8.
(T8) Soil application of wheat bran culture of WT_4+Dipping the tubers with carbendazim.
(T9) Soil application of wheat bran culture of WT_4+Dipping the tubers with MEMC.
(T10) Soil application of wheat bran culture of WT5+Dipping the tubers with carbendazim.
(T11) Soil application of wheat bran culture of WT_5+Dipping the tubers with MEMC.
(T12) Soil application of wheat bran culture of WT_8+Dipping the tubers with carbendazim.
(T13)Soil application of wheat bran culture of WT_8+Dipping the tubers with MEMC.
(T14) Dipping the tubers with carbendazim.
(T15). Dipping the tubers with MEMC.
(T16) Check infested with *R. solani* inoculum only.

The following 32 treatments consisted the second year of experiment:

T1) Check not infested either with pathogen or biocontrol.
(T2) Seed treatment with spores of *T. harzianum* (WT_8).
(T3) Seed treatment with spores of *T. longibrachiatum* (WT_5).

(T4) Seed treatment with spores of *T. longibrachiatum* (WT_4).
(T5) Soil application of wheat bran culture of WT_8.
(T6) Soil application of wheat bran culture of WT_8 + Dipping the tubers with MEMC.
(T7) Soil application of wheat bran culture of WT_8+Dipping the tubers with carbendazim.
(T8) Soil application of wheat bran culture of WT_8+Dipping the tubers with tebuconazole.
(T9) Soil application of wheat bran culture of WT_5.
(T10) Soil application of wheat bran culture of WT_5 + Dipping the tubers with MEMC.
(T11) Soil application of wheat bran culture of WT5+Dipping the tubers with carbendazim.
(T12) Soil application of wheat bran culture of WT_5+Dipping the tubers with tebuconazole.
(T13) Soil application of wheat bran culture of WT_4.
(T14) Soil application of wheat bran culture of WT_4+Dipping the tubers with MEMC.
(T15) Soil application of wheat bran culture of WT_4+Dipping the tubers with carbendazim.
(T16) Soil application of wheat bran culture of WT_4+Dipping the tubers with tebuconazole.
(T17) Soil application of wheat bran culture of EM_{64}. (mutant of WT_4 developed by EMS chemical mutagen).
(T18) Soil application of wheat bran culture of EM_{64} + Dipping the tubers with MEMC.
(T19) Soil application of wheat bran culture of EM_{64} + Dipping the tubers with carbendazim.
(T20) Soil application of wheat bran culture of EM_{64} + Dipping the tubers with tebuconazole.
(T21) Soil application of wheat bran culture of EM_{24}.(mutant of WT_4).
(T22) Soil application of wheat bran culture of EM_{24} + Dipping the tubers with MEMC.
(T23) Soil application of wheat bran culture of EM_{24} + Dipping the tubers with carbendazim.
(T24) Soil application of wheat bran culture of EM_{24} + Dipping the tubers with tebuconazole.
(T25) Soil application of wheat bran culture of EM_{15}. (mutant of WT_5).

(T26) Soil application of wheat bran culture of EM_{15} + Dipping the tubers with MEMC.
(T27) Soil application of wheat bran culture of EM_{15} + Dipping the tubers with carbendazim.
(T28) Soil application of wheat bran culture of EM_{15} + Dipping the tubers with tebuconazole.
(T29) Dipping the tubers with MEMC.
(T30) Dipping the tubers with carbendazim.
(T31) Dipping the tubers with tebuconazole.
(T32) Control infested with *R. solani* inoculum only.

3-12-6. Monitoring disease symptoms

The field was monitored during the growing season for *R. solani* disease symptoms and signs such as:

- Pre-and post-emergence damping-off.
- Root and stem canker.
- Aerial tuberization
- Abnormalities on foliage parts such as yellowing, wrinkling of leaves Stunting.
- Perfect stage of the pathogen
- Reisolation *R. solani* from diseased plant parts.
- Black surf on potato tubers.

3-12-7. Effect of biocontrol on number of emerged seedlings of potato 15 DAS

The number of emerged potato seedlings were recorded 15 days after sowing (DAS). The data were subjected to statistical analysis and means were compared using Duncan multiple range test (5%).

3-12-8. Effect of biocontrol on foliage fresh weight of potato plant:

10 days before harvesting the aerial parts of potato plant were cut (dehalmed) and the fresh weight of foliage of each plot was recorded, data were statistically analysed and they were compared using Duncan multiple range test (5%).

3-12-9. Effect of biocontrol on foliage dry weight of potato plant

The dehalmed parts of plant were shadow dried for 20 days and later they were oven dried at 60 °C for 48 hours, and then weighted. Data were recorded and statistically analysed and compared using Duncan multiple range test (5%).

3-12-10. Effect of biocontrol on incidence of stem canker of potato

At the time of harvesting of potato the under ground parts of stems were collected and number of stems infected with canker recorded and percentage of infected stems over total number of inspected stems for each plot was calculated (disease incidence). Data were statistically analysed and compared using Duncan multiple range test (5%).

3-12-11. Effect of biocontrol on severity of stem canker of potato:

Size of cankers for disease severity based on 0-4 scale (Adams *et al.*,1980) was recorded, where:

0 = Free from diseasc.
1= less than 25% of stem covered by canker.
2= 25-50% of stem covered by canker.
3= 50-75% of stem covered by canker
4= more than 75% of stem covered by canker.

Disease severity was calculated for each treatment using the following formula :

$$\text{Disease severity} = \frac{\Sigma[(A\times0) + (B\times1) + (C\times2)+ (D\times3) + (E\times4)]}{\Sigma\,(A+B+C+D+E)}$$

Where:

A= number of stems free from disease.
B= number of stems infected in scale 1.
C= number of stems infected in scale 2.
D= number of stems infected in scale 3.
E= number of stems infected in scale 4

Data were subjected to statistical analysis using Duncan multiple range test (5%) the means were compared.

3-12-12. Effect of biocontrol on incidence of black scurf of potato:

After harvest number of potato tuber having scurf were recorded (disease incidence) and percentage of infected tuber was calculated for each plot, data recorded and statistically analysed and compered using Duncan multiple range test (5%).

3-12-13. Effect of biocontrol on severity of black scurf of potato:

The severity of black scurf on tubers was determined, using 0-4 scale (Dijst, 1985), where:

0= free from disease.
1= less than 10 small sclerotia on each tuber.
2 = 10-20 sclerotia per tuber.

3 = 21- 50 sclerotia per tuber.
4 = more than 50 sclerotia per tuber.
Disease severity was calculated for each plot by:

$$\text{Disease severity} = \frac{\Sigma[(A\times0) + (B\times1) + (C\times2) + (D\times3) + (E\times4)]}{\Sigma(A+B+C+D+E)}$$

Where:

A= number of tubers free from disease.
B= number of tuber infected in scale 1.
C= number of tuber infeeted in scale 2.
D= number of tuber infected in scale 3.
E= number of tuber infected in scale 4

The data were recorded and they were subjected to statistical analysis and compared using Duncan multiple rang test (5%).

3-12-14. Effect of biocontrol on pereentage weight of scurfed potato tubers

The scurfed potato tubers weight was recorded for each plot and percentage of scurfed tubers over total tuber weight in each plot was calculated. Data were statistically analysed and compared using Duncan multiple range test (5%).

3-12-15. Effect of biocontrol on weight of sclerotia/cm^2 tuber surface

The sclerotia of the infected tubers were scrapped by shaving blade very carefully, and the sclerotia were oven dried at 80°C for 48 hours. The weight of sclerotia/ cm^2 was calculated using the method of Dijst (1985) in this method the surface of potato tuber is estimated by using the fresh weight of potato tubers (in gram) raised by 2/3 power .

$$\text{Surface of potato tubers} = (\text{fresh weight of potato tubers})^{2/3}$$

Data were subjected to statistic analysis and compared using Duncan multiple range test (5%).

3-12-16. Effect of biocontrol on potato yield

After harvesting the potato tuber of each plot was weighted and data were recorded and they were subjected to statistical analysis and compared using Duncan multiple range test (5%).

Chapter 4

Results and Discussions

4-1. Isolation of *Trichoderma* from soil

Isolation of antagonists from the same environment which is going to be used has more advantages over introducing antagonist of different origin. For this purpose it was decided to isolate *Trichoderma* spp. from potato growing fields of Haryana.

Trichoderma was isolated from soil samples No. 4 and 5 from Kurukshetra and sample No. 8 from Karnal , which for the ease are refered hereafter as WT4, WT5, and WT8. Isolates WT4 and WT5 were *Trichoderma longibrachiatum* Rifai (1969), and WT8 was *T. harzianum* Rifai (1969).

In WT4 and WT5 conidiophores were typical *Longibrachiatum* section form, with long primary branches and there was no secondary branches or they were too short. Phialides were solitary growing on the conidiophores (Fig. 1), lageniform 6-10 × 2-3μm, but terminal phialides were 13.5 μm, conidia pale to medium green, ellipsoid 3.6-6× 2.5-3.2μm. With these criteria the two isolates WT4, and WT5 were identified as *Trichoderma longibrachiatum* Rifai (Rifai, Mycol. Pap. 116 :42. 1969) .

Isolate WT8 was fast growing , mycelium cottony shape white in the beginning, soprulation in the ring patern finally coalescent and all the colony turning later to dark green, there was no coconut odour, reverse of the colony colourless. Conidiophores typical of *Trichoderma* branching system, regularly branched in tree like pattern , lower branches longer and frequently rebanched, upper branches shorter and less branched (Fig.2).. Phialides ampulliform usually 3 verticilate 3.7-7.5 × 2.5- 3.7 μm terminally. Conidia subglobose 2.5-3 × 2-2.5 μm With these criteria isolate WT8 was identified as *Trichoderma harzianum* Rifai, (Rifai, Mycol. Pap. 116 :42. 1969) .

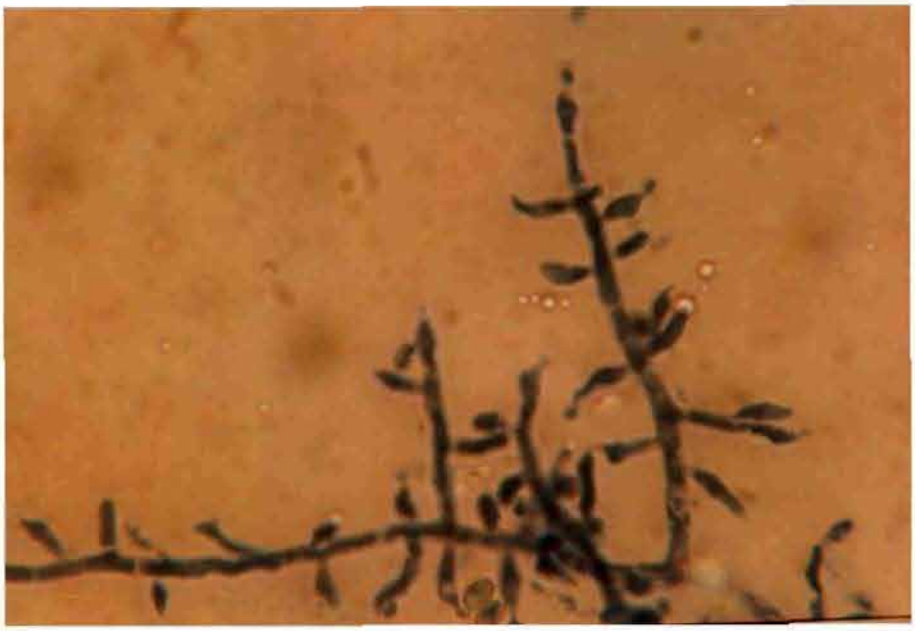


Fig.1 Conidiophore branching system and phialide arrangment of *T. longibrachiatum* (400X magnification).

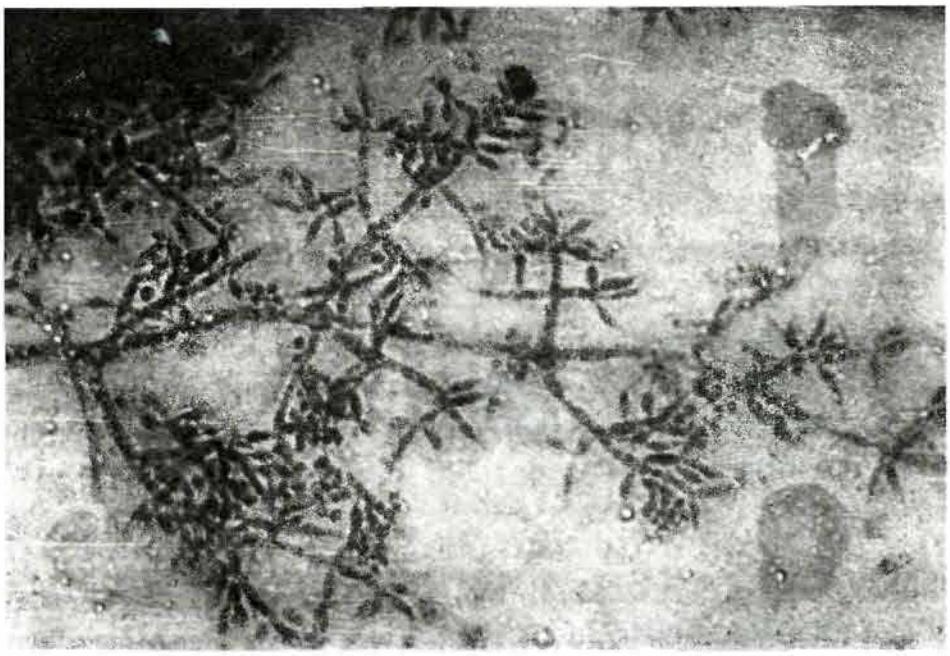


Fig.2 Conidiophore branching system and phialide arrangment of *T. harzianum* (400X).

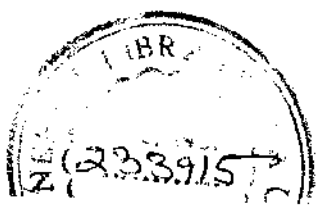
Isolation of *T. longibrachiatum* from the soil samples of Haryna, which is a semitropic rigion is ecologically supported by Turner *et al.* (1997). The ecological preferences of *Trichoderma* are discused in comprehensive reviews of Danilson & Davey(1973 a-c). They mentioned that when dry condition in soil are maintained for long periods of time, the population of *Trichoderma* and *Gliocladium* as a group decrease. They also concluded that certain strains of *T. hamatum and T. pseudokoningi* are adapted to condition of excessive soil moisture and that *T. viride* and *T. polysporim* are restricted to areas where low temperature prevail, whereas *T. harzianum* is commonly found in warm climatic regions and *T. hamatum* and *T. kononingi* are widely distributed in areas of diverse climatic conditions.

Beagle-Ristanio and Papavizas (1985) applied *Trichoderma* spp. for control of *Rhizoctonia* stem canker and Black scurf of potato . Bari (1988) used two *Trichoderma* species viz. *T. harzianum* (IMI 304058), *T. koningii* (IMI 304055) for control of potato black scurf.

4-2 Isolation of *Rhizoctonia solani.*

The fungi isolated from scurfed tubers were fast growing and they covered 9 cm petri plate of PDA in less than 96 hrs at 28 °C. Mycelium buff-coloured to dark brown, showed right angle branching, constriction of branched hyphae at the point of origin, formation of septum in the branch near to the point of branching. The cellular nuclear number (CNN) close to the tips of young hyphae are usually 4-7 when they were stained with alkaline safranin. Sclerotia present, irregular in shape, light to dark brown undifferentiated in to rind and medula. Based on these criteria and guidelines provided by Sneh *et al.*, (1991) all the isolates were identified as *Rhizoctonia solani* Kühn.

Under field condition the perfect stage of the fungus *Tanathephorus cucumeris* was identified on the lower part of potato stems and petioles (up to 7 cm from the ground) as whitish greyish mycelial mat which was the hymenium of the fungus on which barell-shaped basidium were produced bearing four strigmata, basidiospores were 7.5-10 × 4.5-7μm , hyphae 10-14μm buf to dark brown colouer typic *R. solani* form. With these criteria the



fungus was identified as *Thanatephorus cucumeris* (Frank) Donk. This is the first report of the sexual stage of the pathogen from Haryana. Occurrence of *T. cucumeris* has been reported from hilly areas of India by CPRI (1976 and 1981)and from Punjab by Singh *et al.*, (1988).

4-3. Dual Culture of *Trichoderma* vs. *R. solani*

The percentage of growth inhibition of *R. solani* by three isolates of *Trichoderma spp.*, is depicted in Table 1. In dual culture of WT8 (*T. harzianum*) with *R. solani* when the mycelium of the two fungi came in contact to each other, a clear colour changing in *R. solani* mycelium from white to brown was evident and at this contact line the growth of *R. solani* was inhibited by *Trichoderma*. The colour change may be due to the some chemical changes in the cell wall of *R. solani* to defend itself against *Trichoderma*, or may be only because of facing the mycelium with a barrier, though there was no reference in this case. It is clear from the table that all the three isolates has reduced the mycelial growth of *R. solani* even at the periods which there was no direct contact between the antagonists and the pathogen, which indicates the presence of some growth inhibitor for *R. solani* most probably as volatile metabolites which have been produced by *Trichoderma* isolates (Fig. 3).

In dual culture of WT4 (*T. longibrachiatum*) and *R. solani* the mycelial growth of *R. solani* was completely inhibited from 2-5 mm distance from *Trichoderma* this indicates that this isolate produced some diffusable metabolites which were either toxic or inhibitory to *R. solani* far in advance to direct mycelial contact with the antagonist.

The percent of growth inhibition of *R. solani* increased with increase in incubation time and it reached the maximum after direct contact of *Trichoderma spp.* and *R. solani.*, which indicates that *Trichoderma* has several mechanisms for inhibition of mycelial growth of *R. solani* . Similar views are also cited by Denis and Webster 1971 (a-c) ; Papavisaz 1985 ; Kubicek and Harman 1998. After direct contact of *Trichoderm* and *R. solani* mycelial growth of *R. solani* was completly inhibited followed by over

growth *Trichoderma* on *R. solani* mycelium and complete colonization of the *R. solani* mycelium by 144 hours after inoculation in all the three isolates of *Trichoderma*.

Fig 3. Dual culture of *Trichoderma* isolates and *R. solani*

Table 1. Dual culture of *Trichoderma* spp. and *R. Solani* on PDA at 28°C.

	T. harzianum (WT8)			*T. longibrachiatum* (WT5)			*T. longibrachiatum* (WT4)		
Incubation (hr)	72	96	120	72	96hr	120	72	96	120
% inhibition	33	36.3	52.3	55.3	37.6	52	42	48	60

Per cent of inhibition of *R. solani* mycelium calculated as : 100(Growth of *R. solani* in control - Growth of *R. solani* in treatment) / Growth of *R. solani* in control.

Bari (1988) reported that *T. harzianum* (Th- 304058), *T. koningii* (Tk-304055) and *T. viride* (Tv- 304060) were dual cultured against *R. solani* all

the three antagonists first inhibited the growth of the pathogen and colonized the the entire palate later, however *T. viride* was slow growing and covered the entire plate after 84 hrs while the two other antagonsits within 72 hrs . Attempts to reisolate *R. solani* from the zone of interaction and also from the zone where the antagonists has totally overgrown the test fungus failed, revealing that the antagonists are capable of killing the pathogen. At the zone of contact, where both the fungi met , presence of yellow band was observed, which further advanced with the advancing growth of antagonist and this may be due to production of toxins/antibiotics/enzymes by the antagonists for the destruction of the pathogen (Bari, 1988).

The production of yellow band on the reverse of the culture which is due to release of difusible pigments in the media was visible on *T. longibrachiatum* isolates irrespective to the presence or absence of *R. solani* in the present study and this is a criteria of the species which is important in its identification also . Colour changing of the reverse of colony of *Trichoderma* spp. has been reported by Rifai (1969), Domsch and Anderson (1980) and Bissett (1991 a-c).

4-3-1. Light microscopic studies of hyphal interactions

All the isolates of *Trichoderma* were coiling around /growing on or along and or penetrate and grow inside the hyphae of *R. solani* (Fig. 4-5). Appresorium like structures were visible at the end of hyphal side branches of *Trichoderma* when they came in contact with *R. solani* hyphae, through which *Trichoderma* attached to *R. solani* hyphae.

Some abnormalities were observed on hyphae of *R. solani* which have been affected by *Trichoderma* isolates. Cytoplasm of *R. solani* was granulized, sunken discloured spots appeared on the hyphae of *R. solani* which lead to leakage of cytoplasmic materials follwed by hyphal shrinkage and hyphal death. Ultimately the damaged and empty hyphae of *R. solani* were visible. These observations are in accordance with those reported in literature (Bari, 1988 ; Chet and Inbar 1997 ; Chet and Baker 1981 ; Denis and Webster 1971 ; Elad, *et al.*,1982 ; Elad *et al.*, 1983 ; Mukherjee *et al.*, 1995 ; Sivau and Chet , 1989).

Fig.4 Hyphal interaction of *Trichoderma* and *R. solani* (400X).

T. harzianum in addition to the above mentioned symptoms resulted in hyphal disintegration of *R. solani* and it appears that it is able to break the *R. solani* hyphae at the septum points (Fig.5), which leads to disintegration of hyphae of *R. solani* this process is likely related to release of some enzymes which disolve the intermediate cement joining the adjecent cells in the hyphae. This appears to be a unique finding as such reports are not available in literature.

Process of directed growth and coiling of *Trichoderma* spp. hyphae toward / around and running parallel the *R. solani* hyphea is mediated by lectins produced by *R. solani* (Chet and Inbar 1997). Appresorium like structures are used to increase the area of contact of antagtonist and host, which gives more chance for further intercations between the two organisms. Production of lytic enzymes for dissolving and softening of the cell wall of the plant pathogenic fungi including *R. solani* has been reported (Elad *et al.*, 1981), these enzymes pave the way för pentration of the mycoparasite in to the hyphae of host and also by dissolving the cell wall expose the cytoplasmic membrane to other enzymes like proteases and lipases which affect the mememembrane and finally lead to rupturing of the cell and leakage or discharge of cytoplasmic materials from the affected cells .

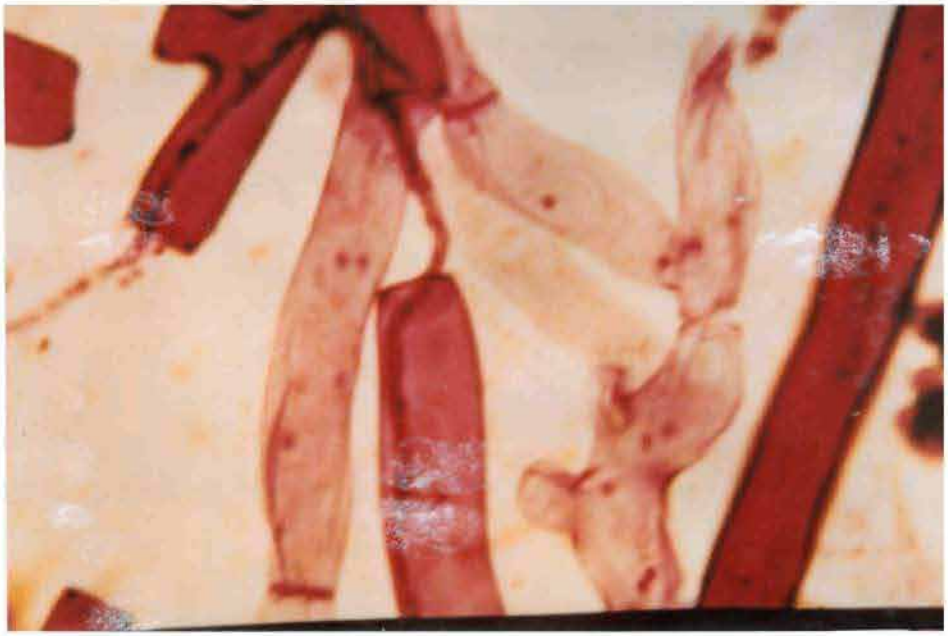


Fig. 5. Breaking the *R. solani* hyphae by *Trichoderma harzianum* (1000X).

