POTENTIATING BIOEFFICACY OF BIOCONTROL AGENTS AND DEVELOPMENT OF INTEGRATED DISEASE MANAGEMENT OF RHIZOME ROT (*PYTHIUM APHANIDERMATUM*) OF GINGER (*ZINGIBER OFFICINALE*)

Thesis submitted to

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In partial fulfil ment of requirements for the award of

Doctor of Philosophy in Botany

By

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Under the guidance of

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2006

DECLARATION

I, Beena.N, honestly declare that the Ph.D thesis entitled "**Potentiating Bioefficacy of biocontrol agents and development of integrated management of rhizome rot (*Pythium aphanidermatum*) of ginger(*Zingiber officinale*)**" is a bonafide record of research work carried out by me at Indian Institute of Spices Research, Calicut under the guidance of Dr Y.R. Sarma , Former Director of IISR. No part of this thesis has been submitted previously to any university for any degree / diploma.

Date: May 2006

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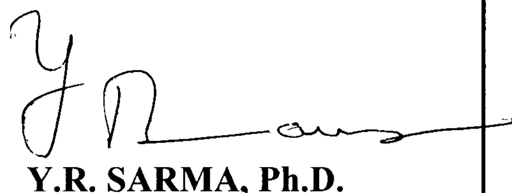
Place: Calicut

CERTIFICATE

This is to certify that the Ph.D thesis entitiled "**Potentiating Bioefficacy of biocontrol agents and development of integrated disease management of rhizomerot (*Pythium aphanidermatum*) of ginger (*Zingiber officinale*)**" is a record of bonafide work carried out by Ms. Beena. N under my supervision and guidance and that it has not previously formed the basis for award of any other degree / diploma.



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DEDICATION

This thesis is dedicated to my

BELOVED PARENTS

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INTRODUCTION

Ginger (*Zingiber officinale* Rosc.) is an annual herb belonging to the family Zingiberaceae. It is an important spice crop, vegetable and medicinal plant which originated in South Asia and the name *Zingiber* is derived from the Sanskrit word``Sringabera" meaning `horse shaped'.

Ginger is cultivated for its rhizome which when raw is used as a vegetable. India is in the unique position of being the largest producer and exporter of ginger producing 1,86,050 MT of ginger annually from an estimated area of 62,090 ha (Anon, 1996). In India, Kerala alone accounts for about 20% of the total production followed by Orissa. Dry ginger has a great market and ginger oleoresin is a value added product that is extracted from the dry ginger and is highly priced in the international market.

Ginger is adapted for cultivation in the tropical as well as the sub tropical humid climate. It prefers soil rich in humus content. Since it is a delicate succulent herb, it cannot withstand waterlogging and hence it is preferably cultivated in well drained soil. Planting of ginger is done during April-June immediately after the onset of the premonsoon showers. Beds are prepared after ploughing and digging. Beds of one metre width and three metres length and 15 cms height are made for planting. FYM is applied @ 20 tonnes / ha and fertilizers @ 75:50:50 (NPK) kg / ha. Mulching with green leaves is done twice, first after 45-60 days after ploughing @ 7.5 tonnes / ha, and the second time, 90-120 days after ploughing. Harvesting is done during the 2nd half of January when the crop is about 8-10 months old when the leaves dry up.

Diseases are the main constraints in the production of ginger. Ginger is affected by fungal, bacterial and viral diseases. It is also affected by leaf spot diseases like *Phyllosticta* leaf spot, anthracnose, *Helminthosporium* leaf spot etc. In India root rot and rhizome diseases incur heavy loss which leads to low productivity and production of ginger.

The term `rhizome rot' is loosely used for all diseases affecting the rhizome irrespective of the pathogens involved since the ultimate result is the partial or complete loss of the rhizome. The diseases include soft rot caused by *Pythium* species, yellows or wilt and sometimes rot caused by species of *Fusarium* and bacterial wilt caused by *Pseudomonas solanacearum* (Thomas, 1941, Dake and Edison, 1988, 1989). These pathogens often form complexes with nematodes leading to synergistic effects on disease incidence. Nematodes like *Meloidogyne incognita* by themselves are also involved in root knot diseases of ginger. Of all the diseases soft rot of ginger is the most destructive in

various stages of its growth. Crop loss to the tune of more than 50 to 80% have been reported (Butler, 1918; Joshi and Sharma, 1982).

The disease is prevalent in almost all ginger growing areas. Though many species of *Pythium* have been associated with the disease, *Pythium aphanidermatum* Edson Fitz. is considered to be the most important and major pathogen. If the disease occurs in the early stages of crop growth, it would result in the total loss of the rhizomes of the affected clumps and loss would be less if affected at the later stages of crop growth. Infection occurs through the roots or collar of the pseudostems as water soaked lesion which gradually spreads both upwards and downwards. Externally foliar yellowing is clearly seen starting from the margins of the lower most leaf and progresses upwards. When the infection spreads to the rhizome, the affected tissues rot emitting a foul smell. Crop loss is thus incurred from the rotting of the rhizome in the field crop, deterioration in the quality of the affected clumps and decay in the storages. Absence of disease resistance, continuous wet period during south west monsoons and leaching off of the proctective fungicides make the crop more vulnerable to the disease.

Intensive methods like selection of healthy seed rhizomes, proper storage, cultural practices such as preparation of raised beds for planting to avoid stagnation and to facilitate drainage, crop rotation or shifting cultivation, application of organic manures and fungicidal treatments of seed pieces and soil are recommended for disease management. Now-a-days eco-friendly methods of disease management are being practiced. As environmental concerns over the use of chemicals for plant disease and pest control continues, the search for biological or sustainable alternatives of control increased.Therefore biological control was suggested as a component of integrated disease management (IDM) in many crops using antagonistic fungi and bacteria. Biological control is the reduction of the amount of inoculum or disease producing activity of a pathogen accomplished by or through one or more organisms other than man (Cook and Baker, 1983). Agrios (1997) defines biological control as the reduction of inoculum density or disease producing activities of a pathogen or parasite in its active or dormant state by one or more organisms accomplished naturally or through manipulation of the environment, host or by mass introduction of one or more antagonists. In the recent years soil solarisation coupled with biocontrol was found to be effective in reducing the disease incidence of rhizome rot (Balakrishnan, 1997; Usman, 1997). *Trichoderma harzianum, Trichoderma virens* and several strains of bacteria have been found to suppress rhizome rot. The efficacy

of the biocontrol agents depend upon the competitive saprophytic ability, hyperparasitic activity, production of volatile and non volatile antibiotics that are detrimental to the pathogen. However the basic studies on the competitive efficacy of the strain, their mode of action and their efficacy in relation to hyperparasitic activity and other parameters received little attention. It is also important to develop a strain with high bioefficacy potential and greater compatibility with the agrochemicals in order to develop integrated disease management. Hence this investigation was undertaken with the following objectives:

1. Isolation, identification and assessment of the bioefficacy of the biocontrol organisms
2. Variability of the organism with respect to bioefficacy, competitive saprophytic ability and hyperparasitic activity.
3. Potentiating the bioefficacy and compatibility with the agrochemicals and other biotechnological approaches and characterizing the same.
4. Testing the developed strain in integrated management of rhizome rot.

REVIEW OF LITERATURE

Rhizome rot or soft rot of ginger and bacterial wilt are the disease problems affecting ginger and are the major production constraints in the production of ginger. Literature on the rhizome rot of ginger has been reviewed in detail (Joshi and Sharma, 1982; Iyer, 1987; and Sarma, 1994). Although crop losses due to the pests and diseases is considered as one of the major reasons for the low productivity, rhizome rot is considered to be a major problem (Butler, 1918; Joshi & Sharma, 1982).

Ginger is grown in almost all regions of India. Area of production is about 62,009 ha and production is about 1,86,050 MT. In Kerala, it is grown over an area of (53,020 ha) and accounting to a production of 1,52,890 MT.

Distribution: The soft rot / rhizome rot of ginger is prevalent all over the world wherever ginger is cultivated intensively. Association of several species of *Pythium* with rhizome rot of ginger has been reported. But the two species viz *Pythium aphanidermatum* and *Pythium myriotylum* are reported to cause severe damage in warm humid climates and these two have been reported in Kerala as soft rot pathogens in addition to *Fusarium oxysporum*, *F. solani* and *Pseudomonas solanacearum* (Dake & Edison, 1988, 1989).

Economic importance: Crop losses due to this malady vary from place to place. Moderate to severe incidence leading to crop loss of more than 50 to 80% have been reported on account of this disease (Butler, 1918; Joshi & Sharma, 1982). Crop loss depends on the stage of crop growth at which the infection starts. If it occurs early, total crop loss of the affected clump results, where as the crop loss is partial if affected at a later stage (Sarma, 1994). In Kerala losses can be as high as 90% during the years of heavy incidence (Rajan & Agnihotri, 1989). *Pythium spp.* also cause huge losses in storage. Instances of storage losses upto 50-90% has been reported (Sinha & Mukhopadhyay, 1988).

Symptoms: Root infection in rhizome rot is also important and has been recognized (Anandaraj and Sarma, 1993; Sarma, 1994). *Pythium spp.* often infects immature and undifferentiated parts of host plant. In ginger both pre-emergence and post emergence rhizome rots are noticed. Pre-emergence rots result when infected seed rhizomes are sown

or through bud infection and subsequent rotting of infected buds. New buds arising from the rhizomes also contract infection leading to total crop loss.

Post-emergence rot can occur at any time after sprouting. The pathogen may penetrate the sprout through the roots or through the collar region finally reaching the rhizome. Initial symptoms appear as water soaked patches at the collar region of the pseudostem. These patches enlarge and the tissues of the collar region becomes soft, watery and rot. Sprouts turn pale and collapse. In well differentiated clumps, the infection starts at the collar region of the pseudostem as water soaked area which generally spreads both upwards and downwards. Foliar yellowing is seen clearly from the margins of the lower most leaves (oldest) and progresses upwards. As the infection spreads gradually to the inner most part of the pseudostem, the intensity of the foliar yellowing increases. Later the infection spreads to the rhizome. The affected rhizomes rot, emit a foul smell and the pseudostems come off with a gentle pull. In the early stages, the root infection often reaches the germinating sprouts leading to the rhizome rot. When the role of root infection in the soft rot of ginger was studied, it was found that pseudostem infection was more when the inoculum was placed at the surface and decreased with the depth of the inoculum source (Anandaraj & Sarma, 1993).

Causal organism: The genus *Pythium* includes a number of readily recognized species with wide distributions and host ranges (Hendrix and Campbell, 1973). It has been reported that several spp. of *Pythium* are involved with the rhizome rot of ginger. They are *Pythium aphanidermatum* (Edson) Fitz. (Mitra and Subramanian, 1928; Vaheduddin, 1955; Sahare and Asthana, 1962; Haware and Joshi, 1974; Sarma *et.al.,* 1979), *P. butleri* Subram (Thomas, 1938), *P. deliense* Meurs (Haware & Joshi, 1974), *P. gracile* (de Bary) Shreak (Butler, 1907), *P. myriotylum* Drechsler (Uppal, 1940; Sahare and Asthana, 1962), *P. pleroticum* (Dohroo & Sharma, 1985), *P. ultimum* (Dohroo *et. al.,* 1987) and *P. vexans* de Bary (Ramakrishnan, 1949). *P. butleri* and *P. gracile* is considered to be identical with *P. aphanidermatum* (Edson) Fitz. (Butler & Bisby, 1931).The most commonly encountered pathogens in the rhizome rot of ginger are *P. aphanidermatum* and *P. myriotylum*. Studies at IISR, Calicut showed that *P. aphanidermatum* is the major spp. involved in the rhizome rot in Kerala state (Anon, 1993).

The Genus *Pythium* Pringsheim: The genus *Pythium* Pringsheim belong to the family Pythiaceae of the order Peronosporales (Ainsworth *et.al.* 1973). The mycelia are well developed often with appressoria and hyphal swellings. Zoosporangia are either filamentous and are not differentiated from the vegetative hyphae. Sporangia are terminal, intercalary or laterally sessile, zoospores are biflagellate. Oogonia (sub) globose, lemon shaped or ellipsoidal, terminal or intercalary with a smooth or ornamental wall. Antheridia are one to several per oogonium, monoclinous, diclinous or hypogynous, stalked or sessile of various shapes. Oospores are usually single, rarely 2-4 in an oogonium, plerotic or aplerotic with a thin or thick (inspissate) wall.

Pythium spp. often occur as saprophytes or parasites in soil, water or on plant or animal substrates. Various colony patterns on agar medium have been recognized. Development of the growth pattern depends on the medium and incubation temperature (Vander Plaats-Niterink, 1981).

Colonies of *Pythium aphanidermatum* (Edson) Fitz. are with a cottony aerial mycelium on corn meal agar and potato dextrose agar. The hyphae are 10 nm wide and the sporangia consists of terminal complexes of swollen hyphal branches of varying length upto 20 nm wide. Zoospores are formed at 20-25^{0}c and encysted zoosporangia are 12µm in diameter. The oogonia are terminal, globose, smooth, 20-25 nm in diameter. Antheridia are mostly intercalary, sometimes terminal, broadly sac shaped 10-14 nm long and 10-14 nm wide, two per oogonium, monoclinous or diclinous. Oospores are aplerotic, 18-22 nm in diameter and wall, 1-2 nm thick.

Pythium spp. are not pathogenic under all environmental conditions, and are more demanding than many other pathogens, particularly as far as temperature and moisture are concerned (Hendrix and Campbell, 1973)..The growth and reproduction of *Pythium* spp. is dependent on various factors. The linear growth of the mycelium is temperature dependent and for *P. aphanidermatum* the optimum temperature for growth ranges between 35 -40^{0}C. *Pythium* spp. can attack a variety of hosts. The plants attacked by *P. aphanidermatum* includes many important cereals and horticultural crops causing root rot, soft rot, fruit rot and cottony blight.

Pathogenesis by *Pythium* spp. is accomplished by cellulolytic enzymes and pectolytic enzymes or phytotoxins which are tissue degrading and *Pythium aphanidermatum* produces these enzymes (Winstead and McCombi, 1961; Janardhan and Hussain, 1974).

Oospores are the major root infecting units (Stanghellini & Burr, 1973), which produces germ tubes and terminate by producing a sporangium. Dissolution of the middle lamelle of the host tissues results in soft rot. Sporangia are produced on the surface of the host when the soil water content is high.

Disease Cycle: The disease is both seed borne (Mc Rae, 1911; Thomas, 1938, Mundkar, 1949) and soil borne. *Pythium* spp. being soil dwellers are known to perennate in the soil in the form of oospores. Sexual reproduction takes place in the host tissue as well as the soil. Oospores present in the soil or infected seed rhizome would serve as the primary source of inoculum (Stanghellini & Burr, 1973).

The rhizome rot infection occurs early if the infection is seed borne. Spread of the disease may be waterborne zoospores or hyphal fragments. Zoospores that are released from the sporangia are chemotactically attracted to the host roots where they encyst and form germtubes which infect the host roots. Under ideal conditions, lesions appear in 72 hours around the penetration point. Sporangia are produced on the surface of the host lesions and zoospores released from the sporangia may be carried passively or by the active movements to healthy plants where it may cause new infection resulting in the spread of the disease.

Epidemiology: The infection and the severity of the *Pythium* disease is known to be influenced by several factors which include environmental and edaphic factors. Greater incidence of the rhizome rot has been reported during the wet years (Rajan and Agnihotri, 1989). Similarly, high relative humidity and low temperature favours the development of the disease (Sarma, 1994).

Excess soil water content favours the infection and severity of the *Pythium* diseases. The influence of the soil temperature varies with the different species of *Pythium* as well as the hosts. The optimum temperature for the germination of the oospores of *Pythium apanidermatum* was reported as 30^0C (Adams, 1971). Similarly the soil pH is also dependent on the spp. involved. The high incidence of *Pythium* induced rhizome rot have been reported during the wet years (Rajan and Agnihotri, 1989) implying the role of high soil water content in the disease onset and spread.

In Kerala, ginger is planted during the months of April-May immediately after the onset of the premonsoon showers and the crop is rainfed. High soil moisture and adequate

temperature (25-30^{0}C) prevailing during the months of July-September, coinciding with the south west monsoon is highly conducive for the disease development of rhizome rot (Sarma, 1994). Since the sprouting of the rhizome bits start in the month of June, juvenile tissues of the host which the pathogen prefers would be readily available throughout the season. Once the disease start, it spreads gradually to the adjacent clumps mostly through the soil water by means of zoospores, hyphal fragments, etc. both under rainfed and irrigated conditions. In general, the disease is less in the hilly slopes because of better drainage (Sarma, 1994).

Constant association of dipteran maggots, *Mimigrella coeruleifrons* and *Eumerus* spp. which infected the seed rhizomes revealed no positive role for them in the disease etiology and were considered as saprophytic colonizers since they were not found to infect the healthy seed rhizomes (Koya, 1988; Premkumar *et.al.*, 1982). However the isolation of the *Pythium* spp. from the foreguts of the adult flies of *M. coeruleifrons* suggested their possible role in disease dissemination (Iyer, *et. al.,* 1981).

Varying degrees of association of *Meloidogyne incognita* was noticed with root system of ginger (Anon, 1993). Interaction of this nematode with *P. aphanidermatum* neither increased the disease nor could pre dispose the plant to infection (Anon, 1993). However increased rhizome rot incidence was reported in association with nematodes viz. *M. incognita* and *Pratylenchus coffeae* (Dohroo *et. al.*, 1987).

Survival of the inoculum and dissemination: *Pythium* spp. survive between crops on infected seed rhizomes. Its survival as oospores in scale leaves and ginger has been reported (Thomas, 1940). *Pythium* spp. are capable of saprophytic survival on plant debris since they are normal inhabitants of many soils. They produce oospores, the perennating structures of the fungi and may reproduce on non host crops and weeds (Jackson, 1995). The saprophytic survival of the fungus in the soil is influenced by environmental factors, soil temperatures, moisture and presence of other microbes.

Infected seed rhizomes are the major sources of disease spread. Apparently normal but infection through contaminated seed rhizomes would serve as the primary source of inoculum. Secondary spread of the disease is mainly through soil water.

Disease Management: Rhizome rot is considered as a complex disease problem. Ginger plant is a delicate succulent herb. It is easily susceptible to stress, both environmental and

biological. Therefore it is essential that the disease management practices are integrated with crop production at all possible stages of crop cycle for reducing the disease development by adopting cultural, chemical and biological control methods (Dake *et. al.*, 1988).

Cultural practices:

a) **Exclusion:** The principle of exclusion is important in the rhizome rot management since most of the rhizome rot pathogens are seed borne. Exclusion of disease from areas not already affected (identified by survey) may be possible by restricting the movement of the seed rhizomes by appropriate measures, specifically through internal quarantine.

b) **Seed selection and storage:** Ginger is mainly propagated through seed rhizomes. Therefore seed health is most important (Park, 1941; Bertus, 1942). Pathogens carried in the seed rhizomes continue to be active in storage under favourable conditions, resulting in drastic reduction in the recovery of the quality seed material. The best method available for the management of seed borne inoculum is the selection of healthy seed rhizomes. Selection of healthy seeds from the disease free gardens and storing them in ideal conditions is an essential pre requisite. Seed storage practise consists of seed treatment with Mancozeb (0.3%) solution and 0.1% Malathion for 30 minutes, air drying and storing in pits lined with sand, in thatched sheds or rooms where the temperatures donot exceed 28-30^{0}C. This procedure ensured optimum seed recovery (Dake *et.al.*, 1989; Mishra & Iyer, 1981, Beena *et.al.*, 1997).

c) **Time of sowing:** Young sprouts being highly susceptible, conducive environmental conditions like high soil moisture and low temperatures would result in high disease incidence during July-August. By early sowing, the pseudostems will become hardy and less vulnerable to infection during critical periods (July-August) of infection. Studies carried out at IISR, Calicut showed that by early planting during May or early June resulted in less disease incidence (Anon, 1984). However the period of sowing would vary for different agroclimatic zones depending upon the early receipt of monsoon showers.

d) Drainage: Stagnation of water in and around the beds would lead to high inoculum build up and also rapid spread of the disease through soil water. Better drainage would thus reduce the chance of infection.

e) Crop rotation: This is an age old practice adopted to reduce the inoculum build up of the soilborne pathogens. Success of crop rotations depend on the survival and longevity of the pathogens concerned. Survival of the pathogens is influenced by its host range, soil type, saprophytic ability, inoculum level, ability to form resting structures and presence of antagonists. These factors in turn influence the type and the length of rotation. Shifting cultivation and crop rotation for three years are followed by the farmers in Kerala. Cucurbitaceous vegetables, Yams (*Amorphophalus esculentus*), Colocasia, Tapioca and Banana are found to be beneficial against soft rot disease of ginger (Rajan & Agnihotri, 1989). Chillies, paddy and gingelly in rainfed areas and ragi, groundnut, maize and vegetables in irrigated conditions are rotated with ginger (Anon, 1995).

f) Site selection and land preparation: Importance of site selection has been emphasized in the package of practices of ginger in Kerala, since the land which has poor physical characteristics can reduce the productivity, impose stress on the host plant and favour the activities of the pathogens. When high soil moisture content due to water stagnation occurs in the field, *Pythium* is able to grow and sporulate. Establishing ginger crops next to infected fields or on land which will intercept run off from the infected field is likely to increase the chances of disease outbreak. Preparation of the land by tillage to create good tilth is recognized as an important practice to loosen the soil, increase the aeration and to increase the root depth. Cultivation of ginger is preferentially done on raised beds to facilitate proper drainage.

g) Weed management: Weeding is essential to improve the growth of ginger by eliminating competition for space and nutrients. Since most of the pathogens of ginger have got wide host range, weeds might play an important role in the carry over of the diseases between crop cycles. Therefore weeding is important in the success of crop rotation.

h) Application of fertililzers: Nitrogen and potassium have been proved to be beneficial in lowering the incidence of *Pythium* and *Fusarium* diseases (Jackson, 1995).

i) After cultivation practices: After cultivation practices such as weeding, mulching with green leaves, earthing up and fertilizer application are required to improve the growth and to maximize the yield. Root injury should be avoided during earthing up, since injury may provide entry points for the pathogen. Rouging should be done in time and the infected soil should be drenched with the fungicides to prevent the spread of the disease. Phytosanitation measures including restricting the movement of people from the infected to healthy fields and disinfestations of the farmers' equipments in infected fields were also recommended to prevent the spread of the disease.

j) Soil management: Since ginger is a well known nutrient exhausting crop, heavy manuring is required to obtain the maximum yield (Perseglove *et.al.,*1981). The effect of different managements with oil cakes viz castor, groundnut, sesamum, margosa, coconut and also saw dust with and without urea were studied and it was found that coconut cake was the best amendment which reduced the disease incidence and increased the yield (Rajan & Singh, 1973). Similarly neemcake @ 2 MT / ha gave 2% reduction of rhizome rot and increased yield by 1.78 MT / ha (Sadanandan and Iyer, 1986). Soil surface burning with dry trash reduced the rhizome rot disease and increased the yield. It increased the availability of the soil nutrients (Sadanandan *et.* al., 1988). Application of soil amendments is known to improve the structure of the soil and increase the microbiological population suppressive to the pathogens (Sarma, 1994).

Soil solarisation: Soil solarisation is a physical disinfestation method of disease control which aims to eradicate or reduce the soil borne pathogens prior to sowing. This technology was first introduced by Katan *et.al.,*(1976). It is essentially a hypothermal disinfestation method accomplished by covering moist soil with transparent polyethylene film during the period of high temperature and intense solar radiation. It should be done when the soil is wet since the resting structures of the pathogens are more sensitive to wet heat. Appropriate soil moisture promotes beneficial soil activity in the soil that enhances pest control (Katan, 1987). Using both transparent and black polythene tarps, the effect of soil solarisation was studied on winter planting of potato, cauliflower and cucumber. Both

the tarps reduced the population of *Alternaria solani, Fusarium oxysporum, F. solani, P. debaryanum* and *Verticillium dahliae.* The respective yield increase in solarisation with transparent and black tarps were nearly 31% and 29% in potato,48% and 10% in cauliflower and only 10% and 0% in cucumber (Abu Blan and Abu Gharbieah, 1994). Weed control is an added advantage of soil solarisation and annual weeds are more sensitive than perennials (Katan, 1987). The possible mechanisms by which the weeds were suppressed were by thermal killing of the germinating seeds, breaking the seed dormancy and consequent killing of the germinating seeds and biological control through weakening or other mechanisms. Volatiles that accumulate under the mulch might be responsible to direct killing or might effect dormancy of germination (Horowitz *et.al.,* 1983; Rubin & Benjamin, 1984). Experiments carried out at the Indian Institute of Spices Research, Calicut also revealed that there was a reduction in the pathogen propagules, disease incidence and increased yield in the solarised ginger fields when compared to the non solarised plots (Balakrishnan *et.al.,* 1996; Usman, *et.al.*, 1996).

Chemical control: Chemical control consists of seed treatment with fungicides to check the seed borne inoculum and soil drench to check the soil borne inoculum. Use of chemical fungicides in the management of rhizome rot consists of seed treatment with or without supplementary soil drenches with fungicide suspension before planting or to the standing crop. In India, the chemical control has been recommended for the management of soft rot and a number of fungicide formulations have been evaluated or recommended for the management of soft rot of ginger. Many organomercurials, copper fungicides, dithiocarbamates and a number of systemics have been effective in improved sprouting, reduced disease incidence of rhizome rot and increased yield. Some of the results obtained for the various tests conducted could be summarized as follows. Seed treatment and soil drenching with Bordeaux mixture-BM (2:2:50) (Bhagawat, 1960) and seed treatment with Ceresan (0.25%) for 30 minutes (Thomas, 1940) were found to effectively control rhizome rot. Pre sowing soil drenching and subsequent treatments every week with Bordeaux Mixture (4:4:50) or Perenox (0.35%) and Dithane D-78 reduced the rhizome rot caused by *P. aphanidermatum* and *P.myriotylum* (Sahare and Astahna, 1962). The on farm evaluation of the antibiotics Aureofungin, Metalaxyl, Captafol and Methoxy-ethyl-mercury chloride (MEMC) with and without Dolomite application demonstrated superiority by MEMC over other fungicides (Sarma *et. al.,* 1979). Metalaxyl was recommended for the seed treatment

and soil drenching based on its performance in disease control after prolonged on farm evaluations of the fungicides at the Indian Institute of Spices Research, Calicut (Sarma, 1994). Effectiveness of Metalaxyl was also reported by other investigators (Koshy *et.al.*, 1988b). Similarly increased germination, reduced disease incidence of rhizome rot and better yield was obtained with captafol when used for seed treatment (Koshy *et.*al, 1988a; Mathur *et.al.*, 1984). Use of seed treatment with Captan, Captafol, Metalaxyl and hot water treatment super imposed by 200ppm Streptocycline were evaluated and it was found that Captan was more effective against rhizome rot (Manmohandas *et.al,* 1990). Five systemic fungicides ie Fosetyl aluminium, Metalaxyl, Oxadixyl, Propamocarb and Ethazole were evaluated against rhizome rot caused by *P. aphanidermatum,* both as seed treatment and soil drench, Metalaxyl formulations viz Ridomil 5-G granules and Apron 35 WS gave the best control (Ramachandran *et.al.*, 1989).

When six non systemic fungicides and four systemic fungicides were tested against *P. aphanidermatum,* Metalaxyl, Captafol, Ziride and Captan and Metalaxyl formulations reduced rhizome rot incidence and increased germination and yield (Thakore *et. al.*, 1988). Rathiah (1987) observed that a mixture of Ridomil and Captafol controlled the disease and increased the yield. Fungicides though effective they are prone for leaching off during heavy monsoon period and also not cost effective (Jackson, 1995).

Biocontrol: It is widely recognised that the increasing use of potentially hazardous fungicides in agriculture has been the cause of growing world wide concern. The resurgence of interest in the use of introduced microorganisms for the biological control of plant pathogens during the past 10-15 years has been driven in past by trends in agriculture towards greater sustainability and increased public concern for the hazards associated with the use of synthetic pesticides. Rapid evolving technologies from molecular biology and genetics have provided new insights into the underlying mechanisms by which biocontrol agents function and have allowed the evaluation of the behaviour of microbial inoculants in natural environments to a degree not previously possible (Thomashow and Weller, 1996)

A modern definition of biological control of plant pathogens has been proposed as "Biological control is the reduction of the amount of inoculum or disease producing activity of a pathogen accomplished by or through one or more organisms other than man" (Cook & Baker, 1983). As defined by the National Academy of Sciences, biological control includes

"the use of natural or modified organisms, genes or gene products to reduce the effects of the undesirable organisms (pests) and to favour desirable organisms such as crops, trees, animals and beneficial insects and microorganisms (National Academy of Sciences, 1987). Biological control of plant diseases involves one or more natural mechanisms like antibiosis, parasitism, lysis, competition, plant growth promotion, predation and induced systemic resistance.Antibiosis plays an important role in biocontrol by the production of secondary metabolites which are toxic to the target pathogen(s) and still some other compounds, promote the plant growth directly (Fravel, 1988; Sivasithamparam and Ghisalberti, 1998). These factors often limit the interaction between plant pathogen and their antagonists resulting in less than acceptable suppression of disease or reduction in pathogen population (Cook and Baker, 1983; Burpee, 1990).

Biological Control of *Pythium* species:

Biological control of *Pythium* species is very difficult because of the rapid germination of sporangia in response to seed or root exudates followed by immediate infection and the ability to cause long term root rots (Whips and Lumsden, 1991). Inspite of these constraints many important diseases have been controlled with the antagonistic fungi, bacteria and actinomycetes (Table 1) and *Pythium* suppressive soils exist and these might be a good source for suitable bioagents. Among the fungal antagonists, the genus *Trichoderma* received much attention, because this species has many advantages as biocontrol agents. First they have fast growth and a great arsenal of inducible polysaccharide-degrading enzymes as a result of which the fungi can be propagated on a wide variety of carbon sources (Tronsmo & Hjeljord, 1992). It is now possible to design media and growth conditions that stimulate the fungi to produce the type of biomass appropriate to the intended application (Harman, 1991; Papavizas, 1985; Tronsmo & Harman, 1992).

Another advantage is the wide range of environmental conditions tolerated by the various *Trichoderma* spp. and isolates. Some isolates can grow at low temperatures and others at high temperatures. Isolates also vary in their tolerance to different chemicals and may show remarkable resistance to fungicides, either inherently or through mutation or adaptation. (Tronsmo, 1989a). It is therefore possible to select fungicide tolerant or resistant biocontrol agents for use in integrated control.

Among the bacteria and actinomycetes, fluorescent pseudomonads, *Bacillus* spp. and *Streptomyces* received maximum attention. These microorganisms were multiplied in large scale and applied to the seed or soil. The genus *Trichoderma* consists of 9 species groups (Rifai, 1969). Some of the species of *Pythium* viz *P. oligandrum, P. nunn, P. periplocum* (Vander Plaats-Niterink, 1981) and *P. acanthophoron* (Lodha & Webster, 1990) are mycoparasites.

Similarly the fluorescent pseudomonads include the species of *P. fluorescens,* (4 biotypes), *P. putida* (2 biotypes), *P. aeroginosa, P. chlororaphus, P. aureifaciens* and *P. syringae* (Schippers *et.al.*, 1987). These species produce a wide variety of secondary metabolites with antagonistic characteristics most of which are nitrogen containing heterocyclic compounds or unusual aminoacids and peptides (Schippers *et.al.,* 1987). Some of the latter group of compounds act as siderophores (Lynch, 1990). But the most widely used biocontrol agents in the world belongs to the fungal genus *Trichoderma.*

Table 1: Biological control of *Pythium* spp.