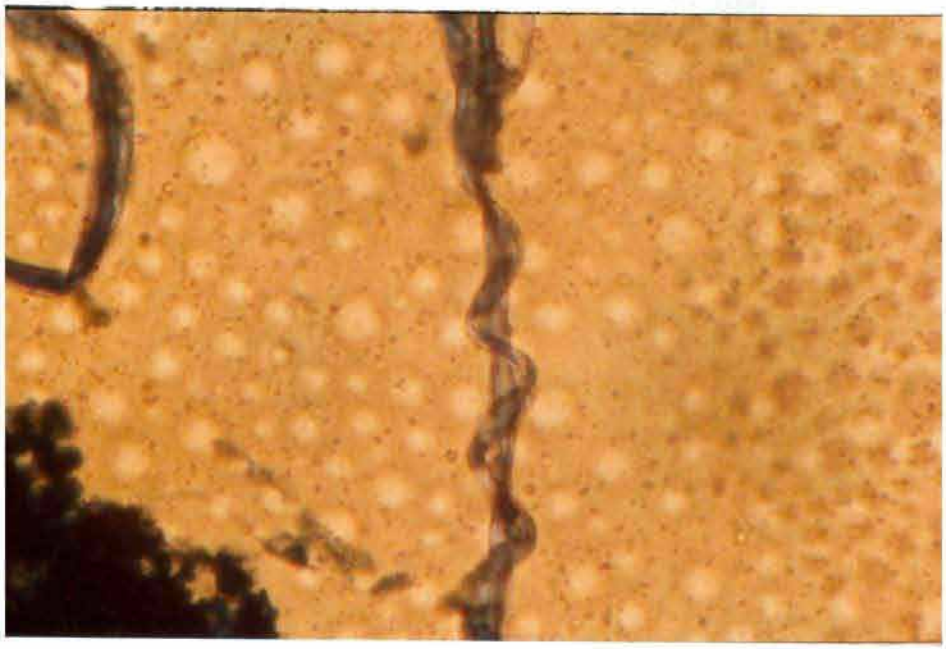


Fig.6. Coiling the *Trichoderma* hyphae around *R. solani* hyphae (400X).

4-3-2. Scanning Electron Microscopy sudies

Hyphal interactions of *R. solani* and *Trichoderma spp.* were more clear under SEM and confirmed light microscopic obsevations. Hyphal coiling, production of appresorium like structure, changinging the cell wall appearance as discoloured sunken spots /area on the cell wall , leakage of cytoplasmic materials from the hyphae , rupturing the cell wall leading to discharge of cytoplasmic materials and finally lysis and disintegration of *R. solani* hyphae were observed under SEM (Fig. 7), which confirm the light microscopic studies and are in consonance with results of other workers (Mukherjee and Mukhopadhyay 1995 ; Elad *et al.*, 1982 ; Bari , 1988).

Mycoparasitism is an exciting phenomenon that plays a role in biological control processes. *Trichoderma* spp. appear to use this mode of action , along with competition and antibiosis, in the course of their biocontrol activity. Myeoparasitism is a complex process and involves several steps (Chet *et al* 1998). The role of lectins in the host- mycoparasite relationship between *T. harzianum* (isolate 203) and *R. solani* has been demonstrated by Elad *et al.*, (1983). They found that *R. solani* hyphae contain a lectin that specifically agglutinates erythrocytes of type O but not those of types A or B. This specific interaction was inhibited by pre-incubation of *R. solani* hyphae with fucose or galactose but was not modified by other sugars tested. These findings led them to assume that a lectin on *R. solani* was involved in the early interaction and that the *Trichoderma* cell walls contained suitable binding sites, such as fucose or galactose, for this lectin. Indeed , they found that *Trichoderma* cell walls contain galactose and suggested that the lectin present in *R. solani* hyphae displays the ability to recognize the galactose residues on the *Trichoderma* cell walls and that this binding plays a key role in prey recognition by the predator. Barak *et al.*(1986) found that methyl-L-fucoside, an inhibitor of *Rhizoctonia* agglutination , prevents coiling of the biocontrol agent around *Rhizoctonia* hyphae. They also demonstrated the presence of L-fucosyl residues on the *Trichoderma* cell wall surface and suggested that these could serve as receptors for *Rhizoctonia* agglutinin, they hypothesized that this recognition was a very early event in the fungus-fungus interactions subsequently leading to mycoparasitism. Following recognition , *Trichoderma* hyphae attach to the

host via the formation of hook-like structures and appressorium-like bodies and coil around the pathogen hyphae (Elad *et al.*, 1983 ; Harman *et al.*, 1981). This typical attachment and coiling appears to be the last step before lytic enzyme activity begins (Chet *et al.*, 1998).

Fig.7. Scanning Electron Microscopy of hyphal interaction of *Trichoderma* and *R. solani* .

4-4. Effect of Culture filtrate of *Trichoderma* on *R. solani* mycelial growth

Culture flitrate of *Trichoderma spp.* inhibited the mycelial growth of *R. solani* and were significantly different from control treatments, however,

their effect was less than that of MEMC and carbendazim. Culture filtrate of *T. longibrachiatum* (WT4) with 36.3 and 43% inhibition at 10 and 20 per cent concentration in the media, respectively was more effective than other isolates of *Trichoderma*. Carbendazim (120ppm) with 100% inhibition was the most effective treatment followed by MEMC (15ppm) . The results are summerized in Table 2.

Table 2. Effect of culture filtrate of *Trichoderma* on mycelial growth of *R. solani* (72 hr) incubation on PDA at 28°C.

Trichoderma isolate[1]	% Filtrate in PDA[2]	Mean[4]	% Inhibition
WT8	10	69[b]	23.3
WT8	20	59[d]	34.4
WT5	10	63.3[c]	29.7
WT5	20	59[d]	34.4
WT4	10	57.3[d]	36.3
WT4	20	51.3[e]	43
MEMC[3]	15ppm	32.3[f]	64
carbedazim[3]	120 ppm	0.0[g]	100
Control 1	10	90[a]	-
Control 2	20	90[a]	-

1 T8 (T.harzianum) and T5 and T4 are two isolates of T. longibrachiatum 2 Filtrate prepred by centrifugation of shake culture of the isolates at 10000 rpm at 4°C followed by filtering through Watman No.1 filter paper . In control only uninoculated shaked media was used. 3 MEMC (Emisan 6% Hg, Excel Industries Limited) and carbendazim (Bavistin 50% WP from BASF). 4 means followed by the same letter are not significantly different (Duncan Test 5%).

Results of this experiment clearly indicates that all the three antagonists do produce non volatile metabolites (antibiotics) which inhibit the mycelial growth of *R. solani* .

Bari (1988) reported 40.96 and 36.70% inhibition of radial growth of *R. solani* by *T. harzianum* and *T.koningii* , respectively after 60 hrs of incubation.

Weindling and Emerson (1932) pioncering works on gliotoxin produced by *Trichoderma lignorum* which was toxic to *R. solani* is the base of further investigation and exploring the biocontrol potential of the genus . Another antibiotic viridin from the same species ascribed by Brain and Heming , (1945), however, later Webster Lomass (1964) demonstrated that these two compounds were actually produced by *Gliocladium virense* , recent results, however, indicate that *G. virens* is much more closely related to *Trichoderma* than previousely thought, and it has been transferred to that genus (Howell, 1998). Other antibiotics reported from *Trichoderma* spp. are trichodermin ,suzukacillin , alamethicin , have been reported by *T. viride* (Bari, 1988 ; Papavizas, 1985 ; Howell, 1998). Howell and Stipanovic ,1983 reported diketopiperazine (gliovirin) produced by *T. harzianum*. In addition to antibiotics *Trichoderma* spp. have been reported to produce lytic enzymes such as β-1,3-glucanase and chitinases (Elad *et al.*, 1982 ; Lorito *et al.*, 1989, 1993 a-b ; Harman *et al.*, 1993) proteases and lipases which affect the cell wall and cytoplasmic membrane. So it isd concluded that the growth inhibition of *Trichoderma* spp. against *R. solani* is due to presence of a collection of anibiotics and enzymes. The synergistic effect of antibiotics with enzymes produced by *Trichoderma* spp. also appears to be important (Belanger *et al.*, 1995 ; Di Pietro *et al.*, 1993 ; Lorito *et al.*, 1994 ; Schirmoback *et al.*, 1994).

4-5. Effect of Culture filtrate on dry weight of *R. solani* :

The culture filtrates of *Trichoderma spp* reduced the dry weight of *R. solani*, however the effects were less than that of MEMC (15 ppm) and carbendazim (125 ppm) but they were significantly superior over the controls. There were significant differences among the culture filtrate of isolates of *Trichoderma* in reduction of dry weight of *R. solani*. *T. longibrachiatum*

(WT4) culture filtrate with 63 and 74 % dry weight reduction of *R. solani* at 10 and 20 per cent concentration in PDB, respectively was more effective and significantly superior to the two other *Trichoderma* isolates when the per cent of dry weight reduction were compared. There were significant differences among the effects of concentration of culture filtrates of *Trichoderma* spp. in PDB also, in other word higher the percentage of culture filtrate in media less the dry weight of *R. solani*. Results of this experiment are summarised in Table 3.

The metabolites produced by *Trichoderma* spp. may affect the pathogenicity of *R. solani* on potato adversely and play an additive role in the reduction of disease severity and incidence under field condition (Bari, 1988). Aluko and Hering (1970) demonstrated that that gliotoxin was the active factor in control of *R. solani* by *G. virens* on seed potatoes.

Table 3. Effect of culture filtrate of *Trichoderma* on dry weight of myceliun of *R. solani* after 7days incubation on PDB at 28°C and 140 rpm .

Trichoderma isolate	% Filtrate in PDB[1]	Mean[3]	% Reduction of weight
T. harzianum (WT8)	10	0.0625[b]	63
	20	0.0478[d]	71
T. longibrachiatum (WT5)	10	0.0624[b]	63
	20	0.0538[c]	68
T. longibrachiatum (WT4)	10	0.0617[b]	63
	20	0.0434[e]	74
MEMC[2]	15 ppm	0.0108[f]	93
carbendazim	125ppm	0.0066[g]	96
Control 1	10	0.1681[a]	-
Control 2	20	0.1673[a]	-

1. Filtrate prepred by centrifugation of shake culture of the isolates at 10000 rpm at 4°C followed by filtering through Watman No.1 filter paper . In control only uninoculated shaked media was used. 2 MEMC (Emisan 6% Hg. Excel Industries Limited) and carbendazim (Bavistin 50% WP , from BASF). 3 means followed by the same letter are not significantly different (Duncan multiple range test 5%).

4-6. Effect of volatile metabolites of *Trichoderma* on *R. solani* .

Volatile metabolites of all the three *Trichoderma* isolates used significantly reduced the mycelial growth of *R. solani.* However, *T. longibrachiatum* (WT4) was the most effective and resulted in 41 % growth reduction of *R. solani* mycelial growth. There was no significant difference between the effects of *T. harzianum* (WT8) and *T. longibrachiatum* (WT5) in reduction of mycelial growth of *R. solani.* Results of these experiment are summarised in table 4. Results of this experiment supported the finding of Bari (1988). He reported significant radial growth inhibition of *R. solani* when it was exposed to vapour action of 4, 8 , 12 and 16 days old cultures of *T. harzianum* and *T. koningii* . The 4 days old culture of the two antagonists produced maximum volatile metabolites compounds, giving 53.47 and 51.47 per cent inhibition in radial growth of *R. solani,* respectively (Bari,1988).

Table 4. Effect of volatile metabolites of *Trichoderma* spp. on growth of *Rhizoctonia solani* (72 hr incubation on PDA at 28 °C).

Trichoderma isolate	Mean	% growth inhibition
T. harzianum (WT8)	62 [b]	31
T. longibrachiatum (WT5)	63 [b]	30
T. longibrachiatum (WT4)	53 [c]	41
control	90 [a]	-

Trichoderma and *R. solani* were inoculated in the centre of separate petri plates of PDA, removing the leads the plate of *R. solani* was reversed on that of *Trichoderma* and the plates were sealed by adhesive tape. In control only uninoculated *Trichoderma* plate of PDA was used. means followed by the same letter are not significantly different (Duncan multiple range test 5%).

Deniss and Webster (1971) studied the production of volatile antibiotics by different isolates and species of *Trichoderma against* several test fungi including R. *solani* . A range of volatile antibiotics was produced by *Trichoderma* spp. having characteristic coconut smell and they reacted differently on different test fungi. The main aroma constituent responsible for coconut odour in *T. viride* has been identified as 6-pentyl-pyrone (Collins and

Halim, 1972) and its antibiotic activity against a number of plant pathogens has been demonstrated (Claydon, *et al.*, 1987; Ghisalberti and Savasithamparam, 1991). Howell (1998) reviewed the role of antibiosis in biocontrol and believed that, although mycoparsitism is usually the first phenomenon that excites the attention of the observer. Further investigation often reveals that the biocontrol agents also produce secondary metabolites with antifungal and / or antibacterial activity. The antimicrobial compounds

Produced by *Trichoderma* and *Gliocladium* constitute a rather diverse group with respect to structure and function and contains both volatile and non volatile compounds. A number of these antibiotics have been related to biocontrol activity, but none has proven to be the arbiter of success or failure in a biocontrol system. More than likely, antibiosis is only one mechanism among the many that constitute a much more complex system, the end product of which is biocontrol (Howell, 1998). The simple aromatic compounds, the pyrones, the butenolides and the isocyano metabolites are low molecular weight, relatively non-polar substances that have a significant vapour pressure. The production of these in the soil environment would be expected to result in high local concentrations of antibiotics that may exert their influence over a "distance". However, members of this group may diffuse through water and also exert effects a small distance from the producing hyphae (Sivasithamparam and Ghissalberti, 1998).

4-7. Effect of Fungicides on *R. solani*

Among the fungicides tested carbendazim was the most effective in reduction of radial growth of *R. solani.* It completely inhibited the mycelial growth of *R. solani* even at 10 ppm concentration. MEMC was effective in control of mycelial growth of *R. solani* , but it completely inhibited the mycelial growth only at 100 ppm concentration . Tebuconazole and chlorothalonil completely inhibited the mycelial growth of *R. solani* at concentration of 200 ppm or more (Table 5). The present findings are similar to those obtained by Kataria, 1973 ; Taneja, 1977 ; Bari 1988 and Yadav, 1996 .

Table 5 . Effect of fungicides on mycelial growth of *Rhizoctonia solani* 96 hrs incubation on PDA at 28 °C.

Fungicide	Radial Growth of *R. solani* (mm)					
	Fungicides concentration (ppm)					
	0	1	10	100	200	500
Tebuconazole	90	87(3)	73(19)	5(94)	0(100)	0(100)
carbendazim	90	38(58)	0(100)	0(100)	0(100)	0(100)
MEMC	90	75(17)	40(55)	0(100)	0(100)	0(100)
Chlorothalonil	90	87(3)	68(24)	7(92)	0(100)	0(100)

Figures are means of 3 replications each, figures in parentheses are % of growth inhibition compared with control which was calculated as [100 x (growth in control – growth in treatment) / Growth in control].

4-8. Effect of Fungicides on *Trichoderma* isolates

The fungicides used in this experiment were: tebuconazole (Folicur^{R} 250 EW , Bayer) @ 100, 200 and 300ppm., carbendazim (Bavistin50% WP, BASF India Ltd.) @ 50, 100, 250 and 500 ppm., MEMC (Emisan 6% Hg Formulation, Excel industries Limited)@ 10, 50and 100 ppm of active ingredients. The fungicides were added to cooling sterilised PDA at the desired concentrations.

None of the isolates was able to grow on carbendazim amended PDA in all the tested concentrations, so it was concluded that all the tested isolates were susceptible to carbendazim. *T. harzianum* (WT8) tolerated 100 ppm MEMC and 200 ppm chlorothalonil,. *T. longibrachiatum* isolates WT5 and WT4 tolerated 50 ppm MEMC and 200 ppm chlorotalonil. So the tolerance to chlorothalonil is common for all the three isolates, but in case of MEMC the tolerance level of WT8 was higher than WT5 and WT4. All the three isolates of *Trichoderma* tolerated tebuconazole only up to 25 ppm concentration and they were not able to grow on 50 ppm and beyond. Results of this experiment are provided in Table 6.

Bari (1988) reported that among the six fungicides (Bavistin, Vitavax-200, Emisan-6, PCNB, Thiram and Dithan M-45) tested against *T. harzianum, T. koningii* and *T. viride* Bavistin was found inhibitory to the growth of all the test species. At concentration of 2.5, 5.0 and 10 μg/ml it

could inhibit the radial growth of *T. harzianum* by 10.23, 72.66 and 100 per cent, respectively.

4-9. Mutation of *Trichoderma* for fungicides resistant:

4-9-1. UV Mutation in *Trichoderma* :

In this experiment out of 40 mutants only 3 mutants namely UM 35, and UM 15 derived from *T. longibrachiatum* (WT5) and UM 14 derived from *T. longibrachiatum* (WT4) tolerated MEMC (100ppm) and chlorothalonil (300ppm) and selected for further tests.

4-9-2. EMS mutation:

In this experiment among 70 mutants EM14, EM15, EM24 and EM64 derived from *T. longibrachiatum* (WT4) and EM 15 and EM25 derived from *T. longibrachiatum* (WT5) tolerated MEMC (100ppm) and chlorothalonil (300ppm) and selected for further tests.

4-9-3. Effect of fungicides on selected mutants of *Trichoderma*

In this experiment all of the tested mutants and wild types but the WT4, WT5 and UM25 tolerated MEMC up to 100ppm and this indicates that both chemical and UV mutation enhanced tolerance to MEMC in mutants. Tolerance to carbendazim at 50 ppm concentration appeared only in EM 15 (mutant of WT5), EM24 and EM 64 (mutants of WT4) which have been developed by EMS mutagen treatment. Tolerance to chlorothalonil at 300ppm concentration was also seen in all of the tested mutants. Results of this experiment are depicted in Table 7.

Table 6. Effect of fungicides on *Trichoderma* spp. 72 hr incubation on PDA at 28°C.

Trichoderma isolate	Fungicide	PPM concentration	Mean [1] (mm)	% Inhibition
WT8 (*T. harzianum*)	MEMC	10	83	7
		50	70	22
		100	23	74
	Carbendazim	10	0	100
		50	0	100
		100	0	100
	chlorothalonil	100	36	60
		200	25	72
	Tebuconazole	10	60	33
		25	50	44
		50	0	100
	Control	-	90	-
WT5 (*T. longibrachiatum*)	MEMC	10	15	83
		50	10	88
		100	0	100
	carbendazim	10	0	100
		50	0	100
		100	0	100
	chlorothalonil	100	20	77
		200	10	88
	Tebuconazole	10	30	66
		25	20	77
		50	0	100
	Control	-	90	-
WT4 (*T. longibrachiatum*)	MEMC	10	30	66
		50	20	77
		100	0	100
	carbendazim	10	0	100
		50	0	100
		100	0	100
	chlorothalonil	100	22	75
		200	10	88
	Tebuconazole	10	60	33
		25	40	55
		50	0	100
	Control	-	90	-

Figures are mean of *R. solani* colony diameter three replicates each. 2.percent inhibition was calculated by: 100 (Growth in control – Growth io treatment) / Growth in control. Concentration of fungicides a.i.

Table 7. Effect of fungicides on mutants and wild types of *Trichoderma* spp. (96 hr incubation on PDA at 28 °C).

	Colony diameter of *Trichoderma* (mm)[1]									
Trichoderma	MEMC[3]			Carbendazim			Chlorothalonil			control
isolate[2]	10	50	100	50	100	200	100	200	300	
WT8	86.3	75	19.7	0	0	0	37	25	10	90
WT5	10	12	0	0	0	0	20	10	0	75
WT4	25	16	0	0	0	0	22	10	0	74.5
UM25	20	17	0	0	0	0	15.7	12	8	80
UM15	77.3	67	27.3	0	0	0	33.3	20.7	14	79
UM35	17.3	12.3	9	0	0	0	21	11.7	9	82
UM14	23.7	18	12.3	0	0	0	31.7	25	15	85
EM24	69	39	21.3	17	0	0	16.7	13.3	9.4	87
EM15	87.7	80.7	27.3	15	0	0	30	20	16	90
EM14	88	81	30.3	0	0	0	15.3	12.3	7.6	88.4
EM25	82.7	55	15.3	0	0	0	36	24.3	11	83
EM64	85	60	32	14	0	0	31.3	28	20	90

1. Figures in the table are means of three replications. 2 WT8 (wild type of *T. harzianum* isolate 8), WT5 and WT4 (wild types of *T. longibrachiatum* isolates WT5 and WT4, respectively). UM35, UM25 and, UM15 (UV induced mutants of WT5), UM14 (UV induced mutant of WT4), EM 64, EM24, and EM14 (EMS induced mutants of WT4), and EM 25 and EM15 (EMS induced mutants of WT5). 3 Concentration of fungicide in medium (ppm).

4-10. Dual culture of some *Trichoderma* mutants and *R. solani*

In this experiment though all of the mutants and wild types inhibited the growth of *R. solani* but EM 64, EM 24 with 47% each , EM15 and EM14 with 40% inhibitory effect against *R. solani* mycelium proved superior over other mutants and wild type isolates. But in EM25 mutant with 11% inhibition effect found inferior when it was compared with its related wild type (WT5) with 29 % inhibition of *R solani* mycelial growth. Results of this experiment are shown in table 8.

Table 8. Effect of mutants on mycelial growth of *R. solani* 72 hours incubation on PDA.

Trichoderma isolate/mutant	Diameter of *R.solani* colony(mm)[1]	% inhibition[2]
control	90	0
WT86	30	33
WT5	64	29
WT4	62	31
UM14	58	35
UM15	62	31
UM 35	60	33
EM 14	54	40
EM15	54	40
EM 24	54	47
EM 25	80	11
Em 64	52	47

1.Figures are mean of three replicates each. 2. percent of inhibition calculated as:

100 (Growth in control- Growth in treatment) / Growth in control.