Biocontrol agent	Target species	References
Bacillus sp.	*P. ultimum* *P. aphanidermatum*	Broadbent *et.al.*, 1971 Wolk & Sarkar, 1994b
Enterobacter cloacae	*P. ultimum*	Hadar *et.al.*, 1983 Nelson & Craft, 1992
Erwinia herbicola	*P.syringae, E.herbicola*	Lindow *et.al.*, 1983
Pseudomonas fluorescens	*P. aphanidermatum*	Elad & Chet, 1987 Wolk & Sarkar, 1994a Rankin & Paulitz, 1994 Dodd & Stewart, 1992 Paul *et.al.*, 1992 Saratchandra *et.al.*, 1993 Zhou & Paultz, 1993
P. fluorescens var putida *P. putida*	*P. aphanidermatum*	Elad & Chet, 1987

Pseudomonads sp.		
P. cepacia	*P. ultimum*	Fridlender *et.al.,*1993
Streptomyces sp.	*P. ultimum*	Bolton, 1980 Dodd & Stewart, 1992
Acrophialophora levis	*P. ultimum*	Harman *et.al.*, 1978
Chaetomium globosum	*P. ultimum*	Kommedahl *et.al.,*1981
T. virens	*P. ultimum*	Lumsden & Locke, 1989 Don Jenson, 1992
Glomus sp.	*P. aphanidermatum*	Iyer & Sunderraju, 1993
VAM	*Pythium* sp.	Hwang *et.al.,*1993
Penicillium frequentans	*Pythium* sp	Liu & Vaughan, 1965
Penicillium sp. *P. stipatum*	*P .indicum*	Krishnamoorthy & Bhaskaran, 1991a & b Sharif *et.al.,*1988
P. oligandrum	*P. ultimum* *P. vexans* *P. graminicola*	Al-hamdani *et.al.,* 1983
P. acanthophoron	*P. myriotylum*	Anon, 1994 Lodha & Webster, 1990
Trichoderma koningii	*Pythium* spp.	Hadar *et.al.,* 1984
T. hamatum *T. harzianum*	*P. aphanidermatum* *P. aphanidermatum* *P. myriotylum* *P. aphanidermatum*	Sivan & Chet, 1982 Bharadwaj & Gupta, 1987; Bharadwaj *et.al.*, 1988 Sivan *et.*al.,1984; Anon, 1994 Xu *et.*al.,1993; Sharif *et.*al.,1988 Anon,1994 Bharadwaj & Gupta,1987; Bharadwaj *et.al.*, 1988
Trichoderma sp.	*Pythium* sp.	Cotes *et.al.*,1996

Biological control of rhizome rot of ginger:

Studies on biocontrol of rhizome rot dates back to the report of Thomas (1938) who suggested the use of *Trichoderma lignorum* against *Pythium* in the dual culture, since the former increased the acidity of the medium which was unfavourable to the growth of *Pythium*. *In vitro* tests using *Trichoderma viride, T. harzianum* and *T. hamatum* against *Pythium aphanidermatum, Fusarium. equiseti* and *Fusarium solani* showed that these antagonists were inhibitory to the pathogens and the efficiency of *Trichoderma* species in checking rhizome rot was established (Bharadwaj & Gupta, 1987).

Sharma and Dohroo (1982) studied the post harvest management of rhizome rot of ginger caused by *F. oxysporum* through chemical control and antagonists. Two fungitoxicants i.e., Bavistin (0.1%) and Bavistin + Dithane–M 45 (0.25%) and an antagonist *Gliocladium virens* were found effective in reducing storage rot of ginger due to *F. oxysporum* f. sp. *zingiberi. T. viride* in various combinations with wood saw dust showed appreciable reduction in rhizome rot incidence (Dataram, 1988). *P.acanthophoron*, a hyperparasite to *P.myriotylum* and *F.solani*, the rhizome rot pathogen of ginger in Rajasthan has been reported (Lodha & Webster, 1990). Biocontrol agents, *T. harzianum, T. viride, T. virens* were isolated and tested both *in vitro* and *in vivo* against the rhizome rot pathogens viz, *F.* equiseti, Mucor spp., *P. ultimum* and *Cylindrocarpon destructans* in Himachal Pradesh. In Udaipur region of Rajasthan integrated application of the bioagents (*T. harzianum, T. viride, T. aureoviride, T. virens* and *P. acanthophoron*) and organic amendments (Oil cakes of Neem, Pongamia and wood sawdust) and rhizome seed dip with Ridomil MZ 72WP (0.625%) + Bavistin (0.1 g /l itre) for 40 minutes was found very effective in suppressing the pathogen viz, *P. myriotylum* and *F. solani* by consequent reduction of disease incidence and increasing the yield (Anon, 1993). VAM fungi viz, *Glomus macrocarpon, G. fasciculatum, Gigaspora* sp. and *Acaulospora laevis* were isolated from the rhizosphere soil of ginger in Kerala (Anon 1994). Pot culture tests with VAM fungi gave beneficial effects in reducing the damage caused by *P. aphanidermatum* and *M. incognita* (Iyer and Sunderaju, 1993). Although a number of biocontrol agents have been reported *Trichoderma* spp. have considerable potential and they can be used to augment the native soil population or seed coating to control soil borne plant pathogens (Mukhopadhyay *et.al.*, 1992). Usman, 1997 reported that the use of antagonistic fungi especially *Trichoderma* species has been more extensive in the control of rhizome rot of ginger.

The Genus *Trichoderma*:

The genus *Trichoderma* belongs to the class hyphomycetes and the order Hypocreales which comes under the Class Deuteromycotina or Fungi Imperfecti. The species of *Trichoderma* have thin loose, floccose or compactly tufted colonies. The colonies have green colour due to pigmentation or phialospores. The complicated and highly ramified conidiophores of *Trichoderma* are conical or pyramidal in outline. The main branch is rebranched into side branches which in turn puts out further side branches and so on.The phialides of the species of *Trichoderma* are mostly flask shaped or nine pin shaped, slightly narrower at the base than above the middle, and from there attenuated towards the apex into a narrow, conical or sub cylindrical neck.

The phialospores may be produced singly or successively and accumulate at the apex of the phialides to form a globose or sub globose conidial head mostly less than 15μ diameter. The phialospores are smooth or minutely rough walled, hyaline or yellowish green to dark green, sub globose, short obovoid or obovoid elliptical or elliptical cylindrical to almost oblong, sometimes appearing angular, their base occasionally distinctly truncate.

Trichoderma possess intercalary or rarely terminal chlamydospores which are mostly globose or less often ellipsoidal.

Based on the type of branching system of the conidiophores the manner of phialides disposition and the character of the phialospores, Rifai in 1969 classified *Trichoderma* into 9 species.

1. *T. aureoviride* Rifai
2. *T. hamatum* (Bon) Bain.
3. *T. harzianum* Rifai
4. *T. koningii* Oud.
5. *T. longibracheatum* Rifai
6. *T. polysporum* (Link ex Pers.) Rifai
7. *T. piluliferum* Webster & Rifai
8. *T. pseudokoningii* Rifai
9. *T.viride* Pers. ex. S.F.Grey

The nine species and a few additional species were keyed out by Domsch, *et.al.,* (1980). Rifai and Webster (1966) established telomorph connections. An intensified investigation of the telomorph taxonomy with cultural studies was initiated by Samuels (1996). The most detailed morphological studies were carried out by Bissett (1991a-c, 1992) and a detailed

key to the identification of *Trichoderma* was given by Gams and Bisset (1998) where 33 species of *Trichoderma* were listed.

***Trichoderma* as a potential biocontrol agent:**

The potential of *Trichoderma* spp. as biocontrol agents was suggested more than 60 years ago by Weindling (1932), who was the first to demonstrate the parasitic activity of the members of this genus to the plant pathogens such as *R. solani.* In particular, isolates of *Trichoderma harzianum, Trichoderma virens* and *T. hamatum* are used against a wide variety of economically important crops (eg.apples, carrots, cotton, grapes, onion, peas, plums and sweet corn). They have been used with success against soil borne diseases, seed borne diseases, in the phyllosphere and against storage rots (Chet *et.al.*, 1982; Papavizas, 1985; Tronsmo, 1986). This wide range of application is due to the various antagonistic mechanisms found in different *Trichoderma* isolates enabling them to function as potent biocontrol agents on many different crops, against a range of pathogens and in several ecological conditions. In some of the successful *in vitro* studies, Dennis and Webster (1971a-c) described the antagonistic properties of *Trichoderma* in terms of antibiotic production and hyphal interaction. *T. lignorum* and *T. viride* completely inhibited the hyphae of *P. debaryanum* (Docea *et. al.,* 1974). *T.viride* could successfully replace the already established cultures of *Ceratocystis ulmi,* the Dutch Elm pathogen both *in vitro* and *in vivo* (Webber & Hedger, 1986). While studying the biocontrol of *Macrophomina phaseolina,* the causal agents of charcoal disease of melons, Elad *et. al.,* (1986) found the inhibition on the growth of the pathogen by *T. harzianum,* in *in vitro,* green house and in the field. Bharadwaj and Gupta (1987) studied the *in vitro* antagonism of *Trichoderma* spp.against *P. aphanidermatum,* the rhizome rot pathogen of ginger. Antagonism of *Trichoderma* spp against *R. solani*, the causal agent of damping off of tomato has been reported by Khara and Hadwan (1990). *T. koningii, T. longibracheatum* and *T. harzianum* gave the highest inhibition against *Armillaria* spp., the tea rot fungus (Osando and Wando, 1994). *T. viride* and *T. harzianum* inhibited *M. phaseolina,* the stem and root rot fungus of cow pea (Singh and Majumdar, 1995). *T. longibracheatum* antagonized *M. phaseolina,* the pathogen of soyabean and formed a clear inhibition zone (Chandel and Chowdhary, 1995). Usman

(1997) and Balakrishnan (1997) established the bioefficacy of *Trichoderma* spp. in controlling the pathogen causing rhizome rot of ginger, *Pythium aphanidermatum.* The

potential of *Trichoderma harzianum* in controlling the foot rot pathogen of black pepper, *Phytophthora capsici* has also been established (Rajan, 2000; Rajan *et. al.,* 2002). Saju, 2004 conducted a study to determine the factors affecting biological control of *Phytophthora capsici* infections in black pepper. Hjeljord and Tronsmo (1998) presented an overview of biocontrol with *Trichoderma* species in which they detailed the criteria for selection, mechanism, formulation etc. Fast growth and multiplication in organic wastes make *Trichoderma* an ideal candidate for biological control of plant pathogens (Prakash *et.al.,* 1999; Saju *et.al.,* 2000; Saju *et.* al., 2002.) Kumar and Marimuthu (1997) reported decomposed coir pith as a conducive medium for colonization of *T. viride. T. hamatum* and *T. virens* could multiply luxuriantly in coconut water upto 50% dilution with tap water (Anandaraj and Sarma, 1997). The reliability of biocontrol systems can be improved by the use of formulations that provide conducive environments for the bioprotectants and fermentation systems that economically produce propagules of high quality (Harman, 1991).

Modes of action of *Trichoderma* spp.:

Suggested modes of action of *Trichoderma* are competition, antibiosis and mycoparasitism (Hennis & Chet, 1975; Papavizas & Lumsden, 1980; Cook & Baker, 1983; Baker & Dickman, 1993). Competition occurs when two or more micro organisms demand more of the same resources i.e., nutrients, space or any other environmental factors provided by the immediate supply (Park, 1960). Both antibiosis and mycoparasitism may be involved in competition for nutrients, indeed production of toxic metabolites is known to be affected by the nutrient status of the growth medium (Fravel, 1988; Ghisalberti and Sivasithamparam, 1991; Howell and Stipanovic, 1995). Competition between the biocontrol agent and the pathogen may lead to disease control.

Antibiosis occurs when the production of the toxic metabolites or the antibiotics by one organism has direct effect on another organism. New insight into the role of antibiotics was gained by the demonstration of synergistic activity of parallel formation of pentabiol antibiotics and hydrolytic enzymes by *T.harzianum* (Schirmbock *et.al.,* 1994). Mycoparasitism is the phenomenon of one fungus parasitising another. It includes chemotropic growth in which a chemical stimulus from the pathogen fungus (host) attracts the parasite (Chet *et.al.,* 1981). Recognition is an essential step in the specificity of mycoparasitism (Chet, 1987). Lectins may play an important role in this specific interaction

(Barak *et.al.,* 1985; Inbar and Chet, 1992, 1994). The third step is the attachment. The *Trichoderma* hyphae can either grow along side the host hyphae or coil around it (Dennis and Webster, 1971c; Harman *et.al.,* 1981; Tronsmo and Dennis, 1978). The last step is the degradation of the host wall by the production of lytic enzymes, such as chitinases and glucan, 1, 3 β glucosidases (Cherif & Benhamou, 1990; Elad *et.al.,* 1983).

Antifungal metabolites of *Trichoderma* spp.

Several toxic metabolites have been reported to be produced by *Trichoderma* spp. *in vitro*. Many of them have been isolated and characterized chemically. The antifungal metabolites of *Trichoderma* have been grouped into pentaketides, terpenoids, octaketides and peptaiobols (Ghisalberti and Sivasithamparam, 1991). In another review, Sivasithamparam and Ghisalberti (1998) discussed the secondary metabolites of *Trichoderma* and *Gliocladium* and listed about 140 compounds. Howell (1998) reviewed the phenomenon of antibiosis and inhibition of the pathogens in detail. The ability to produce the antifungal compounds vary between the isolates of the same species and aggregates and also between the isolates of different species aggregates. (Dennis and Webster, 1971a & b). They studied the volatiles and non volatiles of *Trichoderma* spp. which inhibits a range of pathogens. The volatile producing isolates possessed a characteristic smell especially the members of *T. viride.* The inhibiting metabolites of *T. viride* was identified tentatively as acetaldehyde (Dennis and Webster, 1971 b). The non volatile compounds included chloroform, soluble antibiotics including Trichodermin and peptide antibiotics (Dennis and Webster, 1971a). Upadhyay and Mukhopadhyay (1986), Mukhopadhyay and Kaur (1990) reported the production of antibiotics by *T. harzianum* by inhibiting the growth of *Sclerotium rolfsii, Rhizoctonia solani* and *Fusarium oxysporum. T. virens* produced an antibiotic diketopiperazine (Gliovirin) that was highly inhibiting to *P. ultimun* but not to *R.. solani* (Howell and Stipanovic, 1983).

Several metabolites including lactones, alcohol, terpene derivatives and derivatives of pyrones were isolated from *T.viride* (Zeppa *et. al.*, 1990). They identified that the coconut aroma of this fungus was due to 6 penta α pyrone. Harzianolide, a butenolide and harziandione, a diterpene compound was also isolated from *T. harzianum* (Claydon *et.al.,* 1991; Ghisalberti *et. al.,* 1992).

Rathore *et* al., (1992), reported that the non volatile substance produced by *T. viride* completely inhibited the growth of *P. myriotylum* and its oogonia production and reduced

the colony diameter of *F. solani,* the rhizome rot pathogen. Tricholin, a ribosome inactivating protein isolated from the culture broth of *T. viride* was shown to be antagonistic to *R.. solani* (Lin *et. al.,* 1994). Trichorzianine, a soluble antifungal metabolite isolated from *T. harzianum* was responsible for its biocontrol activity and they are produced during conidiogenesis (Horvath *et.al.,* 1995). Sharma (1998), reported that the antifungal properties of the biocontrol agents *T. harzianum, T. viride, T. virens* and *Absidia cylindrospora* and plant extracts from *Agave americana*, *Azadirachta indica* etc reduced the rhizome rot and yellow of ginger. Some of the antifungal compounds produced by *Trichoderma* spp. are given in the Table 2.

Table 2: Antifungal compounds produced by *Trichoderma* spp.

Compound	Produced by	References
Butenolide	*T.harzianum*	Almassi *et.al.,* 1991
Cyclonerodiol	*T.harzianum* *T.koningii*	Ghisalberti and Rowland, 1993
Dermadin	*T.harzianum* *T.viride*	Pyke and Dietz, 1996 Tamura *et.al.,* 1975
Furanone	*T.harzianum*	Ordentlich *et.al.,*1992
Gliotoxin	*T.lignorum* *T.viride*	Weindling and Emerson, 1936 Wright, 1956 Howell and Stipanovic, 1983
Gliovirin	*T.viride*	Howell and Stipanovic, 1983
Harziandione	*T.harzianum*	Ghisalberti *et.al.*, 1992
Harzianolide(3-(2-hydroxyl propyl-4(hexa-2-dienyl)-2 (5H)furanone	*T.harzianum*	Claydon *et.al.,* 1991
Heptilidic acid	*T.viride* *T.koningii*	Endo *et. al.,*1985
6-n-heptyl pyran 3-one	*T.koningii*	Dennis and Webster, 1971a
Hexahydrobenzo Pyran 5-one	*T.harzianum* *T.koningii*	Simon *et.al.,* 1987 Dunlop *et.al.,* 1989
1-hydroxy&1,8-	*T.harzianum*	Ghisalberti *et.al.,* 1990

dihydroxy-3-methyl anthraquinone		Almassi *et.al.,* 1991
3-(3-isocyano-cyclopent-2-enylidene)propionic acid	*T.harzianum*	Baldwin *et.al.,* 1981
Isonitrile antibiotics	*T.hamatum* *T.koningii* *T.harzianum*	Fujiwara, 1982
Homothallin	*T.harzianum* (UV-mutant)	Graeme Cook & Faull, 1991; Faull *et. al.,* 1994
Isonitrin A	*T.harzianum*	Edenborough and Hebet, 1988
Isonitric acid	*T.harzianum*	Brewer *et.al.,* 1979
6-n-pentyl-2H pyran-2-one	*T.konongii* *T.harzianum* *T.viride*	Okuda *et.al.,* 1982 Simon *et.al.,* 1987 Dunlop *et.al.,* 1989 Worasatit *et.al.,* 1994 Benoni *et.al.,* 1990 Scarselletti and Faull, 1994 Ghisalberti *et.al.,* 1990 Claydon *et.al.,* 1987 Claydon *et.al.,* 1987
Peptaibols	*T.reeesi* (UV-mutant) *T.harzianum*	Bruckner *et.al.,* 1985 Ghisalberti and Sivasithamparan, 1991
Alamethicin	*Trichoderma* spp.	Brewer *et.al.,* 1987
Peptide antibiotics	*T.harzianum, T.hamatum*	Dennis and Webster 1971 a
Trichobrachim	*T.longibrachiatum*	Bruckner *et.al.,* 1990
Trichodermin	*T.viride*	Dennis and Webster 1971 a

	T.polysporum *T.reeesi*	Gotfredson and Vangedan 1965 Watts *et.al.,* 1988
Tricholin	*T.viride*	Lin *et.al.,* 1994
Trichoviridin	*T.hamatum* *Trichoderma* spp.	Baldwin *et.al.,* 1981 Ollis *et.al.,* 1980
Trichorzianin	*T.harzianum*(UVmutant) *T.harzianum*	Bodo *et.al.,* 1985 Schirmbock *et.al.,* 1994 El hajji *et.al.,* 1987 Horvath *et.al.,* 1995
Viridin	*T.viride*	Brian and McGowan, 1945
Viridiol	*Trichoderma* spp.	Moffatt *et.al.,*1969

Enzyme production by *Trichoderma* spp.

Trichoderma is a good source of various toxic metabolites and antibiotics and also enzymes such as exo and endo glucanases, cellobiase, chitinase, protease, lipase and xylanase appropriate to the substrate (Baker and Dickmann, 1993). The major classes of enzymes are cellulolytic (Koivula *et.al.,* 1998), hemicellulolytic (Biely and Tenkanen, 1998), Chitinolytic (Lorito, 1998) and glucanolytic (Benitez *et. al.*, 1998). Mycoparasitism is one of the mechanisms of action of the fungal biocontrol agents and it is presumed that it requires the production of enzymes that digest the fungal cell wall. It seems that the biocontrol strains of *T. harzianum* produced a complex array of chitinolytic and glucanolytic enzymes all of which may be involved in the mycoparasitic activity (Sivan and Chet, 1989; Ulhoa and Peberdy, 1991). Hydrolytic enzymes are induced in mycoparasites like *Trichoderma* spp. by their substrates (Ėlad *et. al.,* 1982). An endo α D (1, 3) glucanase capable of hydrolyzing various α (1, 3) glucanase have been isolated and purified from the culture filtrate of the cellulolytic fungus, *T.viride* (Hasegawa *et. al.,* 1969).

Benomyl tolerant mutants of *T.viride* have produced more cellulase than the wild type and were superior in antagonistic activity (Ahmad and Baker, 1987b). Chitinase is another hydrolytic enzyme which attracted much attention. Chitinase enzymes (including N aetyl glucoseaminidase, Chitobiase and endo chitinase) produced by *T.harzianum* was effective in lysing chitin containing fungi (Harman and Tronsmo, 1992). Lorito *et.al.,* (1994b) studied the synergitic interaction between fungal cell wall degrading enzymes such

as chitinolytic and glucanolytic enzymes and antifungal compounds like gliotoxin, flusilazol, miconazol, captan or benomyl. Belanger *et.al.*, (1995) reported that the strains of *T. harzianum* antagonized first by antibiosis leading to cell death, followed by degradation of the cell wall by means of chitinolytic enzymes. The production of antibiotics may be more important than that of chitinolytic enzymes in conferring superior biochemical properties to *T. harzianum.* The recognition of the target fungus by *Trichoderma* spp. is the basic and first step of mycoparasitism. Inbar and Chet (1994), provided direct evidence for the role of lecithins in the recognition of pathogens by *Trichoderma* spp. Some of the hydrolytic enzymes reported to be involved in mycoparasitism are given in Table 3.

Table 3: Hydrolytic enzymes produced by *Trichoderma* spp.

Enzyme	Produced by	References
Endochitinase	*T.harzianum*	Carsolio *et.al.,* 1994
β 1, 3- glucanase	*T.viride*	Reese and Mandels, 1959 Chesters and Bull., 1963
	T.harzianum	Rudawska and Kamoen, 1992 Chet *et.al.*, 1993 Lorito *et.al.,* 1993a
Exo β-1,3-glucanase	*T.viride*	Jones *et.al.,* 1974
Cellulase	*T.koningii* *T.reesei*	Iwasaki *et.al.,* 1964 Merivuori *et.al.,* 1985
β 1, 3- glucanase and chitinase	*T.viride* *T.harzianum*	Jones & Watson, 1969 Chet and Henis, 1969 Hadar *et.al.,* 1979b Davet, 1987 Sivan & Chet, 1989 Poderes *et.al.*, 1992
	T.longibracheatum	Arlorio *et.al.*, 1992
β 1, 3- glucanase, chitinase and cellulose	*T. hamatum* *Trichoderma* spp.	Chet & Baker, 1980, 1981 Chet, 1987
β 1, 3-glucanase, chitinase, cellulase and xylanase	*T. koningii*	Worasatit *et.al.*, 1994

β 1, 3-glucanase, chitinase, cellulase, protease and lipase	*T. harzianum*	Elad *et.al.*, 1982
Endo 1,4- β-glucanase, 1,4 β-D-glucan, cellobio hydrolase	*Trichoderma* spp.	Blanchette *et.al.*, 1989
Chitinase and chitobiase	*T. harzianum*	Ulhoa & Peberdy, 1993
Chitinase	*Trichoderma* spp.	Cherif & Benhamou, 1990
Endo chitinase	*T. harzianum*	Ulhoa & Peberdy, 1992 de la Cruz *et.al.*, 1992 Harman *et.al.*, 1993
N-acetyl- β-D-glucosaminidase	*T. harzianum*	Ulhoa & Peberdy, 1992
Chitin-1,4 β-chitobiosidase, N-acetyl- β-glucosaminase, Endo chitinase	*T. harzianum*	Harman *et.al.*, 1993 Lorito *et.al.*, 1993a
Glucan- β-1,3-glucosidase, N-acetyl-β-glucosaminidase	*T. harzianum*	Lorito *et.al.*, 1994a
β-1,4-N-acetyl glucosaminidase	*T. harzianum*	Haran *et.al.*, 1995 Inbar and Chet,1995
Poly (1,4- β-(2-acetamido-2-deoxy D-glucoside)-glucan hydrolase β-1,4-N-acetyl glucosaminidase	*Trichoderma* sp.	Shapira *et.al.*, 1989 Lorito *et.al.,* 1993b, 1994
Chitobiohydralase, Endochitinase, β-1,3-glucanase	*T. harzianum*	Schirmbock *et.al.*, 1994
Xylanase	*T. viride* *T. reesei*	Gomes *et.al.*, 1992 Dean and Anderson, 1991 Kubicek, 1993
Xylanase and Cellulase	*T.reesei*	Mach *et.al.*, 1993
Serine protease	*T. harzianum*	Geremia *et.al.*, 1993

Biocontrol and synergism:

Majority of the work done on plant disease biocontrol relate to soil borne diseases using either bacterial or fungal antagonists. But the synergistic interaction between the fungal chitinases and biocontrol strains of bacteria was another avenue explored by Harman's group (Lorito *et.al.*, 1993 b). Their results showed that the inhibiting effects on spore germination and germ tube elongation of *B. cinerea, Fusarium solani* and *Uncinula necator* were synergistically increased by mixing fungal enzymes and cells of *Enterobacter cloaceae* but not of *Pseudomonas* spp. (Lorito *et.al.*, 1993 b). In pot tests seed pelleting with *T.harzianum, Rhizobium* spp. and Carbendazim was found to be highly effective in managing sclerotial root rot of ground nut (Muthumilan and Jeyrajan, 1996). Seed treatment with *Trichoderma* spp. and *B. subtilis* controlled *M. phaseolina* in sesame under field conditions (Sankar and Jeyrajan, 1996).

Lorito *et.al.,* (1993b) reported that the combinations of bacterial culture filtrates with fungal chitinolytic enzymes generated only a moderate response, indicating that the presence of bacterial cells was required for synergistic effect. Chitinolytic enzyme activity in the presence of chitinous substrates enhanced the growth of *E. cloacae* and readily restored the ability of bacterial cells to bind to the hyphae of the pathogens, despite high concentration of D glucose or sucrose in the medium. Chitinolytic enzymes probably help by releasing nutrients from hyphae of target fungi and bacterial cells could utilize these nutrients for proliferation. The subsequent increase in the bacterial population should enhance the ability of bacteria to act as biocontrol agents. Synergism would most likely occur if the bacteria were in a perfect position to utilize the nutrients released by the chitinolytic enzymes (Lorito *et.al.*, 1993b). The results of their study suggest that the potential of the biocontrol agents could be increased and that the combinations of bacteria and fungi may prove to be powerful agents of biocontrol.

Integrated control of plant pathogens:

The advantage of combining plant protection chemicals with biocontrol agents in integrated biological / chemical treatments is that the pathogen can be controlled under climatic conditions beyond the effective range of the bioprotectant. Further more, by replacing some of the chemical treatments, the biological agent reduces both environmental pollution and the danger of the pathogen developing fungicide resistance. Finally the biocontrol agent can establish itself in the infection court, thus providing a localization and

persistence unattainable by chemicals alone. Like non specific resistance, integrated control is seldom complete, but it seldom fails (Bruehl, 1989).Thus according to Papavizas, (1973); Henis & Chet, (1975); Baker & Cook, (1974), integrating biological and chemical control is a very promising way of controlling pathogen with minimal interference with the biological equilibrium.

The potential of *Trichoderma* for biocontrol has been studied largely as synergistic or additive component in integrated pest management system (Papavizas, 1985). Curl *et.al.,* (1977) obtained only a slight additive benefit to biological control of *R. solani* by adding PCNB at doses of one and 10mg / gram soil together with *Trichoderma* in sterilized soil. Hadar *et.al.,* (1979a), applied small non effective doses (1.2 μg / kg) of PCNB to soil along with *Trichoderma* preparation (2g / kg) and found that the incidence of egg plant disease caused by *R. solani* declined from 40 to 13% while *T. harzianum* reduced the disease incidence only by 26%. PCNB alone at this rate had no effect on the disease control of the egg plant seedlings.

Compatibility of the biocontrol agents with agrochemicals became important in integrated disease management (Davet *et.al.,* 1981). The antagonists need to be fungicide tolerant or resistant for use in integrated control. It is feasible to create such isolates by mutation or selecting them on pesticide containing media (Abd-El-Moity *et.al.,* 1982; Tronsmo, 1986, 1991). Ahmad & Baker, (1988b), reported that the sensitivity of *Trichoderma* spp. to benomyl was successfully overcome with the development of mutants, tolerant to benomyl which was successful in rhizosphere colonization even at 10μg / g soil. Integrated control has thus the advantage over exclusively chemical control in that there is less risk of development of fungicide resistance when the combined effect of a biological control agent and a fungicide is used to manage a disease.

Development of fungicidal resistant mutants:

When the naturally available *Trichoderma* is not meeting the required qualities of becoming an efficient biocontrol agent, there is a need to improve the efficient strains for wanted traits. The genetic improvement of *Trichoderma* is generally done to enhance the biocontrol potential to enhance the enzyme production for industrial application, to induce the tolerance for agrochemicals particularly fungicides, to induce the production of novel compounds and to enhance the survival potential in soil and other ecological niches. Abd-El-Moity *et.al.*, (1982), presented evidence that it was feasible to develop strains of

T.harzianum tolerant to fungicides by prolonged and repeated exposure serially from lower to higher concentrations of fungicides. Exposure of four wild type strains of *T.harzianum* to the fungicides, Chlorothalonil, Iprodione, Procymidone and Vinclozolin resulted in the selection of several isolates tolerant to these fungicides (Abd-El Moity, 1982; Papavizas, 1980).

Induced mutagenesis:

Mutation to increase the tolerance of antagonists to fungicides was not used until the earlier 1980s. Papavizas *et.al.,* (1982) was the first to report the induction in *T.harzianum* to benomyl by UV light irradiation and selection that may be used for biological control of soil borne plant pathogens. Mutagenesis may be induced physically by UV and γ rays or chemically by N-Methyl, N-nitro-N-Nitrosoguanidine (NTG), Ethyl Methyl Sulphonate (EMS) and Ethylemine mutagens. Many variants of *T.viride* and *T.harzianum* from their respective wild parents were developed using the physical and chemical mutagens. These new biotypes were tolerant to the fungicides especially the benzimidazole groups (Papavizas and Lewis, 1983; Kumar and Gupta, 1999).

Reports are available on the use of γ rays to improve the biocontrol strains of *Trichoderma* species. Mukherjee and Mukhopadhyay (1993), obtained stable mutants of *T. virens* through gamma irradiation. Ahmad and Baker (1987a) obtained 1000ppm benomyl tolerant mutants by mutating *T. harzianum* using NTG. Papavizas *et.al.,* (1990) obtained mutants of *T. virens* by exposing the conidia of *T. virens* to both UV and EMS three times in succession. Using UV irradiation, Bensaci and Newman (1989) developed mutants of *Trichoderma* spp. which tolerated ipridione and vinclozolin. Viji *et.al.,* (1993), obtained biotypes of *T. harzianum* and

T. longibracheatum using UV irradiation which tolerated 16 µm Carbendazim. Mighelli *et.al.,* (1994) induced hygromycin B or Propiconazole resistance in *Trichoderma* spp. by UV irradiation. Peechia (1994) obtained propiconazol and vinclozolin tolerant mutants of *T. harzianum*. Rajappan (1997) obtained mutants of *T. viride* by UV irradiation with increased biomass, conidia and chlamydospores when compared to the wild type. Kumar (1997), obtained tebuconazole tolerant mutants of *Trichoderma* spp. by UV and gamma irradiation effective against *Macrophomina phaseolina,* the mung bean rot pathogen. Sinha and Upadhyay (1993) could obtain a mutant of *T. viride* by using N-methyl N'-nitro N-nitrosoguanidine (NTG). Strainal improvement in *Trichoderma* both for industrial and

agricultural use was attempted by UV mutation and protoplast regeneration / fusion (Anne, 1992; Davis, 1985; Gracheck and Emert, 1984; Harman and Stasz, 1991; Lalithakumari, 1996; Lalithakumari *et.al.,* 1995).