4-11. Production of lytic enzymes by *Trichoderma*

The cell wall of *R.solani* composed of *β*-1,3- glucan and chitin. The tested isolates of *Trichoderma* viz. WT8, WT5 and WT4 were able *to* grow on *R. solani* cell wall as a sole carbon source as has also been reported by Hadar, 1979. Cell wall is the first protective barrier of fungi against other antagonist and if the cell wall of fungi gets damaged by any means, specially in nature with huge diversity of both micro-organisms and chemicals it will be a soft target for further decomposition. It appears that the main mechanism involved in the antagonism of *T. harzianum* and pathogenic fungi is the release of lytic enzymes (Elad *et al,* 1982). The results on various lytic enzymes have been furnished in Table 9, while for each individual lytic enzyme through histogram on appropriate plate.

4-11-1. Release of N- acetyl –D-glucoseamine colloidal chitin

All of the tested isolates of *Trichoderma* spp. understudy released N-acetyl-D-glucoseamine from colloidal chitin. Isolate EM15 produced the highest amount of chitinase(0.017), followed by WT8, EM64 , EM24, WT4 and WT5 with 0.015, 0.014, 0.014, 0.013 and 0.010 μmole N-acetyl-D-glucoseamine / mg substrate/h , respectively (Table 9 and Fig.8). The activity of chitinase in the tested organisms was not so much different and it ranged from 0.010 and 0.017 unit. Release of N-acetyl-D-glucoseamine from colloidal chitin, which is one of the most important components of fungal cell walls including R. solani, is important. It leads to disintegration of fungal cell wall of the pathogen and make it vulnerable for other enzymes such as proteases. These enzymes affect the plasma membrane and finally leakage and discharge of cytoplasmic materials from the fungal cell which ends with the shrinkage and death of R.solani hyphae and paves the way for penetration of Trichoderma into the R. solani hyphae. It is also important to notice that the chitin used in this experiment is of arthropod origin, and digestion of this kind of chitin by Trichoderma spp. gives a clue that this biocontrol agents may play a role in control of plant pests including insects, nematodes and mites.

4-11-2. Release of N- acetyl –D-glucoseamine from fungal cell wall (FCW)

All the isolates of *Trichoderma* spp. employed in the present investigation produced chitinase and released N- acetyl –D-glucoseamine when their culture filtrate was incubated on fungal cell wall material (FCW). EM64 showed the highest chitinase activity 0.039 followed by EM24 , WT8, EM15, WT4 and WT5 with 0.035, 0.027, 0.020, 0.019 and 0.015 μmole N-acetyl-D-glucoseamine/ mg FCW/h , respectively (Table 9 and Fig. 9). There was a greater difference among the chitinase activity of the tested isolates. Amongst the wild type isolates WT8 with 0.027 was at the top, however mutation in WT4 increased the chitinase activity in its derived mutants namely EM64 and EM24. Release of N- acetyl –D-glucoseamine from CFW of *R. solani* proved that chitinase is involved in mycoparasitism relationship of *Trichoderma* spp. and *R. solani*. It was mentioned earlier that chitin is a major component of *R. solani* cell wall. Disintegration of chitin molecules in the cell wall paves the way for other enzymes such as protease which alter the permeability of plasma membrane and lead to leakage of plasma from the affected cells and finally disintegration of *R. solani* hyphae.

4-11-3. β-1,3- glucanase activity on Laminarin

All of the tested *Trichoderma* spp. isolates released glucose from laminarin by producing β-1,3- glucanase. Isolate EM15 showed the maximum enzyme activity 0.006, followed by EM24, WT5, EM64, WT8 and WT4 with 0.004, 0.004, 0.003,0.002 and 0.0.001 μmole glucose/mg/h (Table 9 and Fig.10). Results of this experiment indicate that there has been variation among the β-1,3- glucanase of isolates and by mutation increased the level of enzyme activity.

4-11-4. β-1,3- glucanase activity on fungal cell wall (FCW) material

All of the tested isolates of *Trichoderma* spp. produced β-1,3- glucanase and released glucose from FCW. EM15 released the maximum amount of glucose 0.006 from FCW followed by EM24 , WT5. EM64, WT4 and WT8 with 0.004, 0.004, 0.003, 0.001 and 0.001μmole glucose/mg/h (Table 9 and Fig.11). Results of this experiment showed that there is difference in β-1,3- glucanase activity of isolates , and mutation increased the activity of the enzyme . The cell wall of *R. solani* is mainly consisted of glucan and there is only 6-8% chitin in the cell wall of *R. solani* (Hadar,1979), this indicates the major role of β-I,3- glucanase in the biocontrol of this pathogen. Release of glucose from the FCW indicates the involving of β-1,3- glucanase as a mechanism for lysis of cell wall of *R.solani*. β-1,3- glucanase is commonly produced by fungi , Chester and Bull (1963) found that out of 21 different fungi , *T. viride* showed the highest glucanase activity. Both β-1,3- glucanase and chitinase must act simultaneously to decompose hyphal walls of *Schizophyllum commune* and it was shown that *T. viride* solubilized the hyphae of *Sclerotium sclerotiorum* by β-1,3- glucanase (Elad *et al*,. 1982). The final result of effect of β-1,3- glucanase on hyphae of *R. solani* is similar to that of chitinase effect on the same, which indicates that lysis of cell wall followed by providing sites of action for other enzymes such as protease lead to leakage of cytoplasmic materials and ultimately shrinkage and death of *R. solani* hyphae.

4-11-4. Endocellulase (Cx) or β- 1,4 glucanase activity

All of the tested isolates produced glucose from cellulose implying break down of this polysaccharide. Isolate WT5 and EM15 with 0.333μmole glucose/mg/h each, produced higher amount of glucose followed by WT8, WT4, EM64 and EM24 with 0.111μmole glucose/mg/h each ,respectively (Table 9 and Fig.13).Chaudhary (1981) has reported 340, 500 and 400 (μmole glucose/g/30 min) enzyme activity of endoglucanase for *Trichoderma reesei*. It is pertinent to mention that *T. reesei* is the test organism fungi for producing the cellulases, and production of cellulase by this fungi have been studied in detail by many workers all around the world and it is important in industry for fine finishing of textiles. Cellulase activity seems to be less important directly against *R.solani*, due to lack of cellulose in the cell wall of this fungus. In nature it may play a role by disintegrating the residue of plants which are carrying the pathogen, and release of glucose from plant residues for *Trichoderma* itself and other micro-organisms which are functioning against *R.solani*. Endoglucanases break down the long chain of cellulose molecules to short chain (oligomers of glucose) and provide more substrate for exoglucanase, which are produced by *Trichoderma* and other micro-organisms.

4-11-5. Exocellulase (C1) or β- 1,4 glucanase activity

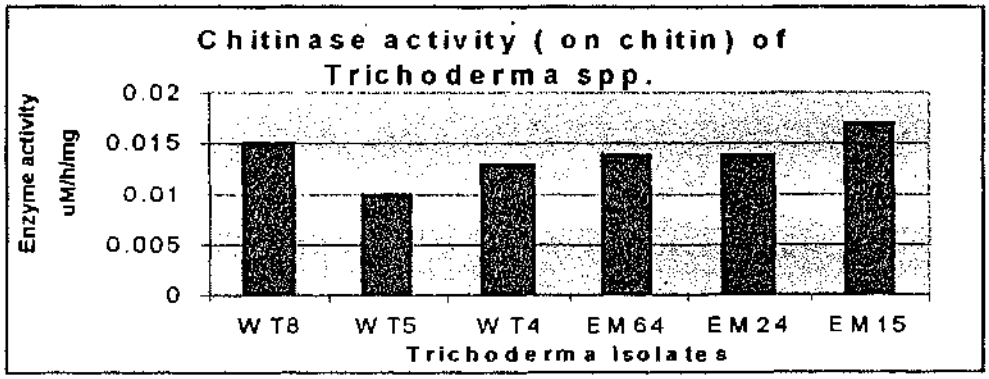
All the tested isolates of *Trichoderma* spp. released glucose from carboxy methyl cellulose (CMC) by producing β- 1,4 glucanase (C1) enzyme. WT8 showed the maximum enzyme activity 0.044, followed by EM24, EM64, EM15, WT5 and WT4 with 0.020. 0.020, 0.002, 0.001 and 0,001μmole glucose/mg/h each, respectively (Table 9 and Fig.12). Chaudhary (1981) has reported the 0, 170 and 72 enzyme activity (μmole glucose/g/30 min) of exoglucanase for *Trichoderma reesei*. Exoglucanases remove the glucose from the ends of cellulose chain gradually. The number of free ends of cellulose chains influences their activity. As it was mentioned earlier on the contrary endoglucanses break down the cellulose chain randomly and provide freer site of action for exoglucanase.

Table 9. Lytic enzyme activity of *Trichoderma* spp.

Trichoderma isolate	Cellulase		β-1,3 Glucanaes		Chitinase	
	Endo β-1,4	Exo β-1,4	Laminarin	FCW	Colloidal chitin	FCW
WT8	0.111	0.044	0.002	0.001	0.015	0.027
WT5	0.333	0.001	0.004	0.004	0.010	0.015
WT4	0.111	0.001	0.001	0.001	0.013	0.019
EM64	0.111	0.020	0.003	0.003	0.014	0.039
EM24	0.111	0.020	0.004	0.004	0.014	0.035
EM15	0.333	0.002	0.006	0.006	0.017	0.020

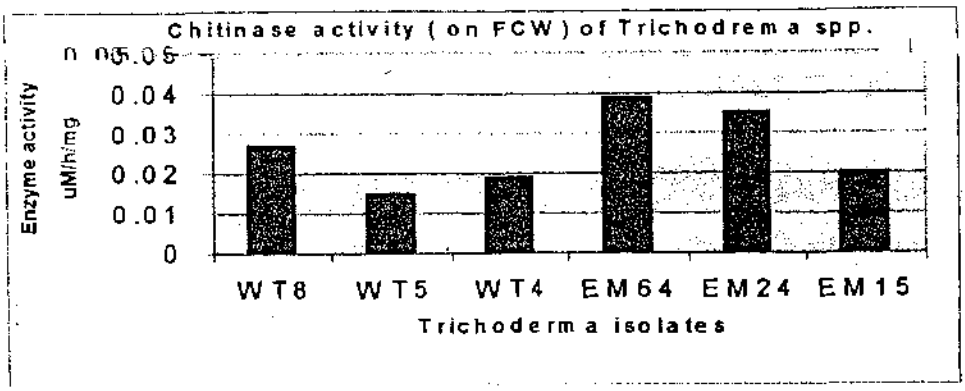
WT8 (*T. harzianum)*, WT5 and WT4 (*T. longibrachiatum)* , EM64 and EM24 mutants of WT4 and EM15 mutant of WT5. Figures are international unit activity of enzyme ($\mu molml^{-1}h^{-1}mg^{-1}$ of substrate). FCW (fungal cell wall material)

Fig.8. Chitinase activity (on chitin) of *Trichoderma* spp.



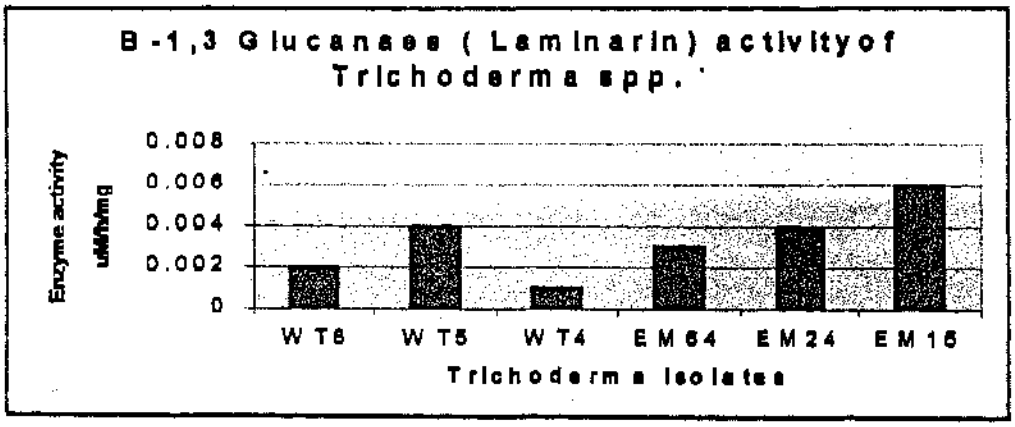
WT8 (*T. harzianum)*, WT4, and WT5 *(T. longibrachiatum)*, EM64 and EM24 mutants of WT4, and EM15 mutant of WT5.

Fig. 9. Chitinase activity (on FCW) of *Trichoderma* spp.



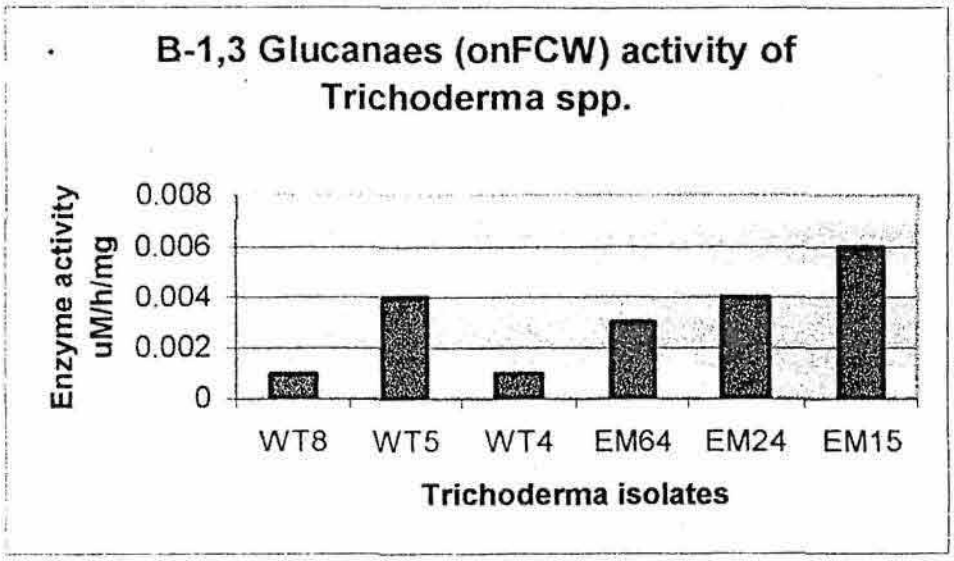
WT8 (*T. harzianum*), WT4 and WT5 (*T. longibrachiatum*), EM64 and EM24 mutants of WT4, and EM15 mutant of WT5.

Fig.10. *β*-1,3 glucanase activity (on laminarin) of*Trichoderma* spp.



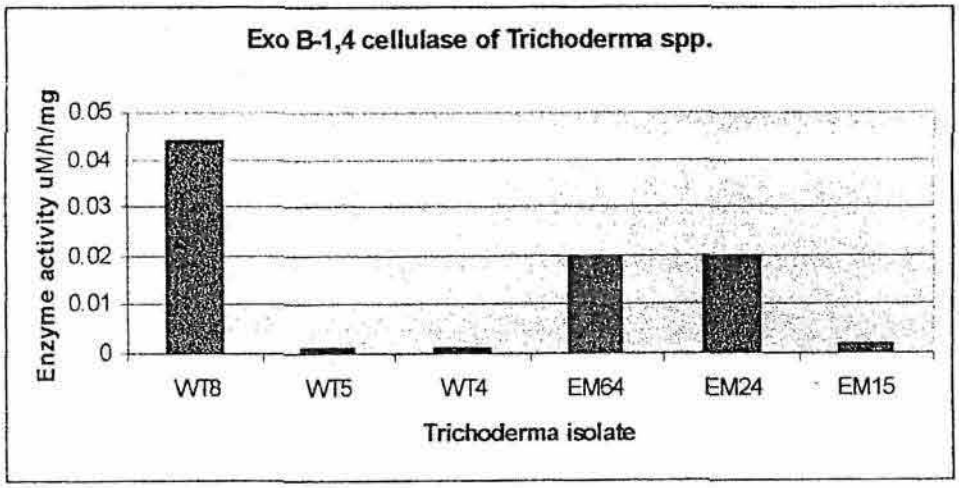
WT8 (*T.harzianum*), WT5 and WT4 (*T. longibrachiatum*), EM64 and EM24 mutants of WT4, EM15 Mutant of WT5.

Fig.11. β-1,3 glucanase activity (on FCW) of *Trichoderma* spp.



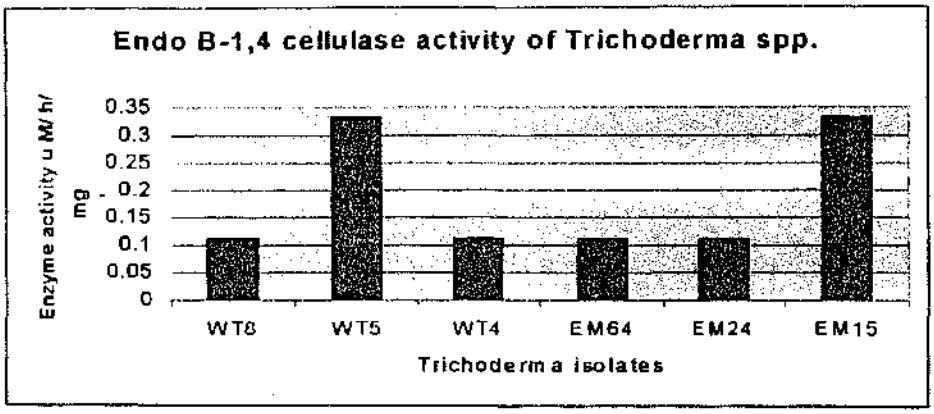
WT8 (*T.harzianum*), WT5 and WT4 (*T.longibrachiatum*), EM64 and EM24 mutants of WT4, EM15 Mutant of WT5.

Fig. 12. Exo β-1,4 cellulase activity of *Trichoderma* spp.



WT8 (T.harzianum), WT5 and WT4 (T.longibrachiatum), EM64 and EM24 mutants of WT4, EM15 Mutant of WT5

Fig. 13. Endo β-1,4 cellulase activity of *Trichoderma* spp.



WT8 (T.harzianum), WT5 and WT4 (T.longibrachiatum), EM64 and EM24 mutants of WT4, EM15 Mutant of WT5.

4-12. Field application of *Trichoderma* to control black scurf of potato.
Inoculum *Trichoderma* isolates namely WT8, WT5, WT4, EM64, EM24 and EM15 were prepared on wheat bran and used for soil application in the desired treatments (Fig. 14). All the isolates of *Trichoderma* were able to grow and colonise the wheat bran substrate as a whitish mycelium in the beginning, which turned green later due to sporulation of *Trichoderma*. Inoculum of *R. solani* isolates (Fig.15) prepared on wheat bran and used for inoculation of all treatments except control not infested with *R. solani*. The *R. solani* isolates were able to grow on wheat bran and colonised the same first as whitish mycelium which later turned to cream and brown colour (Fig.13). *R. solani* isolates produced sclerotia on the substrate too. Tubers were dipped in spore suspension of desired *Trichoderma* isolates namely WT8, WT5, and WT4 for 1 hr before sowing. Tubers for chemical as well as integrated control treatment were dipped in fungicides (MEMC, carbendazim in first year and MEMC, carbendazim and tebuconazole in second year experiments) for 20 minutes before sowing.

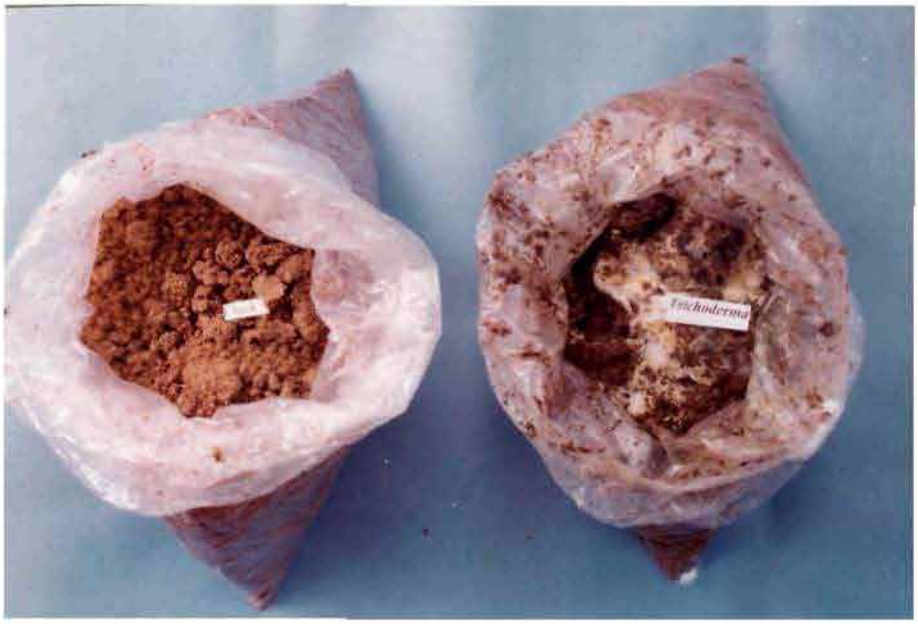


Fig.14. *Trichoderma* growing on wheat bran 20 days incubation.

Fig.15. *R. solani* growing on wheat bran 20 days incubation.

4-12-1. Monitoring disease symptoms and signs

Field observations (Fig.16) revealed that in the first two weeks after sowing it was quite clear that the number of germinated potato seedlings were more in the plots treated with spore suspension of *Trichoderma* spp. (Fig.17). On the contrary number of germinated seedlings were less in control infested with *R. solani.* Which is indicate that *Trichoderma* spores in close contact with tubers in sufficient amount (in compare with soil application of *Trichoderma*) enhanced the germination and emergence of potato seedlings, and *R. solani* hindered or decreased the number of emerged seedlings. The growth promoting capabilities of Trichoderma spp. has been reported on other plants like tomato (Lindsey and Baker 1967) on pepper, perwinkle and chrysantemum (Chang *et al,.* 1986) corn , tomato, tobacco and radish (Windham *et al,.* 1986) sweet corn (Harman *et al,.*1989) pepper and other plants (Kleifeld and Chet , 1992 ; Shivanna *et al,.* 1994). Bari (1988) reported that application of *T. harzianum* increased the number of stems, and plant height of potato.

After 25 five days the ungerminated potato tubers were checked for the cause, and it was found that the young germinating sprouts were infected and in most of the situation appeared dead or diseased by the pathogen(Fig. 19). They looked very weak and distorted. In such tubers after killing the young stems another sprout/stem appeared which again got affected by the pathogen and this process of killing the young stems and regerminating of the sprouts lead to large number of germinated diseased stems on the sprouts. Sprouts were affected at the tip as brown coloured canker in the beginning, which later turned black. The under ground stems of the potato plants were affected by pathogen and symptom of the disease as brown canker of different shape and size were visible on the affected stems (Fig. 18). The canker may enlarge or more often two or more cankers coalesced and girdled the stems, hindering the translocation of metabolites, in the plant leading to symptoms such as leaf rolling, wilting and in some cases aerial tubers formation on the lateral sprouts above the ground level (Fig.20-21). Stems appeared distorted and thickened in such affected plants. The pathogen also affect the roots, and finally death of roots with disruption in uptake of water and minerals from the soil leading to leaf rolling and yellowing which may be confused with some viral diseases. Tubers in affected plants formed near to the ground surface. The fungus in the favourite environmental conditions may

undergo for sexual reproduction and hymenium bearing basidia and basidiospores are produced on the lower parts (up to 7cm) on stems and petioles(Fig.22 and 23). Production of sclerotia as muddy encrustation on the tuber surface (Fig. 24) appeared as final sign of the disease on potatoes produced in *R. solani* infested soils the tuber periderm under such sclerotia remained intake and unaffected. These structures serve as over wintering/ over summering source of the pathogen and as source of transferring the pathogen inoculum to other area and fields. All of these symptoms and signs appeared in the field. Such symptoms have earlier been reported by various workers (CPRI, 1976 and 1981; Hooken, 1981; Dar and Dutta, 1980; Maheshwari and Srivastava, 1991; Mall and Suresh, 1987 and 1988; O' Brien and Rich, 1979; Rich, 1983; Sahi, 1987; Srivastava, 1986). Bari (1988) has noticed only black scurf incidence in his two years studies. He mentioned that other symptoms of the disease (like stem canker) are not common in plains of India, most probably due to climatic factors. However the results of the present experiment showed that all kinds of the disease symptoms and signs of the disease are visible under field condition in Hisar.