Altered metabolite production by mutagenesis:

Although there are very little published reports on the fundamental aspects of mutagenesis performed with the industrial fungi, mutants giving rise to the increased production of various metabolites or catabolic enzymes have been produced (Ball, 1973; 1980; 1984a; Piccatiago, 1983). Improved strains of *T. viride* were isolated by mutation and selection and these produced more cellulase than did the wild strains (Montenecourt and Eveleigh, 1977; Piccatiago, 1983). Mandels *et.al.,* (1971), irradiated *T. viride* with high energy electrons from a linear accelator and produced a new strain that secreted twice as much cellulase as the parental strain. Bien and Witkowska (1991) used ethylene to develop mutants of *T. viride* for enhanced cellulase production. Increased endo and exoglucanase production was observed among the mutants of *Trichoderma* spp. induced by UV and γ rays, NTG and Ethylenemine (Witkowska and Bien, 1991; Witkowska, 1993). Czapowska *et.al.,* (1992) obtained mutants of *T. reesei* producing more cellulase than the wild type. Sinha and Upadhyay (1993) obtained biotypes of *T. viride* using NTG and these sporulated better than the wild type. Using UV rays, Graeme Cook and Faull (1991) and Faull *et.al.,* (1994) mutated *T. harzianum* for altered antibiotic production. Similar mutants of *T. virens* were developed by Howell and Stipanovic (1983). Dinakaran and Marimuthu (1997), obtained nine mutants of *T. viride* using different mutagenic agents with increased antibiotic production. In the recent times genetic improvement of *Trichoderma* as a biocontrol agent using induced mutagenesis has been successfully attempted for fungicidal resistance (Papavizas *et.al.,* 1982), antibiotics production (Faull *et.al.*, 1994), and enzyme secretion (Witkowska and Bien, 1991). Very often the random mutation for fungicidal resistance, using either physical or chemical mutagens are pleiotrophic, which results in alterations in other non targeted traits of an organism. Attempts were made to improve the biocontrol efficacy of the biocontrol agents by mutagenesis. For this both chemical mutagenesis, using the mutagen Ethyl Methyl Sulphonate as well as the physical mutagenesis, using the Ultra Violet Radiation were tried. Experiments conducted by Papavizas *et.al.*, (1990) have revealed that the usage of EMS have produced mutants with greater vigour when compared to the wild type. Studies have also revealed that the

biocontrol efficacy of certain isolates of *Trichoderma* were increased by exposure to ultraviolet radiation (Graeme Cook and Faull, 1991., Viji *et.al.,* 1993, Kay and Stewart, 1994).

Antagonistic potential of fungicidal resistant mutants:

Genetic modifications of BCAs offer the potential for producing improved bioprotectants. In addition to fungicidal resistance, mutation induced other effects in the biotypes such as altered growth and sporulation, rhizosphere competence and enhanced biocontrol capabilities (Papavizas and Lewis, 1983). Papavizas *et.al.*, (1982), used the ability of *Trichoderma* spp. to tolerate fungicides such as benomyl, thiabenzadole (TBZ) as a marker in a selection system following induced mutagenesis with UV light. Wild type strain of the two biocontrol agents were very sensitive to these fungicides. Using this approach, Papavizas and Lewis (1983) and Papavizas *et.al,*. (1982) developed several genetic variants from wild strains of *T. viride* and *T. harzianum* respectively. The new biotypes tolerated high concentrations (upto 500μg / ml) of benomyl as indicated by growth and conidial germination in fungicide amended culture media. Metalaxyl resistant *Trichoderma* performed better than the wild type in the control of *P. aphanidermatum* in sugar beets (Mukhopadhyay and Chandra, 1986) and *F. solani* f. sp. *pisi* in pea (Kraft and Papavizas, 1983). Combinations of iprodione and iprodione tolerant strains of *T. harzianum* suppressed the white rot of onions caused by *Sclerotium cepivorum* more effectively than with the treatment alone (Abd-El-Moity, 1982). The possibility of combining *T. aureoviride* and tebuconazole for the management of *Sclerotium rolfsii* in soyabean was suggested by Dharmaputra and Retnowati, (1994).

Considerable progress has been made in a very short period of time in developing new effective biotypes of biocontrol agents by adaption and genome modification through mutagenesis. It is equally important that it is possible to induce new biotypes of biocontrol agents that differ from wild strains in many respects including the important parameter of compatibility with fungicides for integrated pest management programs. It may be possible to develop multiple pesticide tolerance in strains of biocontrol agents that would be more desirable than single fungicide tolerant strains. The tolerant strains may be thus used successively in association with minimal amounts of chemicals for disease control.

***In vitro* / Green house experiments:**

Several UV induced biotypes or biotypes induced by chemical mutagenesis of *Trichoderma* spp. differed from their respective wild type strains not only in tolerance to benomyl, but also in growth characteristics, sporulation and survival in soil (Papavizas and Lewis, 1983; Papavizas *et.al.,* 1982*).* A few mutants of *T. harzianum* and *T. viride* that were tolerant to benomyl were also more effective than their respective wild type strains in suppressing the competitive saprophytic activity of *R. solani* in soil, damping off of Peas caused by *P. ultimum,* damping off of cotton and radish caused by *R. solani,* white rot of onions caused by *S. cepivorum* and damping off and blight of beans caused by *S. rolfsii.* One UV induced biotype of *T. viride* that possessed tolerance to benomyl effectively suppressed the wilt of Chrysanthemum caused by *Fusarium oxysporum* f. sp. *chrysanthemi* in green house cultures when repeated use of benomyl is critical for the control of foliar pathogen (Locke *et.al.,* 1985). Sinha and Upadhyay (1993), observed that the white coloured mutant of *T. viride* possessed better antagonistic as well as biocontrol ability against *F. udum.* Cassiolato *et.al.*, (1997), reported the parasitism of *Sclerotiana sclerotiarum* and *Sclerotium minor* by *T. harzianum* mutant in the segments of celery. Michalikova and Michrina (1997) reported that the mutant of *T.harzianum* was the most effective mycoparasite that contributed to the biocontrol of *F. culmorum.* Mohammed and Fahmy (1988), reported that the mutants of *T. harzianum* reduced the percentage of white rot infected onion plants, in the pot culture experiments. Studies of Selvakumar *et.al.*, (2000) reported that the mutants of *T. viride* showed better antagonistic potential by reducing the chlamydospore germination. Baby (1998), observed that the mutant strains of *Trichoderma* were able to control *R. .solani* in rice better than the parental strains. Dinakaran and Marimuthu (1997), reported that two mutants with maximum volatiles reduced the mycelial growth of *M. phaseolina.* Mukherjee *et.al.,* (1999), reported that a mutant of *T. pseudokoningii* was very useful for the biological control of the plant pathogen, *Sclerotium rolfsii (Corticium rolfsii)* in ginger rhizomes under post harvest conditions.

Field Experiments:

Many of the promising results obtained in biological control were achieved in the field also. Several fungicide tolerant isolates were obtained through selection and mutagenesis and used in integrated control (Mukherjee *et.al.,* 1997). Similarly instances of

successful management of diseases in the field by the integration of biocontrol agents and the fungicidal seed / soil treatments are available. Application of PCNB and *T. harzianum* improved the control of *Sclerotium rolfsii* in peanuts (Chet *et.al.*, 1979). Likewise, soil application of PCNB @ 4 µg /kg of soil added with wheat bran preparation of *T. harzianum* had an additive effect on the disease control and synergistic effect in reducing the inoculum density of *R. solani* propagules (Henis *et.al.*, 1978). Lifshitz *et.al.,* (1985) obtained additive effect on the control of *Rhizoctonia* pre-emergence damping off by combined seed treatment with *T. harzianum* and soil mix of fungicide Fenodanil. Enhancement in disease control was obtained when soil application of *T. harzianum* was integrated with fungicidal treatment with Triadimenol (Cole & Zvenejka, 1988). Additive effect was also obtained in the control of dry root rot of sugarbeet when Carbendazim soil application was combined with *T. harzianum* or *T. viride* (Vyass, 1994). Howell (1997), evaluated the field efficacy of Viridiol (-) mutants of *T. virens* for biocontrol of cotton seedling diseases. It was found that though these mutants lost their ability to synthesise the phytotoxin, viridiol, they retain their capacity to produce antifungal antibiotics and act as mycoparasites and control cotton seedling disease incited by *Rhizoctonia solani.* Mukherjee *et.al.,* (1997), successfully enhanced the efficacy of benomyl tolerant mutants of *T. viride* for biological control of *Botrytis* grey mould of chickpea. Mukherjee *et.al.*, 1999, evaluated the antagonistic and biocontrol potential of stable benomyl tolerant mutants of *T. pseudokoningii.*

Mass multiplication of fungal antagonists and methods of application:

The development of a stable, cost–effective and easy to apply biocontrol formulation is critical for the advancement of biological control of plant pathogens with introduced antagonists (Lisansky, 1985). The success of biological control lies in the stabilization of the biocontrol organisms in the rhizosphere once it is introduced and this necessitates the development of an economic and easier technique of mass multiplication.

Papavizas and co-workers developed a technically simple system for liquid fermentation and production of alginate pellets that worked well for their biocontrol agents, *T. hamatum, T. harzianum, T. viride* and *G. virens* (Lewis & Papavizas, 1985; Papavizas *et.al.*, 1984). Backman and Rodriguez–Kabana (1975) developed a semisolid fermentation system using molasses enriched clay granules as food base for the antagonists in formulations designed to prevent soil borne diseases. Hadar *et.al* (1979 a, b) used wheat

bran for growth, sporulation and delivery. The preparation contained 2.9 x10^9 conidia / g of dry weight. Sivan *et.al.,* (1984) improved the growth system by adding peat.

Tribe and Ahamed (1975); Tronsmo (1989b) used another fermentation method of mass multiplication using barley autoclaved in plastic bags as the solid support and nutrient for the mass production of the biocontrol agent. The grains create a large surface area and under the right humidity conditions 5 x 10^8 conidiospores / g of barley were produced after 14 days at 21^0c.

Huang *et.al.,* (1981) grew *Trichodedrma* on various autoclaved seeds at room temperatures for several weeks. Dun *et.al.*, (1983), performed liquid fermentation for the large scale production of *Talaromyces* and *T. hamatum.*

Kousalya and Jeyarajan (1990) multiplied *T.viride* and *T.harzianum* on 18 agricultural wastes / by products for investigation. *T .viride* and *T. harzianum* produced maximum colony forming units of 27.2 x10^6 and 26.1 x 10^6 / g respectively on tapioca rind and tapioca refuse. FYM also supported good sporulation of *T. viride* and *T. harzianum.* Panicker and Jeyarajan (1993) used different substrates viz rice bran, wheat bran, peat soil, FYM and rice straw for the mass multiplication of *Trichoderma* spp. *T. viride* population was highest on FYM ie 503 x 10^6 cfu / g compared to 280 x 10^3 cfu /g in peat soil after 7 days of incubation. The efficacy of coffee husk for the multiplication of *Trichoderma*was reported (Indu and Sawant, 1990). Sarma *et.al.*, (1996) reported that the fungal antagonists multiply rapidly on Sorghum meal, coffee husk, sawdust, coir pith and in coconut water.

Shelf life of the biocontrol organism:

For the commercialization of the biocontrol agents the loss of the viability of the propagules is one of the major constraints (Mukhopadhyay *et.al.*, 1992). Fravel *et.al.,* (1985) formulated the sodium alginate *T. harzianum* pellet which was found to be effective even after 2 months of storage. Similarly Jeyarajan *et.al.,* (1994) observed that talc and kaolin clay was found to be the best in retaining *Trichoderma* propagules upto three months.

Methods of application of the biocontrol agents:

According to Chet (1987), there are four different techniques for the application of the biocontrol agents (Table 4) and each has shown to be effective in the field conditions. While the broadcast application is rarely if ever economical, the other three especially those

methods that introduce the antagonists with the planting material are economical. Chet and Baker (1980) found that the minimal effective amount of *Trichoderma* is about 1 x 10^6 cfu/g of soil.

Table 4: Methods of application of the biocontrol agents:

Method	Explanation
Broadcast	*Trichoderma* preparation is broadcast on the surface and incorporated into the infected soil.
Furrow	The preparation is put into the infested soil in the planting furrow.
Root zone	*Trichoderma* is loaded into the root zone by mixing before transplanting seedlings in an infested furrow
Seed coating	Seeds are coated with *Trichoderma* spores using an adhesive.

Generally the different techniques employed for the introduction of antagonists into the rhizosphere are seed application, root application and soil application. Seed application might prove to be the most successful means of biological control of externally seed and soil borne pathogens (Mukhopadhyay *et.al.*, 1992) and it is the cheapest method for the delivery of antagonists to the rhizosphere of the crop plants to be protected from soil borne diseases (Cook & Baker, 1983). Mukhopadhyay *et.al.*, (1992) observed that the integration of the biological and fungicidal seed treatments with *Trichoderma viride / T. virens* improved the biocontrol potential to a greater extent. It involved treating the seeds first with the antagonists, *T. virens* and then with the fungicide carbendazim against the pathogens viz *Sclerotium rolfsii*, *F. solani* and *F. oxysporum* in chick pea, lentil and ground nut. Disease incidence was reduced when the seed bulbs of onion were treated with the antagonistic fungi viz *T. viride, T. harzianum, T. koningii & T. pseudokoningii* tested in both green house and field conditions against *F. oxysporum* f.sp.*cepea* (Flori and Roberti, 1993). Seed dressing with *Trichoderma* reduced the storage rots of ginger seed rhizomes considerably (Sarma *et.al.,* 1996) .

A great deal of work has been done on the biocontrol of soil borne plant pathogens by the incorporation of the biocontrol agents into the soil using organic amendments. Sivan *et.al.,* (1984) reported that the soil application of the wheat bran-peat based inoculum of *Trichoderma* effectively controlled the damping off caused by *Pythium aphanidermatum* in

pea, cucumber, tomato and pepper seedlings. Soil application of *T. harzianum* and *T. viride* significantly reduced the root rot in sesamum (Sethuraman, 1991), root rot of black gram (Rukumani and Mariappan, 1994), capsule rot of small cardamom (Suseela Bhai *et.al.,* 1994) and root rots of ground nut and chick pea and cotton (Jeyarajan & Ramakrishnan, 1995). The disease suppressive role of *Trichoderma* spp. in black pepper, *P. capsici* systems by the soil application was established in the pot culture and in the field conditions (Anandaraj & Sarma, 1994; Rajan, 2000; Rajan *et.al.*, 2002). Application of the biocontrol agents to the soil after solarisation resulted in reduced rhizome rot incidence and increased yield (Usman *et.al.*, 1996). Usman (1997), could also establish the role of *Trichoderma* in controlling rhizome rot of ginger.

Root dip application of the fungal antagonists has not been commonly practiced. However root application in the flower crops such as carnation had controlled the root rot caused by *R.. solani* by more than 70%. Hadar *et.al.,* 1984 reported that this method was superior to and economical when compared to the soil application of the inoculum. In the case of the bacterial antagonists the root dip application is more feasible and economical.

***Trichoderma* as a plant growth promoting microorganism:**

Many rhizobacteria have been reported to stimulate plant growth (Shroth and Hamcock, (1982). Baker *et.al.,* (1984), found that *Trichoderma* can also stimulate the growth in the plants including various floricultural and horticultural plants (Chang *et.al.*, 1986). Pepper seeds germinated two days earlier in raw soil containing the fungus than in untreated controls. Flowering of periwinkle was accelerated, the number of blooms on chrysanthemums was increased and the heights and weights of other plants were greater in either steamed or raw soil infested with *T.harzianum.* If *Trichoderma* is used to promote plant growth, the isolates should be resistant to the common fungicides used in agricultural practices and isolates tolerant to benomyl (Ahmed & Baker, 1987) PCNB or Captan (Elad & Chet, 1983) have been isolated. Growth stimulation by *Trichoderma* could be the result of the production of growth hormones by the fungus, increased uptake of the nutrients by the plants or the control of one or more sub clinical pathogens (Chet, 1990). Kleinfeld 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. Inbar *et.al.*, (1994) were able to demonstrate plant growth promotion by *T.harzianum* isolate T-203 in a commercial green house system.

Shivanna *et.al.,* (1996), observed that many isolates including *Trichoderma* strains were shown to promote growth of wheat and soyabean under greenhouse condition. However, the plant growth promoting fungi were less effective when applied in field, but increased yield was observed in response to some isolates (Shivanna *et.al.,* 1994).

Integrated Disease Management:

The suppression of the pests and plant diseases using the biological control techniques can provide a reliable and alternative aid to pesticide use. Among the various fungal antagonists known, *Trichoderma* spp. have drawn world wide attention and are considered as the most promising biocontrol agents.The potential of *Trichoderma* spp. can be used to augment the native soil population or seed coating to control soil borne pathogens (Papavizas and Lumsden, 1980; Mukhopadhyay *et.al.*, 1992).

The effectiveness of the biological control can be mainly grouped under three categories- the first being the break down of host resistance, secondly the failure of the fungicides in the role of resistance built up on the pathogen and thirdly the deleterious effects of these pesticides, agrochemicals on the beneficial organisms and to the environment.

To overcome these problems and to meet the future challenges, for better production and sustainable agriculture viable approaches such as integrated effective, safe and economical methods are essential.

Integrating BCAs with the sublethal doses of fungicides seems to be a very promising way of controlling the pathogens with minimal interference in the biological equilibrium (Chet, 1987). Combining antagonists with fungicides have yielded significantly more disease control than the other control methods in crops like sugar beet, tobacco, tomato, lentil, potato, chick pea etc (Mukhopadhyay *et.al.*, 1986; Kaur and Mukhopadhyay, 1992). The effectiveness of the integrated disease management trials was also established in Pepper (Sarma *et.al.*, 1994, Rajan *et.al,* 2002., Saju, 2004). The trial carried out at the Indian Institute of spices Research, Calicut also highlights the effectiveness of the Integrated Disease Management in many other crops such as Ginger (Sarma, 1994, Usman *et.al.,* 1996; Balakrishnan, 1997).

Thus a modest level of host resistance combined with other partially effective control measures may be quite essential for effective control. Phytosanitary measures such as use of seeds free of infection, use of vegetative propagative materials, effective sanitation, use of biocontrol agents, application of organic soil amendments, judicious use of contact systemic fungicides may be included in the integrated disease management.

MATERIALS AND METHODS

1. General:

All the *in vitro* experiments were conducted in aseptic conditions. The equipments used for the experiments which included glassware, inoculation loops and needles, syringes etc were sterilized by keeping them in an hot air oven at 180^0C for at least six hours before starting each experiment.

1.1. Glassware:

Borosil and Corning glasswares were used throughout the experiments.Tarson's disposable petriplates and microtips were used for serial dilution.

1.2. Cleaning / Sterilization of the glasswares:

The glasswares used for all the experiments were thoroughly washed with potassium dichromate or cleaning solution and washed with tap water. Later they were rinsed with distilled water, allowed to dry and sterilized in the hot air oven at 180^0C for 6 hours.

1.3. Replication and incubation:

Three / four replications were maintained for each treatment, in the *in vitro* experiments and the petriplates were incubated at room temperatures (28-32 ^{0}C) unless otherwise specified.

1.4. Media used for the different experiments:

The media required for the various experiments were prepared in steel containers and distributed equally into 250 ml. conical flasks @ 100 ml / flask and autoclaved at 15 psi pressure for 20 minutes. The following were the media used for the different experiments.

1.4.1:Potato Dextrose Agar-PDA (Ricker and Ricker,1936)

Composition:

Potato - 200g

Dextrose- 20g

Agar - 17g

Distilled Water- 1000ml

Peeled potato were sliced and boiled in 500ml distilled water for half an hour. It was later filtered using a muslin cloth. Dextrose was added and the extract was made upto 1 litre. Agar powder was added gently and stirred well using a glass rod to get uniform distribution. The media thus obtained was dispensed equally into 250 ml. @ 100 ml / conical flask and sterilized.

1.4.2:Rose Bengal Agar-RBA (Martin, 1950)

Composition:

Agar -	20g
Potassium di hydrogen ortho phosphate-	1.0g
Magnesium sulphate-	0.5g
Peptone-	5.0g
Dextrose-	10.0g
Rose Bengal-	0.033g
Streptocycline-	0.03g
Distilled Water-	1000ml

All the chemicals except Rose Bengal and Streptocycline were dissolved in distilled water. The mixture was heated slowly and stirred with a glass rod until it started to boil. To the mixture Rose Bengal was added and the media was sterlised after it was equally dispensed into 250 ml conical flasks.

1.4.3:*Trichoderma* Specific Medium-TSM (Elad and Chet,1983)

Composition:

Glucose-	3g
Magnesium sulphate-	0.2g
Dipotassium hydrogen phosphate-	0.9g
Ammonium nitrate-	1.0g
Potassium Chloride-	0.5g
Rose Bengal-	0.033g
Metalaxyl-	0.3g
Penta Chloro Nitro Benzene-	0.2g
Chloramphenicol-	0.25g
Agar-	15g

Distilled Water- 1000ml

All the chemicals except Rose Bengal, Metalaxyl, PCNB and Chloramphenicol were dissolved in distilled water. The mixture was heated slowly while stirring until it started boiling. Later on Rose Bengal and Metalaxyl were added and sterilized after it was equally distributed into 250 ml conical flasks. Chloramphenicol was added into the cooled and molten medium before being poured into the petriplates.

1.4.4: P_{10}VP Medium (Tsao and Ocana, 1969)

Composition:

Pimaricin-	10ppm (0.5ml)
Vancomycin-	200ppm (222mg)
or	
Ampicillin-	12mg
PCNB-	100ppm (145mg)

100 ml of distilled water and a reagent bottle covered with carbon paper were sterilized. The chemicals were weighed out into the reagent bottles and it was dissolved by adding sterile distilled water. The preparation was kept in dark to avoid photodegradation of antibiotics.

10 ml of the above solution was added to 90 ml of sterilized potato dextrose agar and stirred thoroughly before pouring the medium.

1.4.5: Czapek Dox Agar-CDA

Composition:

Sodium nitrate ($NaNO_3$) -	2g
Potassium di hydrogen ortho phosphate (KH_2PO_4) -	1.0g
Magnesium sulphate ($MgSO_4.7H_2O$)-	0.5g
Potassium Chloride (KCl) -	0.5g
Ferrous Sulphate ($FeSO_4.7H_2O$) -	0.01g
Sucrose -	30.0g
Bacto Yeast Extract -	0.5g
Agar -	20.0g
Distilled Water -	1000ml

When glass distilled water was used the following trace elements were added.

Zinc Sulphate (Zn $SO_4.7\ H_2O$) -	0.01g
Copper Sulphate ($CuSO_4.5H_2O$ -	0.005g

All the chemicals were added to the distilled water. Agar was added slowly and stirred uniformly. The media was distributed equally into 250ml conical flasks and sterilized at 15 psi pressure for 20 minutes.

1.4.6: King's B - Modified medium (King *et.al.,* 1954)

Composition:

Proteose Peptone -	20g
Dipotassium hydrogen phosphate -	1.5g
Magnesium Sulphate -	1.5g
Glycerol -	10 ml
Agar -	15g
Distilled water -	1000ml
Cyclohexamide -	100ppm (0.1g)
Ampicillin -	50 ppm (0.05g)
Chloramphenicol -	12.5ppm (0.0125g)

All the chemicals except glycerol and the antibiotics were added to the distilled water and boiled. Agar was added slowly and stirred continuously using a glass rod. Glycerol was added to the mixture after boiling. The media was dispensed equally in 250 ml conical flasks and sterilised. The antibiotics were added just before pouring the media into the petriplate

1.4.7: Nutrient Agar Medium (NA)

The Nutrient Agar medium of Hi Media was used in the present study. The medium contained the following ingredients.

Composition:

Agar-	15g
Beef Extract-	3g
Peptone-	5g
Sodium Chloride (NaCl)	5g
Distilled Water-	1000ml

For the preparation of nutrient broth for the culture of bacteria, all the chemicals except agar was weighed out.

1.4.8: YGCA Medium

Composition:

Yeast Extract -	10g
Glucose / Dextrose-	10g
Calcium Carbonate ($CaCO_3$) -	20g
Agar -	20g
Distilled Water -	1000ml

All the chemicals were weighed out into a container containing distilled water. Agar was added slowly and stirred continuosly with a glass rod so as to get uniform mixing. The media was distributed into 20 ml test tubes and sterilized at 15 psi pressure for 20 minutes in the autoclave and maintained as slants for the inoculation of bacteria.

1.4.9: Succinic Acid Medium (SA)

Composition:

Dipotassium Hydrogen Phosphate (K_2HPO_4)-	6g (0.6%)
Potassium Dihydrogen ortho Phosphate (KH_2PO_4)-	3g (0.3%)
Ammonium Sulphate{$(NH_4)_2SO_4$}-	1g (0.1%)
Magnesium Sulphate ($MgSO_4.7H_2O$)-	0.2g (0.02%)
Succinic Acid-	4g (0.4%)
Distilled Water-	1000ml

Weighed out the above chemicals into a container containing distilled water and dissolved. The pH of the medium was adjusted to 7.2 with 2N NaOH.

2. Isolation and identification of pathogens associated with rhizome rot:

2.1. Field Survey:

A field survey was undertaken to record the incidence of rhizome rot disease and also for the isolation of biocontrol agents associated with the ginger crop in some of the major ginger growing areas of Kerala viz. Calicut, Ernakulam, Kottayam, Idukki and Wayanad districts. A total of 150 fields were surveyed in these districts.

2.2. Isolation of pathogen from the infected parts:

The 101 diseased samples collected from the infected fields were thoroughly washed in tap water. The infected plant parts i.e., the parts with the lesions were identified. From these infected areas, small bits were cut out from the advancing margins of the lesions with a small portion of the healthy tissue. Tissue bits were cut out both from the infected rhizomes as well as the pseudostems. These bits were washed in sterile distilled water three times, surface sterilized with 0.1% $HgCl_2$ and plated on Potato Dextrose Agar supplemented with $P_{10}VP$ (Tsao & Ocana,1969) for the isolation of pathogen, *Pythium aphanidermatum,* causing rhizome rot of ginger.

2.3. Isolation of biocontrol agents (BCA).

The roots of the healthy samples were washed in tap water. They were cut into small bits and plated on different media for the isolation of the different biocontrol agents. For the isolation of *Trichoderma* the root bits were washed with sterile distilled water and plated on *Trichoderma* Specific Medium (TSM) (Elad & Chet, 1983). The fluorescent pseudomonads were isolated by plating the healthy root bits on King's B medium (King *et.al.,* 1954).

2.3.1: Isolation of BCAs from Rhizosphere soil (Johnson & Curl, 1972)

Soil samples were collected from the rhizosphere and air dried for 24 hours and sieved through a 2mm sieve. 20 mg of this sieved soil was transferred to a petri plate and spread uniformly under aseptic conditions. Rose Bengal Agar medium (Martin, 1950) was added on to the petriplates and the plates were swirled uniformly to get uniform distribution of the soil. The plates were incubated at 28^0C for three days.

2.3.2: Isolation of BCAs from plant parts:

The healthy root bits and rhizome bits were uniformly cut out and washed with sterile distilled water thrice and blotted on sterile blotting paper. They were subsequently plated on the King's B medium (King *et.al.,* 1954) and incubated for two days. After 48 hours the plates were removed and kept inverted under the UV lamp in the laminar flow. Those bacteria which emitted a flourescence were marked. Those bacteria were subsequently transferred to YGCA slants for further use. Similar bits were plated on *Trichoderma* Specific Medium (Elad & Chet, 1983) for the isolation of *Trichoderma.*

2.4.1: Identification of the fungal biocontrol agents.

All the isolates were grown on PDA in 9 cm petriplates and incubated at 25^0C for two days. They were allowed to sporulate. Slides were prepared from the sporulating area just before the advancement of the colony. The preparation was stained with cotton blue and the different species of *Trichoderma* were identified. Similarly the other fungal agents were also identified.

2.4:2: Identification of the bacterial isolates:

The identity of the fluorescent pseudomonads were further confirmed with the Succinic Acid test. The succinic acid medium was taken in 30 ml vials and sterilized. One loop full of the bacterial inoculum was suspended in the vials containing the medium. The change in the colouration of the medium was noted. Succinic acid medium which was colourless turned green when inoculated with the loop full of bacteria.

2.4.3: Gram's staining technique for bacterial identification:

The bacterial cultures were smeared on separate slides and allowed to dry. The slides were warmed and covered with crystal violet for 30 seconds. The slides were washed with distilled water for a few seconds using wash bottle. Later on they were covered with iodine solution for 30 seconds. Then ethyl alcohol was added drop by drop until no more colour flowed from the smear. The slides were washed with distilled water and allowed to drain. Safranin smear was applied for 30 seconds. Again the slides were washed with distilled water and blotted dry. The slides were allowed to air dry. Observations were made using the immersion objective. Those bacteria that appeared blue were referred to as gram +ve and those appearing pink were described as gram-ve.

2.4.4: KOH Test:

The gram –ve nature of the bacteria were further confirmed with the KOH test. 4% potassium hydroxide solution was prepared. A drop full of the medium was put on a clean glass slide. One loop full of the bacteria was brought to the surface of the slide on which the KOH drop was added and thoroughly mixed. The inoculation loop was then lifted to about 3 cms height to note if there was any thread like appearances.

2.4.5: Sensitivity to antibiotics:

Five different bacterial isolates that were found promising after the initial round of screening were subjected to the antibiotic sensitivity test. 20 ml of the nutrient agar medium was poured into 100ml conical flasks. 20 μl of the antibiotics, Streptomycin, Chloramphenicol, Kanamycin, Ampicillin and Tetracyclin was added to each of the conical flasks containing the nutrient agar medium, so that the composition of Kanamycin was 50ppm, Chloramphenicol - 20ppm, Streptomycin-100ppm, Ampicillin-100ppm and Tetracyclin-15ppm. A single colony of the test bacteria was picked up from each petriplate and dissolved in 400 μl sterile distilled water. A loop full of the bacteria was taken and streaked on the antibiotic amended media. Observations were recorded after 48 hours.

3: Assesment of the bioefficacy of the biocontrol agents (BCA) isolated:

The efficacy of the biocontrol agents agents isolated was tested both *in vitro* and *in vivo*

3.1: *In vitro* screening of the biocontrol agents:

In vitro screening of the organisms for their antagonistic potential was carried out by adopting the dual culture technique (Webber and Hedger, 1986). For this 5mm culture disc of the test organism (fungal biocontrol agent) and the pathogen, *Pythium aphanidermatum* were simultaneously inoculated on the two sides (5 cm apart) in a 9 cm petriplate on PDA under aseptic conditions. The radial growth of the pathogen was measured after 24, 48 and 72 hours. A control was also simultaneously kept. For screening the bacterial biocontrol agents, the pathogen was inoculated at the centre of the petri plate and a loop full of bacteria were streaked on either side of the inoculated pathogen at a distance of 2 cms. A control plate, with only the pathogen inoculated at the centre of the petri plate was also simultaneously kept. Percentage of inhibition was calculated using the formula $\frac{C-T}{C} \times 100$.

Sixty eight bacterial isolates and 109 isolates of *Trichoderma* were screened *in vitro* and the promising isolates were shortlisted.

3.2: *In vivo* screening in green house:

After the preliminary screening of the biocontrol agents *in vitro*, 26 bacterial isolates, 57 isolates of *Trichoderma* (those showing inhibition above 30 % under the lab conditions)

were shortlisted. These isolates, in addition to 116 isolates from the IISR repository of biocontrol agents were screened *in vivo.* The 116 isolates of *Trichoderma* were the isolates obtained from the ginger field survey conducted earlier (Usman, 1996). Potting mixture (Forest soil: sand: FYM) in the ratio of 3:1:1 was filled in poly propylene bags of 6x12" diameter. Plants raised in these bags were used for *in vivo* testing.

3.2.1 :Screening of bacteria

The 26 bacterial isolates that were shortlisted after the preliminary screening by the dual inoculation technique were screened initially in polybags in the greenhouse. From this experiment 10 bacterial isolates (showing a disease incidence of <30%) were shortlisted and they were again subjected to a second round of screening in earthern pots filled with the potting mixture. In this experiment two different experiments were set up.

a) To study the growth promotion effect: By seed treatment with the biocontrol agent alone.

b) To study the disease suppression: By challenging the seed rhizomes with the pathogen and biocontrol agent.

3.2.1.1: Preparation of the test culture:

The different bacterial isolates were grown on nutrient agar medium. From 48 hour old culture, the bacterial growth was gently scraped and suspended in sterile water and was made upto 150 ml The OD values of this bacterial suspension at 660 nm were also recorded. This suspension showed CFU value of 16 $X10^8$. This inoculum was used uniformly for all experiments. Healthy seed rhizomes were dipped in the bacterial spore suspensions for half an hour, air dried and planted in polypropelene bags of size 6 x 12" containing potting mixture. Two bits of the seed rhizomes were planted in each bag. Five replications were maintained for each treatment. The containers were covered over by the green mulch which composed of the green leaves of *Glyricidia maculata.* After 30 days of growth, the sprouts were challenged with the pathogen *Pythium aphanidermatum.*

3.2.1.2: Preparation of *Pythium aphanidermatum* inoculum:

The pathogen inoculum was prepared as follows. 150 ml of Potato Dextrose broth was prepared in Raux bottles of one litre capacity. Three discs of 15 mm diameter from 48 hour old culture of *Pythium aphanidermatum* were cut out from pure culture of the pathogen

grown on petriplate containing PDA medium and inoculated in to the Potato dextrose broth. It was allowed to grow for 7 days. After 7 days the mycelial mat was harvested. It was blended in a mixer and made upto one litre (from 150ml broth). C.F.U determined ($56X10^3$units/ml) by plating 1ml of the serially diluted inoculum on PDA medium amended with P_{10} VP. The plants in polybags were inoculated @10 ml / bag. The inoculum was applied around the base of the pseudostem and later covered with soil.

Observations such as the percentage of germination, percentage of disease incidence, and height of the plants were recorded every week in the poly bag experiment and the results were analysed statistically. In the pot culture experiment, growth parameters like the percentage of germination, no. of sprouts / pot, disease incidence, yield / pot were recorded. The results obtained were statistically analysed using the MSTATC package.

3.2.2: Screening of *Trichoderma* isolates:

The 57 isolates of *Trichoderma,* out of the 109 isolates, obtained from the field survey which were shortlisted by *in vitro* screening were tested *in vivo* in the green house. In addition to this 116 isolates from the IISR repository of biocontrol agents were screened. These included 8 species of *Trichoderma* i.e., 10 isolates of *T. polysporum*, 6 isolates of *T. longibracheatum*, 14 isolates of *T. hamatum*, 26 isolates of *T. aureoviride*, 28 isolates of *T. virens*, 22 isolates of *T. harzianum*, 9 isolates of *T. pseudokonongii* and 1 isolate of *T. viride*. After the initial round of *in vivo* screening (57 isolates from the field survey and 116 from the repository), those showing a disease incidence of <10 % as compared to 80% in control were again screened in the polypropelene bags in the green house.

3.2.2.1: Preparation of the inoculum and *in vivo* testing for the bioefficacy of the antagonists:

Polypropelene bags of size 6x12" were filled with potting mixture. The *Trichoderma* antagonists were grown on PDA medium for four days. From four day old cultures spore / mycelial suspensions were prepared and the no. of colony forming units were determined (150 ml /plate CFU 40-80 $x10^8$ units /ml). Healthy ginger seed rhizomes were dipped in the spore suspension for half an hour, air dried and planted in poly bags filled with 800g potting mixture. Three bits were planted in each bag and three replications were maintained for each treatment. The poly bags were then covered over by green mulch of *Glyricidia maculata*.

3.2.2.2: Preparation of the pathogen inoculum:

The biocontrol treated sprouts were challenged with the pathogen after 30 days of growth. The procedure adopted was as described earlier (3.2.1.2). The growth parameters viz the percentage of germination, height of the plants and disease incidence were recorded and those isolates showing increased bioefficacy were shortlisted.

3. 3: Purification and Maintenance of the Biocontrol Agents:

The fungal isolates which were shortlisted by a preliminary round of *in vitro* and *in vivo* screening were purified on *Trichoderma* Specific Medium (Elad & Chet, 1983) and the cultures were maintained by repeated transfer on potato dextrose agar slants for further use. The bacterial isolates were purified on Nutrient Agar medium and stored on YGCA slants.

3.4: Compatibility of the bacterial and fungal antagonists:

The biocontrol efficacy of the different bacterial as well as the different isolates of *Trichoderma* were determined both *in vitro* and *in vivo.* The shortlisted isolates of bacteria and *Trichoderma* were tested for the compatibility *in vitro. An in vivo* experiment was set up in order to determine whether the different combinations of the bacteria and the fungi showed an increased biocontrol efficacy compared to either of them alone. The experiment was conducted in greenhouse in the polythene bags and repeated in pots.

3.4.1: Greenhouse Experiment:

The bacterial isolates were grown in petriplates on nutrient agar. After 48 hours they were transferred aseptically into containers by gently scraping off the topmost layer into the containers using sterile distilled water. It was made up to 150 ml using sterile distilled water. CFU was determined ($2x10^8$-$16x10^8$/ml). Ginger seed rhizomes were dipped in the suspension for half an hour. They were later on air dried for half an hour and planted in polybags. *Trichoderma* isolates were grown on petriplates on PDA. From four day old cultures the spores were gently scraped into containers and made upto 150 ml using sterile distilled water. CFU was determined ($40 X10^8$ spores / ml). 5ml of this suspension was added to each bit of the seed rhizome. They were later on covered over by green mulch of *Glyricidia maculata.* After 30 days of growth the sprouts were challenged with the pathogen (3.2.1.2). Growth parameters like the germination %, no of sprouts, height,

disease incidence and yield were recorded. The data were statistically analysed by the Duncan's Multiple Range Test and tabulated.

3.4.2: Pot Culture Experiment:

The previous experiment (3.4.1) was repeated in pots also. The bacterial test cultures were prepared as in 3.4.1. The healthy ginger seed rhizomes were selected out and dipped in the bacterial suspension for half an hour and planted in the pots of size 30cm height and 12" diameter filled with 10 kg potting mixture. The *Trichoderma* isolates were grown on sorghum grains. Half broken sorghum grains were soaked in water (40% moisture) and autoclaved in polypropelene bags at 121^0C and 15lb pressure for 15 minutes. Spore suspension of the *Trichoderma* cultures were prepared as in 3.2.2.1 and 5ml of this suspension was inoculated into the autoclaved polypropelene bags containing sorghum meal and the cultures were incubated at 28 ± 2^0 C. After 15 days of growth the inoculum was ready for use. From a fully sporulating culture 1.25 grams of the inoculum was taken and added at the base of each rhizome bit. The pots were covered with the leaves of *Glyricidia maculata.* After the development of the sprouts they were challenged with the pathogen as in 3.2.1.2.

Observations such as germination percentage, sprouts / pot, percentage of disease incidence and yield were recorded. The data obtained were analysed statiscally by the MSTATC package and tabulated.

4: Potentiating the biocontrol efficacy of biocontrol agents:

4.1: Mutation by chemical agents:

An experiment was conducted using EMS adopting three different methods to induce mutation.

4.1.1:

Ethyl Methyl Sulphonate was incorporated into Potato Dextrose Agar Medium to obtain 250ppm and 500 ppm and it was poured uniformly into 6 petri plates. Five species of the *Trichoderma* isolates viz *T. virens*-6, *T. pseudokoningii*-6, *T. hamatum*-6, *T. aureoviride*-21 and *T. harzianum-7* were used in the experiment. A culture disc (5mm) from each of the four day old test cultures raised on PDA was inoculated at the centre of the EMS amended

medium. A control plate i.e., PDA without any amendment was also simultaneously maintained for each of the treatments. Readings were recorded after 24, 48 and 72 hours.

4.1.2:

Four day old cultures of the five different species of *Trichoderma* i.e., *T. virens*-6, *T. pseudokoningii*-6, *T. hamatum*-6, *T. aureoviride*-21 and *T. harzianum-7* were prepared. 5mm discs of each of these isolates were added to vials containing 10 ml sterile distilled water. It was serially diluted upto the fourth dilution. Three replications were maintained for each of the treatment at the fourth dilution. Each of the 10 ml spore suspension was poured into 300ml sterile culture bottles. 1000ppm and 2000ppm of EMS was added to each of the two sets of culture bottles. The third set was kept as a control i.e., without any mutagen. These culture bottles were kept in the rotary shaker for two hours @150 rpm. After two hours the culture was transferred to petriplates @ 1ml / plate and *Trichoderma* Specific Medium was added to each of the plates. The plates were incubated at 28 $^{\pm}2^{0}$C for four days. The number of colony forming units were determined. Observations such as the shape, size, rate of growth of the colony and texture of the spores were noted.

4.2: Mutation by Ultra Violet radiation:

4.2.1

Culture plates of the different isolates of *Trichoderma* i.e., *T. virens*-6, *T. pseudokoningii*-6, *T. hamatum*-6, *T. aureoviride*-21 and *T. harzianum-7* were prepared. 5mm diameter culture discs from 48 hour old culture of different isolates were cut out and added to 10 ml sterile distilled water. They were serially diluted upto the fourth dilution. An aliquot of 1ml spore suspension was poured onto petriplates containing TSM (previously poured and solidified medium) and the aliquot was uniformly spread by using a spreader. The plates were exposed to the ultra violet rays with the lids of the petriplates open. The petriplates were kept at a distance of 30 cms from the germicidal lamp (Single; 40W 254nm). The plates were exposed to different durations of 30, 60, 90, & 150 minutes respectively. Three replications were maintained for each treatment. A control was also simultaneously kept. The pertri plates were incubated at 28$^{\pm}2^{0}$Cand observations were recorded after 5 days. The number of colony forming units in each plate was counted and it was compared to the number of colonies in the control plate. The minimum survival percentage was calculated using the formula-

$$\frac{\text{No. of colonies in the treated plates}}{\text{No. of colonies in untreated plates}} \times 100$$

The colour of the colonies, the shape, size and texture of the spores were also noted.

4.2.2: Irradiation of the first generation mutants:

One of the mutants of the first generation which was fast growing was selected out for the second round of mutation. The procedure adopted for the second round of irradiation was the same as above (4.2.1). Observations such as the no. of colony forming units, rate of growth of the colony, size of the colony, shape and the colour of the spores were also noted.

4.3: Test with copper sulphate amended media:

Five day old culture of *Trichoderma* was taken. The mycelia along with the spores were scraped into sterile distilled water using sterile spatula. The suspension was filtered using sterile muslin cloth. It was serially diluted upto the fifth dilution (CFU 67×10^5). This aliquot was poured into the petriplates. It was exposed to the UV rays at a distance of 30 cms for 30 minutes from the germicidal lamp. One ml each of the irradiated spore suspension was poured into (1) three petriplates containing TSM amended with 250ppm $CuSO_4$ (2) in the next three plates TSM was amended with 500ppm of $CuSO_4$ and (3) the remaining three plates were kept as unamended control. Another set of three plates without any irradiation was kept as absolute control. The plates were incubated for five days. Observations such as the CFU / plate, size, shape and colour of the colony was noted and the minimum survival percentage was calculated as in 4.2.1.

4.4: Exposure of culture of different age groups to UV rays:

T. harzianum was grown on petriplates. The mycelia and the spores were gently scraped using a sterile spatula and filtered using a sterile muslin cloth. 10 ml of this aliquot was exposed to UV rays and then one ml of the irradiated solution was poured on to the TSM plates amended with $CuSO_4$ and EMS. The experiments were tabulated as follows.

Table 5: Exposure of culture of different age groups to UV rays:

Age of culture	Distance from the UV source	Time of exposure	Media amended with
48 hours	20 cms	30 minutes	$CuSO_4$ (100ppm & 200ppm)
96 hours	20 cms	30 minutes	EMS (1000ppm & 2000ppm)

4.5: Temperature treatment to obtain mutants:

Trichoderma harzianum–7 disc of 5 mm diameter, from 72 hour old culture was added to four vials containing sterile distilled water with 0.02% dextrose in it. It was kept overnight to allow the initiation of the germtubes. After 24 hours, it was kept in a water bath, (temperature between 80-100^{0}C and heated for different time intervals, i.e., 10, 20, 30, & 40 minutes. After the heat treatment, the mycelial debris from each vial was plated on PDA. Observations such rate of growth, were recorded after 24, 48 & 72 hours.

4.6: *In vitro* screening of the mutants by dual inoculation:

The mutants that were obtained by irradiation were screened rapidly against *Pythium aphanidermatum* by the dual inoculation technique (Webber & Hedger, 1986) as mentioned earlier and the percentage of inhibition was calculated as in 3.1.

4.7: *In vivo* screening of the mutants for bioefficacy:

The selected mutants showing a greater inhibition percentage were screened *in vivo* in the green house conditions in polypropelene bags and observations were recorded as per the procedure (3.2.2).

Observations such as the germination percentage, no. of sprouts, height, and disease incidence were recorded periodically and the effective isolates were shortlisted.

5: Characterisation of *T.harzianum* and the mutants:

5.1: Rate of growth on different media:

Potato Dextrose Agar, Corn Meal Agar, Czapex Dox Agar & *Trichoderma* Specific Medium were prepared as per the method mentioned earlier (1.4). Thr ee day old culture of the different isolates, i.e., the four different mutants obtained by the UV irradiation of the wild type *T. harzianum*-7 were taken. 5mm discs were cut out and plated on the different media. Rate of growth and sporulation of the mutants were studied.

5.2: Rate of spore germination:

Potato Dextrose Broth was prepared. The spores of *T.harzianum* and the mutants were inoculated into the broth in the cavity slides. Observations were recorded. The germination percentage, the time taken for the spores to put forward its germtube and the length of the germtube was measured using the graduation on the ocular of the graduated eye piece of the microscope.

5.3: Effect of light on the sporulation of *Trichoderma harzianum-7* (wild type) and the mutants:

Different media viz PDA, CMA, & CDA were prepared. 5mm discs of *Trichoderma harzianum-7* and the mutants were cut out from four day old cultures and plated on the petriplates containing the different media. Of these half of the plates were kept exposed to the light while the remaining half were kept in darkness. Observations such as spore colour, nature of the spores and the no. of colony forming units were recorded after 72 & 96 hours.

5.4: Growth of *Trichoderma* and the mutants on sorghum grains:

Mass multiplication of *Trichoderma* was standardized using Sorghum meal. Sorghum grains were broken in a mill into coarse particles and moistened with water (400ml / kg of sorghum grains) and packed in polypropelene bags. These were autoclaved at 121^0C and 15 lb pressure for 45 minutes. The *Trichoderma* culture was grown in 250 ml conical flasks. After 96 hours of growth, spore suspensions were prepared by scraping the conidia using sterile distilled water. Its CFU was determined (10^7 units / ml). Five ml of this spore suspension were injected into the autoclaved bags using a sterile syringe. The bags were incubated at 28 ± 2^0C for 15 days. The CFU were determined after 5, 10, 15, 20 & 25 days.

5.5: Bioassay of the culture filtrates:

100ml of the potato dextrose broth was dispensed into four conical flasks @ 25 ml / flask. 5mm discs from 48 hour old pure culture of the wild type isolate *T.harzianum*-7, and the mutants M_1-25, M_1-10 & M_2-3 were inoculated into the broth and allowed to grow for 7 days. The mycelia were then separated using Whatman No.1 filter paper. The filtrate was extracted using ethyl acetate. It was concentrated in the rotaevaporator. 50μl of the ethyl

acetate extract was sterilized with 70% ethyl alcohol (950µl ethyl alcohol was added) 50µl of this mixture was pipetted out into previously poured petriplates containing PDA. A control (with only 50µl of the ethyl acetate) was also simultaneously kept. 5 mm discs of the pathogen *Pythium aphanidermatum* was inoculated on the above plates after the extract completely dried. The growth of the pathogen was measured after 48 hours and the percentage of inhibition was calculated. The same experiment was repeated with a higher concentration of the culture filtrate i.e., 100µl of the ethyl acetate extract.

5.6: Mechanism of interaction:

The three mutants obtained by the UV irradiation, i.e., M_1-25, M_1-10 & M_2-3 and the wild type, *T. harzianum* were used for the interaction study. Three types of tests were conducted for the study. They were

1. Hyphal interaction test
2. Volatile antibiotic test
3. Non volatile antibiotic test.

5.6.1: Hyphal interaction test:

5mm culture discs of *Pythium aphanidermatum* and each of the test fungi were paired in 9cm petriplates containing PDA medium, keeping them at a distance of 5 cms from each other. The paired cultures were incubated at 25 ± 2^0C. Colony diameter was measured after 48 & 72 hours of incubation. Control plates were inoculated with the pathogen alone and was kept for comparison. The percentage of inhibition of the pathogen was calculated by measuring the colony diameter. Two methods of inoculation were followed.

a. Pre inoculation of the antagonist 24 hours prior to the pathogen.

b. Simultaneous inoculation of the pathogen and the test fungi.

Observations were carried out both visually and microscopically. Reisolation of the pathogen after 7 days of treatment was also tested on the P_{10}VP medium.

5.6.2: Test for Volatile Antibiotics:

To test the volatile antibiotics of the test fungi, the procedure of Dennis & Webster (1971-b) was followed. Test isolates of *T.harzianum*-7 and the mutants were inoculated on the petriplates containing PDA and incubated for 48 hours at 25 ± 2^0C. After incubation, the lid of each of the test isolate was replaced by the bottom plate of another petriplate inoculated

with fresh disc of *P. aphanidermatum* on PDA. The two petri plates were sealed together with adhesive tapes to avoid leakage of volatiles. Plain PDA bottom covered with the bottom plates containing *P. aphanidermatum* served as control. After 24 hours and 48 hours of incubation the colony diameter of the pathogen was measured and the percentage of inhibition was calculated.

5.6.3: Test for Non Volatile Antibiotics:

For the nonvolatile test the method of Dennis & Webster (1971-c) was followed. Sterile cellophane discs (9cm) were transferred aseptically into petriplates containing PDA and left overnight to allow the excess moisture to evaporate. Culture discs (5mm) of *Trichoderma* and its mutants were inoculated on the cellophane discs at the centre of the petriplates and incubated at 25 ± 2^0C. After 48 hours of incubation the cellophane discs containing the fungal growth were completely removed and 5mm discs of the pathogen *P. aphanidermatum* was inoculated at the center of the petriplates and was incubated at a temperature of 25 ± 2^0C. Cellophane discs removed from uninoculated plates and inoculated with the culture disc of *P. aphanidermatum* served as control.

5.7: Effect of temperature on the growth of *Trichoderma* and its mutants:

Potato Dextrose Agar plates were prepared. The wild type of the isolate of *Trichoderma* i.e., *T.harzianum* –7 and its mutants M_1-25, M_1-10 and M_2-3 were plated on these plates. The plates were kept at different temperatures ie 15^0C, 20^0C, 28^oC (room temperature) and 45^0C and allowed to incubate. Readings i.e., the radial growth of the test organisms were recorded after 24, 48 and 72 hours and tabulated.

5.8: Sensitivity to Agrochemicals:

In vitro compatibility of the different agrochemicals viz fungicides like Metalaxyl mancozeb (Ridomil MZ), Copper Oxy Chloride (COC) and Mancozeb (Dithane M-45) with *Trichoderma* and its mutants were studied as follows.

5.8.1: Rate of growth on PDA amended with the agrochemicals:

The agrochemicals i.e., Metalaxyl mancozeb (100ppm, 200ppm, 300ppm, 400ppm & 500ppm), Copper Oxy Chloride (0.2%, 0.4%, 0.6% & 0.8%) and Mancozeb (0.3%, 0.6%, 0.9% & 1.2%), according to the recommended dosage, were amended in different

proportions in PDA and autoclaved. Later on they were poured on to the petriplates and allowed to solidify. The wild type *T.harzianum*–7 and its mutants M_1-25, M_1-10 and M_2-3 were plated on it. The readings were recorded after 48 hours and one week and tabulated.

5.8.2: Mycelial growth on PD Broth with the agrochemicals:

The agrochemicals i.e., Metalaxyl Mancozeb (100ppm, 200ppm, 400ppm & 500ppm), Copper-Oxy-Chloride (0.2%, 0.4%, 0.6% & 0.8%) & Mancozeb (0.3%, 0.6%, 0.9% & 1.2%) were amended in different proportions as per the concentrations mentioned above in PD broth and autoclaved. 5mm discs of the wild type *T.harzianum* –7 and its mutants M_1-25, M_1-10 and M_2-3, from 48 hour old culture were inoculated into it at room temperature (28^{0}C). After one week of growth, the mycelia were harvested and its dry weight was determined.

5.9.1: Rhizosphere Competence Assay (Ahmad & Baker, 1987a):

The rhizosphere Competence Assay of *Trichoderma* and its mutants were determined according the method described by Ahmad & Baker (1987a).

Potting mixture (Forest Soil: Sand: FYM in the ratio 3:1:1) was taken and moistened with water (20%). Its pH was determined using the pH meter. The soil was mixed thoroughly and crushed using a mortar and pestle. Its pH was also determined.

Cultures of the wild isolate i.e., *T.harzianum-7* and its mutants M_1-25, M_1-10 and M_2-3 were plated on PDA at 25^{0}C. The four day old sporulating cultures were flooded with sterile distilled water and the conidia were gently scraped off from the surface using a sterile scalpel. The suspension was filtered through a sterile muslin cloth. The spore suspension was made upto 300ml using sterile distilled water and the spore count was adjusted to 10^7 spores / mycelial bit, with a haemocytometer. Ginger seed rhizomes were surface sterilized in 1% $HgCl_2$ and washed with distilled water and air dried. Seeds were treated with the conidial suspensions of the *Trichoderma* spp. in water containing 2 % boiled starch (w / v) as a spreader or sticker. Controls were treated with the starch solution alone. To determine the conidium density per seed, the seed rhizomes were suspended in water and the conidium density in the water wash was determined by the serial dilution technique.

Tea cups of size 3x3" were slit longitudinally into two halves. Each half was filled with the moistened soil and preincubated in plastic bags. One treated seed was placed on one half of the tube. The unseeded half was placed on the seeded portion and secured with rubber bands. The cups were completely randomized and placed vertically in plastic trays containing the same soil as in the teacups. No water was poured into the pots after the seeds were sown. The trays were covered with polythene covers to maintain constant humidity. The trays were kept in the green house at 28^0C.

After 60 days of growth, (till the roots grew to a considerable height), the cups were removed from the trays. After the unseeded half was carefully removed from the pits, the roots in the seeded half was excised in 2cm / 3cms segments, starting from the crown. The scalpel was flamed in between the cuts. The loosely adhering soil was shaken off from the root bits. The roots with the adhering soil was air dried for 30 minutes. Each unit was weighed and transferred to a 30 ml vial containing 10 ml sterile distilled water. The contents of the vials were stirred. The CFU was determined by plating the spores on TSM. Root segments were removed from the dilution flasks and blotted dry on a paper and weighed to determine the dry weight of the soil removed through washing. The plates were incubated at 25^0C for 5 days. Counts of *Trichoderma* CFU per mg of the rhizosphere soil for each root segment were made. Statistical analysis were done by transforming the data to log (cfu count+1).

5.9.2: Test for Rhizosphere Competence (Ahmad & Baker, 1988a):

Czapex Dox Agar medium was prepared as mentioned earlier (1.4.5) and poured into petriplates. Another modified medium of Czapek Dox Agar was prepared in which the carbon source was replaced by Carboxy Methyl Cellulose (CMC). This medium was also poured onto the petriplates. Similarly CD broth with glucose as the carbon source and CMC as the carbon source was prepared. *Trichoderma* and its mutants were grown on the PDA plates. 5 mm discs were cut out from 2 day old culture and plated on the two sets of plates (one set containing glucose and another containing CMC as the carbon source) and also inoculated into the two sets of broth containing CD broth. Observations were recorded in both cases. In the plated the radial growth of the fungus was measured and in the broth the dry weight of the mycelia were recorded after 6 days.

5.10.1: Competitive Saprophytic Ability (Ahmad & Baker 1987b):

To test the competitive saprophytic ability of *Trichoderma* and its mutants, the Cambridge method (Garret, 1970) was modified and adopted. The wild type *Trichoderma, T.harzianum-7* and its mutants M_1-25, M_1-10 and M_2-3 were grown on half broken Sorghum meal as described in 5.4. The CFU was determined ($2X10^8$ units / g). 500 g each of sterile soil moistened with sterile water (25% moisture content) was taken polythene containers. 1, 2, 3 and 4 grams of the inoculum mixture of *Trichoderma* and its mutants were added to each of the plastic containers. 20 cellophane discs (the discs were obtained by punching holes of 6mm diameter) cut out from sterile cellophane paper were added to each container. Three replications were maintained for each treatment. The containers were arranged in a randomized design, covered with plastic sheet to conserve moisture and incubated in the green house. An absolute control was also maintained. Soil sampling was done after 5, 10, 15 and 20 days. All the 15 cellophane discs, from each treatment including the non infested control, were removed from the pots after 10 days, washed in tap water to remove all the adhering soil and debris, and sterilized with 1% $HgCl_2$. The discs were plated on TSM. The CSA index for each strain was developed as follows:

$$i=1$$

$$\text{CSA index} = \varepsilon_n\ [\text{In}(1/1-C_i)/(t_i)(\log p_i)]/n$$

where C=frequency of isolation of a specific strain of *Trichoderma* from the segments, t= time of incubation, p=population density of conidia added to the soil, n= no. of treatments.The RC index for each strain was developed from data reported previously using the equation

$$i=1$$

$$\text{RC index} = \varepsilon_n[\log(p_i+1)\text{In}(d_i+1)]/n$$

where p=population density per milligram of rhizosphere soil, d=root depth, and n=total root length.

5.10.2: Competitive Saprophytic Ability by Agar Plate Method (Rao, 1959)

Sorghum – sand mixture (3g sorghum: 100 g dry sand: 15 ml distilled water) was autoclaved in lots of 170 g in 250 ml conical flasks for 1 hour at 121^{0}C and 15 lb pressure pressure. After inoculation with three 6mm discs (of *Trichoderma* and the mutants), the flasks were incubated for a period of 28 days at 25^{0}C.

Potting mixture with Forest soil: Sand: FYM in the ratio 3:1:1 was taken. Moisture content was adjusted to 40 % after determining its pH (5.1)

Inoculum-soil mixture were prepared in the following ratio

Sorghum : Sand

1- 24.5g : 0.5g

2- 22.5g : 2.5g

3- 18.75g : 6.25g

4- 12.5g : 12.5g

5- 6.25g : 18.75g

6- 2.5g : 22.5g

Slightly modified CDA + yeast agar was prepared. The medium was autoclaved at 121^{0}C and 15 lb pressure for 20 minutes. It was cooled to 40^{0}C and poured into the petridishes.

10 g of the inoculum : sand mixture of the above composition was evenly spread on a petriplate and plain water agar medium was added on it and allowed to solidify so that agar plugs could be cut out from these plates.

10 discs of the above combinations were cut out from each plate and plated as it is on two CDA plates at equal distances.

Recognisable colonies were recorded after 2-3 days, since *Trichoderma* were fast growing. For each inoculum disc, the inoculant fungus was assigned the rating of 1, 3/4, 1/2, 1/4 or 0 according to the total proportion of the fungal colony it occupied. The rating of each of the 10 discs were added together and the total multiplied by 10 to give the percentage figure.

5.11: Enzyme Activity of *T.harzianum*-7 and its biotypes (Elad *et.al.*, 1982)

The ability of *T.harzianum* and its mutants to produce hydrolytic enzymes such as β 1, 4 endoglucanase and β 1,3 glucanase are of great significance as they are implicated in biological control. Hence the enzymatic activity was assayed in 10 days old culture filtrate of *T.harzianum* and its mutants using standard protocols.

5.11.1: Preparation of autoclaved mycelial mat or fresh mycelial mat of *Pythium aphanidermatum*:

Ehrlenmeyer flasks (100ml) containing PD broth (20 ml each) were inoculated with three day old mycelial disc (5mm) of *Pythium aphanidermatum* grown on PDA and incubated at 28 ± 1^0C for 10 days in a shaker at 150 rpm. After allowing the growth of the pathogen for 10 days, the mycelial mat was washed with sterile distilled water. Five washings were given. It was then homogenized using a lyophilizer at 150 rpm for 10 minutes. 20 ml of Czapek Dox salt without sucrose (carbon source replaced by the homogenized mycelia) was added to each flask and it was autoclaved at 15 lb pressure for 20 minutes. Three replications were maintained for each treatment. To another set of flasks in which the mycelia were grown, the mycelial mat was washed with sterile distilled water five times under aseptic conditions. 20 ml of autoclaved Czapek Dox salt solution (in which the carbon source was replaced by the live mycelia) was poured into it under aseptic conditions. Another set of flasks containing Czapek Dox Broth alone was also prepared.

5.11.2: Preparation of the enzyme source:

Spore suspension was prepared from three day old culture plates of *Trichoderma harzianum-7* and its mutants. One disc of 5mm was cut out and added to 10 ml sterile distilled water. It was shaken well. One ml of this spore suspension was poured into the three sets of flasks prepared above (5.11.1). Three replications were maintained for each treatment.

The flasks were incubated at 28 ± 1^0C for 10 days. After 10 days of incubation, the contents of the flasks were passed through a Whatman No.1 filter paper. The culture filtrate

was concentrated to about 5 ml at 40^0C in a rota evaporator. The culture filtrate thus obtained was kept at 4^0C after adding sodium azide (0.02%) as preservative.

5.11.3: β1, 4 endoglucanase Assay

Enzymatic hydrolysis of Carboxy Methyl Cellulose was assayed by the dinitro salicylic acid method (Miller, 1959). The reaction mixture ie 450μ l of 1% CMC 50μ l (9:1) of the crude enzyme extract were incubated at 55^0 C for 30 minutes. 500μ l (dinitro salicylic) DNS was added and incubated in the water bath for 5 minutes. While the test tubes were warm, 1ml Sodium potassium tartarate was added. The whole mixture was cooled to room temperature and made upto 6 ml using sterile distilled water. The absorbance at 540 nm was recorded. The enzyme activity (EGU) was expressed as the release of μ mol glucose / ml of culture filtrate / min / mg protein.

5.11.4: β 1, 3 glucanase Assay

Enzymatic hydrolysis of laminarin (Sigma) was assayed by the dinitrosalicylic acid method (Miller, 1959). The reaction mixture ie 62.5 μ l enzyme extract + 62.5 μ l 4% laminarin was incubated at 40^0C for 60 minutes. 375 μ l of DNS reagent was added and heated for 5 minutes in a boiling water bath. It was then diluted with 1 ml distilled water and vortexed. Absorbance was recorded at 500nm.The enzyme activity (GU) was expressed as the release of μ mol glucose / ml of culture filtrate / min / mg protein.

6: Integrated Management of Rhizome rot disease:

The efficacyof the different mutants were exhibited by the enzyme assay and other *in vitro* experiments. The biocontrol potential of the different bacterial as well as the fungal agents were further stabilized by its capacity to reduce the rhizome rot disease of ginger caused by *Pythium aphanidermatum* by a preliminary round of *in vivo* screening of the different isolates in the green house. Also the compatibility of the different agrochemicals with the BCAs were established. So pot culture experiment as well as field experiments were conducted to evaluate the bioefficacy of the different strains of *Trichoderma* (obtained by UV mutation) in the management of the rhizome rot disease.

6: Experimentation under *in vivo* conditions:

6.1: Pot Culture Experiment:

6.1.1: General:

Pot culture experiments were conducted to assess the disease suppressive effects of the test biocontrol agents. The experiments were conducted in earthern pots of 12" diameter. The pots were cleaned with water and uniformly filled with potting mixture at the rate of 3:1:1 (Soil: Sand: FYM). The experiments were conducted for two consecutive years during the June-December periods. The soil was artificially infested with the pathogen after the initiation of the sprouts in the ginger seed rhizomes. Biocontrol agents, which included the wild type of the isolate, *T. harzianum*-7 and its mutants were mass multiplied on Sorghum grains and used for both the seed treatment as well as the soil application. Care was taken to ensure that the same spore load (**$2x10^8$** cfu / g) was applied both for the seed treatment as well as the soil application. Healthy seed rhizomes weighing 20-25 grams with 2-3 good buds were planted in equidistant portions @ 3 pieces / pot. Six replications were maintained for each treatment for all the experiments. All the cultural and agronomical practices like mulching, fertilizer application, weeding etc were done at appropriate times as per the package of practices. Leaves of *Glyricidia maculata* were used uniformly for mulching in all the treatments and for all the experiments. Germination %, disease incidence & yield were recorded separately for comparison.

6.1.2: Inoculum Preparation:

Potato Dextrose broth (with 20% potato & 2% dextrose) was prepared to raise the pathogen *Pythium aphanidermatum* in Raux bottles. The broth was distributed uniformly in.the Raux bottles @150 ml / bottle and sterilized at 15 lb pressure for 20 minutes. Each bottle was inoculated with 2 discs of 6mm size taken from 48 hour old culture of the pathogen grown on PDA. The inoculated bottles were kept horizontally to get maximum surface area for development of the mycelial mat. The culture bottles were incubated for 7 days in the room temperature at 28-30^0 C. Later the mycelia were carefully removed, washed with water and blended thoroughly. Each mat was made upto 1000ml. This inoculum was applied @ 30 ml / pot. After the application of the inoculum the soil was mixed well.