Occurence of sexual stage of the pathogen in plains of India has only been reported from Punjab (Singh *et al*,. 1988), which was prevalent in our field experiments also. Presence of sexual stage of the pathogen under field condition is very important. Because in addition to anastomosis which is common in *R. solani* the sexual compatibility will increase the chance of more heterogenicity which makes the pathogen more difficult to control by fungicides. The occurrence of sexual stage was during January, indicated that sexual reproduction needs cold weather around 18°C and high relative humidity which was prevalent in this month.

4-12-2a. Effect of biocontrol on emergence of potato seedlings 15 DAS (1998-1999)

Result presented in Table 12a.and Fig.27, reveal that seed application of all the three isolates of *Trichoderma* spp. significantly increased the number of emerged seedlings 15 DAS, and they were found superior over other treatments including check not infested with *R. solani*. This indicated that *Trichoderma* spp. were capable of producing some metabolites which enhanced or promoted the germination and emergence of potato seedlings, or they had checked the elements, which hinder the seedling emergence. Soil application of WT8 and WT5 also increased the number of emerged seedlings

Fig. 16. Field experiment on biological control of potato black scurf by *Trichoderma* spp.

Fig. 17. Promoting seedlings emergence of potato by tuber treated with 3 isolates of *Trichoderma* spp.

Fig.18. Stems canker of potato caused by *R. solani.*

Fig.19. Potato sprouts affected or killed by *R. solani.*

Fig.20. Aerial tuberization of potato caused by *R. solani.*

Fig.21. Aerial tuberization and stem canker of potato caused by *R. solani.*

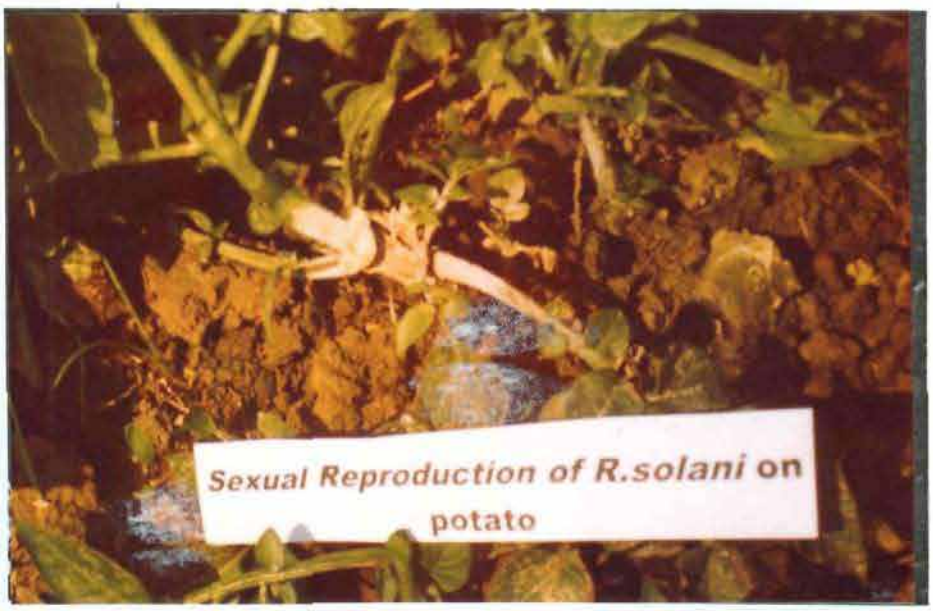


Fig. 20. Hymenium of *Thanatephorus cucumeris* (= teleomorph of *R. solani*) on potato stems under field condition.

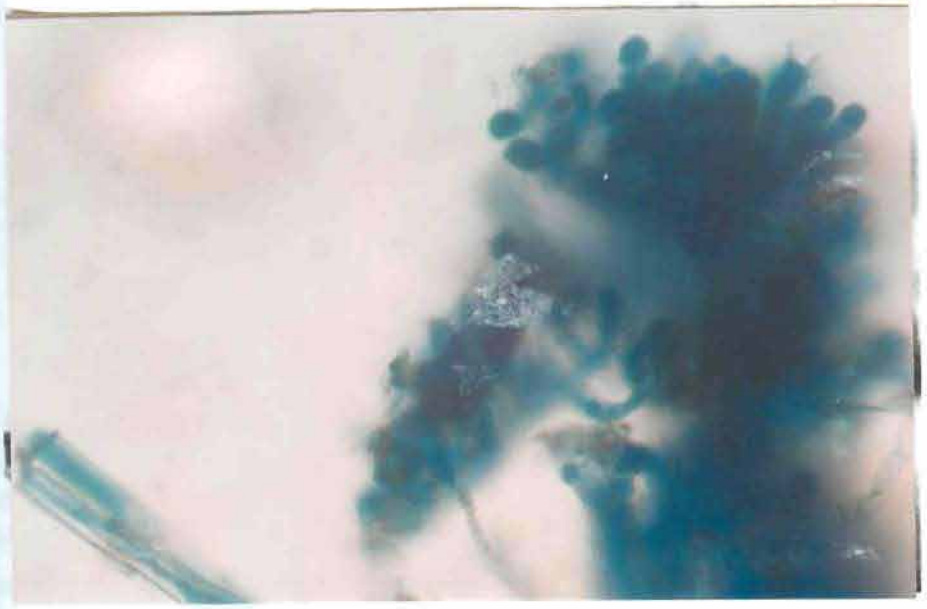


Fig. 21. Basidium of *Thanatephorus cucumeris* (1000X)

Fig.24. Sclerotia (black scurf symptom) of *R. solani* on potato tuber.

and along with check not infested ranked the second. Carbendazim and MEMC though appeared significantly better than pathogen infested check treatment, but were inferior to seed application of the three isolates and soil application of WT5 and WT8 isolates of *Trichoderma* spp. Inoculation of soil with *R. solani* showed 39% decrease in seedling emergence as compared with non inoculated soil .

There was a positive significant correlation between number of emerged seedlings and potato yield. The corrlateions between number of emerged seedlings with foliage fresh weight (0.453), Foliage dry weight (0.469), stem canker incidence (-0.044), stem canker severity (- 0.187), black scurf incidence (-0.006), % weight of scurfed tubers (- 0.155) , black scurf severity (-0.040), and mg sclerotia weight/ cm^2 (-0.062) were found non significant (Table. 13).

4-12-2b. Effect of biocontrol on emergence of potato seedlings 15 DAS (1999-2000)

Inoculation of soil with *R. solani* resulted in decreased emergence of seedlings (10.67), while in non inoculated control the number was 17. 67 (Table 14a, and Fig. 30), which indicates that inoculation of *R. solani* caused 40% decrease of seedling emergence.

It was found that seed application of WT4, WT5, and integration of EM64+carbendazim, seed application of WT8, EM24 +carbendazim, EM15+carbendazim , WT5+carbendazim ·, WT4+carbendazim ,WT8+carbendazim , soil application of WT8 and tuber dipping with carbendazim were the superior treatments. Effects of these treatments were statistically equal to that of control not infested with *R. solani*. However the percentage of seedling emergence of the first 6 treatments were higher than that of the control not infested treatment, thus these treatments collectively ranked first.

It is clear that seed application of *Trichoderma* spp. were more effective in increasing the number of emerged seedlings, which indicates that close contact of larger number of propagules of *Trichoderma spp* with tuber and the kind of propagules used in these treatments have resulted to increase of seedling number. This is most probably due to some chemical metabolites produced by *Trichoderma* spores or germlings, which stimulated germination of potato seedlings from tubers in early stage of sprouting. It may also protect the seedlings from some soil and tuber borne (minor) pathogens also, which hinder the germination and emergence of seedlings.

Soil application of *Trichoderma* isolates, also significantly increased the number of emerged seedlings. In general, all the treatments having the biocontrol agents alone or in combination with carbendazim showed increased number of emerging seedlings. Tuber treatment with carbendazim also increased the number of emerged seedlings when used alone.

Soil application of WT4, WT5 and EM15, integration of EM64+MEMC, EM24+MEMC, EM24+tebuconazole and tebuconazole ranked the second. However, WT8+MEMC, and EM15 +MEMC and also WT8 +tebuconazole and control infested with *R. solani* were found not

significantly different to increase the number of emerged seedlings and these treatments ranked the last.

Seed application of *Trichoderma* spp. with spore suspension and integration of EM64 + carbendazim and EM15 + carbendazim promoted the emergence of potato seedlings. The mean number of emerged seedlings out of 20 were 19.33 in case of WT5 and WT4 seed application and EM64 + carbendazim and 18.67 in WT8 seed application and 18.33 in case of EM24 + carbendazim were found superior treatments. Soil application of wild types and mutants of *Trichoderma* spp. and integration of carbendazim and biocontrol agents also significantly increased the number of emerged seedlings. Integration of biocontrol agents with MEMC and tebuconazole and application of the two fungicides alone were found inferior in increasing the number of emerged seedlings. Number of emerged seedlings was the least in control infested with *R. solani* inoculum only.

There was highly significant positive correlation between number of emerged seedlings and foliage fresh weight, (0.488), and foliage dry weight (0.465). However the correlations between number of emerged seedlings and stem canker incidence (-0.158), stem canker severity (-0.065), black scurf incidence (-0.212), percentage weight of scurfed tubers (-0.103), black scurf severity (-0.114), mg weight of sclerotia/ cm^2 tuber surface(-0.197) and potato yield (0.118) were found non significant (Table 13).

Lindsey and Baker (1967) reported that *T. viride* increased plant height, shoot weight, root weight and total weight of tomato under gnotobiotic condition. However, they demonstrated that the phenomenon was not restricted to *Trichoderma* spp. Chang *et al.*, (1986) observed plant growth promotion resulting in enhanced germination, more rapid flowering, increased flowering, increased height and fresh weight in pepper, perwinkle, chrysanteinum and/or other plants after treatment of soil with peat/bran inoculum or conidial suspension of *T. harzianum*. Windham *et al.*, (1986) demonstrated that *T. harzianum* and *T. koningii* promoted the growth of corn, tomato, tobacco and radish including increased germination rates, dry weight and emergence. Harman *et al.*, (1989) working with *T. harzianum* (T12), reported that T12 promoted higher seedling emergence, final stand and dry weight in plots of sweet corn. The possibility of plant growth promotion resulting from control of other undiagnosed diseases was not eliminated (Bailey and Lumsden , 1998). Kleifeld and Chet (1992) found that a peat/bran

preparation of *T. harzianum* isolate T-203 was more effective at inducing plant growth promotion (germination, seedling length, dry weight and leaf area) in pepper and other plant species than conidial suspension or seed coatings. They suggested that the peat/bran served as food reservoir for isolate T-203. Plant growth promotion was demonstrated in plants grown in semi-strile Hougland's solution, suggesting the effect was not entirely associated with suppression of minor pathogens. Shivanna *et al.*, (1994) reported that the plant growth promoting fungi were less effective when applied in field tests, but increased yield was observed in response to some isolates of *Trichoderma* spp.

Trichoderma has a real potential for plant growth promotion and induced resistance. A great deal of effort should be put into understanding the mechanisms involved in these effects so that their potential can be fully realised (Baily and Lumsden, 1998). It is evident that addition of specific *Trichoderma* isolates to the rhizosphere can result in plant growth promotion. The plant growth promoting effect in some systems are prolonged even to the point of increasing yield (Ahmad and Baker, 1988 ; Harman *et al.*, 1989). What is unclear at this point is how *Trichoderma* species promote plant growth and how the effect can be predicted and exploited. The mechanism involved in plant growth promotion by *Trichoderma* species are only now being investigated (Baily and Lumsden, 1998).

It has been proposed that plant growth promotion in response to treatment with *Trichoderma* species is the result of indirect effect through the control of minor pathogens. This hypothesis suggests that all plants grown in the open environment are to some extent diseased and unable to reach their maximum growth potential. The addition of *Trichoderma* species to these plants, with undefined disease potential, result, in disease suppression and enhanced plant growth (Baily and Lumsden, 1998).

Another hypothesis of plant growth promotion by *Trichoderma* spp. is their direct effect. It has been demonstrated that under gnotobiotic condition, plant growth promotion achieved under condition where only known organisms involved were the plant and *Trichoderma* isolate. Evidence supporting the presence of growth promoting diffusable factor was obtained by separating germinating maize, tomato and tobacco seeds from homogenized mycelial preparation of *T. koningii* and *T. harzianum* with a cellophane membrane. This diffusable factor was not identified (Windham,

1986). The evidence suggested that the *T. harzianum* isolate was limiting and even reversing the effects of oxidative damage to the roots. Addition of *T. harzianum* strain 1295-22 to hypochloride- damaged seedlings restored seedling vigour. Hypochloride is reported to cause oxidative damage to plant tissue (Baily and Lumsden, 1998).

4-12-3a. Effect on foliage fresh weight of potato (1998-1999)

A perusal of data in Table 12a. and Fig. 27 could reveal, that inoculation of soil with *R. solani* resulted in 34% reduction of foliage fresh weight of potato at the time of harvest. All the treatments significantly reduced the reduction of fresh weight of foliage of potato caused by *R. solani*. Integration of WT4 with carbendazim supported the highest foliage fresh weight (65.92 q/ha) which is 11.9 q/ha or 22% more than control not infested and 30.47 q/ha (88%) more than control infested with *R. solani* and it ranked first. Seed application of WT4, WT5 and WT8 resulted in 63, 50 and 39 %, more foliage fresh weight than control infested with *R. solani*, respectively. Soil application of WT4, WT5 and WT8 produced 73, 70 and 63% more foliage fresh weight than control infested with *R. solani* respectively. Integration of carbendazim with WT4, WT5 and WT8 resulted to 85, 45 and 63% more foliage fresh weight than control infested with *R. solani*, respectively. Integration of MEMC with WT4, WT5 and WT8 lead to 67, 31 and 56% more foliage fresh weight than control infested with *R. solani*, respectively.

In general soil application of *Trichoderma* spp. was more effective in preventing foliage fresh weight reduction by *R. solani* than seed application which is most probably due to higher population and evenly distribution of biocontrol agents when they were used as soil application. Integration of carbendazim with biocontrol agents performed better than integration of MEMC with the same. Application of carbendazim was superior to MEMC when the two chemicals used alone.

The correlations between foliage fresh weight of potato with foliage dry weight and yield were positive and highly significant. While the correlations between foliage fresh weight with stem canker incidence, black scurf incidence, black scurf severity and mg sclerotia/ cm^2 tuber surface were negative and significant, and the correlations of foliage fresh weight with stem canker severity, % weight of scurfed tuber were negatively highly significant.

The correlations of foliage fresh weight with number of emerged seedlings (0.453) was not significant (Table 13).

4-12-3b. Effect on foliage fresh weight of potato (1999-2000)

Foliage fresh weight in control infested with *R. solani* was 36.11q/ha that is 18.33q/ha (33%) less than that of the control not infested plots. WT4+carbendazim, Em64+carbendazim, EM24+carbendazim and WT8 soil application with 68.33, 65.00, 63.89 and 60 q/ha foliage fresh weight, respectively, significantly produced higher foliage fresh weight than other treatments and ranked first. It is interesting that the foliage weight of all these four treatments are 25, 19, 17 and 10 per cent more than that of control not infested with *R. solani*. This indicated that the antagonists play a positive role in enhancing the growth of potato plant in addition to check the pathogen. The first three of these treatments have carbendazim as chemical component and the biological agent component is WT4 (*T. longibrachiatum*) or its derived mutants in common .

EM 15 soil application, WT4+MEMC, WT4+tebuconazole, WT8+carbendazim, EM15+carbendazim, EM24+tebuconazole, carbendazim, WT4 seed application, WT8+MEMC, Control not infested, EM64 soil application, WT5 seed application, WT8+tebuconazole, EM24+MEMC, WT5+carbendazim, WT4 soil application, EM64+MEMC, and EM15+MEMC supported 59.44, 58.89, 58.89, 57.78, 57.78, 57.22, 57.22, 56.11, **54.44**, 55.00, 53.33, 53.33, 53.33, 51.67, 51.67, 51.67, 51.11 q/ha foliage fresh weight, respectively. There was no significant difference among these treatments and they collectively ranked the second. However in this group the first eight treatments produced higher foliage fresh weight than control not infested with *R. solani*. It is evident that all of these treatments except carbendazim have either biological agent alone or integration of biological control with fungicides.

WT8 seed application, MEMC, EM15+tebuconazole, WT5+tebuconazole, EM64+tebuconazole, WT5+MEMC, WT5 seed application , with 49.44, 49.44, 48.89, 48.89, 47.22, 46.67, 46.11 q/ha foliage fresh weight, respectively, showed no significant difference among themselves and collectively ranked third.

Tebuconazole and control infested with *R. solani* with 38.33 and 36.11 q/ha foliage fresh weight, respectively, showed no significant difference and collectively ranked fourth or the last (Table 14a, and Fig. 30).

From the result of this experiment it may be concluded that among the three fungicides used carbendazim increased the foliage fresh weight when used either alone or in combination with biocontrol agents. Tebuconazole was not effective in increasing the foliage fresh weight. Plots treated with tebuconazole either alone or in combination with biocontrol agents were quite distinct from others in the field, due to retarded growth and dark green colour foliage of the plants due to enhanced chlorophyll content of these plants. MEMC performed better than tebuconazole but less than carbendazim in increasing the foliage fresh weight of potato plants.

Integration of biocontrol agents with fungicides appeared better than application of each component alone. So, there may be a synergistic effect between fungicides and biocontrol agent, for example in case of WT4 and carbendazim when used separately supported 56.11, and 57.22 q/ha foliage fresh weight, respectively and ranked second. But when they were combined 63.89 q/ha foliage fresh weight was obtained and it ranked first. Same is in case of tebuconazole which ranked in the last group along with control infested with *R. solani*, but in combination with biocontrol agents it came to 2^{nd} or 3^{rd} rank.

The positive correlation between foliage fresh weight with number of emerged seedlings (0.488), foliage dry weight (0.925), and potato yield (0.525) were highly significant. The negative correlation of foliage fresh weight with stem canker incidence (-0.367), and percentage of scurfed tuber (-0.389) were significant. While the negative correlation of foliage fresh weight with stem canker severity (-0.320), black scurf incidence (-0.264), black scurf severity (-0.232) and mg weight of sclerotia /cm^2 tuber surface (-0.320) were not significant (Table 15).

4-12-4a. Effect on foliage dry weight of potato (1998-1999)

Since dry weight is a component of fresh weight of foliage parts, so it is natural that it follows the pattern of foliage fresh weight. It was found that

in *R. solani* inoculated plots the foliage dry weight is reduced to 65% of the same of the control not inoculated with *R. solani*. Integration of carbendazim

With WT4 supported the highest foliage dry weight 6.576 Kg/ha which was 1.237 Kg/ha (23%) more than that of control not infested and 2.931 Kg/ha (80%) more than control infested with *R. solani*. All of the treatments significantly increased the foliage dry weight over that of control infested plots. Soil application of biocontrol agents was more effective than seed application of the same, and integration of carbendazim with biocontrol agents was found more effective than integration of MEMC with biocontrol agents. Tuber dipping with carbendazim was superior to MEMC. Soil application of WT4, WT5 and WT8 lead to 66, 65 and 58% more foliage dry weight than that of control infested, respectively. Seed application of WT4, WT5 and WT8 resulted to 54, 46 and 35% more foliage dry weight than the same of control not infested with *R.solani*. Integration of carbendazim with WT4, WT5 and WT8 produced 80, 42 and 49 % more foliage dry weight than that of control infested with *R. solani*, respectively. Integration of MEMC with WT4, WT5 and WT8 lead to 63, 28 and 52% more dry weight over that of control infested with *R. solani*, respectively. Tuber dipping in carbendazim and MEMC resulted to 55 and 35% foliage dry weight more than that of control infested with *R.solani* (Table 12a, and Fig. 27).

Foliage dry weight was positively and significantly correlated with potato yield and highly significantly correlated with foliage fresh weight of potato. However there were negative highly significant correlations between foliage dry weight with stem canker severity, %weight of scurfed tubers, and negative significant correlation between foliage dry weight with stem canker incidence, incidence of black scurf, severity of black scurf, and mg sclerotia/ cm^2 tuber surface. The correlations between foliage dry weight and number of emerged seedlings (0.453) was not significant (Table 13).

4-12-4b. Effect on foliage dry weight of potato (1998-2000)

Foliage dry weight of potato plant in control infested with *R. solani* was 3.79 q/ha which is 1.71 q/ ha (31%) less than control not infested with *R. solani*. Foliage dry weight in control not infested with pathogen was 5.50 q/ha. WT4+carbendazim, EM64+carbendazim with 6.92 and 6.72 q/ha supported 82.6 and 77.3% higher foliage dry weight over control infested with *R. soluni*, respectively, and ranked first. WT8 soil application, EM15

soil application, WT4+tebuconazole, EM15+tebuconazole, WT4+MEMC, WT8+carbendazim, EM24+carbendazim, EM15+carbendazim, and carbendazim with 6.05, 6.04, 5.97, 5.95, 5.91, 5.89, 5.88, 5.88 and 5.88 q/ha foliage dry weight ranked second. Growth promoting effect of these treatments is evident when compared with control not infested with the pathogen. WT5+carbendazim with 5.78 q/ha foliage dry weight ranked third, WT4 seed application, WT8+MEMC, EM24+carbendazim, EM64soil application, WT5 seed application and control not infested with *R. solani*, supported 5.65, 5.64, 5.59, 5.55, 5.51 and 5.50 q/ha foliage dry weight and ranked fourth. EM24+MEMC, WT8+tebuconazole, EM24 soil application and EM15+MEMC with 5.47, 5.44, 5.29 and 5.29 q/ha foliage dry weight ranked fifth. EM64+MEMC, WT5+tebuconazole and MEMC with 5.18, 5.10 and 5.04 q/ha foliage dry weight ranked sixth. WT8 seed application, EM64 tebuconazole and WT5 soil with 4.90, 4.80 and 4.73 q/ha foliage dry weight ranked seventh. WT5MEMC with 4.72 q/ha foliage dry weight ranked eighth, and finally tebuconazole and control infested with *R. solani* with 3.89 and 3.79 q/ha foliage dry weight, respectively ranked the last (Table 14a, and Fig. 30).

Soil inoculation with *R. solani* in second year experiment resulted in less foliage fresh weight reduction (31%) when compared with the first year experiment (64%). The difference may be due to the, longer preservation of the pathogen on PDA which may reduced the virulence of the pathogen. Or it may be due to changing the soil plots in second year and sowing potato in plots which previously were not under potato cultivation, and application of NPK fertilisers in the second year experiment.

The two treatments (WT4+carbendazim, EM64+carbendazim) resulted in 25.8 and 22.2% more foliage dry weight over control not infested with *R. solani*, which indicates that they have growth promoting effect in addition to control of the reduction of growth due to pathogen. WT8 soil application, EM15 soil application, WT4+tebuconazole, EM15+tebuconazole, WT4+MEMC, WT8+carbendazim, EM24+carbendazim, EM15+carbendazim, and carbendazim, WT5+carbendazim, WT4 seed application, WT8+MEMC, EM24+carbendazim, EM64soil application, WT5 seed application also supported more foliage dry weight than control not infested with *R. solani* and exhibited growth promoting effect. EM24+MEMC, WT8+tebuconazole, EM24 soil application, EM15+MEMC, EM64+MEMC, WT5+tebuconazole, MEMC, WT8 seed application, EM64

tebuconazole, WT5 soil, and WT5+MEMC did not resulted in higher foliage dry weight over control not infested with *R. solani.* However, when the impact of pathogen in reducing the plant growth is considered it is obvious that all of these treatments had increased the foliage dry weight of potato in field. The only treatment which did not significantly increased the foliage dry weight was tebuconazole, but when it integrated with biocontrol agent the growth retarding effect of this fungicide was reduced.

In general it is concluded that application of biocontrol agents either alone or in combination with fungicides promoted the growth of potato plant. Carbendazim was found the superior fungicide followed by MEMC, however tebuconazole exhibited growth-retarding effect and found the inferior fungicide in this experiment. Integration treatments performed better than application of each component alone.

The correlation of foliage dry weight with number emerged seedlings (0.465), and foliage fresh weight (0.925) were highly significant, the correlation of foliage dry weight with stem canker incidence (-0.387), stem canker severity (-0.356), percentage weight of scurfed tubers (-0.365), and potato yield (0.397) were significant. There was no significant correlation between foliage dry weight with black scurf incidence (-0.260), black scurf severity (-0.243), and mg weight of sclerotia/cm^2 tuber surface of potato (Table 15).

4-12-5a. Effect on incidence of stem canker of potato (1998-1999)

Inoculation of soil with *R.solani* lead to 85.39 % Stem canker incidence. While in control not inoculated with *R. solani* stem canker incidence was 39.15%, which indicated that the population of pathogen in natural soil is considerably high, but inoculation of soil with the pathogen significantly increased the incidence of stem canker. All the treatments significantly reduced the incidence of stem canker, data are presented in Table 12. Integration of carbendazim with WT8 completely checked the incidence of stem canker. Tuber dipping with carbendazim and MEMC showed 17.67 and 24.32 % stem canker incidence (79 and 71 % reduction of disease incidence), respectively. Soil application of WT4, WT5 and WT8 resulted to 23.64, 22.86 and 22.35 % incidence of stem canker incidence (72, 73 and 74 % reduction of disease incidence), respectively. Seed application of WT4, WT5 and WT8 lead to 55.18, 54.27 and 59.85% incidence of stem

canker incidence (35, 36 and 30 % reduction of disease incidence), respectively. Integration of carbendazim with WT4, WT5 and WT8 showed 9.58, 3.02 and 0.00 % of stem canker incidence (89, 97 and 100 % reduction of disease incidence), respectively. Integration of MEMC with WT4, WT5, and WT8 resulted to 13.62, 7.48 and 10.12 % incidence of stem canker incidence (84, 91 and 88% reduction of disease incidence), respectively. In general integration of biocontrol agent with fungicides was superior to application of chemical and biocontrol separately. Integration of carbendazim was more effective than MEMC with biocontrol agents. Tuber dipping with carbendazim was better than MEMC in reducing the incidence of stem canker disease of potato caused by *R. solani*. Soil application of the antagonists performed better than seed application of the same, which is most probably due to higher amount of population and type of inoculum of biological agent used (Table 12a, and Fig. 29).

There were negative significant correlations between stem canker incidence and foliage fresh and foliage dry weight of potato. The correlations between stem canker incidence with stem canker severity, black scurf incidence, %weight of scurfed potato, black scurf severity and mg sclerotia/ cm^2 were positive and highly significant. The correlations between stem canker incidence and potato yield (-0.321) and number of emerged seedlings (0.044) were not significant (Table 13).

4-12-5b. Effect on incidence of stem canker of potato (1999-2000)

In control plots infested with *R. solani* there was 80.41% incidence of stem canker, while it was 46.13% in control not infested with *R. solani*. This indicates that pathogen population in natural soil is quite high, but artificial inoculation significantly increased the disease incidence as it was observed in the 1st year.

WT8+carbendazim and EM15+MEMC completely inhibited stem canker incidence and found the most effective treatments to check the incidence of stem canker. EM15+carbendazim, WT5carbendazim, carbendazim, WT5+MEMC, EM24+carbendazim, EM64+tebucanazol, WT8+MEMC, EM24+tebuconazole with 16.04, 15.65, 15.31, 14.75, 13.29, 12.77, 8.85, and 8.56 per cent stem canker incidence (80, 81, 81, 82, 83, 84, 89 and 89% reduction of disease incidence), respectively, appeared significantly different from other treatments and ranked second. MEMC, WT4+tebuconazole, EM64+carbendazim, EM15+tebuconazole,

WT5+tebuconazole, WT4+carbendazim and WT4+MEMC with 22.85, 21.33, 21.09, 20.79, 20.25, 20.25, 19.33 and 17.29 per cent stem canker incidence (72, 73, 74, 74, 75, 75, 76 and 79% reduction of disease incidence) ranked third and appeared at par with each other. EWT5 soil application, WT8 soil application, EM24+MEMC, WT8+tebuconazole, and tebuconazole with 34.83, 34.02, 33.00, 32.07 and 27.91 percent stem canker incidence (57, 58, 59, 60 and65% reduction of disease incidence) , respectively ranked fourth. EM54 soil application, WT4 seed application, EM15 soil application, and EM24 soil application with 44.03, 41.88, 39.99 and 36.22 per cent disease incidence (45, 48, 50 and 55% reduction of disease incidence), respectively, ranked fifth. WT5 seed application, control not infested, WT4 soil application, with 48.43, 46.13 and 45.36 per cent stem canker incidence (40, 43,and 44% reduction of disease incidence), respectively, ranked sixth . WT8 seed application with 61.19 (24% reduction of disease incidence) and control infested with *R. solani* with 80.41 (0% reduction of disease incidence), respectively, ranked seventh and eighth respectively (Table 14a, anf Fig. 32).

In general as in the 1st year experiment the integration of biocontrol agents with fungicides was superior to application of chemical and biocontrol separately. Integration of carbendazim was more effective than MEMC with biocontrol agents. Tuber dipping with carbendazim was better than MEMC in reducing the incidence of stem canker disease of potato caused by *R. solani*. Soil application of the antagonists performed better than seed application of the same, which is most probably due to higher amount of population and type of inoculum of biological agent used.

There was highly significant positive correlation between incidence of stem canker and stem canker severity (0.916), black scurf incidence (0.847), percentage weight of scurfed tubers (0.865), black surf severity (0.592), mg sclerotia/cm^2 tuber surface (0.837). However, the negative correlation between incidence of stem canker and yield of potato (-0.521) was highly significant. There was negative significant correlation between stem canker incidence and foliage fresh weight (-0.367), foliage dry weight (-0.387). But the correlation between stem canker incidence and number of emerged seedlings was not significant and followed the same trend as in the first year experiment (Table 15).

4-12-6a. Effect on severity of stem canker of potato (1998-1999)

In control infested with *R. solani* stem canker severity was 2.83 while in control not infested it was 0.85. These figures indicated that the population of pathogen in natural soil is considerable and artificial inoculation of soil with *R. solani* has significantly increased the disease severity. All the treatments significantly reduced the severity of stem canker incidence (Table 12). WT8 +carbendazim though, was the most effective treatment which completely checked the disease severity. Yet it was significantly equal to WT5+carbendazim, WT5+MEMC, WT4+carbendazim, WT4+MEMC, carbendazim, WT5 soil application, MEMC, WT8+MEMC, WT8 soil application and control not infested with *R. solani* treatments with disease severity of less than 0.89 and collectively all of these treatments ranked the first. Seed application of WT4, WT5 and WT8 showed 1.12, 1.39 and 1.52 disease severity and ranked the second category along with WT4 soil application but they were significantly different from control infested with *R. solani*(Table 12a, and Fig. 28).

In general integration of biocontrol with carbendazim and MEMC and soil application of the antagonists performed better than seed application of the antagonists alone. Carbendazim performed better than MEMC when the two fungicides used alone.

The correlations between severity of stem canker and foliage fresh weight, foliage dry weight were negative and highly significant, while the correlation of stem canker severity and potato yield was negative and significant. The correlations between stem canker severity, black scurf incidence, % weight of scurfed potato, black scurf severity and mg sclerotia/ cm^2 of tuber surface were positive and highly significant. The correlation between stem canker severity and number of emerged seedlings (–0.187) was not significant (Table 13).

4-12-6b. Effect on severity of stem canker of potato (1999-2000)

Severity of stem canker in infested control was 2.37 and it was 1 in control not infested with the pathogen, which again shows the presence of *R. solani* in the field soil not inoculated with the same and efficacy of artificial inoculation in increasing the disease severity. All of the treatments reduced

the severity of the disease. WT8 + carbendazim, EM15 + MEMC and WT5 +carbendazim completely checked the severity of stem canker. However they were not significantly different from WT5+carbendazim, WT4+MEMC, EM64+carbendazim, EM64+carbendazim, EM24+carbendazim, Em15+carbendazim, EM15+tebuconazole, carbendazim, WT5+MEMC, WT4+tebuconazole and EM64+MEMC which were significantly superior than other treatments in reducing the severity of stem canker and collectively ranked first. MEMC, WT4+carbendazim, WT5 soil application, WT8+tebuconazole, WT8+MEMC and EM24+tebuconazole with 0.37, 0.40, 0.60, 0.63, 0.67 and 0.67 disease severity showed no significant difference among themselves and ranked second. EM24 soil application, EM24+MEMC, WT8 soil application, EM15 soil application, WT4seed application, control not infested with *R. Solani*, and EM64 soil application with 0.70, 0.73, 0.90, 0.93, 0.97, 1.00, and 1.00 severity of stem canker, respectively ranked third, followed by WT4 soil application with 1.03 disease severity in rank fourth. WT5 seed application and WT8 seed application with 1.46 and 1.50 disease severity in rank fifth. Ultimately control infested with *R. solani* with 2.36 disease severity ranked last (Table 14a, and Fig.31).

In general integration of biocontrol with carbendazim and MEMC and soil application of the antagonists performed better than seed application of the antagonists alone. Carbendazim performed better than MEMC when the two fungicides nsed alone. Soil application of biocontrol agent performed better than seed application of the saine most probably because of higher population density of the same in soil application and the type of inoculum used. Better performance of carbendazim may be related to the nature of this fungicide, which is systemic and can be translocated through out the plant and thus it may protect the plant far from the site of application of the fungicide a criteria, which is not present in MEMC.

There was highly significant positive correlation between stem canker severity and stem canker incidence (0.916), black scurf incidence (0.866), and percentage weight of scurfed potato (0.848), black scurf severity (0.678), mg weight of sclerotia/ cm^2 (0.856). But there was highly significant negative correlation between stem canker severity and potato yield (-0.484). The correlation between stem canker severity and foliage dry weight (-0.356) was negative and significant. However, the correlation between stem canker severity and number of emerged seedling (0.065), and foliage fresh weight (-0.356) was not significant (Table 15).

4-12-7a. Effect on incidence of black scurf on potato tubers (1998-1999)

Incidence of black scurf was 85.33 in control infested with *R. solani.* It was 22.42 in control not infested with the pathogen, which again indicated to the presence of considerably high population of the pathogen in natural soil, and effectiveness of artificial inoculation to increase the incidence of black scurf on tubers. All treatments significantly reduced the incidence of the disease. Integration of WT4 and WT5 with carbendazim and MEMC completely checked the incidence of the disease and they were ranked the first category. Tuber dipping in MEMC with 1.33% scurf incidence (98% reduction of disease incidence) ranked the second followed by WT8+carbendazim and tuber dipping in carbendazim with 3.52 and 4.70 % disease incidence (96 and 95% reduction of disease incidence), respectively in the third rank. WT8+MEMC with 6.25% disease incidence (93% reduction of disease incidence) ranked fourth followed by WT5soil application with 12.23% disease incidence (86% reduction of disease incidence) as fifth. WT8 soil application with 22.31 (74% reduction of disease incidence) and control not infested with *R. solani* with 22.42% disease incidence (69% reduction of disease incidence) ranked the sixth. WT8 seed application showed 26.78% disease incidence (67% reduction of disease incidence) at seventh. WT4 soil application with 29.93 disease incidence (66% reduction of disease incidence) ranked at eighth. WT5 seed application with 40.32% disease incidence (53% reduction of disease incidence) at ninth, WT4 seed application with 53.57 disease incidence (37% reduction of disease incidence) at tenth. Finally control infested with *R. solani* with 85.33% incidence of black scurf at eleventh or last ranks (Table 12b, and Fig. 29).

Here again it is clear that integration of chemicals with biological control is more effective in checking the incidence of black scurf highlighting the concept of integrated approach in disease management. Soil application of biological agents is more effective than seed application of the same most probably due to higher population and type of inoculum of antagonists. Tuber dipping with MEMC was more effective than dipping the tubers in carbendazim. A phenomenon, which may be related to the nature of these two chemicals, MEMC is non-systemic and may be more stable than carbendazim in the soil, so it may protect the plant against the pathogen longer than carbendazim.

There was negative significant correlation between black scurf incidence and foliage fresh and foliage dry weight of potato plants. The correlation's between black scurf incidence and stem canker incidence, stem canker severity, mg sclerotia/ cm^2 tuber surface, was positive and highly significant. The correlation between incidence of black scurf and potato yield was –0.487 and it was not significant .The correlation between incidence of black scurf and number of emerged seedlings 15 DAS (–0.006) was not significant (Table 13).

4-12-7b. Effect on incidence of black scurf on potato tubers (1999-2000)

There was 64 per cent disease incidence in control plots infested with *R. solani* while it was 21.33 in control not infested with *R. solani*, this indicated the presence of fairly high population of pathogen in the soil. However, artificial inoculation of soil with *R. solani* increased the disease incidence. All of the treatments reduced the incidence of disease in compare with control infested. Tuber dipping in MEMC and carbendazim suspension and integration of EM24+carbendazim, EM15+MEMC ,WT5+MEMC, WT5+carbendazim and WT4+MEMC , WT4+tebuconazole. completely checked the incidence of the disease. However, effect of these treatments were not significantly different from EM64+carbendazim, EM24+tebuconazole, tebuconazole, WT5+tebuconazole, WT5+carbendazim, WT4+carbendazim, EM24+MEMC, EM15+carbendazim, EM64+tebuconazole, EM64+MEMC, and WT8+MEMC with, 0.67, 2.33, 3.00, 3.33, 3.33, 3.33, 5.00, 5.00, 6.00, and 6.33 percent black scurf incidence (99, 98.95, 96, 95, 95, 95, 95, 92, 91, 91 and 90% reduction of disease incidence), respectively, and these group of treatments ranked first. WT8+tebuconazole, WT5 soil application and EM15+tebuconazole with 10.33, 11.00 and 13.33 per cent disease incidence (84, 83 and 79% reduction of disease incidence), respectively ranked second. Control not infested with *R. solani*, WT8 soil application WT8 seed application, EM24 soil application, EM64 soil application, and EM15 soil application with 21.33, 24.33, 26.67, 27.62, 28.33 and 28.33 per cent disease incidence (67, 62, 58, 57, 56, and 56% reduction of disease incidence) at third place. WT4 soil application with 31% (52% reduction of disease incidence) and WT5 seed application with 41% disease incidence (36% reduction of disease incidence) at fourth and fifth ranks, respectively. Finally WT4 seed application with 54.33% (15% reduction of disease incidence) at sixth and control infested with *R. solani* with 64 % disease incidence at the last rank (Table 14b, and Fig. 32).

In general it can be concluded that application of carbendazim and MEMC either alone or in combination with some biocontrol agents completely checked or very effectively reduced the incidence of black scurf of potato under field condition. Soil application of antagonists were more effective than seed application of the same to reduce the incidence of the disease. Mutant EM15 was inferior than its related wild type WT5 when the two used as soil application alone or in combination with carbendazim and tebuconazole, however the mutant EM64 and EM24 showed no significant difference from the wild type WT4 to control the incidence of black scurf.

There was highly significant positive correlation between black scurf incidence and stem canker incidence (0.847), stem canker severity (0.869), percentage weight of scurfed tubers (0.926), black scurf severity (0.713), mg weight of sclerotia/ cm^2 tuber surface (0.919). There was highly significant negative correlation between black scurf incidence and potato yield (-0.525). But the correlation between black scurf incidence and number of emerged seedlings (0.212), foliage fresh weight (-0.264) and foliage dry weight (-0.260) were not significant (Table 15).

Bari (1988) reported that Emisan-6 and Bavistin reduced the sclerotial index on potato tubers from 90.88 in check to 50.88 and 59.91 percent respectively. He reported that the disease control was maximum in Emisan- 6(44.61 per cent).

4-12-8a. Effect on severity of black scurf on potato tubers (1998-1999)

Severity of potato black scurf in soil inoculated with *R. solani* was 2.32, while it was 0.72 in control not infested with the pathogen, which indicted the presence of considerable population of *R. solani* in non inoculated soil and effectiveness of artificial inoculation to increase the severity of the disease. All treatments significantly reduced the severity of the disease (Table 12). WT4+ carbendazim and WT5 +carbendazim and MEMC completely inhibited the severity of the disease. However, there was no significant difference between the effects of these treatments and that of the WT8 +carbendazim, so these four treatments lopped the first. Tuber dipping with carbendazim, WT8+MEMC, MEMC, WT4 soil application, WT5 soil application and control not infested with *R. solani* with 0.43, 0.45, 0.58, 0.58,

0.65 0.72 degree of disease severity, respectively showed no significant difference and they ranked second. Seed application of WT5, WT8 and WT4 with 1.38, 1.52 and 1.57 scale of disease severity, respectively occupied the third place and finally control infested with *R solani* with 2.32 was ranked the last.

Again from the results of this experiment it is evident that integration of chemical and biological control is superior than application of each one individually, similarly soil application of biocontrol agents was found more effective than seed application of the same (Table 12b, and Fig. 28).

There was negative significant correlation between severity of black scurf with foliage fresh and dry weight of potato. But there were highly significant positive correlation between severity of black scurf with incidence of stem canker , severity of stem canker ,incidence of black scurf and percentage weight of scurfed tubers. The correlation between severity of black scurf (-0.410) and also severity of black scurf and number of emerged seedlings 15 DAS (0.040) were found not significant (Table 13).

4-12-8b. Effect on severity of black scurf on potato tubers (1999-2000)

A critical appraisal of data presented in Table 14 indicate the efficacy of treatments in relation to management of black scurf followed same trend as in 1st year experimentation.

Severity of black scurf was 1.82 in control infested with *R. solani* and 0.24 in control not infested with *R. solani*. All the treatments significantly reduced the severity of black scurf. However there was significant difference among the effects of different treatments on the severity of black scurf and based on these differences the treatments were ranked.

WT5+MEMC, WT5+carbendazim, WT4+MEMC, EM15+MEMC, MEMC, and carbendazim completely reduced the severity of black scurf, however there was no significant difference between these treatments and EM15+carbendazim, tebuconazole,, WT4+tebuconazole, WT8+tebuconazole,EM15+tebuconazole, EM24+tebuconazole, EM24+MEMC, and WT5+tebuconazole with 0.06, 0.10, 0.14, 0.14, 0.17, 0.17, and 0.17 disease severity and all of these treatments were ranked in first group. Control not infested with *R. solani*, EM64soil application, WT8soil

application, WT4 soil application, WT5 soil application, EM24 soil application, EM15 soil application and WT4+carbendazim with 0.24, 0.26, 0.28, 0.28, 0.33, 0.33, 0.39 and 0.40 disease severity, respectively ranked in the second group. WT8+MEMC, EM64+MEMC and EM64+tebuconazole with 0.47, 0.47 and 0.67 disease severity, respectively ranked third. EM64+ carbendazim, WT8+carbendazim, WT5seed application, EM24+carbendazim, WT4 seed application and WT8 seed application with 0.77, 0.87, 0.88, 0.90, 0.98, and 0.98 disease severity respectively, placed in fourth rank. Finally control infested with *R. solani* with 1.82 disease severity in the last rank (Table 14b, and 31).

The positive correlation between black scurf severity and stem canker incidence (0.592), stem canker severity (0.687), black scurf incidence (0.713), percentage weight of scurfed tubers (0.720), mg weight of sclerotia/cm^2 tuber surface was highly significant. But the negative correlation between black scurf severity and number of emerged seedlings (-0.114), foliage fresh weight (-0.232), foliage dry weight (-0.243), and potato yield (-0.232) was not significant (Table 15).