6.1.3: Multiplication of the Biocontrol Agents:

Trichoderma harzianum-7 and its mutants were multiplied on sterilized sorghum grains in polypropelene bags (20x20cm). The method of mass multiplication was as mentioned earlier (5.4). For the seed treatment with the bacterial isolates, the bacterial cultures were grown on petriplates on Nutrient Agar medium. After 48 hours of growth, the spore suspensions of the bacterial isolate were prepared by gently scraping the surface of the medium of the petriplates on which the bacteria had grown. The suspensions were made upto 150 ml from each plate. Its CFU was determined (**16×10^8** units / ml).

6.1.4: Seed treatment:

Spore load of the biocontrol agents were determined both by the haemocytometer count as well as by determining the colony forming units / g of the culture. For the seed treatment with starch, 2% of boiled starch solution was prepared. The *Trichoderma* culture that was mass multiplied on the sorghum grains were added to it @ 1.25g of the inoculum mixture / bit of seed rhizome and thoroughly mixed. Ginger seed rhizomes were dipped in the suspension for 30 minutes, air dried for 24 hours and planted in pots. For the seed treatment with the bacterial isolates, the inoculum was prepared as mentioned in 6.1.3. Seed rhizomes were dipped in the suspension for ½ hour, air dried and planted in the pots. For the seed treatment with the fungicides Metalaxyl Mancozeb (200ppm) and Mancozeb (0.3%), the respective fungicidal solutions were prepared. Healthy seed rhizomes were dipped in the suspension for ½ hour and air dried for 24 hours and later on planted in the pots.

6.1.5: Soil application of BCAs:

The biocontrol agents which were mass multiplied on the sorghum grains were mixed with neem cake @ 1.25 g of the inoculum mixture / bit of the seed rhizome and applied at the base of each pit.

6.1.6: Determination of the spore load:

The BCAs grown on sorghum grains were suspended in 10 ml sterile water @ 1g / 10 ml. It was diluted thrice and plated on TSM and incubated for four days. After 4 days the CFU / g of sorghum grains was determined.

The haemocytometer was cleaned with alcohol and dried. A small drop of the well suspended spore suspension was placed at the center of the slide and covered with a coverslip. The preparation was allowed to stand for 2 minutes before counting so that the spores settled down on the bottom of the square. The spores inside the small squares were counted. The spores on the top and left touching the middle line of the perimeter of each square was also counted.

Cell concentration /ml was determined by the following calculation.

Conidia / ml = Average count per square X dilution factor X 10^4

6.1.7: Pot Culture Experiments: The following experiments were conducted.

6.1.7.1: Integrated Management of Rhizome rot:

Treatments:

1. RMZ + M_1-25
2. RMZ + M_1-10
3. RMZ + M_2-3
4. RMZ + *T.h-7*
5. RMZ alone
6. DM-45 + M_1-25
7. DM-45 + M_1-10
8. DM-45 + M_2-3
9. DM-45 + *T.h-7*
10. DM-45 alone

11. Starch + M_1-25

12. Starch + M_1-10

13. Starch + M_2-3

14. Starch + *T.h-7*

15. M_1-25 alone

16. M_1-10 alone

17. M_2-3 alone

18. *T.h-7* alone

19. Control

6.1.7.2:Combinations of bacterial isolates and mutants:

Treatments:

1. FP1+ M_1-25
2. FP1+ M_2-3
3. FP1+ M_1-10
4. FPI + *T.h -7*
5. FP 1 alone
6. FP 43 + M_1-25
7. FP 43 + M_2-3
8. FP 43 + M_1-10
9. FP 43 + *T.h -7*
10. FP 43 alone
11. FP 44 + M_1-25
12. FP 44 + M_2-3
13. FP 44 + M1-10
14. FP 44 + *T.h -7*
15. FP 44 alone
16. FP100 + M_1-25
17. FP100+ M_2-3
18. FP100+ M_1-10
19. FP100 + *T.h -7*

20. FP100 alone

21. FP113 + M_1-25

22. FP113 + M_2-3

23. FP113 + M_1-10

24. FP113 + *T.h -7*

25. FP113 alone

26. M_1-25 alone

27. M_2-3 alone

28. M_1-10 alone

29. *T.h -7* alone

30. Control

6.1.8: Observations:

The following observations were made periodically every week.

1. Germination %: The total no. of seed rhizomes germinated / the total no. of seed rhizomes sown

2. Height of the plants (in cms).

3. Diease Incidence: No. of clumps infected out of the total germinated clumps.

4. Yield: Fresh weight of the rhizomes.

6.2: Field Trials:

6.2.1: Field Trials at theIISR Experimental Farm, Peruvannamuzhy:

Field trials were conducted at the IISR Experimental farm, Peruvannamuzhy for two consecutive cropping seasons in the *Pythium* sick soils

6.2.1.1: Climate & Soil Composition:

A warm humid tropical climate was prevalent in the experimental area. The soil type was loamy laterite having moderate fertility with pH 6.3.

6.2.1.2: Preparation of the land:

Preparation of the land started in the month of May-June. The land was ploughed 4 to 5 times to bring the soil to fine tilth. Weeds, stubbles, roots etc were removed. Beds of size 3x1x 0.15m were prepared. 40 plants in four rows were accommodated in a bed with 20 cms in between plants and 25 cms between rows. Between the beds 50 cms gap was maintained.

6.2.1.3: Mass multiplication of Biocontrol Agents:

The biocontrol agents were mass multiplied on sorghum grains. The method adopted for mass multiplication was as described earlier in 5.4

6.2.1.4: Seed treatment:

Seed treatment of the rhizomes were as discussed earlier in the pot culture experiment (6.1.4). For the seed treatment of the bacterial cultures, the cultures were grown in Nutrient broth in Raux bottles. The method of seed treatment was as discussed earlier (6.1.4).

6.2.1.5: Soil Application of the BCAs:

To 100 kg of neemcake, 10 litres of water was added and kept overnight. This resulted in the formation of a powdered mixture. The biocontrol agents were mass multiplied on sorghum grains and 50 g of the inoculum was mixed with 1 kg of neem cake and applied @ 1 kg / bed. The mixture (50 g inoculum mixed with one kg neemcake) was uniformly distributed to the 40 pits of the bed @ 1.25 g / pit at the time of planting.

6.2.1.6: Treatment & Design of the experiments:

The experiments were conducted with the completely randomized block design with 6 replications for each treatment and a plot size of 6 beds. The treatments were the same as discussed in the pot culture experiment (6.1.7.1& 6.1.7.2).

6.2.1.7: Planting :

The variety Himachal was used throughout the experiment. Seed rhizomes weighing 20-25 grams each having 2-3 good buds were plated in four rows at a spacing of 20 cms X 25 cms. The seed rhizomes were planted in shallow pits prepared with a hand hoe and covered over with FYM after the application of neem cake into the pit. After covering the pit with another layer of soil, the bed was levelled and mulched with green leaves. Fertilizer applications and other cultural operations were carried out according to the package of practices

6.2.1.8: Observations:

6.2.1.8.1: Germination:

Germination of the rhizomes was recorded 45 days after planting. From this the percentage of germination was calculated as follows.

$$\frac{\text{No. of seed rhizomes germinated}}{\text{Total no. of seed rhizomes planted}} \times 100$$

6.2.1.8.2: Disease Incidence:

The number of clumps infected out of the total no. of clumps germinated were recorded periodically. The percentage of disease incidence was calculated as follows:

$$\frac{\text{No. of clumps infected}}{\text{Total no. of clumps germinated}} \times 100$$

6.2.1.9: Yield:

After eight months of planting, the yield / bed was recorded. The yield was taken as the fresh weight of the rhizomes. Weight of the good and marketable rhizomes harvested from each bed was recorded and the mean of all the replications of each treatment was taken.

6.2.1.10: Statistical Analysis:

The data obtained from all the experiments were analysed statistically by the MSTATC package using the Duncan's Multiple Range Test and tabulated.

6.2.1.10.1: Comparison of the bioefficacy of the mutants:

A comparative study of the mutants obtained was done in order to determine their bioefficacy and superiority over the wild type. For this the different growth parameters like height of the plant, No. of sprouts, % of disease incidence and yield were compared. Another comparison in terms of the rhizosphere competence, competitive saprophytic ability, enzyme activity, disease suppression and yield was also made.

6.2.1.11: Monitoring the population of the pathogen *Pythium aphanidermatum* and the biocontrol agents in the experimental soil:

The initial population of *Pythium* in the soil prior to planting of the ginger seed rhizomes was determined. Similarly the population of the biocontrol organisms were also determined. The population of *Pythium* and the introduced organisms were frequently monitored during 30, 60, 90 & 150 days after germination of the seeds.

6.2.1.11.1 Soil sampling and method of processing:

Soil samples were collected from each bed at 0-15 cm depth and the soils in each replication were composited to form a composite sample. Moistore content was determined by taking the fresh weight and dry weight of the soil samples separately. Soil samples for each treatment were analysed separately. The soil samples were air dried for 24 hours and was sieved through a 2 mm sieve. Soil dilution plate method (Johnson & Curl, 1972) was employed for counting the colony forming units in the *Pythium* Specific Medium i.e., PDA / CMA supplemented with $P_{10}VP$ (Tsao & Ocana, 1969) and *Trichoderma* Specific Medium (Elad & Chet, 1983). Three replications were maintained for each treatment. After incubation for four days, the colony count / plate was taken and the colony forming units / gram of the soil was determined.

EXPERIMENTAL RESULTS

1.1: Field Survey:

During the survey, 150 fields were surveyed. 250 samples were collected from the fields which included 101 diseased and 149 healthy samples. The pathogen *Pythium aphanidermatunm* was isolated from all the diseased samples. 68 isolates of bacteria and 109 isolates of *Trichoderma* were isolated from the different plant parts of the healthy samples (Table 6). Out of the 68 bacterial isolates 41 (60%) were obtained by plating the root bits while the remaining 27 (40 %) were from the rhizome bits. Out of the 109 isolates of *Trichoderma*, 20 (18%) were from the root samples, 57 isolates (53%) from the rhizosphere while the remaining 32 isolates (30%) were from the rhizome bits. The percentage of rhizome rot disease incidence of ginger was more in Ernakulam area (14.7%) while Kottayam recorded the least disease incidence (1.0%). Rhizome rot disease caused by *Pythium apanidermatum* was predominant in most of the fields. However three fields in Ernakulam district were infested with the bacterial wilt pathogen *Ralstonia solanacearum* (Biotype III). Diseased as well as healthy samples were collected from the fields surveyed (Table 6).

Table 6: Field Survey:

Sl.No.	Name of the district	No.of fields Surveyed	No.of samples collected	Diseased Samples	Healthy samples	% DI
1	Ernakulam	36	62*	23	36	14.7
2	Kottayam	18	20	2	18	1.0
3	Kozhikode	10	20	10	10	10.7
4	Idukki	68	118	51	67	10.7
5	Wayanad	18	33	15	18	12
	Total	150	250	101	149	

*3 fields were infected with bacterial wilt (*Ralstonia solanacearum*)

1.2 : Isolation of the efficient biocontrol agents:

All the microorganisms that were isolated using the different media were screened rapidly for the bioefficacy to suppress ***Pythium aphanidermatum*** by the dual culture technique (Webber & Hedger, 1986) in order to shortlist the efficient isolates. Out of the 68 bacterial isolates 26 were found promising as they exhibited an inhibition percentage ranging from 35-70%. Among the different isolates of *Trichoderma*, 57 isolates showed inhibition ranging from 25-70%.These isolates were further tested *in vivo* for their biocontrol efficacy and the most efficient ones were isolated (Table 7).

Table 7: Screening of biocontrol agents against *Pythium aphanidermatum*

Sl.No.	Organisms tested	No. of isolates tested	No. of isolates inhibiting *Pythium* *(in vitro)*	% of inhibition	No. of isolates showing disease suppression *(in vivo)*	Disease incidence (%)
1	Bacterial isolates	68	26	35-70	10	0-30
2	*Trichoderma* spp	109	57	25-70	3	0-10
3	*Trichoderma* isolates from IISR repository		116	25-50	17	0-10

1.3: Identification of the biocontrol agents:

Bacteria:

When the 26 efficient isolates were subjected to the Succinic acid test mentioned earlier (2.5.1), ten isolates exhibited a green colouration when the bacteria were inoculated into the medium, while the other ten isolates showed a pale green colouration. The remaining 6 isolates remained colourless in the medium. However all the isolates were found to be gram

negative when they were subjected to the Gram's staining test although there were differences in the size of the rods. Thread like appearance was obtained from all the isolates when they were subjected to the KOH test. It was thus confirmed that all the isolates belonged to the fluorescent pseudomonads.

***Trichoderma* spp.:**

Out of the 109 isolates of *Trichoderma*, 57 were found promising by the dual culture and these were identified. Among the 57 isolates 19 isolates were identified as *T. virens* Muller, Gidder & Foster, 4 isolates were identified as *T. aureoviride* Rifai. and the remaining 34 were identified as *T. harzianum* Rifai. Majority of the isolates of *T.virens* (90%) were obtained from the root samples, while most of the *T.harzianum* isolates were obtained from the rhizosphere (50%) as well as rhizome bits (30%).

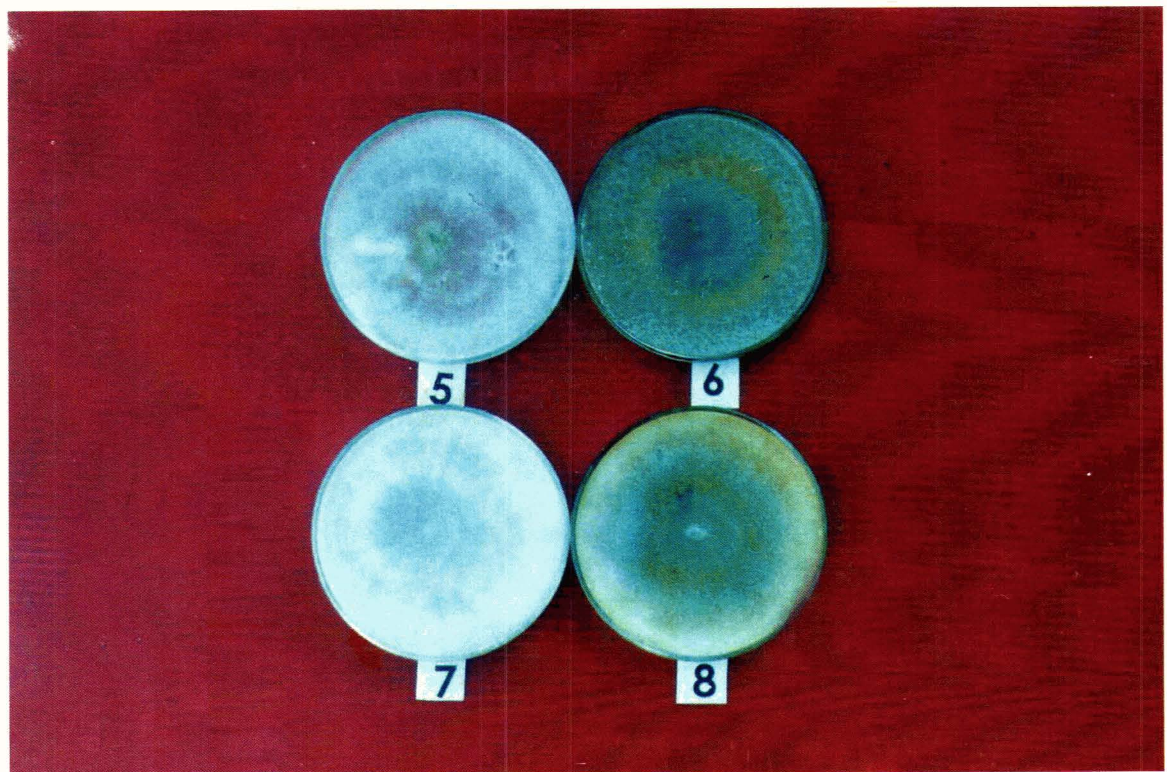
1.4: Sensitivity of bacterial isolates to antibiotics:

The five efficient strains of the bacterial isolates, when subjected to the antibiotic test (2.5.4) revealed that none of the isolates were sensitive to Tetracycline and Ampicillin. Similarly Isolate No. 44 was not sensitive to any of the antibiotics. Isolate No. 1 was sensitive to Streptomycin, while Isolate Nos. 43 & 100 were sensitive to Chloramphenicol. Isolate No.113 was sensitive to Streptomycin, Kanamycin and Chloramphenicol. Sensitivity to the antibiotics could be used as the markers to identifty the bacteria retrived from the soil during the analyses of the soil samples. (Table 8).

Table 8: Sensitivity of bacterial isolates to antibiotics:

Isolate No.	Control	Strepomycin	Tetracyclin	Kanamycin	Chloramphenicol	Ampicillin
FP1	+	-	+	+	+	+
FP43	+	+	+	+	-	+
FP44	+	+	+	+	+	+
FP100	+	+	+	+	-	+
FP113	+	-	+	-	-	+

1: *1-Trichoderma harzianum* *2- Trichoderma aureoviride*
3-Trichoderma polysporum *4-Trichoderma viride*



2: *5- Trichoderma koningii* *6-Trichoderma pseudokoningii*
7-Trichoderma hamatum *8-Trichoderma virens*

Plates 1 & 2: *Trichoderma* isolates from the IISR Repository of biocontrol

2: Assessment of the bioefficacy of the biocontrol agents isolated:

2.1: *In vitro* screening of the biocontrol agents against *Pythium aphanidermatum*:

All the biocontrol agents ie 68 bacterial isolates and 109 isolates of *Trichoderma* were screened rapidly by the dual inoculation technique (Webber & Hedger, 1986) as mentioned earlier. From these 26 bacterial and 57 *Trichoderma* isolates were shortlisted. Among the bacterial isolates, the inhibition percentage varied from 29-64% after 24 hours and from 32-54% after 48 hours, and from 30-70% after 72 hours (Table 9). Among the 57 isolates of *Trichoderma* shortlisted the percentage of inhbition varied from 10 –71 % after 24 hours, 15 -70 % after 48 hours and from 25- 71.5 % after 72 hours. Most of the fungal antagonists grew over the pathogen without forming an inhibition zone (Table 10).

Table 9: *In vitro* screening of bacterial isolates:

Sl.No.	Isolate No.	% of inhibition after		
		24 hours	48 hours	72 hours
1	FP1	31.3	35.2	35.2
2	FP2	35.9	37.6	37.6
3	FP5(1)	34.6	40.1	40.1
4	FP5(2)	35.9	42.0	42.6
5	FP6	34.6	37.0	37.6
6	FP7	42.5	42.6	42.6
7	FP10	35.9	37.0	37.7
8	FP13	35.9	40.1	40.1
9	FP29	30.4	30.5	31.0
10	FP39	45.0	45.8	45.8
11	FP43	34.6	43.6	45.8
12	FP44	35.9	42.5	42.6
13	FP46	47.7	49.2	49.2
14	FP50	35.9	38.4	38.4
15	FP62	39.2	43.0	43.3
16	FP76	35.9	38.4	38.4
17	FP81	33.6	33.3	33.3
18	FP88	42.5	51.2	52.0
19	FP95	64.1	69.2	70.0

20	FP98	34.6	34.6	36.9
21	FP100	50.0	52.0	52.4
22	FP104	34.6	40.6	40.1
23	FP110	31.0	31.0	31.0
24	FP113	37.9	47.9	48.2
25	FP118	34.6	39.0	39.6
26	FP119	41.1	50.0	50.0

Table 10: *In vitro* screening of *Trichoderma* isolates against *P. aphanidermatum*:

Sl.No.	Isolate No.	*Trichoderma* spp.	% of inhibition after		
			24 hours	48 hours	72 hours
1	2	*T.virens*	15.6	25.6	30.2
2	6	*T.virens*	70.3	70.7	71.3
3	11	*T.virens*	15.0	15.7	25.0
4	16	*T.virens*	30.3	30.8	30.8
5	18	*T. virens*	49.1	49.1	69.7
6	21	*T. virens*	17.0	17.3	27.2
7	17	*T.harzianum*	38.0	41.3	46.5
8	27	*T. virens*	23.0	23.9	31.1
9	7	*T.harzianum*	16.6	24.7	29.5
10	22	*T.harzianum*	48.0	52.3	56.0
11	10	*T.harzianum*	23.0	23.9	30.2
12	31	*T.harzianum*	20.1	20.1	28.5
13	32	*T.harzianum*	30.0	30.7	31.9
14	33	*T.virens*	36.6	43.2	48.8
15	34	*T.harzianum*	25.0	25.0	25.6
16	35	*T.harzianum*	20.0	20.0	31.9
17	38(1)	*T.harzianum*	19.0	27.5	37.2
18	38(2)	*T.harzianum*	20.0	22.8	31.9
19	40	*T.virens*	20.0	20.2	27.9
20	41	*T.virens*	33.0	33.8	38.1
21	42	*T.harzianum*	13.3	20.1	25.0
22	43(1)	*T.virens*	29.0	29.4	34.8
23	43(2)	*T.harzianum*	22.3	22.6	27.9
24	45	*T.harzianum*	16.6	18.4	26.5
25	46	*T.harzianum*	10.0	17.7	25.0
26	48	*T.harzianum*	30.3	31.0	31.8
27	49	*T.harzianum*	26.6	26.7	29.5
28	50	*T.harzianum*	33.3	41.0	41.0
29	51	*T.harzianum*	21.0	21.0	27.9
30	53	*T.virens*	30.0	33.8	40.5
31	54	*T.harzianum*	56.6	57.8	62.0
32	57	*T.virens*	15.6	15.7	26.5
33	59	*T.harzianum*	31.3	34.9	39.5

34	63	*T.virens*	52.3	52.3	57.4
35	64	*T.harzianum*	29.0	34.9	38.1
36	70	*T.harzianum*	34.0	42.1	46.5
37	75	*T.harzianum*	13.3	22.8	27.2
38	76(1)	*T.virens*	18.0	22.0	30.2
39	76(2)	*T.harzianum*	16.6	25.6	34.1
40	77	*T. virens*	29.0	29.4	34.8
41	78	*T.harzianum*	34.6	35.8	39.6
42	79	*T.virens*	54.6	55.9	56.0
43	84	*T.harzianum*	23.0	23.9	27.9
44	87	*T.virens*	36.6	46.0	41.8
45	90	*T.aureoviride*	23.3	25.0	25.0
46	94	*T.aureoviride*	39.3	39.3	44.2
47	96	*T.harzianum*	20.0	21.1	26.8
48	98	*T.aureoviride*	40.0	40.2	43.5
49	103	*T.virens*	15.6	15.7	25.0
50	104	*T.harzianum*	30.9	39.3	40.1
51	105	*T.harzianum*	39.3	39.3	42.7
52	106	*T.virens*	46.0	46.0	46.5
53	107	*T.harzianum*	25.0	25.6	34.8
54	112	*T.harzianum*	28.0	31.8	31.8
55	114	*T.virens*	20.0	20.1	27.9
56	116	*T.virens*	33.3	38.3	38.8
57	118	*T.harzianum*	20.0	22.0	27.2

2.2: *In vivo* screening of the biocontrol agents:

2.2.1: Screening of bacterial isolates:

The 26 bacterial isolates that were shortlisted by the dual culture technique were screened *in vivo* under the green house conditions. Of these, 10 isolates showed a disease suppression of > 70% when compared to control which recorded a disease suppression of 40%. The disease incidence in the treated plants varied from 9 to 87.5% while in the control a disease incidence of 60% was recorded (Table 11). Among the 26 isolates only those (10 isolates) showing a reduced disease incidence of 9.5 to 28 % were selected for further studies. Two different pot culture experiments were set up in order to evaluate the growth promoting effect as well as the biocontrol efficacy. In the first experiment when the growth promoting effects were studied it was found that all the isolates showed an increasing germination % of 81-100 %, increased height (30-56 cms), increased number of sprouts (13-18) and increased yield (275-380 grams / pot) when compared to control in which a germination % of 81%, height of 21.7 cms, 12 sprouts / pot and yield of 230 grams / pot was recorded (Table 12). In the second experiment when the bio efficacy of the isolates was

studied the disease incidence ranged from 8.5 to 25% when compared to 60% in the control (Table 13). But by comparing all the growth parameters, out of the 26 isolates, five different isolates of fluorescemt pseudomonads (FP1, FP43, FP44, FP100 & FP113) were selected for further studies.

Table 11: ***In vivo*** **screening of the bacterial isolates against** ***P. aphanidermatum***

Sl.No	Isolate No.	Germ %	No. of sprouts	D.I (%)	Difference in D.I over control
1	FP1	70.0^{b}	10.0^{cd}	20.0^{k}	+40.0
2	FP2	80.0^{a}	11.0^{bc}	36.0^{f}	+24.0
3	FP 5(1)	60.0^{c}	8.0^{ef}	37.8^{f}	+22.2
4	FP 5(2)	60.0^{c}	16.0^{a}	31.3^{h}	+28.7
5	FP 6	70.0^{b}	10.0^{cd}	30.0^{h}	+30.0
6	FP 7	30.0^{g}	5.0^{h}	40.0^{e}	+20.0
7	FP 10	40.0^{f}	9.0^{de}	11.0^{lm}	+49.0
8	FP 13	50.0^{e}	10.75^{bc}	40.0^{e}	+20.0
9	FP 29	60.0^{c}	7.0^{fg}	57.0^{d}	+3.0
10	FP 39	60.0^{c}	10.0^{cd}	40.0^{e}	+20.0
11	FP 43	70.0^{b}	10.0^{cd}	20.0^{k}	+40.0
12	FP 44	80.0^{a}	9.0^{de}	22.2^{j}	+37.8
13	FP 46	40.0^{f}	6.0^{gh}	33.3^{g}	+26.7
14	FP 50	70.0^{b}	11.5^{b}	9.5^{m}	+50.5
15	FP 62	40.0^{f}	$8.o^{ef}$	75.0^{b}	-15.0
16	FP 76	50.0^{e}	8.0^{ef}	12.5^{l}	+47.5
17	FP 81	50.0e	9.0^{de}	11.0^{lm}	+49.0
18	FP 88	40.0^{f}	8.75^{de}	37.8^{f}	+22.2
19	FP 95	60.0^{c}	7.0fg	28.0^{i}	+32.0
20	FP 98	60.5^{c}	7.0^{fg}	57.0^{d}	+3.0
21	FP 100	60.0^{c}	9.0^{de}	11.0^{lm}	+49.0
22	FP 104	40.0^{f}	7.0^{fg}	57.0^{d}	+3.0
23	FP 110	40.0^{f}	8.0^{ef}	87.5^{a}	-27.5
24	FP 113	70.0^{b}	11.0^{bc}	27.2^{i}	+32.8
25	FP 118	40.0^{f}	8.0^{ef}	12.5^{l}	+47.5
26	FP 119	30.0^{g}	8.0^{ef}	37.0^{f}	+23.0
27	Control	55.0^{d}	8.75^{de}	60.0^{c}	

+decrease in disease incidence over control
-increase in disease incidence over control

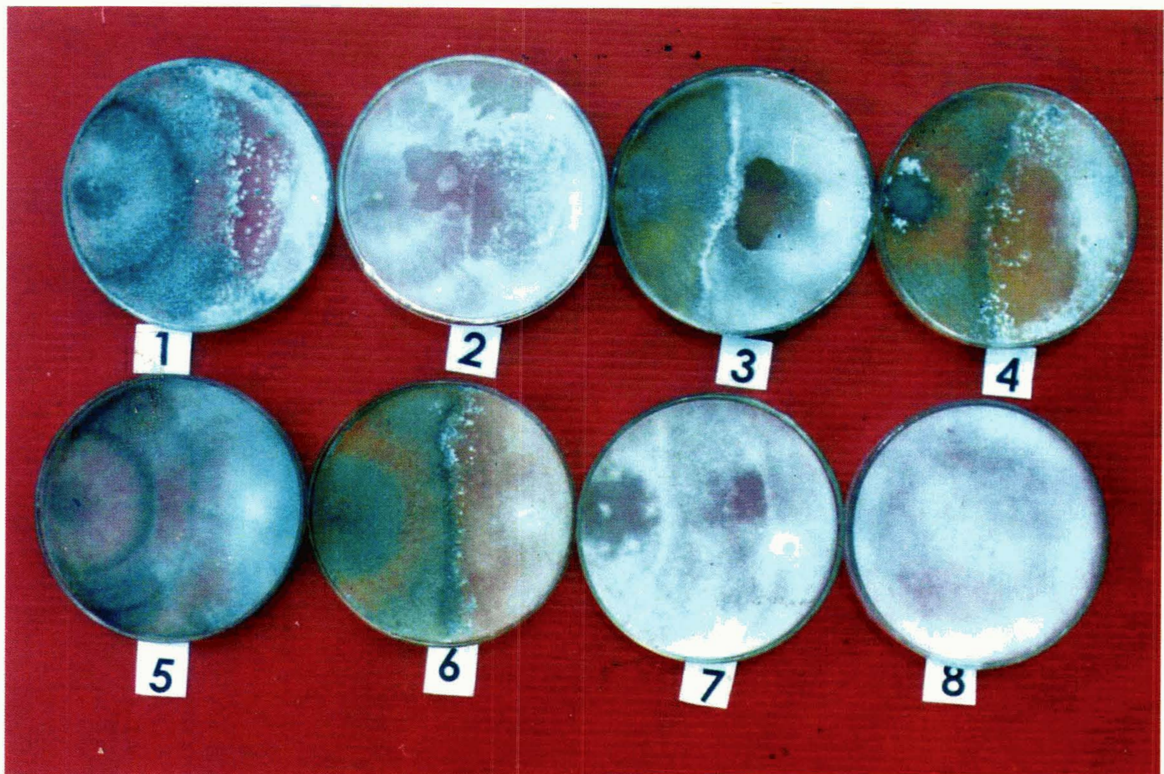


Plate 3: *In vitro* screening of the fungal antagonists against *Pythium aphanidermatum*

1-T. harzianum 2-T. hamatum 3-T. aureoviride 4-T. viride
5- T. virens 6-T. pseudokoningii 7-T. polysporum 8- P.aphanidermatum (Control)

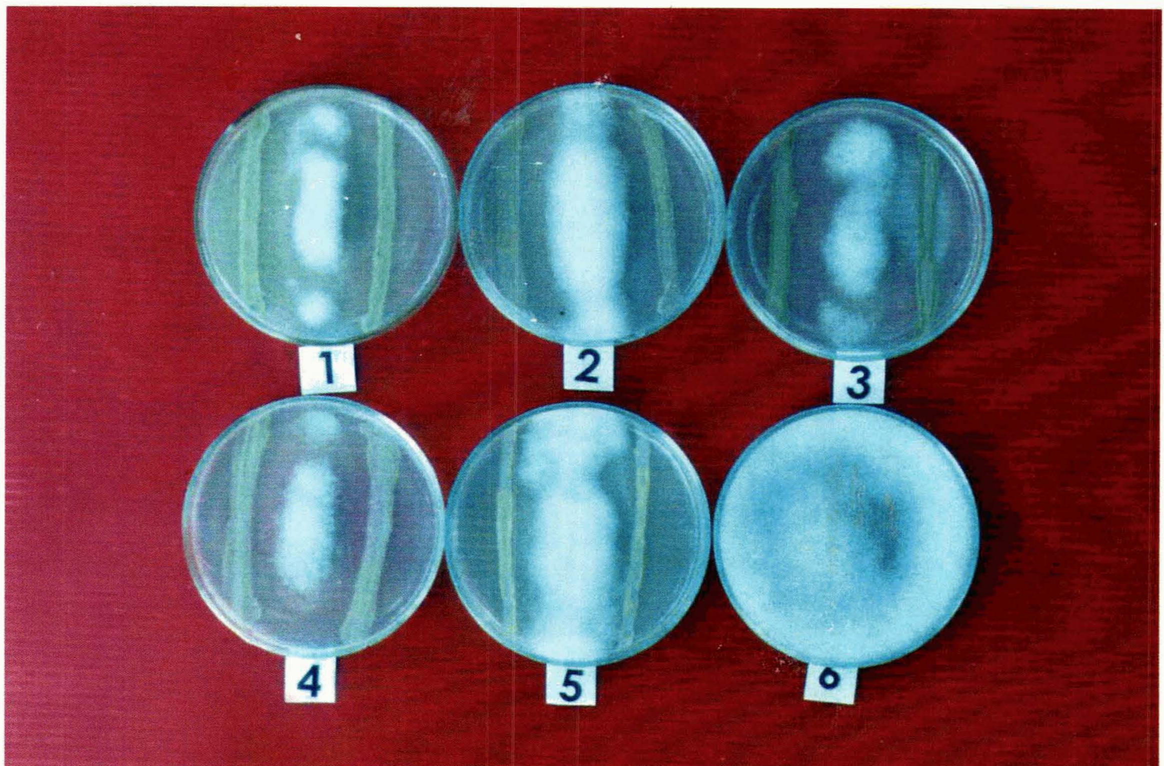


Plate 4: *In vitro* screening of the bacterial antagonists (Fluorescent Pseudomonads) against *Pythium aphanidermatum*

1-FP1 2-FP43 3-FP44 4-FP100 5-FP113 6- *Pythium aphanidermatum* (Control)

Plate 5: *In vivo* screening of the biocontrol agents in the green house.