4-12-9a. Effect on weight percentage of scurfed potato tubers(1998-1999)

The percentage weight of scurfed tubers in control infested with R. *solani* was 86.20, which was significantly higher than all other treatments. While that of control not infested with R. *solani* was 29.05 per cent, which indicates that artificial inoculation increased the percentage of scurfed tubers and there is fairly high population of pathogen in the soil itself. Integration of WT4 + carbendazim, WT5+carbendazim and MEMC completely reduced the percentage weight of scurfed tubers and ranked the first. Integration of WT8+carbendazim, and tuber dipping in carbendazim with 5.98 and 6.82% weight of scurfed tubers, respectively found not significantly different and ranked second. Tuber dipping in MEMC and integration of WT8+MEMC with 7.85 and 8.32% weight of scurfed tubers, respectively ranked third. Soil application of WT5 showed 15.96% weight of scurfed tubers was significantly different from all other treatments and ranked fourth. Soil application of WT8 and WT4 with 18.25 and 18.95 % weight of scurfed tubers, respectively ranked fifth. Seed application of WT5 and WT8 with 24.72 and 25.65% weight of scurfed tubers, respectively, placed in sixth rank. Control not infested with 22.42 and seed application of WT4 with 38.98% weight of scurfed tubers were significantly different and

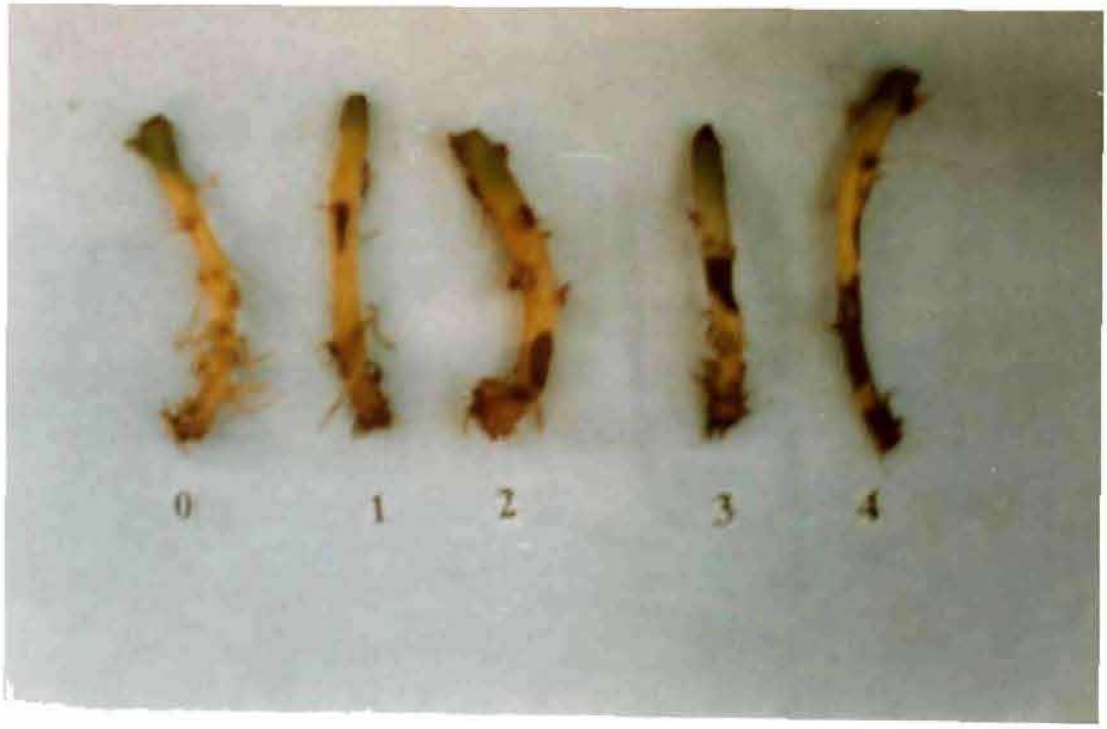


Fig.25 . Severity of stem canker disease caused by *R. solani* on potato.

Fig. 26 . Severity of black scurf disease caused by *R. solani* on potato.

ranked seventh and eight, respectively. Finally control infested with R. solani with 86.20% weight of scurfed tubers was significantly different from other treatments and ranked ninth or the last (Table 12b).

There were highly significant negative correlation between percentage weight of scurfed tubers with foliage fresh and foliage dry weight of potato. The negative correlation of percentage weight of scurfed tubers and potato yield was significant. However the positive correlation between percentage weight of scurfed tubers with incidence of stem canker, severity of stem canker, incidence of black scurf, severity of black scurf and mg sclerotia/ cm^2 potato tuber surface were highly significant. The negative correlation between percentage weight of scurfed tubers and number of emerged seedlings (-0.155) was not significant (Table 13).

4-12-9b. Effect on weight percentage of scurfed potato tubers(1999-2000)

The percentage weight of scurfed tuber was 60.52 and 30.63 in control infested and control not infested with *R.solani*, respectively. All the treatments reduced the percentage weight of scurfed tubers, but there was significant difference among the effects of these treatments, and based on these differences the treatments were classified to 5 groups. WT5+carbendazim,WT5+MEMC, WT4+MEMC, WT4+tebuconazole, EM24+carbendazim, EM15+MEMC, MEMC, and carendazim completely reduced the percentage weight of scurfed tubers. However there was no significant difference amongst the effects of these treatments and EM24+tebuconazole, EM64+carbendazim, WT4+carbendazim, WT8+MEMC, tebuconazole, WT8+carbendazim and EM15+carbendazim with 2.71, 3.32, 3.82, 3.84, 4.31, 6.14 and 7.43 percent weight of scurfed tubers (96, 95, 94, 94, 93, 90, 89 and 88% reduction of weight of scurfed tubers), respectively, were ranked the first. EM24+MEMC, WT5+tebuconazole, EM64+tebuconazole and EM64+MEMC with 8.96, 8.57, 9.83 and 13.72 percent weight of scurfed tubers (85, 86, 84 and 77% reduction of weight of scurfed tubers) , respectively ranked second. EM24 soil application, WT8+tebuconazole, WT5 soil application, WT4 soil application, EM15 soil application, WT8 soil application,

.EM15+tebuconazole, EM64 soil application, WT5 seed application, WT8 seed application, and control not infested with *R. solani* with 17.04, 18.37, 19.55, 19.78, 19.96, 21.18, 23.55, 24.06, 25.77, 27.74 and 30.63 per cent weight of scurfed tubers (72, 70, 68, 67, 65, 61, 60, 57, 54 and 49% reduction of weight of scurfed tubers), respectively ranked second, followed by WT4 seed application with 40.57 (35% reduction of weight of scurfed tubers) and control infested with *R. solani* with 60.52 per cent weight of scurfed tubes at third and forth ranks, respectively (Table 14b).

The correlation between percentage weight of scurfed tubers and stem canker incidence (0.865), stem canker severity (0.848), black scurf incidence (0.928), black scurf severity (0.720), mg sclerotia/cm^2 tuber surface(0.923) and potato yield (-0.522) was highly significant, but there was significant correlation between percentage weight of scurfed tubers and foliage fresh weight (-0.389) and foliage dry weight (-0.356), however the correlation of percentage weight of scurfed tubers and number of emerged seedlings was not significant (Table 15).

4-12-10a. Effect on mg sclerotia /cm 2 tuber surface (1998-1999)

Inoculation of soil with *R. solani* resulted in 18.22 mg sclerotia/ cm^2 potato tuber surface, while in control not infested the same was 7.00 mg. Integration of WT4 and WT5 with carbendazim and MEMC completely reduced the mg sclerotia/ cm^2 potato tuber surface. The effects of these treatments were, however, not significantly different from integration of WT8+MEMC (1.25 mg sclerotia/ cm^2 potato tuber surface or 93% reduction of sclerotia weight/ cm^2), and these five treatments ranked the first. WT8+carbendazim,and soil application of WT8 and WT4 with , 2.15, 2.53 and 3.39 , mg sclerotia/ cm^2 potato tuber surface (88, 86 and 81 % reduction of sclerotia weight/ cm^2) , respectively ranked second. Soil application of WT5 with 4.95 mg sclerotia/ cm^2 potato tuber surface ranked third followed by control not infested with *R. solani* at fourth place. Soil application of WT8, WT5, and WT4 , with 9.82, 9.76 and 10.39 mg sclerotia/ cm^2 potato tuber surface (46, 46 and 43% reduction of sclerotia weight/ cm^2), respectively ranked fifth followed by control infested with *R. solani* at the last rank (Table 12b , and Fig. 28).

Weight of sclerotia/ cm^2 showed negative significant correlation with foliage fresh and highly significant negative correlation with foliage dry weight. It has highly significant positive correlations with incidence and severity of stem canker and incidence and severity of black scurf. However, the negative correlation between weight of sclerotia/ cm^2 and potato yield (–0.383) was not significant (Table 13).

4-12-10b. Effect on mg sclerotia /cm 2 tuber surface (1999-2000)

In control infested with *R. solani* there was 17.08 mg sclerotia/ cm^2, while it was 5.25 mg sclerotia/ cm^2 in control not infested, which indicates fairly high population of *R. solani* in naturally not inoculated soil, and efficacy of artificial inoculation to increase the amount of sclerotia on tuber surface. All the treatments significantly reduced the amount of sclerotia/ cm^2 of tuber surface as compared with control infested with *R. solani*. WT5+MEMC, WT5+carbendazim, WT4+MEMC, WT4+tebuconazole, EM24+carbendazim, EM15+MEMC, MEMC, and carbendazim completely reduced the amount of sclerotia/ cm^2. However, there was not significant difference between the effect of these treatments and EM24+tebuconazole, tebuconazole, WT8+MEMC, WT5+tebuconazole, EM64+tebuconazole, WT4+carbendazim, WT8+tebuconazole, WT8+carbendazim, EM15+carbendazim, EM24+MEMC and WT8 soil application with 0.60, 1.06, 1.18, 1.50, 1.74, 1.84, 1.87, 2.02, 2.39, 2.54 and 2.69 mg sclerotia/ cm^2 (96, 93, 91, , 90, 89, 89, 88, 86, 85, and 84% reduction of sclerotia weight/ cm^2), respectively, thus these treatments were kept in first rank. WT4 soil application, EM64+MEMC, EM64+carbendazim, EM15+tebuconazole, EM24 soil application, EM64 soil application, WT5 soil application, EM15 soil application and control not infested with *R. solani* with 3.24, 3.25, 3.26, 3.39, 4.13, 4.25, 4.89, 5.14 and 5.25 mg sclerotia/ cm^2 (81, 81, 81, 80, 76, 75, 71, 70 and 69 % reduction of sclerotia weight/ cm^2), respectively were significantly different from other treatments and consisted the second rank. Seed application of three wild type isolates of *Trichoderma* spp. namely WT8, WT5and WT4 with 8.25, 10.69 and 11.59 mg sclerotia/ cm^2 (52, 37 and 32% reduction of sclerotia weight/ cm^2), respectively ranked third followed by control infested with *R. solani* with 17.08 as the last (Table 14b, and Fig. 31).

The positive correlation between mg sclerotia/ $cm^{2\ tuber}$ surface and stem canker incidence (0.837), stem canker severity (0.856), black scurf incidence (0.919), percentage weight of scurfed tubers (0.923), black scurf severity (0.856) and negative correlation of the same with potato yield (-0.479) were highly significant, but there was not a significant correlation between mg sclerotia/ cm^2 tuber surface and number of emerged seedlings(0.197), foliage fresh weight (-0.320) and foliage dry weight (-0.311) (Table 15).

4-12-11a. Effect on potato yield (1998-1999)

Inoculation of field plots with *R. solani* reduced the potato yield from 93.24 in control not inoculated plots to 64.63 q/ha in control plots inoculated with the pathogen, which implies 30% yield reduction due to inoculation of the pathogen. All the treatments significantly prevented the yield reduction caused by *R. solani*. WT5+carbendazim resulted to 99.54 q/ha that was 6.3 q/ha or 6% more yield over control not infested and 34.91 q/ha or 54% more yield over control infested with *R.solani*. WT5+carbendazim, WT4 soil application, WT8 soil application, WT5 soil application, WT5+MEMC, WT4 seed application with 99.54, 98.15, 94,44, 93.24, 93.24, 92.32 and 89.98 q/ha yield (54, 52, 46, 44, 44, 43, 39% more yield over control infested with *R. solani* plots were significantly superior treatments. There were no significant difference amongst the effect of these treatments and control not infested with *R.solani* though in first three treatments the yield figures were more than that of control not infested, thus all of these treatments ranked in the first group. WT4+carbendazim, WT8 seed application, WT4+MEMC, carbendazim, WT8+carbendazim, WT5 seed application, MEMC, and WT8+MEMC with 88.15, 87.5, 87.5, 86.94, 86.58, 85.65, 84.44, and 80.19 q/ha yield (36, 35, 35, 35, 34, 33, 31 and 24% more yield over control infested with *R. solani* plots), were significantly different from control infested with *R.solani* and ranked second. Control infested with *R. solani* ranked the last and least yield producing treatments (Table 12b, and Fig. 29).

The correlation coefficient between yield and other parameters clearly indicated that yield has positive significant correlation with number of emerged seedlings, and dry weight and highly significant positive correlation with foliage fresh weight of potato plant, but there was significant negative correlation between yield and stem canker severity and percentage weight

of scurfed tubers. Though there was negative correlations between yield and scurf index, mg sclerotia/ cm^2 of tuber surface, incidence of black scurf and incidence of stem canker, but these correlations were not significant The correlation coefficient between yield and other parameters are presented in Table 13.

In general it is concluded that biocontrol agents alone or in combination with fungicides increased the potato yield. Soil application of the biocontrol agents found more effective to increase the potato yield than that of seed application of the same, most probably due to higher population density of biocontrol agent in soil application method. Though carbendazim and MEMC did not showed significant difference but the yield figure of carbendazim were 4.25 q/ha more than that of MEMC plots. Soil application of all the three antagonists performed far much better than fungicide application and all the treatments having antagonists performed better than chemical control in respect to increase the yield, which indicates the growth promoting role of the *Trichoderma* spp. isolates, which is supported by findings of other workers (Bari, 1988 ; Lindsey and Baker, 1967 ;Chang *et al.*, 1986 ; Baker *et al,* 1984 ; Windham *et al.*, 1986 ; Harman *et al.*,1989 ; Lynch *et al.*, 1991 ; Calvet *et al.*, 1993 ; Inbar *et al.*,1994 ; Shivana *et al* ., 1996).

4-12-11b. Effect on potato yield (1999-2000)

Potato yield in control infested with *R. solani* was 62.22, while in not infested control it was 90 q/ha, which implies that inoculation of soil with *R. solani* was responsible for yield reduction by 30% . All the treatments increased the potato yield, however there were significant difference among the effects of treatments and based on these differences the treatments ranked in 8 groups. The first and most superior treatment was EM24+carbendazim with 173.33q/ha or 179% more yield over control infested with pathogen, which was significantly higher than all other treatments and ranked first. This was followed by WT8+carbendazim, EM64+carbendazim, EM24+tebuconazole and WT8+tebuconazole with 148.89, 146.11, 141.67 and 137.78 q/ha (139, 135, 128 and 121% more yield over control infested with pathogen) , respectively ,consisted the second group. WT5+tebuconazole, WT4+carbendazim, WT4+MEMC, EM64+MEMC, EM64+tebuconazole, and EM24+MEMC with 132.78, 131.67, 130.56, 128.33, 126.67 and 123.89 q/ha (113, 112, 110, 106, 104 and 99% more

yield over control infested with pathogen) , respectively, were the third group. EM24soil application, EM15+carbendazim, WT5+carbendazim, WT8+MEMC and WT5soil application with 120.56, 120.00, 117.78, 115.00 and 113.89, q/ha (94, 93, 89, 85 and 83% more yield over control infested with pathogen) consisted the fourth group. EM15 soil application, WT4 soil application, WT5+MEMC, WT8 soil application and carbendazim with 107.00, 105.00, 100.00, 98.33 and 97.22 q/ha (72, 69, 61, 58 and 56%, more yield over control infested with pathogen), respectively, ranked fifth. WT4+tebuconazole, EM64 soil application, control not infested with *R.solani*, tebuconazole, WT8 seed application, and EM15+MEMC with 91.67, 91.67, 90.00, 87.22, 86.67 and 84.44 q/ha (47, 47, 45, 40, 39, and 36% %, more yield over control infested with pathogen), respectively, ranked sixth. WT8 seed application, WT4 seed application and EM15+tebuconazole with 78.89, 76.11 and 74.44 q/ha (27, 22and 20% more yield over control infested with pathogen), respectively, placed in group seventh. Finally MEMC with 65.00 q/ha (4% more yield over control infested with pathogen) and control inoculated with *R. solani* with 62.22 q/ha found the last and least yield producing treatments (Table 14b, and Fig. 32).

There was a highly significant positive correlation between potato yield and foliage fresh weight (0.525), and highly negative correlation between potato yield with stem canker incidence (-0.521), stem canker severity (-0.484), black scurf incidence (-0.525), percentage weight of scurfed potato (-0.522), mg weight of sclerotia/cm^2 tuber surface (-0.479). However potato yield was significantly correlated with foliage dry weight (0.397), but there was no significant correlation between potato yield and number of emerged seedlings 15DAS (0.118) and also with black scurf severity (-0.232) data are presented in Table 15.

Bari (1980) reported that Bavistin significantly increased the yield of potato tubers. The yield in Bavistin treated plots was 36.11 tone / ha as against 29.63 in check plots. However, Bavistin did not significantly differed from other treatments in terms of yield in the first year experiment but in the second year experiment only Bavistin significantly increased the yield of potato. As high as 28.27 percent (9.55 tone/ ha) increase in tuber yield was recorded in soil application of *T. harzianum* +Bavistin, 13.81 per cent (4.66 tone /ha) in *T. harzianum* +Emisan-6, 11.60 per cent (3.92 tone/ ha) in broadcasting application of *T. harzianum* which were significantly higher

than check plot, however, seed coating with *T.harzianum* did not significantly increased the potato yield (Bari, 1988).

Table 10. Analysis of variance for biological, chemical and integrated control on potato black scurf (1998-1999).

S.V.	df	MS									
		Seedling Emergence 15 DAS	Foliage Fresh weight	Foliage Dry weight	Stem canker incidence	Stem canker severity	Black scurf incidence	Black scurf severity	% weight of surfed tubers	Weight of sclerotia /cm^2	Potato yield
Replication	2	0.271	0.259	0.124	0.098	0.003	0.007	0.007	0.007	0.009	0.079
Treatment	15	50.734	150.435	1.518	877.380	12.273	19.389	16.586	19.389	0.364	0.0254
Error	30	0.698	0.417	0.082	0.728	0.026	0.014	0.007	0.014	0.005	0.060
C.V. %	-	3.5	1.21	5.24	2.85	3.22	3.45	1.91	3.45	7.92	7.73

S.V. (Sources of variance), df (degree of freedom), C.V. (covariance) .F= MS treatment/ MS Error,

Ft (α=0.05) =2.01, Ft (α=0.01) =2.70.

Table 11. Analysis of variance for biological, chemical and integrated control on potato black scurf (1999-2000).

S.V.	df	MS									
		Seedling Emergence 15 DAS	Foliage Fresh weight	Foliage Dry weight	Stem canker incidence	Stem canker severity	Black scurf incidence	Black scurf severity	% weight of surfed tubers	Weight of sclerotia /cm^2	Potato yield
Replication	2	0.406	0.016	0.000007	0.381	0.168	1.018	74.595	10.154	75.222	3099921
Treatment	15	21.522	0.125	0.002	15.747	11.295	3.555	154.742	60.514	165.480	2443088
Error	30	2.686	0.025	0.000016	0.505	0.871	0.560	24.792	9.764	37.097	7256208
C.V. %	-	9.72	9.76	1.77	16.45	6.94	8.14	8.43	8.25	8.93	8.3

S.V. (Sources of variance), df (degree of freedom), C.V. (covariance) .F= MS treatment/ MS Error,

Ft (α=0.05) =2.01, Ft (α=0.01) =2.70.

Table 12.a. Biological, chemical and integrated control of potato black scurf (1998-99 experiment).

Treatment[1]	Emerged seedlings (15 DAS) 2		Foliage fresh weight		Foliage dry weight		Stem canker		Stem Canker index
	Number	%increas e/ control	q/ha	%increas e/control	q/ha	%increas e/control	incidence	%decrease /control	
Control-	26.30	65.41	54.02	52.38	5.339	46.47	39.15	54.15	0.85
WT8 seed	28.01	76.16	49.45	39.49	4.950	35.80	59.85	29.91	1.52
WT5 seed	28.60	79.87	53.33	50.44	5.338	46.45	54.27	36.44	1.39
WT4 seed	28.70	80.50	56.15	58.39	5.615	54.05	55.18	35.38	1.12
WT8 soil	25.98	63.40	57.80	63.05	5.787	58.77	22.35	73.83	0.85
WT5 soil	24.45	53.77	60.31	70.13	5.998	64.55	22.86	73.23	0.58
WT4 soil	24.60	54.72	61.67	73.96	6.053	66.06	23.64	72.31	0.97
WT8 carbe	25.16	58.24	57.95	63.47	5.663	55.36	0.00	100	0.00
WT8MEMC	17.80	11.95	55.49	56.53	5.543	52.07	10.12	88.15	0.79
WT5 carbe	26.51	66.73	51.62	45.61	5.162	41.62	3.02	96.46	0.11
WT5MEMC	19.32	21.51	46.67	31.65	4.669	28.09	7.48	91.24	0.24
WT4 carbe	25.90	15.9	65.92	85.95	6.576	80.41	9.58	88.78	0.39
WT4MEMC	20.23	27.23	59.43	67.64	5.945	63.10	13.62	84.05	0.48
Carbe	24.6	54.72	56.65	59.80	5.679	55.80	17.67	79.31	0.53
MEMC	19.01	19.56	49.28	39.01	4.922	35.03	24.32	71.52	0.62
Control+	15.90	0.00	35.45	0.00	3.645	0.00	85.39	0.00	2.83
CD (5%)	1.39	-	1.07	-	0.477	-	1.66	-	0.89

1check- (Control not infested), check + (control infested with *R. solani* only), WT8 (*Trichoderma harzianum*), WT5 and WT4 two isolates of *T. longibrachiatum*, Seed (seed treatment in 10^7 spore/ ml of *Trichoderma* isolates) , soil (soil application of wheat bran culture of *Trichoderma* isolates or mutants @ 83 g/m^2),. All the plots except Check - were inoculated with wheat bran culture of *R. solani* @ 75 g weight/ m^2. 2 Chemical treatments MEMC (Emisan 6% Hg formulation), carb (carbendazim, Bavistin 50% WP). 3 figures are mean of 3 replication for each treatment.

Table 12.b. Biological, chemical and integrated control of potato black scurf (1998-99 experiment).

Treatment[1]	Black scurf		weight of (infected tubers/ total)		Black scurf Severity	Weight sclerotia /cm2 tuber surface		Yield	
	% incidence	% decrease/ control	percent	% decrease/ control		mg	% decrease/ control	(q/ha)	% increase/ control
Control-	22.42	73.73	29.05	66.30	0.72	7.00	61.58	93.24	44.27
WT8 seed	26.78	68.62	25.65	70.24	1.52	9.82	46.10	87.50	35.39
WT5 seed	40.32	52.75	24.72	71.32	1.38	9.76	46.43	85.65	32.52
WT4 seed	53.57	37.22	38.98	54.78	1.57	10.39	42.97	89.98	39.22
WT8 soil	22.31	73.85	18.25	78.83	0.65	2.53	86.11	94.44	46.12
WT5 soil	12.23	85.67	15.96	81.49	0.58	4.95	72.83	93.24	44.27
WT4 soil	29.93	64.92	18.95	78.02	0.68	3.39	81.39	98.15	51.86
WT8 carbe	3.52	23.24	5.98	93.06	0.32	1.25	92.99	86.58	33.96
WT8MEMC	6.25	92.68	8.32	90.35	0.45	2.15	88.20	80.19	24.08
WT5 carbe	0.00	100	0.00	100	0.00	0.00	100	99.54	54.02
WT5MEMC	0.00	100	0.00	100	0.00	0.00	100	92.32	42.84
WT4 carbe	0.00	100	0.00	100	0.00	0.00	100	88.15	36.39
WT4MEMC	0.00	100	0.00	100	0.00	0.00	100	87.50	35.39
Carbe	4.70	94.49	6.82	92.09	0.43	0.80	95.61	86.94	34.52
MEMC	1.33	98.44	7.85	90.89	0.58	0.92	94.95	84.44	30.65
Control+	85.33	0.00	86.20	0.00	2.32	18.22	0.00	64.63	0.00
CD (5%)	1.18	-	1.48	-	0.41	1.26	-	11.37	-

1check- (Control not infested), check + (control infested with *R. solani* only), WT8 (*Trichoderma harzianum*), WT5 and WT4 two isolates of *T. longibrachiatum*, Seed (seed treatment in 10^7 spore/ ml of *Trichoderma* isolates) . soil (soil application of wheat bran culture of *Trichoderma* isolates or mutants @ 83 g/m^2).. All the plots except Check - were inoculated with wheat bran culture of *R. solani* @ 75 g weight/ m^2. 2 Chemical treatments MEMC (Emisan 6% Hg formulation), carb (carbendazim, Bavistin 50% WP), 3 figures are mean of 3 replication for each treatment.

Table 13. Correlation of results of field experiment (biological, chemical and integrated control on potato black scurf ,1998-1999).

	No. of seedlings	Foliage fresh weight	Foliage dry weight	Stem canker incidence	Stem canker severity	Black scurf incidence	% weight of scurfed tubers	Black scurf severity	Mg sclerotia/ cm^2	yield
No. of seedlings	1.000	0.453	0.469	0.044	-0.187	0.006	-0.155	0.040	0.062	0.523*
Foliage fresh weight	0.453	1.000	0.916**	-0.555*	-0.624**	-0.585*	-0.632**	-0.567*	-0.541*	0.628**
Foliage dry weight	0.469	0.916**	1.00	-0.620*	-0.653**	-0.555*	-0.631**	-0.578*	-0.563*	0.572*
Stem canker incidence	0.044	-0.555	-0.620*	1.000	0.935**	0.910**	0.902**	0.964**	0.959**	-0.321
Stem canker severity	-0.187	-0.624**	-0.653**	0.935**	1.000	0.911**	0.930**	0.926**	0.912**	-0.501*
Black scurf incidence	-0.006	-0.585*	-0.555*	0.910**	0.911**	1.000	0.962**	0.937**	0.947**	-0.487
% weight of scurfed tubers	-0.155	-0.632**	-0.631**	0.902**	0.930**	0.962**	1.000	0.919**	0.941**	-0.563*
Black scurf severity	0.040	-.567*	-0.578*	0.964**	0.926**	0.937**	0.919**	1.000	0.966**	-0.410
Mg sclerotia/ cm^2	0.062	-0.541*	-0.563*	0.959**	0.912**	0.947**	0.941**	0.966**	1.000	-0.383
yield	0.523*	0.628**	0.572*	-0.321	-0.501*	-0.487	-0.563*	-0.410	-0.383	1.000

** Correlation significant at the 0.01level (2-tailed). *correlation significant at 0.05 level (2-tailed).

Table 14a. Biological, chemical and integrated control of potato black scurf (1999-2000).

Treatment[1]	seedlings emergence (15DAS)		Foliage fresh weight		Foliage dry weight		Stem canker		Stem Canker index
	Number	% increase	q/ha	% increase	q/ha	% increase	incidence	% decrease	
Control-	17.67	65.60	54.44	50.76	5.50	45.12	46.13	42.63	1
WT8 seed	18.67	74.98	49.44	36.91	4.90	29.29	61.19	23.90	1.50
WT5 seed	19.33	81.17	53.33	47.69	5.51	45.38	48.43	39.77	1.46
WT4 seed	19.33	81.17	56.11	55.39	5.65	49.08	41.88	47.92	0.97
WT8 soil	17.33	62.42	60.00	66.16	6.05	59.63	34.02	57.69	0.90
WT8MEMC	11.67	9.37	55.56	53.86	5.64	48.81	8.85	88.99	0.67
WT8 carbe	17.00	59.33	57.78	60.01	5.89	55.41	0.00	100	0.00
WT8 Tebu	11.00	3.09	53.33	47.69	5.44	43.55	32.07	60.12	0.63
WT5 soil	16.33	53.05	46.11	27.69	4.73	24.80	34.83	56.68	0.60
WT5MEMC	12.67	18.74	46.67	29.24	4.72	24.54	14.75	81.66	0.20
WT5 carbe	17.67	65.60	51.67	43.09	5.78	52.51	15.65	80.54	0.10
WT5 tebu	12.67	18.74	48.89	35.39	5.10	34.56	20.25	74.82	0.23
WT4 soil	16.33	53.05	51.67	43.09	5.38	41.95	45.36	43.59	1.03
WT4MEMC	13.33	24.93	58.89	63.08	5.91	55.94	17.29	78.50	0.10
WT4 carbe	17.33	62.42	68.33	89.23	6.92	82.59	19.33	75.96	0.40
WT4 tebu	13.33	24.93	58.89	63.08	5.97	57.52	21.33	73.47	0.30
EM64 soil	17.00	59.33	55.00	52.31	5.55	46.44	44.03	45.24	1.00
EM64 EMC	15.00	40.58	51.67	43.09	5.18	36.68	21.09	73.77	0.30
EM64 carbe	19.33	81.16	65.00	80.00	6.72	77.31	20.79	74.41	0.17
EM64 tebu	13.00	21.84	47.22	30.77	4.80	26.65	12.77	83.87	0.17
EM24 soil	17.33	62.42	52.22	44.61	5.29	39.58	36.22	54.96	0.70
EM24 EMC	13.67	28.12	53.33	47.68	5.47	44.33	33	58.96	0.73
EM24 carbe	18.33	71.79	63.89	76.93	5.59	47.49	13.29	83.47	0.17
EM24 tebu	14.67	37.48	57.22	58.46	5.88	55.15	8.56	89.35	0.67
EM15 soil	16.33	53.05	59.44	64.61	6.04	59.37	39.99	50.27	0.93
EM15 EMC	11.33	6.18	51.11	41.54	5.29	39.58	0.00	100	0.00
EM15 carbe	18.00	68.70	57.78	60.01	5.88	55.15	16.04	80.05	0.17
EM15 Tebu	13.00	21.84	48.89	35.39	5.95	57.78	20.25	74.82	0.17
MEMC[2]	12.67	18.74	49.44	36.92	5.04	32.98	22.85	71.58	0.37
Carbe	17.33	62.42	57.22	58.46	5.88	55.15	15.31	80.96	0.17
Tebu	14.00	31.21	38.33	6.15	3.89	2.64	27.91	65.29	0.37
Control+	10.67	0.00	36.11	0.00	3.79	0.00	80.41	0.00	2.36
CD (5%)	2.67	-	8.54	-	0.17	-	7.91	-	0.32

1check- (Control not infested), check + (control infested with *R. solani* only), T8 (*Trichoderma harzianum*), T5 and T4 two isolates of *T. longibrachiatum*, Seed (seed treatment in 10^7 spore/ ml of *Trichoderma* isolates) , soil (soil application of wheat bran culture of *Trichoderma* isolates or mutants @ 83 g/m^2), EM64, EM24 two mutants of isolate T4 of *T. longibrachiatum*, EM15 mutant of isolate T5 of *T. longibrachiatum*. All the plots except Check - were inoculated with wheat bran culture of *R. solani* @ 75 g wet weight/ m^2. 2 Chemical treatments MEMC (Emisan 6% Hg formulation), carb (carbendazim= Bavistin 50% WP), tebu (Tebuconazol= Folicur 250 EM),. 3 figures are mean of 3 replication for each treatment.

Table 14b. Biological, chemical and integrated control of potato black scurf (1999-2000 experiment).

Treatment[1]	Black scurf		weight of scurfed tubers/total weight		Scurf index	Weight sclerotia/ cm^2 tuber surface		Yield	
	incidence	%Decrease	Per cent	%Decrease/ control		mg	%Reduction/ control	(q/ha)	% increase/ control
Control-	20.72	66.92	30.63	49.39	0.24	5.25	69.32	90.00	44.65
WT8 seed .	26.04	58.43	27.74	54.16	0.98	8.82	48.36	78.89	26.79
WT5 seed	39.58	36.81	25.77	57.42	0.88	10.69	37.41	86.67	39.30
WT4 seed	54.04	13.73	40.57	32.96	0.98	11.59	32.14	76.11	22.32
WT8 soil	24.30	61.21	21.18	65.00	0.28	2.69	84.25	98.33	58.04
WT8MEMC	3.67	94.14	3.84	93.65	0.47	1.18	93.09	115.00	84.83
WT8 carbe	2.16	96.55	6.14	89.85	0.87	2.02	88.17	148.89	139.30
WT8 Tebu	10.29	83.57	18.37	69.64	0.14	1.87	89.05	137.78	121.44
WT5 soil	10.83	82.71	19.55	67.70	0.33	4.89	71.37	113.89	83.04
WT5MEMC	0.00	100	0.00	100	0.00	0.00	100	100.00	60.72
WT5 carbe	0.00	100	0.00	100	0.00	0.00	100	117.78	89.30
WT5 tebu	2.53	95.96	8.57	85.84	0.17	1.50	91.22	132.78	113.40
WT4 soil	30.58	51.18	19.78	67.32	0.28	3.24	81.03	105.56	69.65
WT4MEMC	0.00	100	0.00	100	0.00	0.00	100	130.56	109.84
WT4 carbe	2.76	95.59	3.82	93.69	0.40	1.84	89.23	131.67	111.62
WT4 tebu	0.00	100	0.00	100	0.00	0.00	100	91.67	47.33
EM64 soil	28.27	54.87	24.06	60.02	0.26	4.25	.75.12	91.67	47.33
EM64 EMC	5.97	90.47	13.72	77.33	0.47	2.36	86.62	128.33	106.25
EM64 carbe	0.54	99.14	3.32	94.51	0.77	3.26	80.91	146.11	134.83
EM64 tebu	4.62	92.62	9.83	83.76	0.67	1.74	89.81	126.67	103.58
EM24 soil	27.18	56.61	17.04	71.84	0.33	4.13	75.82	120.56	93.76
EM24 EMC	2.88	95.40	8.96	85.19	0.17	2.54	85.13	123.89	99.12
EM24 carbe	0.00	100	0.00	100	0.00	0.00	100	173.33	178.57
EM24 tebu	0.54	99.14	2.71	95.52	0.17	0.60	96.48	141.67	127.69
EM15 soil	28.27	54.87	19.96	67.02	0.39	5.14	69.91	107.78	73.22
EM15 EMC	0.00	100	0.00	100	0.00	0.00	100	84.44	35.71
EM15 carbe	3.67	94.14	7.43	87.72	0.06	2.39	86.01	120.00	92.86
EM15 tebu	13.29	79.10	23.55	61.09	0.14	3.39	80.15	74.44	19.64
MEMC[2]	0.00	100	0.00	100	0.00	0.00	100	65.00	4.47
Carbe	0.00	100	0.00	100	0.00	0.00	100	97.22	56.25
Tebu	1.59	97.47	4.31	92.87	0.10	1.06	93.79	87.22	40.18
Control+	62.84	0.00	60.52	0.00	1.82	17.08	0.00	62.22	0.00
CD (5%)	1.44		8.17	-	0.22	2.93	-	11.59	-

1check- (Control not infested), check + (control infested with *R. solani* only), T8 (*Trichoderma harzianum*), T5 and T4 two isolates of *T. longibrachiatum*, Seed (seed treatment in 10^7 spore/ ml of *Trichoderma* isolates) , soil (soil application of wheat bran culture of *Trichoderma* isolates or mutants @ 83 g/m^2), EM64, EM24 two mutants of isolate T4 of *T. longibrachiatum*, EM15 mutant of isolate T5 of *T. longibrachiatum*. All the plots except Check - were inoculated with wheat bran culture of *R. solani* @ 75 g wet weight/ m^2. 2 Chemical treatments MEMC (Emisan 6% Hg formulation), carb (carbendazim= Bavistin 50% WP), tebu (Tebuconazol= Folicur 250 EM),. 3 figures are mean of 3 replications for each treatment.

Table 15 . Correlation of results of field experiment (biological, chemical and integrated control on potato black scurf 1999-2000).

	No. of seedlings	Foliage fresh weight	Foliage dry weight	Stem canker incidence	Stem canker severity	Black scurf incidence	% weight of scurfed tubers	Black scurf severity	Mg sclerotia/ cm^2	yield
No. of seedlings	1.000	0.488**	0.465**	0.158	0.065	0.212	0.103	0.114	0.197	0.118
Foliage fresh weight	0.488**	1.000	0.925**	-0.367*	-0.320	-0.264	-0.389*	-0.232	-0.320	0.525**
Foliage dry weight	0.465**	0.925**	1.000	-0.387*	-0.356*	-0.260	-0.356*	-0.243	-0.311	0.397*
Stem canker incidence	0.158	-0.367*	-0.387*	1.000	0.916**	0.847**	0.865**	0.592**	0.837**	-0.521**
Stem canker severity	0.065	-0.320	-0.356*	0.916**	1.000	0.869**	0.848**	0.687**	0.856**	-0.484**
Black scurf incidence	0.212	-0.264	-0.260	0.847**	0.869**	1.00	0.928**	0.713**	0.919**	-0.525**
% weight of scurfed tubers	0.103	-0.389*	-0.356*	0.865**	0.848**	0.928**	1.000	0.720**	0.923**	-0.522**
Black scurf severity	0.114	-0.232	-0.243	0.592**	0.687**	0.713**	0.720**	1.000	0.856**	-0.232
Mg sclerotia/ cm^2	0.197	-0.320	-0.311	0.837**	0.856**	0.919**	0.923**	0.856**	1.000	-0.479**
yield	0.118	0.525**	0.397*	-0.521**	-0.484**	-0.525**	-0.522**	-0.232	-0.479**	1.000

** Correlation significant at the 0.01level (2-tailed), *correlation significant at 0.05 level (2-tailed).

Fig.27. Effect of biological, chemical and integrated control of potato black scurf on seedling emergence 15DAS (seedling number) , Foliage Fresh Weight (Fol f W) and Foliage dry weight (Fol dry W) of potato in field 1998-1999.

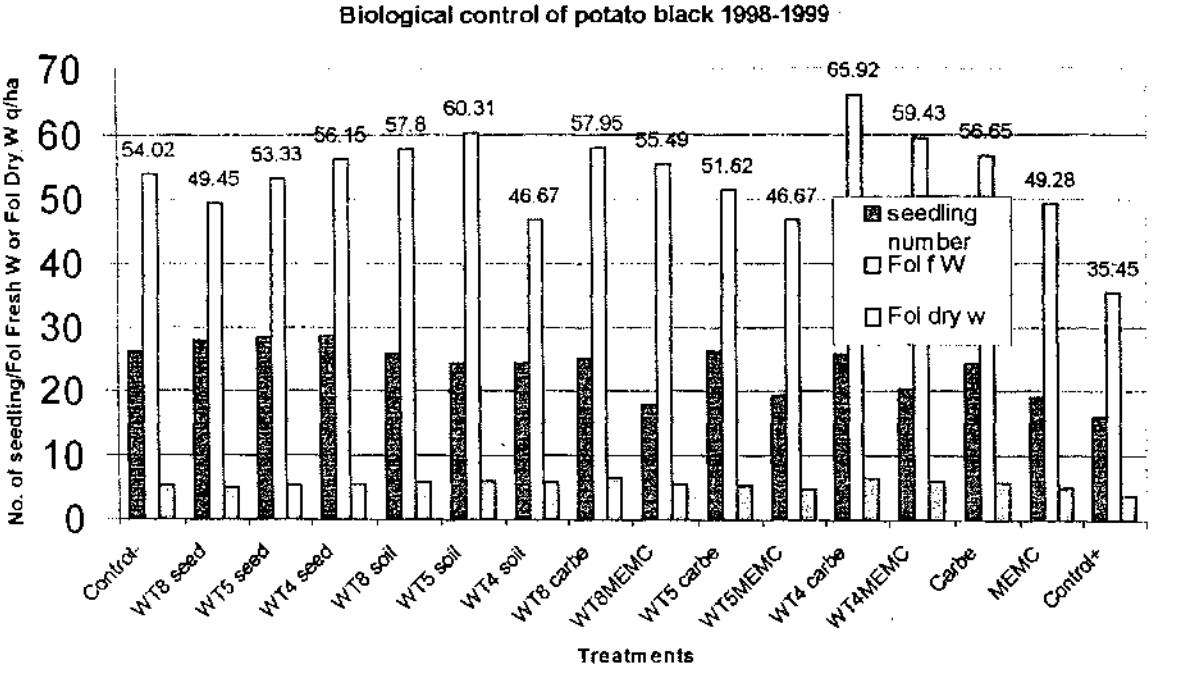


Fig.28. Effect of biological, chemical and integrated control of potato black scurf on black scurf severity (scur severity) , mg weight of sclerotia/cm^2 (mg scl), and stem canker severity (Cank Sever) in field (1998-1999).

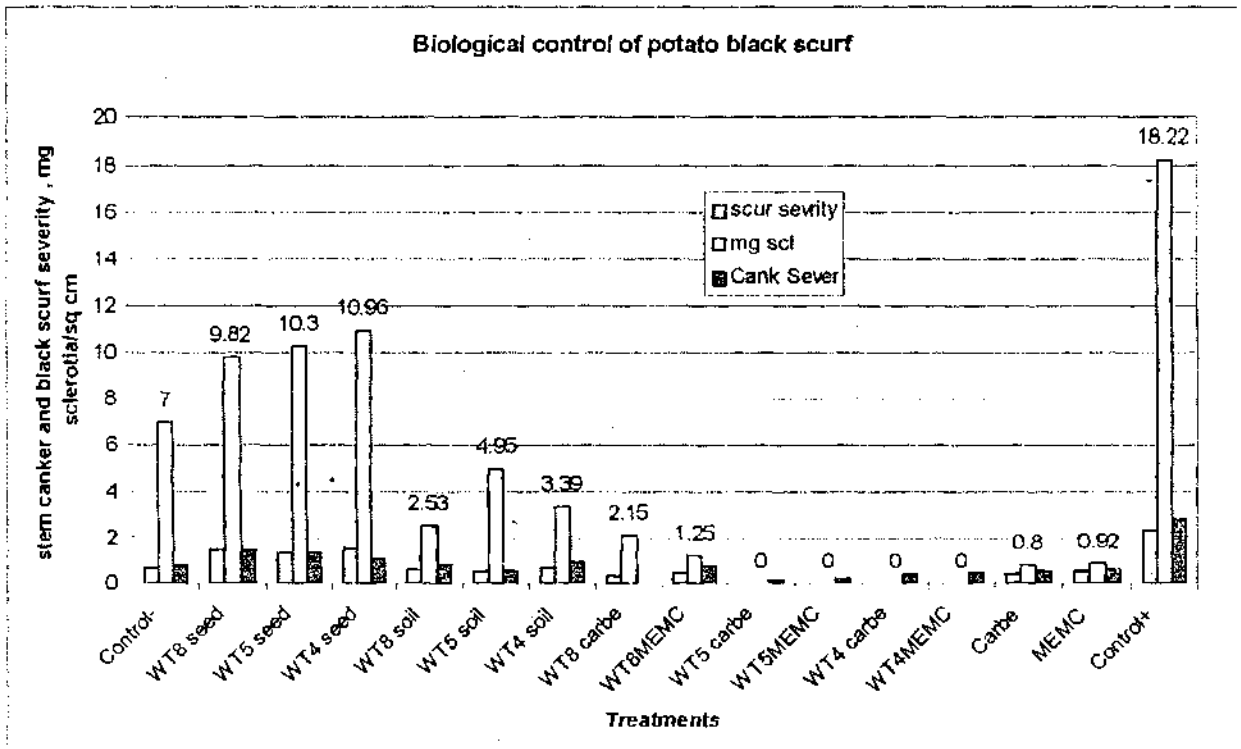


Fig.29. Effect of biological, chemical and integrated control of potato black scurf on stem canker incidence (Cank inci), potato yield (Yield q/ha) and black scurf incidence in field (1998-1999).

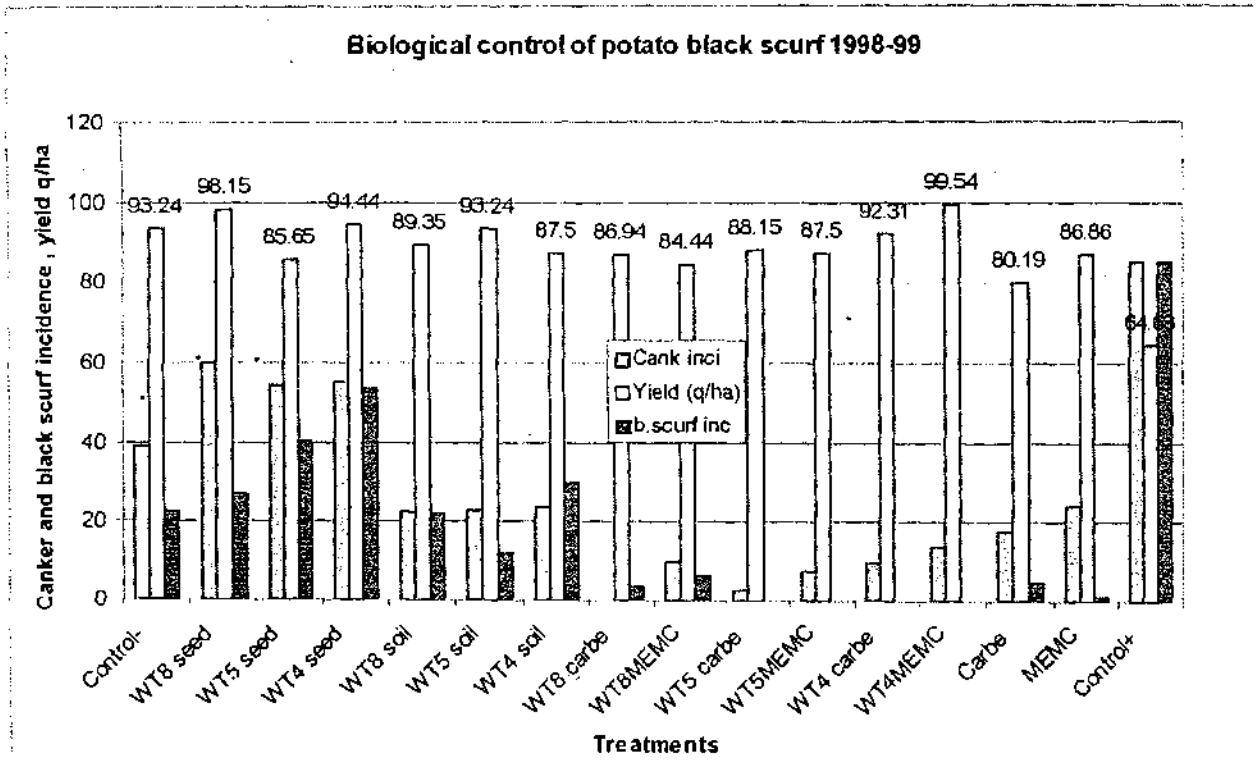


Fig. 30. Effect of biological, chemical and integrated control of potato black scurf on number of emerged seedlings(emergence), Foliage fresh weight (FFW)and foliage dry weight (FDW) in field (1999-2000).