Plate 6: *In vivo* screening of the bacterial antagonists against rhizome rot of ginger

Table 12: Effect of bacteria on growth of ginger:

Sl.No	Isolate No.	Germ%	Sprouts /pot	Height (Cms)	Yield/pot (g)	Difference in yield over control
1	FP 1	93.8^{a}	16.0^{ab}	37.0^{bc}	355.0^{a}	+125.0
2	FP 10	87.5^{a}	13.5^{bc}	55.5^{a}	292.5^{abc}	+62.5
3	FP 43	100.0^{a}	15.8^{ab}	47.6^{ab}	335.0^{ab}	+105.0
4	FP 44	93.8^{a}	15.3^{ab}	42.9^{abc}	380.0a	+150.0
5	FP 50	81.3^{a}	15.3^{ab}	39.2^{bc}	310.0^{abc}	+80.0
6	FP 76	87.5^{a}	15.0^{abc}	43.8^{abc}	275.0^{abc}	+45.0
7	FP 81	81.3^{a}	13.5^{abc}	46.9^{ab}	282.5^{abc}	+52.5
8	FP 100	87.5^{a}	14.0^{bc}	42.4^{abc}	280.0^{abc}	+50.0
9	FP 113	93.8^{a}	15.3^{ab}	38.6^{bc}	315.0^{abc}	+85.0
10	FP 118	93.8^{a}	17.5^{a}	30.8^{cd}	217.5^{c}	-12.5
11	Control	81.3^{a}	12.0^{c}	21.7^{d}	230.0^{bc}	

\+ increase in yield
-decrease in yield

Table 13: Bioefficacy of bacterial isolates:

Sl. No	Isolate No.	Germ %	Sprouts/pot	D.I (%)	Yield/pot (g)	Difference in yield over control
1	FP 1	60.4^{ab}	7.8^{a}	21.5^{b}	57.8^{ab}	+47.3
2	FP 10	54.2^{ab}	7.7^{a}	13.3^{b}	49.3^{ab}	+38.8
3	FP 43	77.1^{a}	9.9^{a}	16.8^{b}	89.8^{a}	+79.3
4	FP 44	64.6^{ab}	9.9^{a}	24.4^{b}	77.1^{ab}	+66.6
5	FP 50	66.7^{ab}	9.4^{a}	23.7^{b}	60.8^{ab}	+50.3
6	FP 76	52.1^{b}	10.0^{a}	8.6^{b}	58.3^{ab}	+47.8
7	FP 81	68.8^{ab}	10.0^{a}	18.1^{b}	59.5^{ab}	+49.0
8	FP 100	77.1^{a}	12.1^{a}	5.8^{b}	84.8^{a}	+74.3
9	FP 113	70.8^{ab}	12.5^{a}	23.0^{b}	45.7^{ab}	+35.2
10	FP 118	75.0^{a}	9.5^{a}	18.4^{b}	61.5^{ab}	+51.0
11	Control	25.0^{c}	4.2^{a}	59.9^{a}	10.5^{b}	

*+ increase in yield
-decrease in yield

2.2.2: *In vivo* screening of *Trichoderma* isolates for bioefficacy:

The 57 isolates of *Trichoderma* that were shortlisted by the dual inoculation technique were screened *in vivo* in the green house conditions. The growth parameters like the germination percentage, height of the sprouts and disease incidence (%) was recorded. The disease incidence ranged from 5 % to 93.3% in the treated plants while in the control the disease incidence recorded was 82.0%. After the preliminary round of *in vivo* screening three

different isolates i.e., Isolate Nos. 6, 38 and 103 were selected. These three isolates showed a reduced disease incidence of <10% when compared to 82 % D.I. in the control (Table 14).

Table 14: *In vivo* screening of *Trichoderma* isolates obtained from the field survey

Sl. No	Isolate No.	*Trichoderma* spp.	Germ%	Height (cms)	D.I (%)	Difference in D.I over control
1	2	*T.virens*	100	20.7	24.4	+57.6
2	**6**	***T.virens***	**100**	**18.0**	**7.9**	**+74.1**
3	7	*T.harzianum*	75	14.0	64.2	+17.8
4	10	*T.harzianum*	50.0	11.7	25.2	+56.8
5	11	*T.virens*	100	22.3	72.7	+9.3
6	16	*T.virens*	50	10.7	50.0	+32.0
7	17	*T.harzianum*	100	22.7	19.1	+62.9
8	18	*T.virens*	100	21.3	16.9	+65.1
9	21	*T.virens*	50.0	11.0	45.3	+36.7
10	22	*T.harzianum*	75	16.7	24.4	+57.6
11	27	*T.virens*	100	21.0	17.7	+64.3
12	31	*T.harzianum*	100	3.0	14.7	+67.3
13	32	*T.harzianum*	75	13.7	92.2	-10.2
14	33	*T.virens*	100	19.7	33.4	+40.6
15	34	*T.harzianum*	100	13.7	59.7	+22.3
16	35	*T.harzianum*	100	19.3	25.2	+56.8
17	38(1)	*T.harzianum*	75	16.0	16.7	+65.3
18	**38(2)**	***T.harzianum***	**75**	**19**	**6.9**	**+75.1**
19	40	*T.virens*	50.0	12.3	75.7	+6.3
20	41	*T.virens*	100	2.3	11.5	+70.5
21	42	*T.harzianum*	75	10.3	68.2	+13.8
22	43(1)	*T.virens*	75	17.7	16.9	+65.1
23	43(2)	*T.harzianum*	50	15.7	23.2	+58.8
24	45	*T.harzianum*	100	23.7	18.2	+63.8
25	46	*T.harzianum*	50	11.0	78.6	+3.4
26	48	*T.harzianum*	100	20.0	58.5	+23.5
27	49	*T.harzianum*	75	15.7	62.2	+19.8
28	50	*T.harzianum*	75	12.0	61.0	+21.0
29	51	*T.harzianum*	75	18.7	24.0	+58.0
30	53	*T.virens*	50.0	9.3	53.3	+28.7
31	54	*T.harzianum*	75.0	15.6	21.5	+60.5
32	57	*T.virens*	100	18.7	16.1	+65.0
33	59	*T.harzianum*	100	17.7	15.2	+66.8
34	63	*T.virens*	100	22.0	20.7	+61.3
35	64	*T.harzianum*	100	3.0	14.7	+67.3
36	70	*T.harzianum*	100	20.0	20.7	+61.3
37	75	*T.harzianum*	100	19.3	22.1	+59.9

38	76(1)	*T.virens*	75	13.7	15.2	+66.8
39	76(2)	*T.harzianum*	100	19.3	23.3	+58.7
40	77	*T.virens*	50	10.3	32.2	+49.8
41	78	*T.harzianum*	100	19.3	13.9	+68.1
42	79	*T.virens*	100	20.7	11.0	+71.0
43	84	*T.harzianum*	100	18.3	80.0	+2.0
44	87	*T.virens*	50	11.3	49.9	+32.1
45	90	*T.aureoviride*	50	13.3	32.0	+50.0
46	94	*T.aureoviride*	100	17.7	21.4	+60.6
47	96	*T.harzianum*	75	16.3	67.1	+14.9
48	98	*T.aureoviride*	100	21.3	18.1	+63.9
49	**103**	***T.virens***	**100**	**20.0**	**5.0**	**+77.0**
50	104	*T.harzianum*	75	14.0	25.7	+56.3
51	105	*T.harzianum*	50	10.0	29.6	+52.4
52	106	*T.virens*	100	23.7	19.7	+62.3
53	107	*T.harzianum*	50	9.3	93.3	-11.3
54	112	*T.harzianum*	100	15.7	68.9	+13.1
55	114	*T.virens*	50	12.0	64.0	+18.0
56	116	*T.virens*	50.0	13.3	57.3	+24.7
57	118	*T.harzianum*	75	12.0	30.6	+51.4
58	Control		83.3	17.3	82.0	
CD at 5%			12.26	4.6	8.5	

+decrease in disease incidence over control

-increase in disease incidence over control

From the IISR repository of biocontrol agents, 116 isolates of *Trichoderma* obtained from the healthy root samples of ginger were screened *in vivo* in polybags. Among these biocontrol agents, the disease incidence ranged from 0-60% compared to 78.3% in untreated control. 17 isolates, which recorded a disease incidence of < 10% were shortlisted from these different isolates (Table 15). The different isolates shortlisted were *T. polysporum*-8, *T. longibracheatum*-4., *T. hamatum*-6, *T. hamatum*-11, *T. hamatum*-13, *T. hamatum*-25, *T. aureoviride*-21, *T. aureoviride*-24, *T. virens*-9, *T. virens*-17, *T. harzianum*-6, *T. harzianum*-7, *T. harzianum*-11, *T. harzianum*-39, *T. harzianum*-40, *T. pseudokoningii*-4 and *T. pseudokoningii*-6

Table 15: Bioefficacy of biocontrol agents on growth and rhizome rot of ginger:

Sl. No.	Name of the isolate	Germ %	Height Cms	DI (%)	Difference in D.I over control
1	*T. polysporum-1*	100	18.0	11.2	+67.1
2	*T. polysporum-2*	100	18.7	21.7	+56.6
3	*T. polysporum-3*	66.7	15	30.2	+48.1
4	*T. polysporum-4*	66.7	14.3	54.6	+23.7
5	*T. polysporum-5*	100	14.0	33.3	+43.0
6	*T. polysporum-6*	33.3	6.3	26.1	+52.2
7	*T. polysporum-7*	66.7	13.7	16.9	+65.1
8	***T. polysporum-8***	**100**	**15.3**	**7.1**	**+71.2**
9	*T. polysporum-9*	50	7.3	53.3	+25.0
10	*T. polysporum-10*	100	17.0	23.3	+54.7
11	*T. longibracheatum-1*	100	23.3	11.3	+67.0
12	*T. longibracheatum-2*	50	9.7	10.6	+67.7
13	*T. longibracheatum-3*	100	17.0	23.6	+54.7
14	***T. longibracheatum-4***	**50**	**7.3**	**0**	**+78.3**
15	*T. longibracheatum-8*	100	17.0	23.3	+55.0
16	*T. longibracheatum-10*	66.7	12.7	23.7	+54.6
17	***T. hamatum -6***	**50**	**11.3**	**8.9**	**+69.4**
18	*T. hamatum –7*	50	8.7	23.3	+55.0
19	*T. hamatum -8*	100	13.3	12.6	+65.7
20	*T. hamatum -9*	50	8.7	50.0	+28.3
21	*T. hamatum –10*	83.3	10.3	58.2	+20.1
22	***T. hamatum –11***	**100**	**20.0**	**0.0**	**+78.3**
23	***T. hamatum –13***	**100**	**12.7**	**8.5**	**+69.8**
24	*T. hamatum –15*	100	21.0	12.5	+65.8
25	*T. hamatum –16*	100	15.7	18.9	+59.4
26	*T. hamatum –17*	66.7	9.3	31.7	+46.6
27	*T. hamatum –22*	100	16.7	32.2	+46.1
28	*T. hamatum –23*	100	13.3	16.7	+61.6
29	***T. hamatum –25***	**100**	**16.3**	**0**	**+78.3**
30	*T. hamatum –28*	100	15.7	21.0	+57.3
31	*T. aureoviride-1*	100	20.7	11.2	+67.1
32	*T. aureoviride-3*	100	18.3	63.4	+14.9
33	*T. aureoviride-4*	100	12.3	27.2	+51.1
34	*T. aureoviride-6*	100	12.7	29.9	+48.4
35	*T. aureoviride-7*	100	17.7	13.2	+65.1
36	*T. aureoviride-8*	100	15.3	32.1	+46.2
37	*T. aureoviride-9*	100	9.3	29.4	+48.9
38	*T. aureoviride-12*	100	10.0	16.3	+62.0
39	*T. aureoviride-13*	50	10.7	21.7	+56.6
40	*T. aureoviride-15*	100	18.7	26.0	+52.3
41	*T. aureoviride-16*	50	7.0	32.7	+45.6
42	*T. aureoviride-17*	100	16.3	24.5	+53.8
43	*T. aureoviride-18*	100	14.0	16.2	+62.1

44	*T. aureoviride-19*	83.3	18.4	18.4	+59.9
45	*T. aureoviride-20*	100	11.7	14.4	+63.9
46	***T. aureoviride-21***	**100**	**20.7**	**3.6**	**+74.3**
47	*T. aureoviride-22*	100	19.0	15.7	+62.6
48	*T. aureoviride-23*	100	15.6	20.7	+61.3
49	***T. aureoviride-24***	**50**	**10.0**	**0.0**	**+78.3**
50	*T. aureoviride-26*	100	16.7	21.7	+56.6
51	*T. aureoviride-27*	100	12.7	23.3	+55.0
52	*T. aureoviride-28*	100	15.0	20.0	+58.3
53	*T. aureoviride-29*	100	14.7	53.3	+25.0
54	*T. aureoviride-30*	100	13.0	15.3	+63.0
55	*T. aureoviride-40*	100	11.7	43.3	+35.0
56	*T. aureoviride-41*	100	11.3	44.3	+34.0
57	*T. virens –1*	50	10.7	37.2	+41.1
58	*T. virens -2*	50	7.3	41.8	+36.5
59	*T. virens -4*	50	7.7	30.0	+48.3
60	*T. virens –5*	100	11.3	14.7	+63.6
61	*T. virens –6*	50	10.0	43.3	+35.0
62	*T. virens –7*	100	19.3	15.5	+62.8
63	***T. virens –9***	**100**	**22.7**	**1.7**	**+76.6**
64	*T. virens –10*	50	9.3	21.5	+56.8
65	*T. virens –11*	50	9.1	33.3	+45.0
66	*T. virens –12*	50	8.7	31.1	+47.2
67	*T. virens –13*	100	11.7	48.7	+29.6
68	*T. virens –14*	100	13.3	12.5	+65.8
69	*T. virens –15*	100	15.6	15.6	+62.7
70	*T. virens –16*	66.7	13.3	15.9	+62.4
71	***T. virens –17***	**100**	**24.7**	**5.3**	**+73.0**
72	*T. virens –18*	100	16.7	30.0	+48.3
73	*T. virens –19*	100	12.0	40.0	+38.3
74	*T. virens –20*	100	11.0	57.2	+21.1
75	*T. virens –21*	100	22.0	14.8	+63.5
76	*T. virens –22*	100	11.3	53.3	+25.0
77	*T. virens –23*	50	11.3	23.7	+54.6
78	*T. virens-24*	50	8.3	11.8	+66.5
79	*T. virens –25*	100	12.0	30.0	+48.3
80	*T. virens –26*	83.3	9.4	34.3	+44.0
81	*T. virens –27*	100	16.0	18.0	+60.3
82	*T. virens –28*	100	15.3	32.2	+46.1
83	*T. virens –29*	50	10.0	13.3	+65.0
84	*T. virens –30*	66.7	13.3	46.7	+31.6
85	*T. harzianum –1*	100	11.7	16.7	+61.6
86	*T. harzianum –5*	100	15.0	23.6	+54.7
87	***T. harzianum –6***	**100**	**21.3**	**9.4**	**+68.9**
88	***T. harzianum-7***	**100**	**19.7**	**5.1**	**+73.2**
89	*T. harzianum –9*	100	15.0	23.6	+54.7
90	*T. harzianum –10*	100	18.0	14.7	+63.6

91	***T. harzianum –11***	**100**	**12.7**	**8.2**	**+70.1**
92	*T. harzianum –12*	50	9.7	24.1	+54.2
93	*T. harzianum –13*	100	15.3	13.2	+65.1
94	*T. harzianum –14*	50	10.0	13.3	+65.0
95	*T. harzianum –20*	50	10.0	26.7	+51.6
96	*T. harzianum –21*	100	19.0	21.2	+57.1
97	*T. harzianum –25*	100	21.0	19.1	+59.2
98	*T. harzianum –30*	100	20.0	10.0	+68.3
99	*T. harzianum –29*	100	16.7	24.4	+53.9
100	*T. harzianum –32*	66.7	10.7	50.5	+27.8
101	***T. harzianum-39***	**100**	**12.6**	**8.1**	**+70.2**
102	***T. harzianum-40***	**100**	**22.3**	**5.8**	**+72.5**
103	*T. harzianum-42*	100	19.3	15.8	+62.5
104	*T. harzianum-43*	100	17.0	14.1	+64.2
105	*T. harzianum-44*	66.7	9.3	11.1	+67.2
106	*T. harzianum-45*	100	20.7	9.7	+68.6
107	*T. pseudokoningii-1*	66.7	8.7	30.5	+47.8
108	*T. pseudokoningii-2*	100	12.3	29.7	+48.6
109	*T. pseudokoningii-3*	100	17.7	27.4	+50.9
110	***T. pseudokoningii-4***	**100**	**19.7**	**0**	**+78.3**
111	***T. pseudokoningii-6***	**100**	**11.3**	**3.3**	**+75.0**
112	*T. pseudokoningii-7*	100	13.3	50.0	+28.3
113	*T. pseudokoningii-8*	50	10.0	40.0	+38.3
114	*T. pseudokoningii-9*	100	12.3	54.3	+24.0
115	*T. pseudokoningii-10*	100	14.0	20.7	+57.6
116	*T. viride*	100	16.0	60.0	+18.3
117	Control	75	16.0	78.3	
CD at 5%		16.2	5.5	6.5,11.9	

+ decrease in disease incidence over control

After the preliminary round of *in vivo* screening, those isolates of *Trichoderma* exhibiting a disease incidence of <10 % were again screened *in vivo*. The different growth parameters i.e., the germination percentage, sprouts / bag, disease incidence and yield were recorded. From these, those isolates showing a reduced disease incidence and increased yield were shortlisted for further studies (Table 16). The shortlisted isolates were ***T. virens-6, T. hamatum-6, T. harzianum-7, T. aureoviride-24 & T. pseudokoningii-6.***

Table 16: *In vivo* screening of the shortlisted *Trichoderma* isolates:

Sl. No	Isolate No.	Germ %	Sprouts/bag	D.I (%)	Yield/ bag(g)	Difference in yield over control
1	***T.virens-6***	**75.0**	**12.3**	**7.5**	**26.3**	**+22.3**
2	*T.harzianum-38*	58.3	6.3	22.8	15.5	+11.5
3	*T.virens-103*	58.3	6.3	41.0	14.5	+10.5
4	*T.polysporum-8*	50.0	5.3	58.3	9.3	+5.3
5	***T.hamatum-6***	**83.3**	**12.7**	**8.1**	**26.0**	**+22.0**
6	***T.hamatum-11***	50.0	9.7	48.3	13.7	+9.7
7	*T.hamatum-13*	66.7	8.7	18.9	8.3	+4.3
8	*T.hamatum-25*	75.0	6.3	41.0	10.7	+6.7
9	*T.aureoviride-21*	66.7	8.7	19.2	9.8	+5.8
10	***T.aureoviride-24***	**75.0**	**9.0**	**14.7**	**15.3**	**+11.3**
11	*T.virens-9*	41.7	8.7	19.2	8.8	+4.8
12	*T.virens-17*	75.0	13.0	12.5	15.7	+11.7
13	***T.harzianum-6***	75.0	18.7	50.0	8.7	+4.7
14	***T.harzianum-7***	**91.6**	**15.0**	**11.1**	**25.3**	**+21.3**
15	*T.harzianum-11*	100.0	14.0	23.1	14.3	+10.3
16	*T.harzianum-39*	50.0	8.0	29.3	15.3	+11.3
17	*T.harzianum-40*	83.3	11.3	24.6	11.8	+7.8
18	*T.pseudokoningii-4*	91.7	10.0	26.7	14.3	+10.3
19	***T.pseudokoningii-6***	**83.3**	**14.3**	**9.2**	**23.7**	**+19.7**
20	*T.longibracheatum-4*	100.0	14.0	32.2	12.0	+8.0
21	*Control*	66.7	9.3	70.5	4.0	
	CD at 5%	19.42	4.8	13.7	5.16	

3: Compatibility of the bacterial and fungal antagonists:

When the different combinations of the bacterial isolates i.e., FP1, FP43, FP44, FP100 & FP113 and fungal antagonists i.e., *T. aureoviride*-24, *T. harzianum*-7, *T. virens*–6, *T. hamatum*-6 and *T. pseudokoningii*-6 were screened *in vivo* in the green house, it was found that the combinations gave a reduced disease and increased yield than when treated alone. The combination of the different fungal agents i.e., *T. aureoviride*-24, *T. harzianum*-7, *T. hamatum*-6 and *T. pseudokoningii*-6 with the bacterial isolate No. FP 113 was found to be more superior than the rest of the combinations since an increased yield was obtained in these combinations.However it was found that in the case of the fungal isolate, *T. virens*-6, the combinations with the bacterial isolates FP 1 and FP 44 were found to be superior as an increased yield was obtained in these combinations (Table 17 & 18).

Table 17:Combinations of bacteria and fungus-Polybag Experiment:

Sl. No	Isolate No	Sprouts/ Bag	Height (cms)	DI (%)	Yield/bag (g)	Difference in yield over control
1	FP 1	3.4	32.8	14.6	50.0	+38.0
2	FP 43	3.0	51.8	15.6	66.0	+54.0
3	FP44	3.6	49.0	15.0	78.0	+66.0
4	FP100	3.0	45.8	3.4	54.4	+42.4
5	FP113	3.6	42.4	5.6	50.0	+38.0
6	*T.av-24*	3.2	53.0	20.0	58.0	+46.0
7	*T. h-7*	3.4	50.6	34.6	54.0	+42.0
8	*T.v-6*	4.0	47.2	3.4	100.0	+88.0
9	*T.pk-6*	3.4	42.8	5.0	76.0	+64.0
10	*T.ham-6*	2.6	32.6	25.0	52.0	+40.0
11	FP1+*T.av-24*	3.8	37.8	10.0	95.0	+83.0
12	FP43+*T.av-24*	2.8	50.2	13.4	84.0	+72.0
13	FP44+ *T.av-24*	2.6	55.2	20.0	98.0	+86.0
14	FP100+*T.av-24*	5.6	47.6	2.5	94.0	+82.0
15	FP113+*T.av-24*	3.6	60.4	3.4	112.0	+100.0
16	FP1+ *T. h-7*	3.8	45.4	5.0	94.0	+82.0
17	FP 43+ *T. h-7*	4.8	55.2	17.6	100.0	+88.0
18	FP44+ *T. h-7*	3.0	52.0	47.2	54.0	+42.0
19	FP100+ *T. h-7*	3.6	45.6	13.0	62.0	+50.0
20	FP113+ *T. h-7*	4.8	52.4	23.2	108.0	+96.0
21	FP 1 + *T.v-6*	3.0	51.0	8.4	96.0	+84.0
22	FP 43+ *T.v-6*	4.4	38.6	8.0	78.0	+66.0
23	FP44+ *T.v-6*	4.0	48.0	13.3	96.0	+84.0
24	FP100+ *T.v-6*	3.0	52.0	20.0	78.0	+66.0
25	FP113+ *T.v-6*	2.8	50.2	23.4	62.0	+50.0
26	FP 1 + *T.pk-6*	3.4	46.8	15.0	60.0	+48.0
27	FP 43+ *T.pk-6*	4.4	43.8	10.6	72.0	+69.0
28	FP44+ *T.pk-6*	4.2	43.4	10.0	100.0	+88.0
29	FP100+ *T.pk-6*	3.4	53.0	13.2	74.0	+62.0
30	FP113+ *T.pk-6*	3.4	57.8	16.6	106.0	+94.0
31	FP 1 + *T.ham-6*	3.2	54.8	13.4	78.0	+66.0
32	FP 43+ *T.ham-6*	3.2	42.4	8.0	70.0	+58.0
33	FP44+ *T.ham-6*	3.6	50.0	6.8	100.0	+88.0
34	FP100+ *T.ham-6*	2.8	59.2	11.6	62.0	+50.0
35	FP113+ *T.ham-6*	2.8	66.4	15.0	110.0	+98.0
36	Control	2.6	32.6	50.0	12.0	
CD at 5%		1.881	13.13	29.5	46.21	

Table 18: Combinations of bacteria and fungus-Pot culture Experiment:

Sl. No	Isolate No	Germ%	Sprouts /pot	DI (%)	Height (cms)	Yield/pot (g)	Difference in yield over control
1	FP 1	85.0	8.6	13.5	30.2	100.0	+48.0
2	FP 43	80.0	7.0	31.6	24.1	132.0	+80.0
3	FP 44	65.0	6.4	11.5	30.7	155.8	+103.8
4	FP100	70.0	7.0	17.8	28.3	108.2	+56.2
5	FP113	60.0	5.2	17.8	23.5	100.8	+48.8
6	*T.av-24*	60.0	6.6	11.6	32.8	116.0	+64.0
7	*T. h-7*	65.0	7.2	23.7	30.1	108.0	+56.0
8	*T.v-6*	50.0	5.6	19.2	29.6	200.0	+148.0
9	*T.pk-6*	35.0	8.6	16.2	22.4	104.0	+52.0
10	*T.ham-6*	45.0	5.8	21.4	33.7	152.4	+100.4
11	FP 1+ *T.av-24*	65.0	9.2	5.0	26.6	200.0	+148.0
12	FP43+ *T.av-24*	70.0	7.4	2.8	31.7	168.0	+116.0
13	FP44+ *T.av-24*	60.0	9.8	0.0	38.1	176.0	+124.0
14	FP100+*T.av-24*	75.0	10.0	2.2	34.8	188.1	+136.1
15	FP113+*T.av-24*	55.0	9.2	2.2	34.8	223.0	+171.0
16	FP 1+ *T. h-7*	70.0	9.8	8.5	28.3	184.0	+132.0
17	FP 43+ *T. h-7*	60.0	8.6	0.0	30.4	200.0	+148.0
18	FP44+ *T. h-7*	60.0	11.2	3.3	37.7	108.0	+56.0
19	FP100+ *T. h-7*	70.0	8.4	0.0	34.4	127.2	+75.2
20	FP113+ *T. h-7*	70.0	9.0	6.0	30.9	216.0	+164.0
21	FP 1 + *T.v-6*	60.0	7.8	6.2	24.6	192.0	+140.0
22	FP 43+ *T.v-6*	45.0	7.0	6.7	30.8	156.0	+104.0
23	FP44+ *T.v-6*	50.0	6.4	9.4	19.4	192.4	+140.4
24	FP100+ *T.v-6*	50.0	8.4	5.8	25.8	156.0	+104.0
25	FP113+ *T.v-6*	45.0	10.0	5.8	33.9	132.0	+80.0
26	FP 1 + *T.pk-6*	40.0	5.8	8.2	22.5	156.0	+104.0
27	FP 43+ *T.pk-6*	40.0	5.6	8.0	25.8	140.0	+88.0
28	FP44+ *T.pk-6*	60.0	6.8	15.4	18.5	200.0	+148.0
29	FP100+ *T.pk-6*	45.0	6.2	10.0	16.9	134.0	+82.0
30	FP113+ *T.pk-6*	60.0	7.8	9.3	24.2	220.0	+168.0
31	FP 1 +*T.ham-6*	60.0	7.2	13.0	27.4	136.0	+84.0
32	FP 43+*T.ham-6*	50.0	7.6	11.8	29.9	144.0	+92.0
33	FP44+*T.ham-6*	65.0	9.8	21.1	28.4	202.0	+150.0
34	FP100+*T.ham-6*	75.0	12.6	18.8	33.2	148.0	+96.0
35	FP113+ *T.ham-6*	45.0	10.4	5.9	33.2	212.0	+160.0
36	Control	45.0	6.2	50.6	28.5	52.0	
CD at 5%		26.8	2.9	15.1	8.8	10.9	

7: Poly bag Experiment- Combinations of bacteria (Fluorescent Pseudomonads) and fungi *(Trichoderma spp.)*

8: Pot Culture Experiment- Combinations of bacteria (Fluorescent Pseudomonads) and Fungi *(Trichoderma* spp.*)*

Plates 7 & 8: *In vivo* screening of the biocontrol agents in the green house

4: Potentiating biocontrol efficacy of biocontrol agents:

4.1.1: Mutation by chemical agents:

When the different isolates of *Trichoderma i.e., T. harzianum*–7, *T. hamatum*-6, *T. pseudokoningii*-6 & *T. aureoviride*-24 were plated on PDA amended with 250 ppm and 500ppm EMS, the rate of growth of the test organisms in both the treatments i.e., 250 ppm and 500 ppm Ethyl Methyl Sulphonate were almost on par. Full growth of the test organisms were obtained after 72 hours in all the treated plates (Table19). Similarly there was no significant difference between the isolates in the treated and control plates and there was no phenotypic difference between the isolates obtained.

Table 19: Growth (in cms) of *Trichoderma* on EMS amended media:

Treatment	250 ppm			500ppm			Control		
	24 hrs.	48 hrs.	72hrs.	24hrs.	48hrs.	72hrs.	24hrs.	48hrs.	72hrs.
T.virens-6	3.0	6.2	Full*	3.0	6.2	Full	3.0	6.2	Full
T.pseudokoningii-6	2.9	3.0	8.0	2.9	3.0	8.0	2.9	3.0	8.0
T.hamatum-6	2.5	4.2	8.0	2.5	4.2	8.0	2.5	4.2	8.0
T.aureoviride-24	3.0	6.1	Full	3.0	6.1	Full	3.0	6.1	Full
T.harzianum-7	3.1	6.3	Full	3.1	6.3	Full	3.1	6.3	Full

*Full- completely covered the petriplates.

4.1.2: When the spore suspensions of the different isolates of *Trichoderma* were treated with 1000ppm & 2000ppm EMS, the number of colony forming units obtained in the treated as well as the control plates were the same and also there was no phenotypic difference between the colonies obtained in the control as well as the treated plates (Table 20). The size of the colonies as well as the growth pattern was also the same. The texture of the spores obtained in the treated as well as the control plates were also not different.The behavioural pattern of the mutants thus obtained were also similar to that of the wild type. Thus the result obtained was negative.

Table 20: CFU on TSM after treatment with EMS:

Treatment	CFU X10^4		
	1000 ppm	2000ppm	Control
T.virens-6	680	600	612
T.pseudokoningii-6	420	400	412
T.hamatum-6	450	421	430
T.aureoviride-24	690	700	690
T.harzianum-7	780	740	700

4.2: Mutation by UV irradiation:

Colonies were not obtained in any of the petri plates plated with the different species of *Trichoderma.* However in the treatment with *T.harzianum–7*, a few colonies were obtained. The number of colonies obtained in the treated as well as the control plates treated with *T.harzianum–7*, were counted and the minimum survival percentage was calculated. From the colonies obtained after irradiation, those showing difference in phenotype, growth pattern, rate of growth were isolated and for convenience labeled from M_1 upto M_1-25. It was found that the survival percentage steadily decreased as the duration of the exposure to the ultra violet rays were increased. In the plates exposed to UV for 120 minutes, a minimum survival percentage of 0.33% was obtained (Table21). In one of the replications of the plates exposed to 120 minutes irradiation, an albino colony was obtained. This colony was similar to the wild type and the mycelia also appeared to be similar to the wild type i.e., *T.harzianum* when viewed under the microscope, except that it was colourless. Another colony which was fast growing than the wild type was also picked up. But this colony was phenotypically similar to the wild type although it was fast growing. This colony was named as M_1-10.