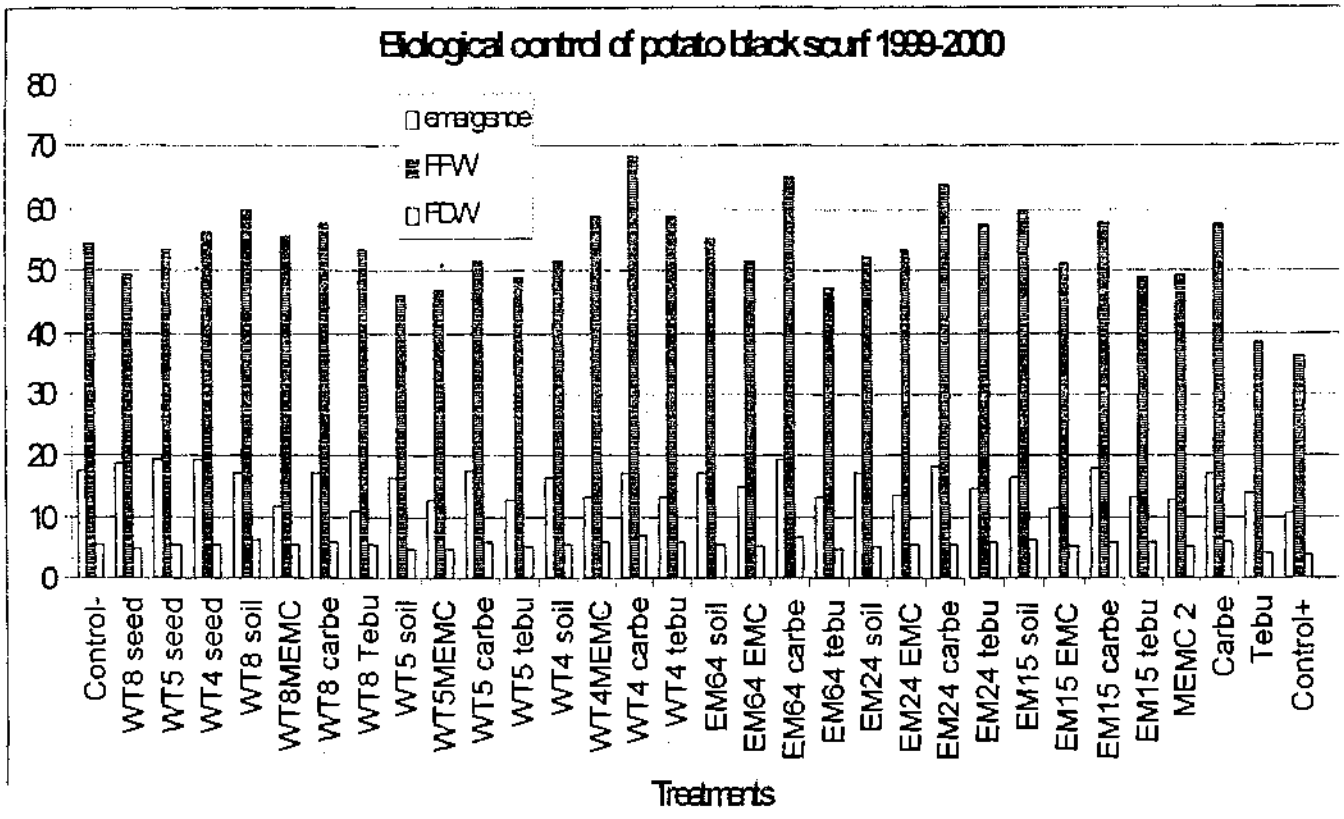


Fig. 31. Effect of biological, chemical and integrated control of potato black scurf on black scurf severity(BSS), mg weight of sclerotia / cm^2 severityof tuber surface (Mg scl) and stem canker (SCS) in field (1999-2000).

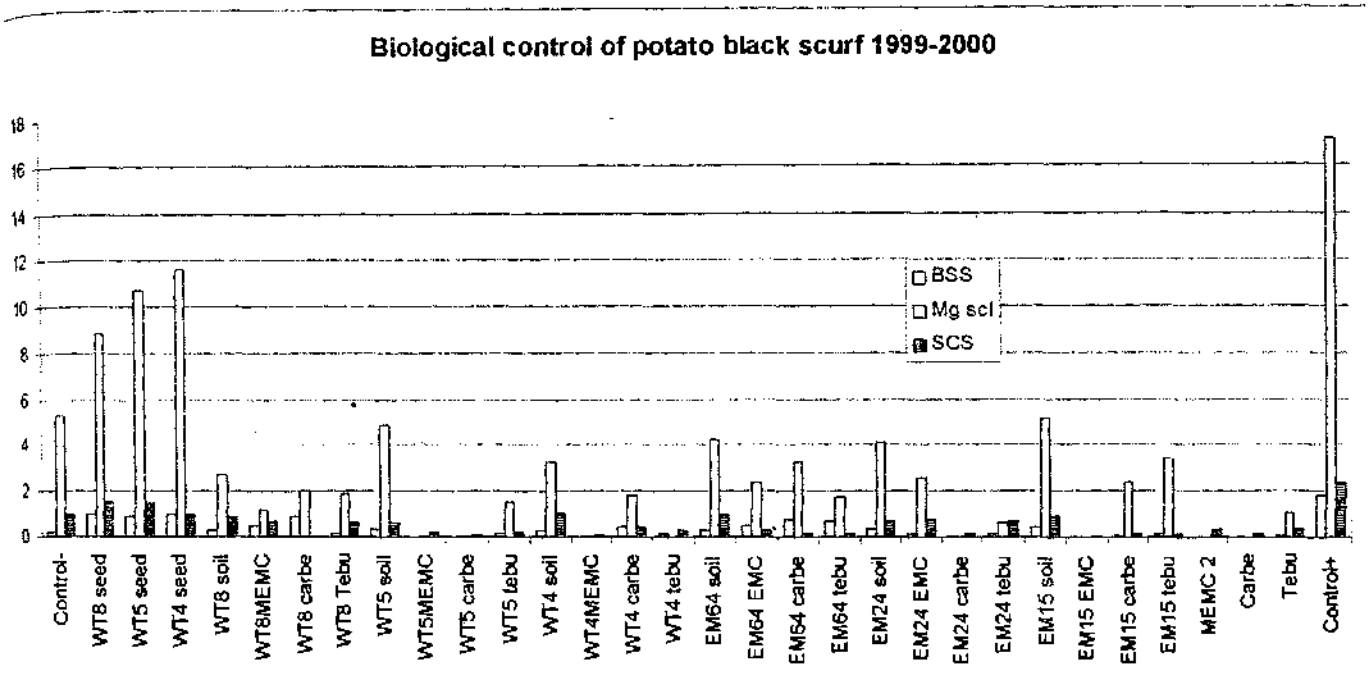
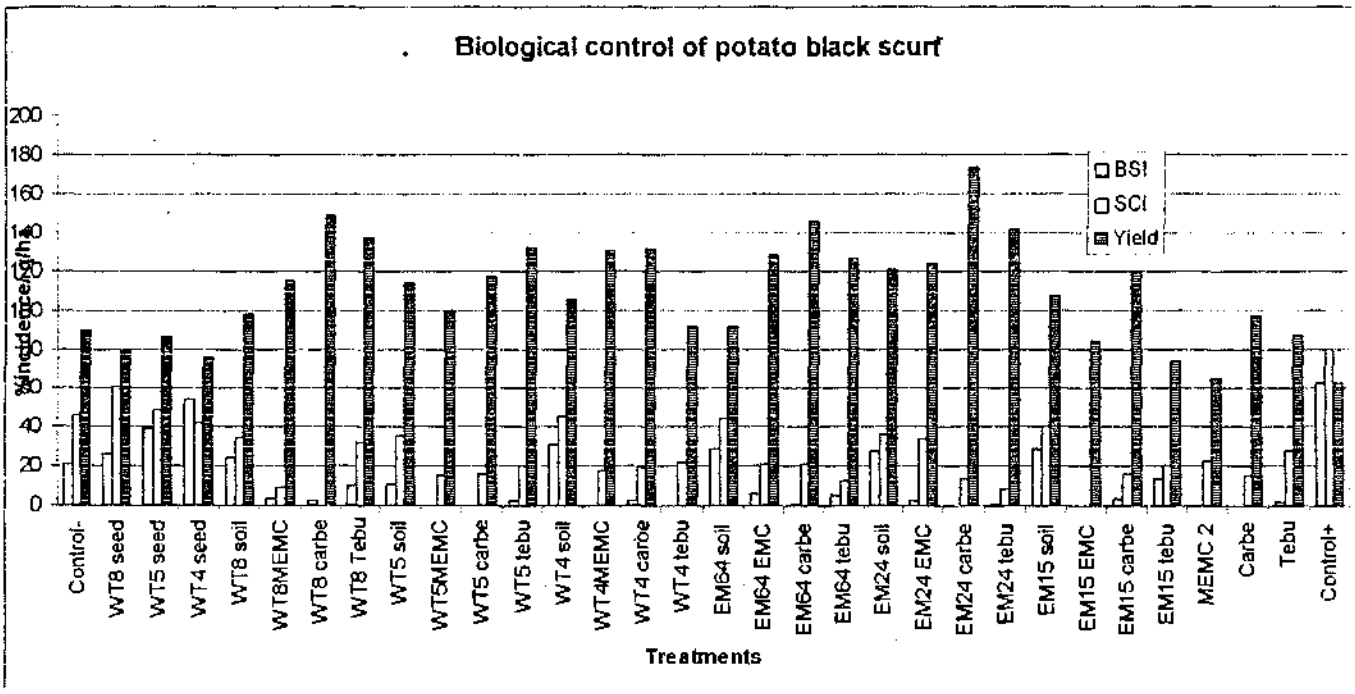


Fig. 32. Effect of biological, chemical and integrated control of potato black scurf on black scurf incidence(BSI), stem canker incidence (SCI), and yield in field (1999-2000).



Chapter 5

Summary and conclusion

Two isolates (WT5 and WT4) of *Trichoderma longibrachiatum* Rifai (1969) were isolated from soil samples of Kurukshetra . While one isolate (WT8) of *T. harzianum* Rifai (1969) was isolated from soil samples of Karnal. *Rhizoctonia solani* was isolated from scurfed tubers.

All the three isolates of *Trichoderma* inhibited 52 to 60% mycelial growth of *R. solani* in dual culture plates. The growth inhibition of *R. solani* increased with increase in incubation period. The growth of *R. solani* was completly inhibited after it came in direct contact with *Trichoderma* followed by over growth of *Trichoderma* on *R. solani* mycelium. *Trichoderma* isolates were found coiling around /growing on or along and or penetrating and growing inside the hyphae of *R. solani*. Appresorium like structures were visible at the end of hyphal side branches of *Trichoderma* when they came in contact with *R. solani* hyphae, through which *Trichoderma* attached to *R. solani* hyphae. There were abnormalities viz. sunken discloured spots on hyphae, rupturing the cell wall, granulization and leakage of cytoplasmic materials followed by shrinkage , lysis and death of *R. solani* hyphae which have been affected by *Trichoderma* isolates. *T. harzianum* in addition to the above mentioned symptoms also caused hyphal disintegration of *R. solani* and it appears that it is able to break the *R. solani* hyphae at the septum points.

Culture filtrate and volatile metabolites of *Trichoderma* isolates inhibited the mycelial growth of *R. solani*. *T. longibrachiatum* (WT4) culture filtrate caused 63 and 74 % dry weight reduction of *R. solani* at 10 and 20 per cent concentration in PDB. The cell wall of *R.solani* composed of β-1,3- glucan and chitin. The tested isolates of *Trichoderma* were able to grow on *R. solani* cell wall as a sole carbon source. All of them released N-acetyl-D-glucoseamine from colloidal chitin / *R. solani* cell wall and also reducing sugars from laminarin/ *R. solani* cell wall Release of N-acetyl-D-glucoseamine from colloidal chitin/ *R. solani* cell wall and reducing sugars fro laminarin / *R. solani* cell wall, indicated the chitinase and β-1,3- glucanse activity , which leads to disintegration of fungal cell wall of the pathogen

and make it vulnerable for other enzymes such as proteases, which affect the plasma membrane and finally leakage and discharge of cytoplasmic materials from the fungal cell which ends with the shrinkage and death of *R.solani* hyphae and paves the way for penetration of *Trichoderma* into the *R. solani* hyphae. So, it is concluded that *Trichoderma* isolates are effective against *R. solani* by parasitism and antibiosis. All of the tested isolates produced reducing sugars from cellulose and carboxy methyl cellulose implying release of cellulases, however these enzymes are not directly effective against *R. solani.*

Among the fungicides tested carbendazim completely inhibited the mycelium of *R. solani* at 10 ppm concentration followed by MEMC. None of the three isolates of *Trichoderma* was able to grow on carbendazim amended PDA in all the tested concentrations, so it is concluded that all the tested isolates were susceptible to carbendazim. *T. harzianum* (WT8) tolerated 100 ppm MEMC and 200 ppm chlorothalonil. Tolerance to chlorothalonil was common for all the three isolates, but in case of MEMC the tolerance level of WT8 was higher than WT5 and WT4. All the three isolates of *Trichoderma* tolerated tebuconazole only up to 25 ppm.

Three UV induced mutants namely UM 35, and UM 15 derived from *T. longibrachiatum* (WT5) and UM 14 derived from *T. longibrachiatum* (WT4) mutants and EMS induced mutants namely EM14, EM15, EM24 EM64 derived from *T. longibrachiatum* (WT4) and EM 15 and EM25 derived from *T. longibrachiatum* (WT5) tolerated MEMC (100ppm) and chlorothalonil (300ppm) . EM15, EM24 and EM64 were tolerant to carbendazim at 50 ppm.

All of the mutants and wild types inhibited the growth of *R. solani* but EM 64, EM 24 with 47% each , EM15 and EM14 with 40% inhibitory effect against *R. solani* mycelium proved superior over other mutants and wild type isolates in dual culture plates.

Selected isolates of *Trichoderma* (WT8, WT5, WT4, EM64, EM24 and EM15) were able to grow on wheat bran as substrate, which used for soil application of the antagonists. Inoculum of *R. solani* isolates prepared on wheat bran and used for soil inoculation.

The under ground stems and roots of the potato plants were affected by pathogen. Brown canker of different shape and size were visible on the affected stems. The canker may enlarge or more often two or more cankers coalesced and girdled the stems, hindering the translocation of metabolites, in the plant leading to symptoms such as leaf rolling, wilting and in some

cases aerial tubers formation on the lateral sprouts above the ground level. Stems appeared distorted and thickened in such affected plants. Tubers in affected plants formed near to the ground surface. Sexual reproduction of *R. solani* appeared as hymenium bearing basidia and basidiospores on the lower parts (up to 7cm) on stems and petioles in field. Ultimately black scurf as muddy encrustation on the tuber surface appeared as final sign of the disease. Occurence of sexual stage of the pathogen in Haryana has been observed for the first time. Presence of sexual stage of the pathogen under field condition is very important. Because in addition to anastomosis which is common in *R. solani* the sexual compatibility will increase the chance of more heterogenicity which makes the pathogen more difficult to control by fungicides.

Inoculation of soil with *R. solani* showed 39 and 40% decrease in seedling emergence in the 1998-99 and 1999-2000 experiments, respectively. Tuber application of *Trichoderma* spp. promoted emergence of potato seedlings in the first two weeks after sowing. This is most probably due to some chemical metabolites produced by *Trichoderma* spores or germlings, which stimulated germination of potato seedlings from tubers in early stage of sprouting. It may also protect the seedlings from some soil and tuber borne (minor) pathogens, which hinder the germination and emergence of seedlings. Carbendazim and MEMC though appeared significantly better than pathogen infested plots, yet , were inferior to seed application of the three isolates and soil application of WT5 and WT8 isolates of *Trichoderma* spp. In general, all the treatments having the biocontrol agents alone or in combination with carbendazim showed increased number of emerging seedlings. Tuber treatment with carbendazim also increased the number of emerged seedlings when used alone.

Inoculation of soil with *R. solani* resulted in more than 30 % reduction of foliage fresh and dry weight of potato. Soil application of antagonists lead to more foliage fresh/dry weight over seed application of the same. In general , it is concluded that application of biocontrol agents either alone or in combination with fungicides promoted the growth of potato plant. Carbendazim was found the best fungicide followed by MEMC, however tebuconazole exhibited growth-retarding effect and was not effective.

Inoculation of soil with *R. solani* lead to 85.39 and 80.41 % Stem canker incidence in 1998-99 and 1999-2000 experiments, respectively. All the treatments significantly reduced the incidence of stem canker. Integration of carbendazim with WT8 completely checked the incidence of

stem canker. Tuber dipping in carbendazim and MEMC suspension showed 79 and 71 % reduction of disease incidence, respectively. Soil application of *Trichoderma* isolates resultedt in 72- 74 % reduction of disease incidence. Seed application of *Trichoderma* isolates provided 30-36%, reduction of disease incidence. In general, integration of biocontrol agent with fungicides was superior to application of each component separately. Integration of carbendazim was more effective than MEMC and tebuconazole. Tuber dipping in carbendazim was better than MEMC and tebuconazole in reducing the incidence of stem canker disease of potato caused by *R. solani.* Soil application of the antagonists performed better than seed application of the same, which is most probably due to higher amount of population and type of inoculum of biological agent used.

In control infested with *R. solani* stem canker severity was 2.83 and 2.37 in 1998-99 and 1999-2000 experiments, respectively. All the treatments significantly reduced the severity of stem canker incidence WT8 +carbendazim was the most effective treatment which completely checked the disease severity. Integration of biocontrol agents with carbendazim and MEMC and soil application of the antagonists appeared better than seed application of the antagonists alone. Carbendazim performed better than MEMC when the two fungicides were used alone.

Incidence of black scurf was 85.33 and 64 % in *R. solani* infested in 1998-99 and 1999-2000 experiments. All treatments significantly reduced the incidence of the disease. Integration of WT4 and WT5 with carbendazim or MEMC completely checked the incidence of the disease in 1998-99 experiment. Tuber dipping in MEMC and carbendazim suspension and integration of EM24+carbendazim, EM15+MEMC, WT5+MEMC, WT5+carbendazim and WT4+MEMC, WT4+tebuconazole completely checked the incidence of the disease in 1999-2000. It is concluded that application of carbendazim and MEMC either alone or in combination with some biocontrol agents completely checked or very effectively reduced the incidence of black scurf of potato under field condition. Soil application of antagonists were more effective than seed application of the same to reduce the incidence of the disease. EM64 and EM24 mutants showed no significant difference from the wild type WT4 to control the incidence of black scurf while EM15 appeared inferior to WT5.

Severity of potato black scurf in *R. solani* inoculated plots were 2.32 and 1.82 in 1998-99 and 1999-2000 experiments, respectively. All treatments significantly reduced the severity of the disease. It is evident that

integration of chemical and biological control is superior than application of each one individually. Similarly soil application of biocontrol agents was found more effective than seed application of the same.

The percentage weight of scurfed tubers in control infested with R. *solani* was 86.20 and 60.52 in 1998-1999 and 1999-2000 experiments, respectively. All the treatments reduced the percentage weight of scurfed tubers. Integration of WT4 + carbendazim, WT5+carbendazim and MEMC completely reduced the percentage weight of scurfed tubers. WT5+carbendazim,WT5+MEMC, WT4+MEMC, WT4+tebuconazole, EM24+carbendazim, EM15+MEMC, MEMC, and carendazim completely reduced the percentage weight of scurfed tubers in 1999-2000 experiment.

Inoculation of soil with *R. solani* resulted in 18.22 and 17.08 mg sclerotia/ cm^2 potato tuber surface, 1998-1999 and 1999-2000 experiments respectively. All the treatments significantly reduced the amount of sclerotia/ cm^2 of tuber surface as compared with control infested with *R. solani.* Soil application of antagonists performed better than seed application of the same to reduce sclerotial weight/cm^2. WT5+MEMC, WT5+carbendazim, WT4+MEMC, WT4+tebuconazole, EM24+carbendazim, EM15+MEMC, MEMC, and carbendazim completely reduced the amount of sclerotia/ cm^2. Soil application of antagonists resulted in more reduction of mg sclerotia/ cm^2 over soil application of the same.

Inoculation of field plots with *R. solani* lead to 30% yield reduction. All the treatments significantly prevented the yield reduction caused by *R. solani.* WT5+carbendazim resulted in 54% more yield over control infested with *R.solani.* In general it is concluded that biocontrol agents alone or in combination with fungicides increased the potato yield. Soil application of the biocontrol agents was found more effective in augmenting the potato yield over that of seed application of the same, most probably due to higher population density of biocontrol agent. Soil application of antagonists performed better than fungicide application and all the treatments having antagonists performed better than chemical control in respect to increase in yield, which indicates the growth promoting role of the *Trichoderma* spp. isolates. The most superior treatment was EM24+carbendazim with 173.33q/ha or 179 % more yield over control infested with pathogen. Among the fungicide treatments carbendazim resulted in more potato yield either in combination or alone.There was a highly significant positive correlation between potato yield and foliage fresh weight. Potato yield was

significantly correlated with foliage dry weight .There were highly negative correlation between potato yield with stem canker incidence, stem canker severity , black scurf incidence , percentage weight of scurfed potato , mg weight of sclerotia/cm^2 tuber surface.

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ABSTRACT

(An abstract of the thesis submitted in partial fulfilment of requirement for the degree of Ph.D., in subject of Plant Pathology of the CCS Haryana Agricultural University, Hisar)

Using Davet (1971) selective media two isolates of *Trichoderma longibrachiatum* and an isolate of *Trichoderma harzianum* were isolated from soil samples of potato fields of Kurukshetra and Kamal (Haryana) ,India ,respectively. All the isolates of

Trichoderma inhibited the growth and colonised *Rhizoctonia solani* mycelium on dual culture plates on PDA. Light microscopic studies showed that hyphal coiling, parasitism and lysis are common mode of action of *Trichoderma* isolates against *R.solani*. SEM supported the light microscopic studies and revealed that *Trichoderma* spp. induced sunken discoloured spots, leakage of cytoplasmic materials on *R. solani* hyphae. *T. harzianum* in addition to the mentioned effects breaks the *R. solani* hyphae at septum points. Culture filtrate of *Trichoderma* isolates reduced both the radial growth and mycelial weight of *R. solani* in PDA and PDB respectively. Volatile metabolites of *Trichoderma* isolates strongly inhibited the mycelial growth of *R.solani*. *Trichoderma* isolates released lytic enzymes β - 1,3 glucanase, chitinase and cellulase, the first two enzymes are responsible for degradation of cell wall of *R.solani*. *R.solani* induced pre and post emergence damping-off, stem and root canker, aerial tuberization, black scurf, and reduced the growth and yield of potato under field condition. Perfect stage of the pathogen occurred on the lower part of stems, petioles and leaflets of potato plants up to 7 cm from the soil surface, which was observed for the first time in Haryana. Inoculation of soil with *R. solani* resulted in 30 per cent reduction of potato yield in field experiments. Integration of wheat bran culture of *Trichoderma* isolates with fungicides (carbendazim, tebuconazole and MEMC) to *R solani*, infested soil reduced the incidence and severity of stem canker and black scurf of potato under field condition. Applications of biocontrol agents reduced the incidence and severity of stem canker and black scurf of potato and promoted the plant growth and yield either alone or in combination with fungicides. Seed application of *Trichoderma* spp. increased the emrgence of potato seedlings 15 days after sowing. Carbendazim was the superior fungicide, which

effectively controlled the diseases and increased the yield. Tebuconazole showed growth retarding effect on potato and did not significantly increased the yield. Integration of the fungicides with biocontrol agents showed synergistic effect and boosted the potato yield.