Table 21: CFU after exposure to ultraviolet radiation:

Treatment	Time of exposure	No of colonies obtained	Minimum Survival %
T_1R_1 T_1R_2 T_1R_3	30minutes ,, ,,	21 20 22	3.465
T_2R_1 T_2R_2 T_2R_3	60 minutes ,, ,,	4 5 4	0.7095
T_3R_1 T_3R_2 T_3R_3	90 minutes ,, ,,	8 10 6	1.3
T_4R_1 T_4R_2 T_4R_3	120 minutes ,, ,,	1 1 0	0.33
T_5R_1 T_5R_2 T_5R_3	150 minutes ,, ,,	0 0 0	0
T_6R_1 T_6R_2 T_6R_3	Control ,, ,,	616 596 600	

4.2.2: Irradiation of the first generation of mutants:

When one of the mutants of the first generation was irradiated for the second time under the UV rays, a minimum survival percentage of 0.6 % was obtained when the plates were exposed to the UV rays for 90 minutes. Similarly one colony was obtained when the plates were exposed to UV for 120 minutes and it exhibited a minimum survival percentage of 0.02% (Table 22). The colonies which were slightly more dark than the wild type were picked up. Also some colonies of the mutants were bigger than the parents. Since only a few colonies were obtained in this experiment all the colonies were picked up.

Table 22:Irradiation of the first generation mutants:

Treatment	Time of exposure	No of colonies obtained	Minimum Survival %
T_1R_1 T_1R_2 T_1R_3	30minutes ,, ,,	30 10 11	3.4
T_2R_1 T_2R_2 T_2R_3	60 minutes ,, ,,	4 5 10	1.2
T_3R_1 T_3R_2 T_3R_3	90 minutes ,, ,,	1 1 1	0.6
T_4R_1 T_4R_2 T_4R_3	120 minutes ,, ,,	1 0 0	0.02
T_5R_1 T_5R_2 T_5R_3	150 minutes ,, ,,	0 0 0	0
T_6R_1 T_6R_2 T_6R_3	Control ,, ,,	500 495 500	

4.3: Test with copper sulphate amended media:

When the *Trichoderma* spores were exposed to UV rays and plated on the petriplates in which the TSM was amended with copper sulphate (250 ppm and 500 ppm), no colonies were obtained in both the dilutions i.e., the second and the fifth dilution. However in the fifth dilution, in the untreated plates, $45X10^5$ colonies were obtained (Table 23).

Table 23:Test with copper sulphate amended media and colonies obtained:

Treatment	10^2 dilution		10^5 dilution	
	Irradiated	Unirradiated	Irradiated	Unirradiated
250ppm	0,0,0	1,0,0	0,0,0	0,0,0
500ppm	0,0,0	0,0,0	0,0,0	0,0,0
Control	0,0,0	Uncountable	0,0,0	$45x10^5$

4.4: Exposure of two day old mycelia to UV rays:

When the two day old culture of *Trichoderma harzianum*-7 was gently scraped into sterile distilled water and serially diluted upto 10^2 dilution, it was found that 20×10^5 spores were present in the sterile water solution. Upon exposure to UV rays and plating on TSM amended with 100ppm & 250ppm $CuSO_4$ one colony was obtained in each treatment. Colonies were obtained in the different replications also as well as the irradiated and unirradiated plates. There were no phenotypic differences between the wild type and the mutants. But some of them were found to be fast growing. The colonies that were obtained in the irradiated and copper sulphate amended plates were picked up and designated from SSM_1-1 upto SSM_1-6 (Table 24).

Table 24:Exposure of two day old mycelia to UV rays:

Treatment	10^2 dilution	
	Irradiated	Unirradiated
100ppm	1,1,1	1,0,0
250ppm	0,0,0	1,1,1
Control	5,2,3	Uncountable

4.5: Temperature treatment to obtain mutants:

When the culture disc of *Trichoderma harzianum-7* was heated in the water bath for different time intervals and plated on PDA, it was found that the debris of the mycelial fragments of the disc which was heated for 30 minutes produced mycelia on PDA. Later on this temperature tolerant isolate was dual cultured against *Pythium aphanidermatum.* But no inhibition was obtained. The result was thus negative. Hence this isolate was discarded.

4.6: *In vitro* screening of the mutants obtained.

All the mutants obtained from the different experiments were screened *in vitro* by the dual inoculation technique (Webber & Hedger, 1986) in order to determine its biocontrol efficacy. The growth of the pathogen was measured after 24, 48 & 72 hours and the inhibition percentage was recorded (Table 25).The different mutants exhibited an inhibition % ranging from 0-51.2%. However only nine isolates showed an inhibition percentage more than the wild type which was 39.0 %. These isolates were shortlisted for further studies.

Table 25: ***In vitro*** **screening of the mutants:**

Sl.No.	Isolate No	Inhibition % after		
		24 hours	48 hours	72 hours
1	M_1-1	0.9	1.8	2.4
2	M_1-2	0.9	2.1	4.8
3	M_1-3	4.5	4.8	7.3
4	M_1-4	10.1	12.4	29.2
5	M_1-5	10.1	10.1	12.1
6	M_1-6	4.4	4.8	7.3
7	M_1-7	4.4	4.8	7.3
8	M_1-8	2.2	3.2	4.8
9	M_1-9	0.9	2.2	4.8
10	M_1-10	25.0	58.1	51.2
11	M_1-11	2.4	2.8	4.8
12	M_1-12	3.8	4.6	7.3
13	M_1-13	2.1	2.3	2.4
14	M_1-14	0.0	0.0	0.0
15	M_1-15	0.0	0.0	0.0
16	M_1-16	0.0	0.0	0.0
17	M_1-17	0.0	0.0	0.0
18	M_1-18	0.9	1.2	4.8
19	M_1-19	3.6	4.8	7.3
20	M_1-20	25.0	30.0	39.8
21	M_1-21	4.8	6.5	7.3
22	M_1-22	2.4	2.4	2.4
23	M_1-23	4.0	4.5	4.8
24	M_1-24	3.2	3.6	4.8
25	M_1-25	32.0	35.0	51.2
26	M_2-1	10.1	12.1	14.2
27	M_2-2	2.4	2.5	3.4
28	M_2-3	35.0	40.8	43.8
29	M_2-4	0.0	0.0	0.0
30	SSM_I-1	45.0	50.0	51.2
31	SSM_I-2	30.2	35.0	39.0
32	SSM_I-3	25.8	38.9	48.7
33	SSM_I-4	0.0	0.0	0.0
34	SSM_I-5	35.0	38.0	46.3
35	SSM_I-6	0.9	1.8	2.4
36	*T.h*-7	25.8	34.0	39.8

4.7: ***Invivo*** **screening of the mutants.**

The nine different mutants which were shortlisted by the *in vitro* screening were screened *in vivo* in poly bags in the green house conditions. The results obtained

indicated that an increased height, more number of sprouts and a disease incidence of <40 % were obtained in three of the mutants ie M_1-10, M_1-25 & M_2-3 (Table 26). In the wild type a disease incidence of 50 % was recorded while in the absolute control a disease incidence of 80 % was obtained. The mutants M_1-10, M_1-25 & M_2-3 were selected for further studies.

Table 26: *Invivo* screening of the mutants

Sl.No.	Isolate No.	Germ%	Sprouts /bag	Height (cms)	DI(%)	Yield/bag(g)
1	**M_1-10**	**100^a**	**8^a**	**39^a**	**10^d**	**$45a^b$**
2	M_1-20	50^c	2^a	25^{abc}	80^a	15^{de}
3	**M_1-25**	**100^a**	**6^a**	**38^{ab}**	**0^d**	**60^a**
4	**M_2-3**	**100^a**	**7^a**	**35^{ab}**	**10^d**	**58^a**
5	SSM_1-1	80^b	5^a	30^{abc}	50^{bc}	30^{bcd}
6	SSM_1-2	100^a	2^a	25^{abc}	40^c	25^{cde}
7	SSM_1-3	50^c	3^a	26^{abc}	60^b	25^{cde}
8	SSM_1-5	40^c	6^a	20^{bc}	60^b	20^{cde}
9	SSM_1-6	100^a	5^a	12^c	35^c	20^{cde}
10	*T.h*-7(wild type)	80^b	5^a	20^{bc}	40^c	35^{bc}
11	Control	50^c	2^a	16^c	80^a	10^e

5: Characterisation of the developed strains:

5.1:Rate of growth on different media:

The wild isolate, *Trichoderma harzianum*-7 and its mutants i.e., M_1-25, M_1-10 & M_2-3 were grown on different media, ie PDA, CMA and CDA. The rate of growth (diameter) on these different media was measured. The spore characters were also determined .The observations were tabulated as in the Table 27.

Table 27: Rate of growth of the mutants on different media:

Media	Isolate No	Rate of growth (in cms) after			Spore characters
		24 hrs.	48hrs.	72 hrs.	
PDA	M_1-25	3.65	7.1	Full*	After 24 hrs only mycelial growth was observed. Sporulation started after 48 hrs. After 72 hrs. two zones of sporulation was seen. Diameter of inner sporulation zone was 3.6 cms. Spores were white in colour.

	M_1-10	2.9	7.75	Full	The growth of the mycelia was upwards. After 48 hours sporulation started. After 72 hours the spores were observed in two concentric rows. Diameter of inner sporulation zone was 2.9 cms. Spores were light green.
	M_2-3	3.8	8.75	Full	Mycelial growth was upwards. Spores were dark green in colour. Sporulation zone was observed at a diameter of 3.8 cms. Two concentric zones of spores were found after 72 hours. Spores were dark green.
	T.h-7	3.3	5.9	8.5	Sporulation started after 72 hours. Diameters of inner sporulation zone was 3.3 cms and the outer one was 7.3 cms. The spores were alternately light green and dark green in colour.
CMA	M_1-25	3.0	6.1	8.5	Sporulation started after 48 hours. But the spores were scanty. After 72 hours 2 rings of spores were visible. The inner sporulation zone was at a diameter of 3.0 cms while the outer zone was at 6.1 cms.
	M_1-10	3.1	6.8	Full	Mycelial growth was upwards. After 48 hours the sporulation zone was visible at a diameter of 2.8 cms. The spores were light green in colour. After 72 hours two concentric zones of spores were obtained with alternate light and dark spores.
	M_2-3	3.15	6.6	Full	The sporulation started after 48 hours at a distance of 2.8 cms diameter. After 72 hours the sporulation was complete and the spores were visible in two concentric zones, the inner one at a diameter of 2.5 cms and the outer one at a distance of 6.8 cms.

	T.h-7	3.1	7.3	Full	After 72 hours sporulation was complete and the spores were found in two concentric circles, the inner sporulation zone was at a distance of 3.1cms diameter while the outer one was at a distance of 7.3 cms. Spores were alternately light green and dark green in colour.
CDA	M_1-25	2.8	6.0	8.2	The growth of the mycelia was scanty. Sporulation was less when compared to the spores in PDA and CMA. Spores were scattered and white in colour.
	M_1-10	3.2	6.5	Full	Spores were scanty and light green. A clear sporulation zone was not visible but the spores developed in concentric circles.
	M_2-3	3.3	6.8	Full	Spores were scanty and light green in colour. The spores were scattered and light green in colour. But they became dark green when the age of the culture increased.
	T.h-7	3.1	6.35	Full	Spores were less than those found in PDA and concentric but not in clear rings.

*Full- completely covered the petriplate

5.2: Comparison of spore germination of *Trichoderma harzianum-7* and the developed strains: When the wild type i.e., *T. harzianum-7* and its mutants M_1-25, M_2-3 & M_1-10 were plated in the cavity slides containing PD broth, it was found that the rate of growth of the mutants were on par with the wild type i.e., it took 15-18 hours for the isolates to put forward its germ tube. The percentage of germination of the mutants (M_1-10 and M_2-3) were slightly more than the wild type. However the percentage of germination of the mutant M_1-25 was lesser than the wild type. This mutant took 19 hours to put forth its germ tube. The length of the germ tube at the time of germination were tabulated as follows (Table 28).

632.3 BEE/P TH
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Table 28: Rate of spore germination:

Sl. No.	Isolate No.	%of germination	Time taken for germination	Length of germ tube at the time of germination
1	*T.h*-7	60	16 hours	18.8μm
2	M_1-25	55	19 hours	15.26μm
3	M_2-3	62	15 hours	20.71μm
4	M_1-10	65	16 hours	19.62μm

5.3: Effect of light on the sporulation of *Trichoderma* and the mutants:

When the cultures of *Trichoderma* and its mutants were plated on different media and kept in both conditions i.e., in the light as well as in the darkness, it was found that light ensures profuse sporulation because in the plates kept in the dark the spores were less and scattered. However when intermittent light was given to the cultures, at an interval of 48 hours, rapid sporulation was observed. The readings were tabulated as in Table 29. In CDA the mycelia were less dense, but the conidia were more dense and green. In PDA the sporulation zone was visible in the plates kept in the light while there was no sporulation zone in the plates kept in the darkness. The CFU in one 5mm disc of the sporulating culture was recorded after 96 hours of growth.

Table 29: Effect of light on sporulation of *Trichoderma*:

Media	Isolate No	72 hours		96 hours			
		Light	Dark	Light	CFU	Dark	CFU*
PDA	*T.h*-7	+++++	-	+++++	$6X10^5$	-	$3 X10^5$
	M_1-10	+++++	-	+++++	$8X10^5$	-	$3.5 X10^5$
	M_2-3	+++++	-	+++++	$6.5X10^5$	-	$3 X10^5$
	M_1-25	++	-	+++	$4.5X10^5$	-	$2.5 X10^5$
CMA	*T.h*-7	++++	-	+++++	$6.5X10^5$	-	$3.5 X10^5$
	M_1-10	++++	-	+++++	$7X10^5$	-	$3.5 X10^5$
	M_2-3	++++	-	++++	$6.5X10^5$	-	$3 X10^5$
	M_1-25	++	-	+++	$5X10^5$	-	$2.5 X10^5$
CDA	*T.h*-7	++	-	+++	$3.5X10^5$	-	$2.5 X10^5$
	M_1-10	++	+-	+++	$4.5X10^5$	+-	$2.7 X10^5$
	M_2-3	++	+-	+++	$4.3X10^5$	+-	$2.6 X10^5$
	M_1-25	+	-	++	$3X10^5$	-	$2.3 X10^5$

+ indicates amount of sporulation
- indicates no sporulation.
*-CFU/5mm disc

TH 584.39 BEE/P

NB 5622

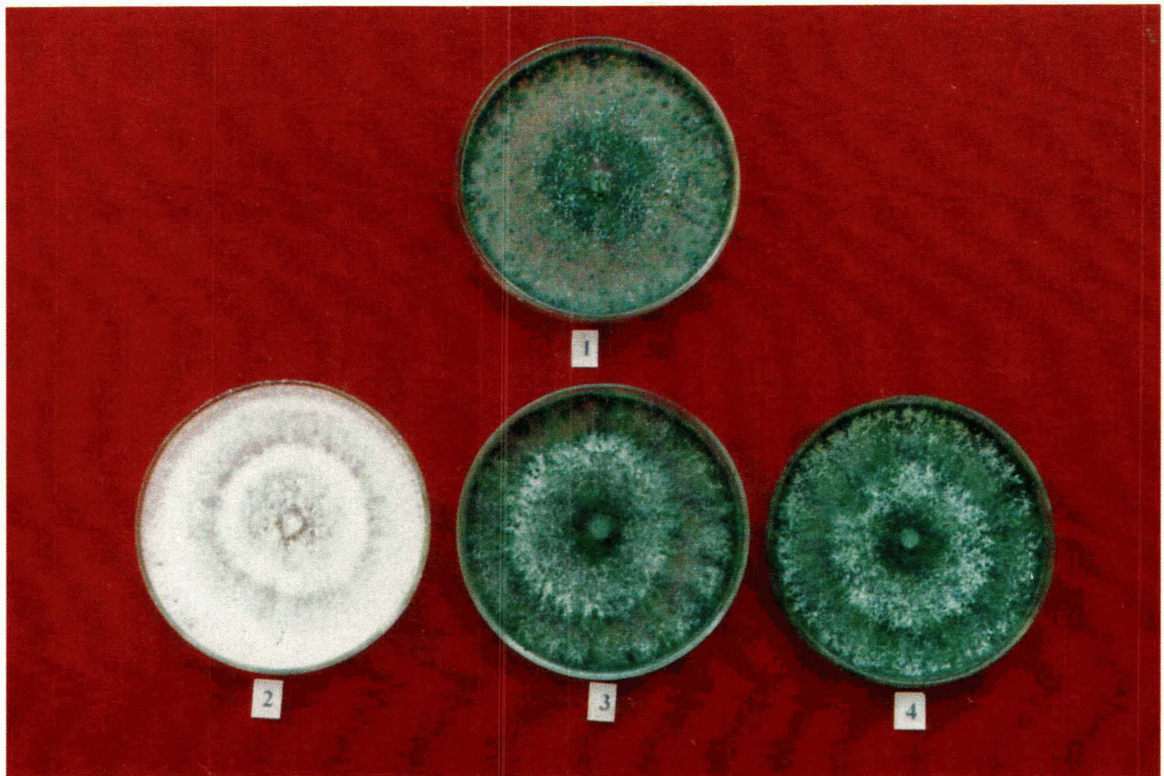


Plate 9: The strains of *Trichoderma* developed by irradiation with UV rays

1-*Trichoderma harzianum*-7 (Wild type)   2-M_1-25

3-M_1-10   4-M_2-3

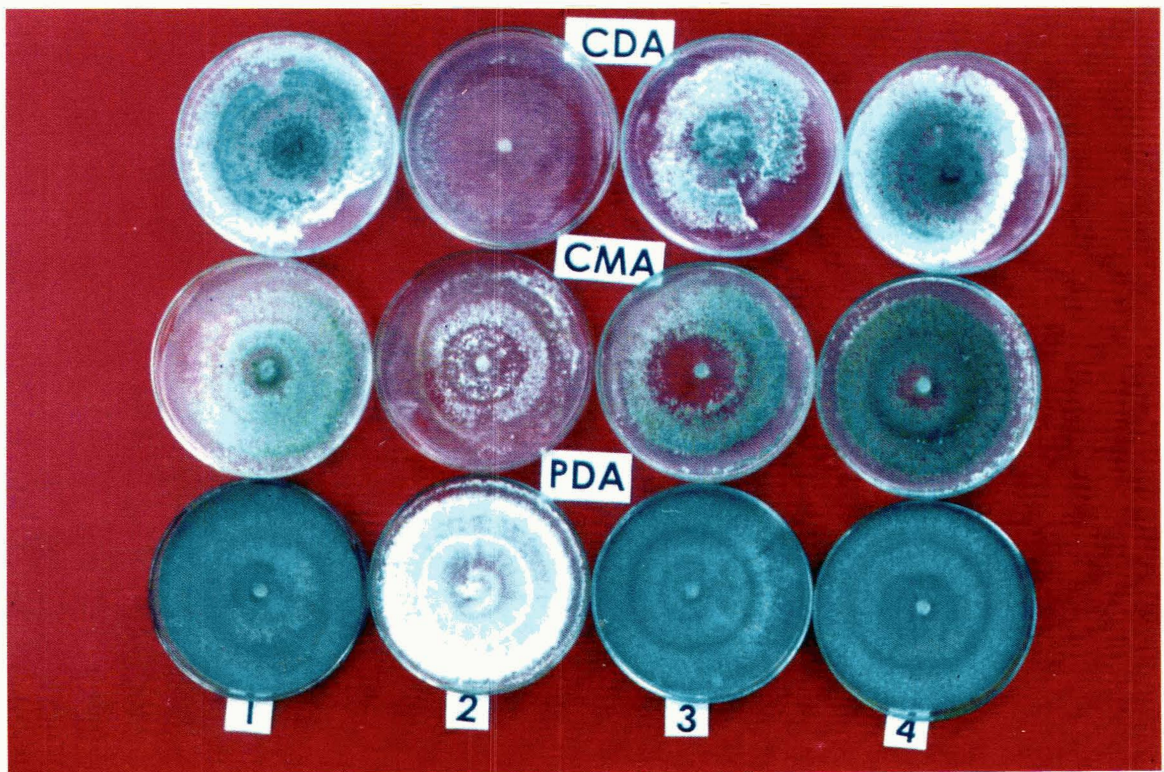


Plate 10:Growth of Trichoderma and the developed strains on different media

1-*Trichoderma harzianum*-7 (Wild type)   2-M_1-25

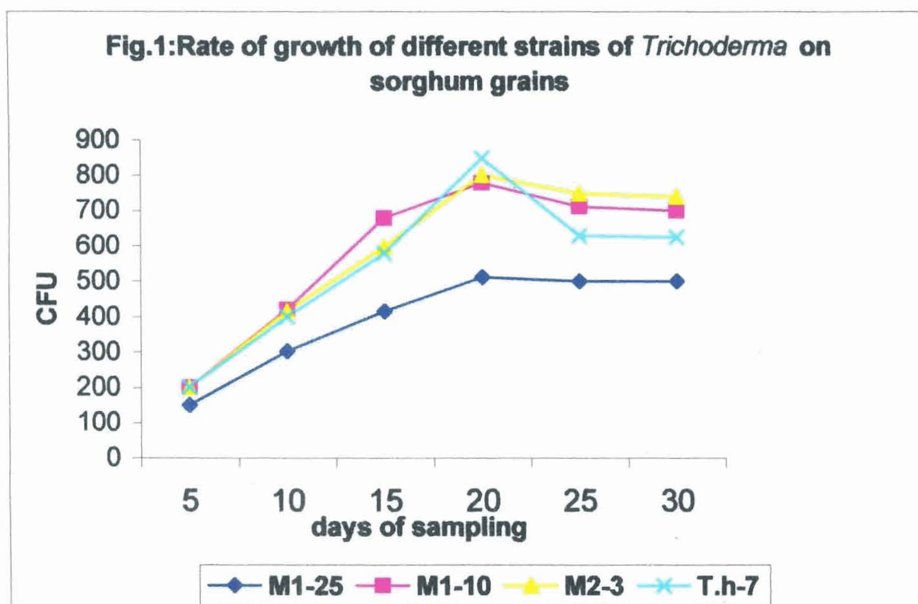
3-M_1-10   4-M_2-3

5.4: Rate of growth of *Trichoderma* and the mutants on sorghum grains:

When the different mutants were grown on broken sorghum meal in polypropelene bags, it was found that the colony forming units started steadily increasing from the 5th day, reached the peak after the 20th day, then reduced a little and remained constant from the 30th day onwards. Though the maximum number of colonies were obtained in the wild type, there was a sharp decline in the number of spores after the 20th day.(Table 30) (Fig1).

Table 30:Rate of growth of *Trichoderma* and the mutants on sorghum grains:

Treatment	5th day	10th day	15th day	20th day	25th day	30th day
M_1-25	150	302	415	512	500	500
M_1-10	200	420	680	780	712	700
M_2-3	199	415	598	801	749	740
T.h-7	201	399	580	850	630	625
CFU X10^5						



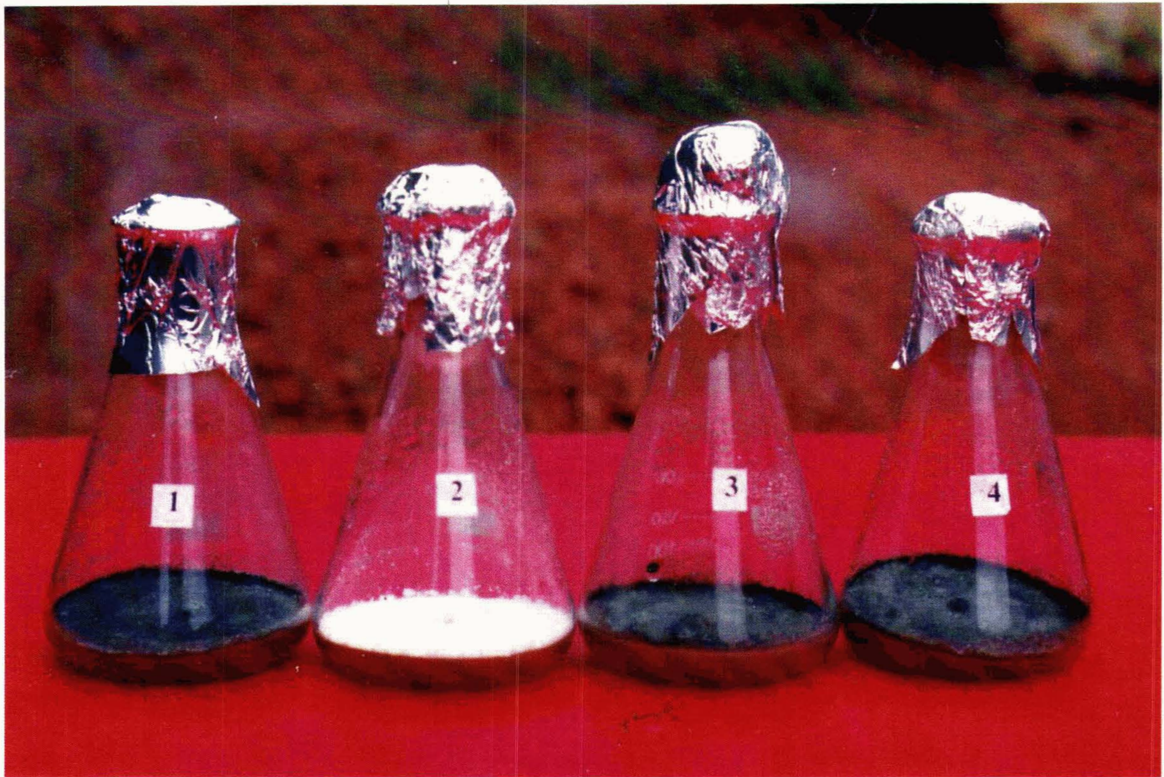


Plate 11: Rate of growth of the developed strains of *Trichoderma* for mass multiplication

1-*Trichoderma harzianum-7* (Wild type) 2-M_1-25

3-M_1-10 4-M_2-3

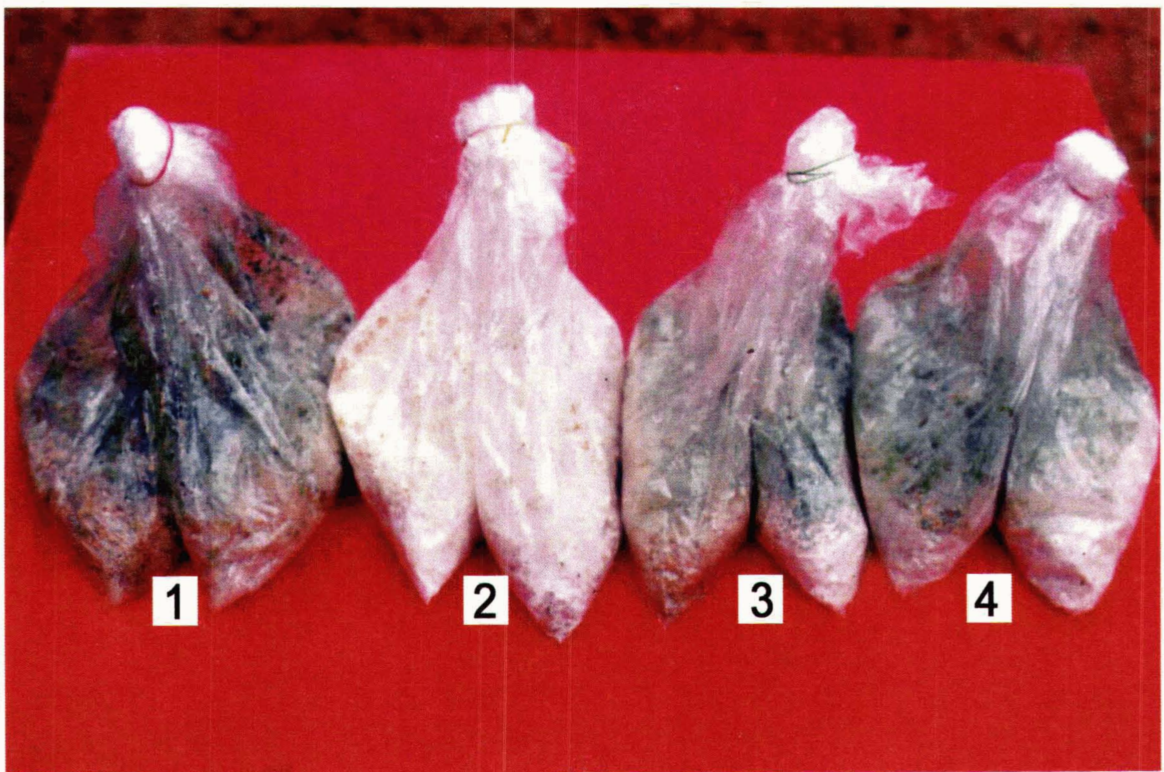


Plate 12: Mass multiplication of *Trichoderma* and the developed strains on sorghum grains

1-*Trichoderma harzianum-7* (Wild type) 2-M_1-25

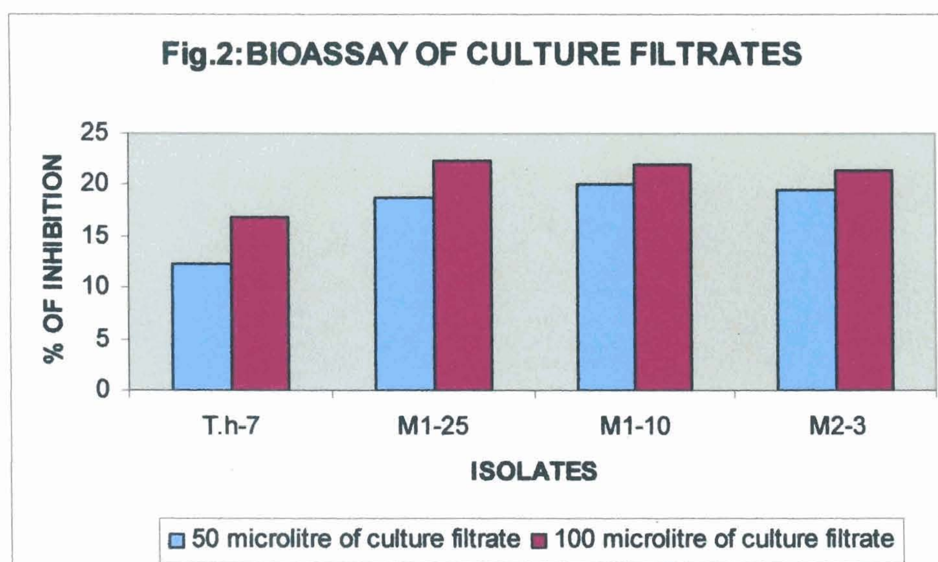
3-M_1-10 4-M_2-3

5.5: Bioassay of the culture filtrates:

The culture filtrates (50μ l and 100μ l) of *Trichoderma* and its mutants were amended with PDA and plated on petriplates. The rhizome rot pathogen *Pythium aphanidermatum* was plated on the plates and the percentage of inhibition was calculated after 48 hours. The results obtained indicated that the mutants showed an inhibition percentage ranging from 18 to 20 % while the wild type recorded an inhibition percentage of 12.3% when the media was amended with 50μ l of the culture filtrate. However when 100μ l of the culture filtrate was added to the medium the inhibition percentage ranged from 21.4 to 22 % when compared to 16.9% in the wild type (Table 31)(Fig 2).

Table 31: Bioassay of the culture filtrates:

Sl.No	Isolate No.	% Inhibition when 50μ l was added	% Inhibition when 100μ l was added
1	*T.h*-7	12.3^{d}	16.9^{d}
2	M_1-25	18.8^{c}	22.4^{a}
3	M_1-10	20.1^{a}	22.0^{b}
4	M_2-3	19.6^{b}	21.4^{c}



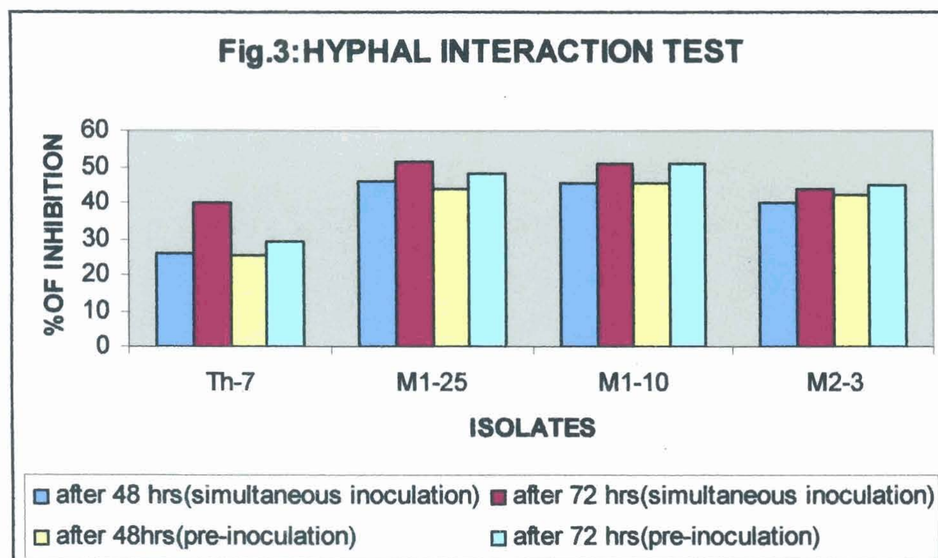
5.6: Mechanism of interaction:

5.6.1: Hyphal interaction test:

Two types of tests were conducted in order to determine the rate at which the growth of the pathogen was inhibited. When the pathogen and the test organisms were simultaneously inoculated the percentage of inhibition ranged from 39% to 51.2% after 72 hours while in the second test where the test organism was inoculated prior to the pathogen the percentage of inhibition ranged from 29 to 51%. In all the isolates except M_1-25, where a clear inhibition zone was obtained, the test organism over grew the pathogen. The percentage of inhibition of the mutants was significantly higher than the wild type (Table 32) (Fig.3).

Table 32: Hyphal interaction test:

Sl.No.	Isolate No.	Simultaneous inoculation % of inhibition after		Pre-inoculation % of inhibition after	
		48 hours	72 hours	48 hours	72 hours
1	*T.h*-7	26.1	39.8[c]	25.2	29.1[d]
2	M_1-25	46.0	51.2[a]	44.0	48.0[b]
3	M_1-10	45.5	51.0[a]	45.5	51.0[a]
4	M_2-3	40.0	43.8[b]	42.0	44.8[c]

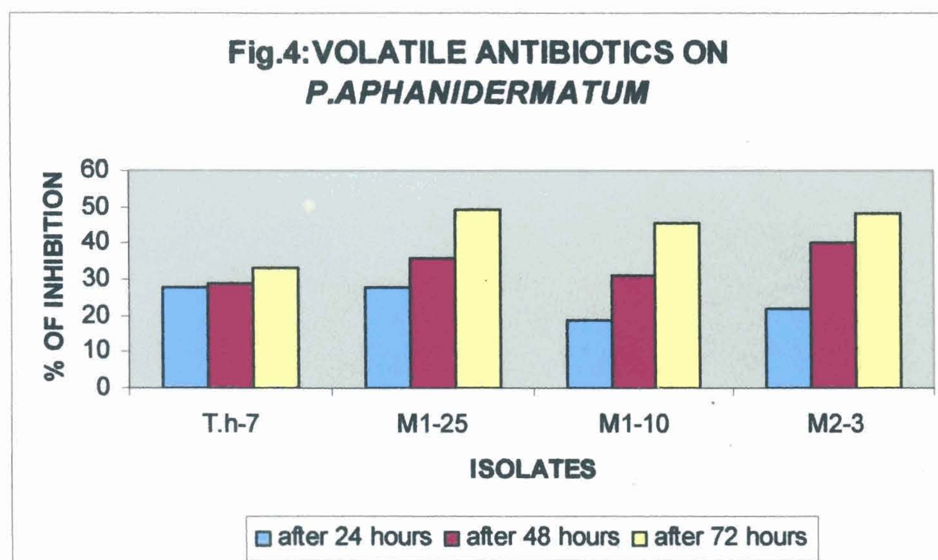


5.6.2: Volatile antibiotic test:

In the volatile inhibition test the rate of growth of the pathogen was measured after 24, 48 and 72 hours. It was found that the rate of inhibiton ranged between 33.0% to 49.2% after 72 hours. However the percentage of inhibition of the mutants were significantly greater (45-49.2%) than the wild type which was only 33.0% (Table 33)(Fig.4).

Table 33: Volatile antibiotic test:

Sl.No.	Isolate No.	% of inhibition after		
		24 hours	48 hours	72 hours
1	*T.h*-7	28.1	28.8	33.0^{c}
1	M_1-25	28.1	36.1	49.2^{a}
2	M_1-10	18.75	31.3	45.6^{b}
3	M_2-3	21.8	40.1	48.0^{ab}

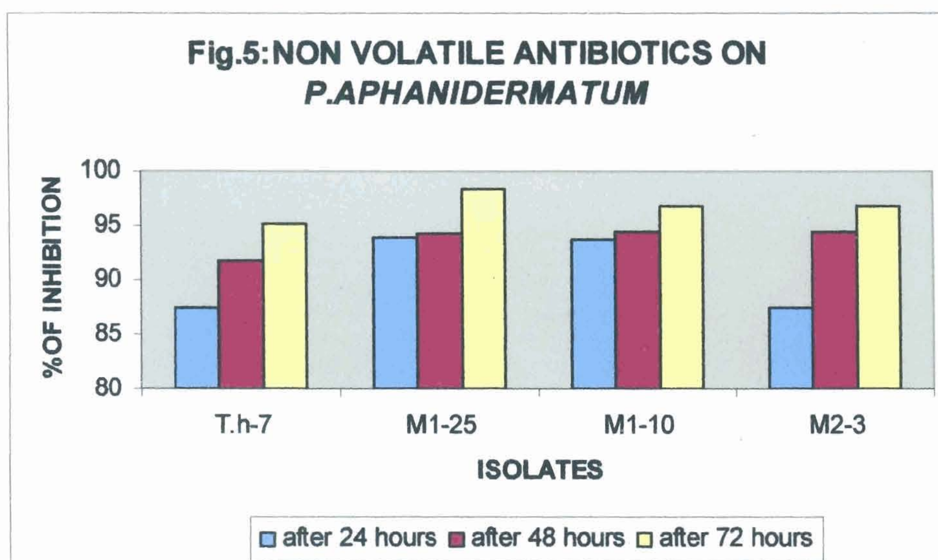


5.6.3: Non Volatile antibiotic test:

In the non volatile antibiotic test all the isolates showed a complete inhibition of the pathogen. The growth of the pathogen was visible only around 0.1 or 0.2 mms around the disc. In all the isolates the percentage of inhibition was above 95% and the growth of only the test organisms were visible after 72 hours (Plate 14).

Table 34: Non volatile antibiotic test:

Sl.No.	Isolate No.	% of inhibition after		
		24 hours	48 hours	72 hours
1	Th-7	87.5	91.8	95.2[c]
2	M_1-25	94.0	94.28	98.4[a]
3	M_1-10	93.75	94.48	96.8[b]
4	M_2-3	87.5	94.48	96.8[b]



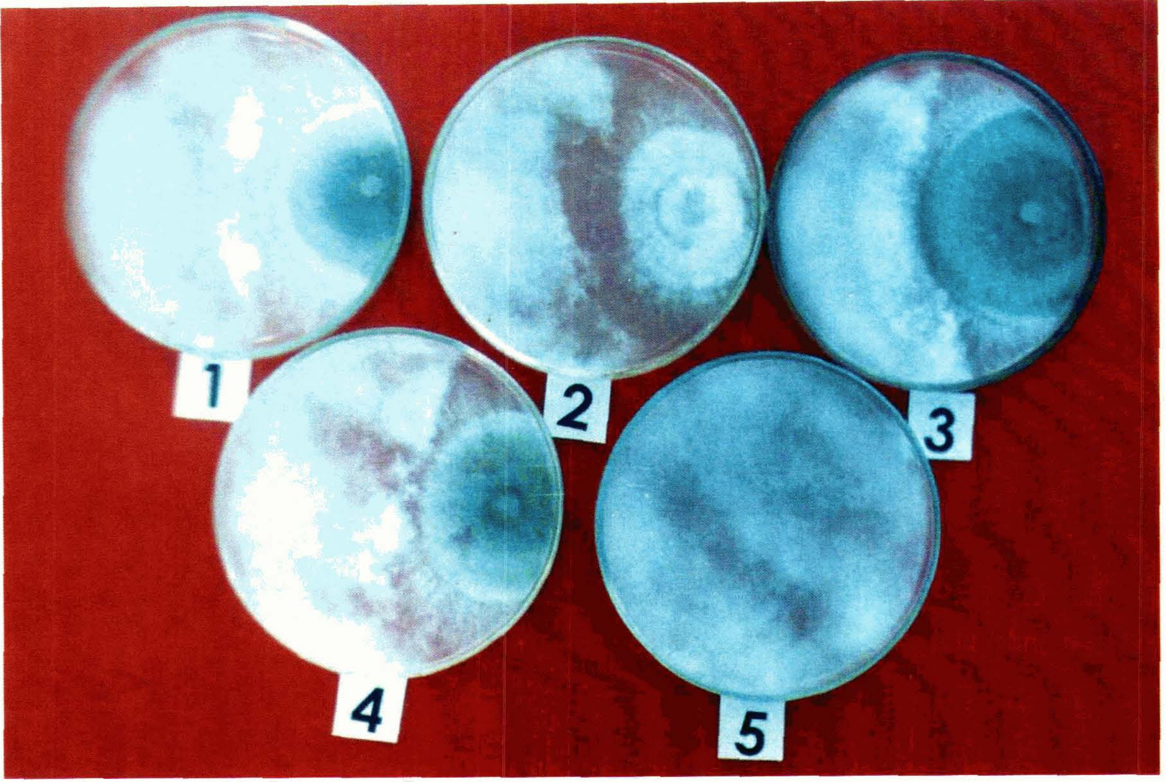


Plate 13: Inhibition of *Pythium aphanidermatum* by dual culture

1-*T. harzianum*-7 X *P. aphanidermatum* 2-M_1-25 X *P. aphanidermatum*
3-M_1-10 X *P. aphanidermatum* 4-M_2-3 X *P. aphanidermatum*
5- *P. aphanidermatum alone* (Control)

Plate 14: Inhibition of *Pythium aphanidermatum* by non volatile antibiotics

1-M_1-25 (mutant) 2-M_2-3 (mutant) 3-M_1-10 (mutant) 4-*T. harzianum*-7 (wild type)
5-*Pythium aphanidermatum* alone (Control)

Plate 15: Inhibition of *Pythium aphanidermatum* by volatile antibiotics (Petriplates sealed together)

1-*T. harzianum-7* X *P. aphanidermatum* 2-M_1-25 X *P. aphanidermatum*
3-M_1-10 X *P. aphanidermatum* 4-M_2-3 X *P. aphanidermatum*

Plate16: Inhibition of *Pythium aphanidermatum* by volatile antibiotics (Petriplates kept open)

1-*T. harzianum-7* X *P. aphanidermatum* 2-M_1-25 X *P. aphanidermatum*
3-M_1-10 X *P. aphanidermatum* 4-M_2-3 X *P. aphanidermatum*

5.7: Effect of temperature on the growth of *Trichoderma* and its mutants:

The rate of growth of *Trichoderma* and its mutants at different temperatures indicated that there was complete inhibition of the test organisms at 45^0c.However the growth of the organisms at 20^0c was on par with the growth at room temperature. There was no growth rate at 15^0c (Table 35). The radial growth was measured in mms.

Table 35:Rate of growth (in mm) of mutants at different temperatures:

Isolate No.	24 hours				48 hours				72 hours			
	15^0c	20^0c	30^0c	45^0c	15^0c	20^0c	30^0c	45^0c	15^0c	20^0c	30^0c	45^0c
M_1-25	-	21	23	1	-	35	33	2	-	36	41	2
M_1-10	-	22	26	0	-	35	40	1	-	38	45	1
M_2-3	-	23	25	3	-	36	40	3	-	39	45	3
T.h-7	-	20	23	2	-	30	33	2	-	39	43	2

5.8.1: Sensitivity to agrochemicals:

The rate of growth of the different mutants was on par with the rate of growth of the wild type (Table 36). As the concentration of the agrochemicals increased, there was reduction in the growth of the isolates. The rate of growth of the albino mutant M_1-25 was less than the growth of the wild type. However the growth of the other two mutants M_2-3 and M_1-10 was slightly more than the wild type in the untreated plates.

When treated with the fungicides viz Copper oxy chloride, the growth of the mutants M_2-3 and M_1-10 was more than the wild type. M_2-3 exhibited only 13% inhibition after 48 hours at 0.2 % concentration of COC while M_1-10 exhibited only 10.8% inhibition. The wild type exhibited 29.3 % inhibition after 48 hours. The albino mutant, M_1-25 grew faster than the wild type at 0.4 % and 0.6% concentration. It exhibited growth upto 0.8% concentration while in the other isolates the growth was arrested after 0.6% concentration. When treated with Mancozeb, the growth was observed only at 0.3% concentration. At 0.6 % concentration the growth was visible only in the wild type as well as the mutant M_1-10. When treated with the fungicide Metalaxyl Mancozeb the growth was observed only after one week in 500 ppm of the fungicide amended solution. As such it was found that all the mutants were compatible with the different agrochemicals at different proportions. The observations were tabulated as in Table 36.

Table 36: Compatibility of the mutants with the agrochemicals.

	Growth (Diameter) in cms							
	T.h-7		M_1-25		M_2-3		M_1-10	
	48 hrs	168 hrs	48 hrs	168 hrs	48 hrs	168 hrs	48 hrs	168 hrs
Control	6.5	Full*	5.6	Full	8.0	Full	8.2	Full
0.2%COC	2.9	Full	3.56	Full	3.0	Full	4.0	Full
0.4%COC	1.95	Full	2.45	Full	1.9	Full	2.1	Full
0.6%COC	1.8	Full	1.2	Full	1.8	Full	1.9	Full
0.8%COC	-	-	1.2	1.2	-	-	-	-
0.3%DM-45	2.8	Full	1.1	Full	2.9	Full	2.8	Full
0.6%DM-45	1.0	6.0	-	-	-	-	1.2	5.2
0.9%DM-45	1.6	-	-	-	-	-	1.0	-
1.2%DM-45	-	-	-	-	-	-	-	-
100ppm RMZ	-	7.2	-	6.9	-	6.5	-	6.9
200ppm RMZ	-	1.4	-	-	-	1.2	-	1.4
400ppm RMZ	-	-	-	-	-	-	-	-
500ppm RMZ	-	-	-	-	-	-	-	-

Full *-completely covered the petriplates

5.8.2: Mycelial growth on PD Broth amended with the fungicidal agrochemicals:

After one week, mycelial growth was visible in all the flasks in which the fungi were inoculated. The mycelial weight of the of the mutants was significantly more than the mycelial weight of the wild type in all treatments except at 0.3% Mancozeb concentration. When treated with COC the mycelial growth at 0.4% COC concentration was more than the mycelial weight at 0.2% concentration. When treated with Metalaxyl Mancozeb the mycelia grew at all concentrations from 100ppm to 500ppm. In Mancozeb also, the mycelia grew at all concentrations from 0.3% to 1.2%. The mycelial weight of the mutant M_1-25 was significantly more than the wild type at 0.4% and 0.6% concentration of COC and in the untreated flasks. The mycelial weight of the mutant, M_1-10 was more than the rest at 0.8% COC, 0.6%, 0.9% & 1.2% Mancozeb concentration. Similarly the mycelial weight of the mutant M_2-3 was more than the others at 0.2% COC and 200 ppm RMZ concentration (Table 37).

Table 37: Growth of mutants on PD Broth:

Treatments	*T.h-7* (mg)	Diff. over control (%)	$M_1 25$ (mg)	Diff. over control (%)	$M_2 3$ (mg)	Diff. over control (%)	$M_1 10$ (mg)	Diff. over control (%)
Control	145.6^{d}		218.4^{a}	-20.9	152.7^{c}		183.9^{b}	
0.2%COC	92.3^{d}	-36.6	172.7^{b}	+18.4	173.8^{a}	+13.8	148.9^{c}	-19.0
0.4%COC	215.9^{b}	+48.2	258.8^{a}	+66.8	169.3^{d}	+10.8	206.6^{c}	+12.3
0.6%COC	188.2^{d}	+29.2	364.5^{a}	-29.1	289.1^{b}	+89.3	269.7^{c}	+46.6
0.8%COC	40.3^{d}	-72.3	154.9^{c}		174.5^{b}	+14.2	229.3^{a}	+24.7
0.3%DM-45	250.0^{a}	+71.7	230.3^{c}	+5.4	247.5^{b}	+62.1	232.4^{d}	+26.4
0.6%DM-45	180.4^{d}	+23.9	223.3^{c}	+2.2	284.9^{b}	+86.5	287.8^{a}	+56.5
0.9%DM-45	171.3^{d}	+17.6	220.2^{c}	+0.8	249.2^{b}	+63.2	256.0^{a}	+39.2
1.2%DM-45	92.74^{d}	-36.3	127.8^{c}	-41.5	228.6^{b}	+79.7	272.9^{a}	+48.4
100ppm RMZ	196.1^{b}	+34.6	193.0^{c}	-11.6	187.7^{d}	+22.9	196.6^{a}	+6.9
200ppm RMZ	188.7^{c}	+29.6	147.4^{d}	-32.5	215.1^{a}	+40.9	206.5^{b}	+12.2
400ppm RMZ	151.2^{d}	+4.12	235.5^{a}	+7.8	227.3^{b}	+48.8	226.4^{c}	+23.1
500ppm RMZ	162.2^{d}	+11.6	194.6^{b}	-10.9	182.7^{c}	+19.6	226.0^{a}	+22.8

- decrease in mycelial weight over control
+ increase in mycelial weight over control

17: Growth of wild type and mutants in copper-oxy-chloride amended medium

18: Growth of wild type and mutants in Mancozeb amended medium

19: Growth of wild type and mutants in Metalaxyl Mancozeb amended medium

Plates 17-19: Compatibility of *Trichoderma* and the developed strains with agrochemicals

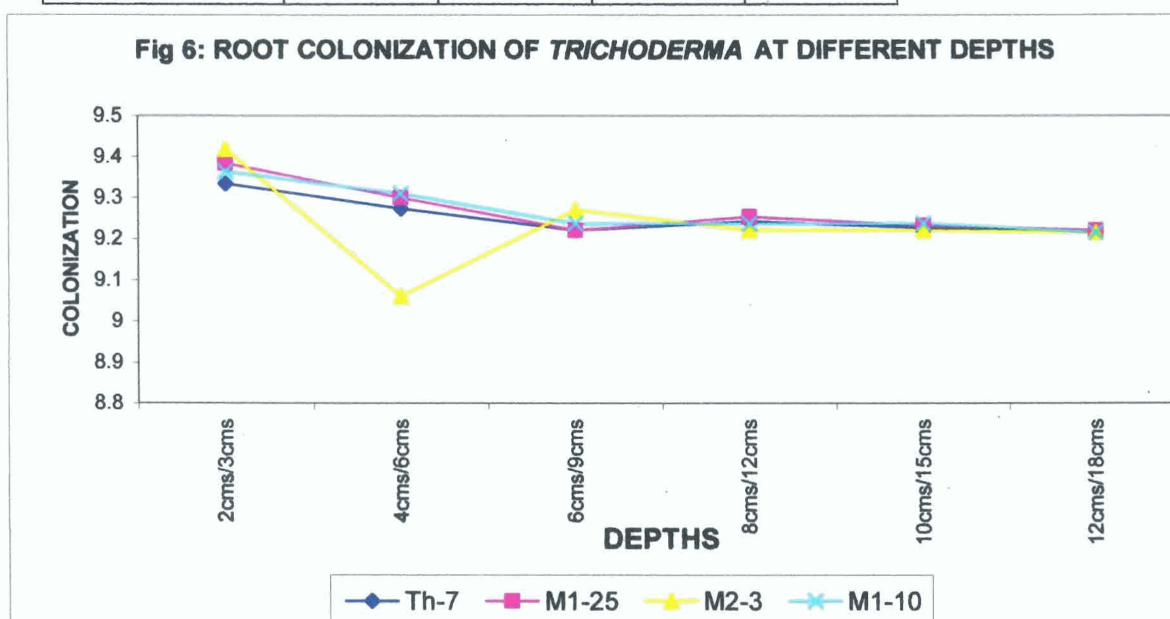
1-*Trichoderma harzianum-7* (Wild type) 2-M_1-25 3-M_1-10 4-M_2-3

5.9.1: Rhizosphere Competence Assay

The ginger seed rhizomes were allowed to grow till the roots grew to a considerable length. After allowing the growth for 60 days, the cups were removed. The length of the roots were measured. It was then excised at 2cm or 3cms into 6 equal parts with sterile scalpel. The rhizosphere soil were plated on TSM and the CFU per mg of the soil for each root segment was determined. The data obtained revealed that the biocontrol agents could be detected in the soil at any depth. However the population density was maximum at the upper 4cm depth. At the 6cm depth there was a reduction in the number of colonies obtained in the wild type, *T.h-7* and the mutant M_1-25. In the other isolates the number of colonies obtained gradually reduced as the depth of the roots increased. The log transformed CFU of each isolate at 2cm / 3 cm depths is recorded in the Table 38.

Table 38: Population levels of *Trichoderma* at different root depths:

Root depth	*T.h-7*	M_1-25	M_2-3	M_1-10
2cms/3cms	9.3448	9.3838	9.418	9.3631
4cms/6cms	9.2743	9.3003	9.2602	9.31
6cms/9cms	9.2213	9.2213	9.2696	9.2377
8cms/12cms	9.2428	9.2533	9.2213	9.2377
10cms/15cms	9.227	9.232	9.2213	9.2372
12cms/18cms	9.2151	9.2213	9.2151	9.2151



20-Ginger rhizomes treated with different strains of *Trichoderma* in tea cups kept in plastic trays covered with polythene sheet

21-Sprouted seed rhizomes of ginger in tea cups kept in plastic trays

22-Tea cups with sprouted rhizomes removed from the plastic trays

Plates 20-22:Rhizosphere Competence Assay

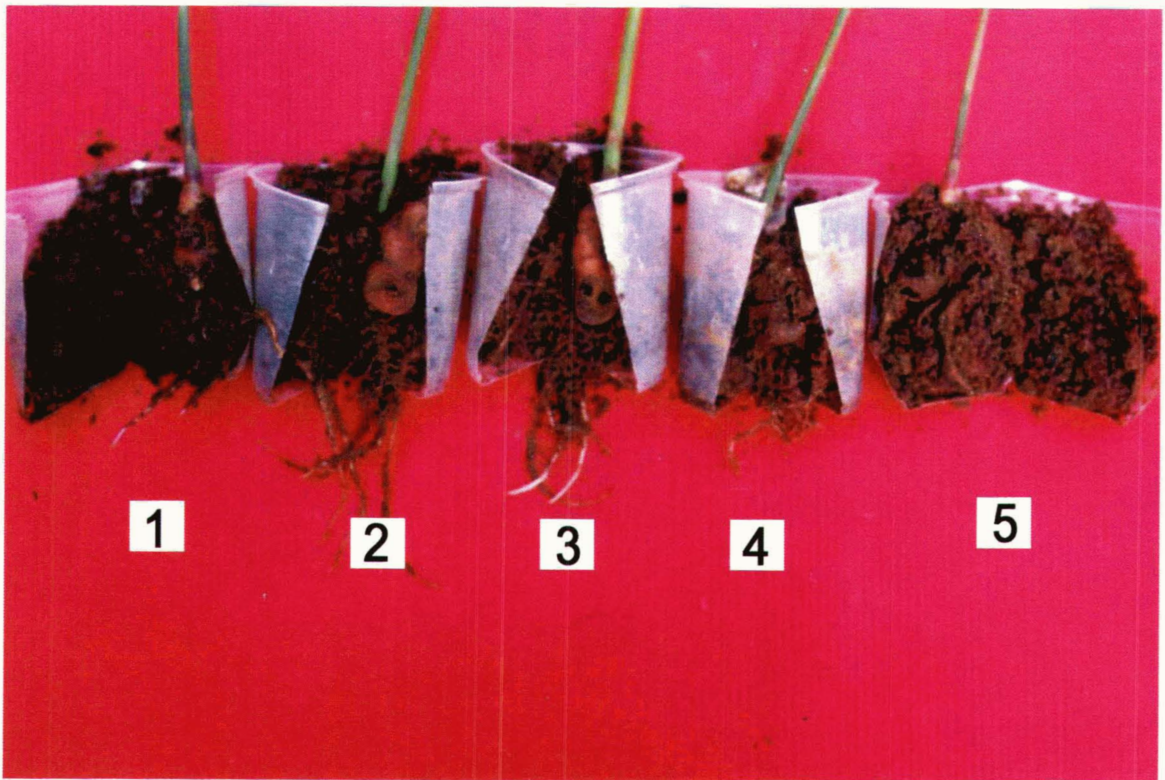


Plate 23-Tea cups with the sprouted seed rhizomes split open

Plate 24-Ginger rhizomes with roots colonized by different strains of *Trichoderma*

1-*Control (untreated rhizome)* 2- *Trichoderma harzianum-7*
3-M_1-25 4-M_1-10 5-M_2-3

5.9.2: Test to determine the rhizosphere competence:

When the different mutants were plated on petriplates containing CDA with glucose as the carbon source, the rate of growth of the mutant M_1-25 was lesser than the wild type. But the mutants M_1-10 and M_2-3 grew faster when compared to the wild type after 48 hours. After 72 hours all the isolates showed uniform growth because the entire plate was covered with the fungus. The cfu in the wild type was almost on par with the mutants M_1-10 and M_2-3. The number of spores in the albino mutant was comparatively lesser (900 $X10^4$ units / ml) than the wild type (1160 X 10^4 units / ml). When the isolates were grown on petriplates containing CDA with CMC as the carbon source, the rate of growth of the mutants M_1-10 and M_2-3 was greater than the wild type after 48 hours even though uniform growth was obtained after 72.hours. In the CD broth amended with glucose or CMC as the carbon source, the rate of growth of the mutants M_1-25, M_2-3 and M_1-10 was greater than the wild type (Table 39). Thus the data obtained revealed that the mutants exhibited increased growth on CDA amended with CMC and this could be due to the rhizosphere competence by the secretion of β 1, 4 endo glucanase.

Table 39: Test to determine the rhizosphere competence:

Isolate No	Radial growth in CDA (in cms)			CFU $X10^4$	Mycelial wt. in CDB	Radial growth in CDA+CMC(in cms)			CFU $X\ 10^4$	Mycelial wt in CDB+ CMC
	24 hrs.	48hrs.	72 hrs.			24hrs.	48hrs.	72hrs.		
T.h-7	2.0	3.6	Full*	1160	148.0	1.7	3.6	Full	396	150.0
M_1-25	1.9	3.6	7.8	900	218.3	1.8	3.0	7.5	350.0	215.2
M_1-10	2.2	5.3	Full	1165	183.8	2.4	4.6	Full	480	168.0
M_2-3	2.2	3.8	Full	1100	152.7	2.3	4.5	Full	405	161.6

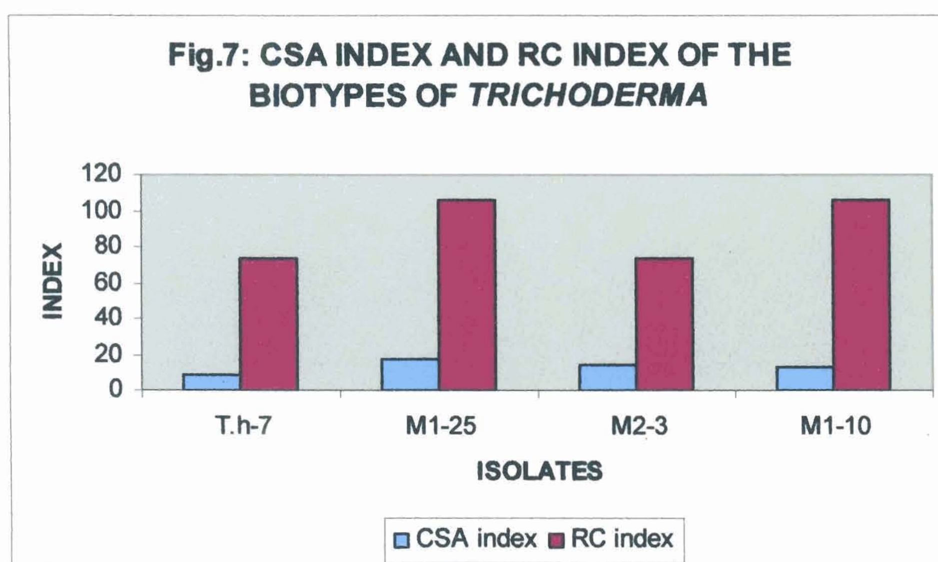
*-completely covered the petriplates

5.10.1: Competitive Saprophytic Ability:

The containers in the experiment was incubated for a period of 10 days. After 10 days the cellophane discs were retrieved from the soil and plated on TSM in order to determine the % of colonization. From the data obtained, the CSA was determined. The observations revealed that the mutants and the wild type could be isolated from the soil at any population density. The percentage of colonization of the cellophane discs was not proportional to the conidial density in the soil. The RC index was also calculated for the different strains and tabulated (Table 40). The CSA index of the mutant M_1-25 was found to be significantly greater than the wild type.

Table 40: CSA index and RC index of the different strains of *Trichoderma:*

Treatments	CSA index	RC index
T.h-7	8.5164	73.90
M_1-25	17.624	106.009
M_2-3	13.9038	73.96
M_1-10	12.44	106.35
CD at 5%	5.181	52.69

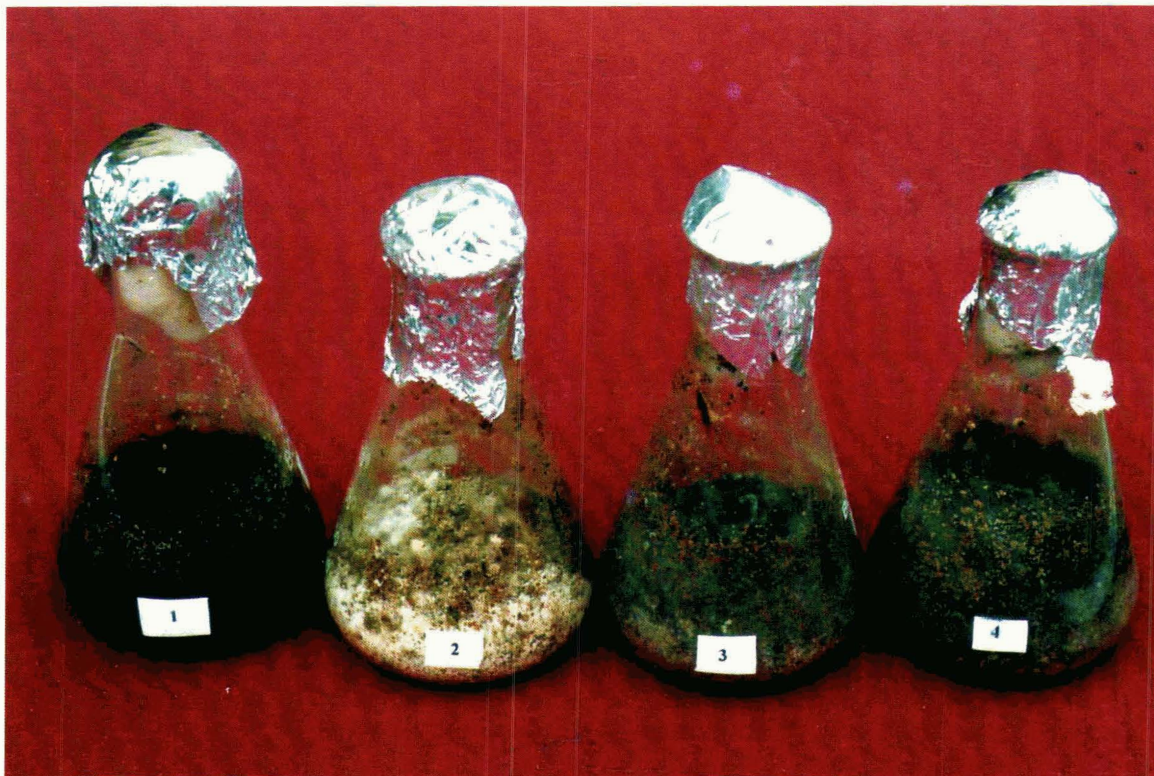


5.10.2: Competitive Saprophytic Ability by Agar plate method:

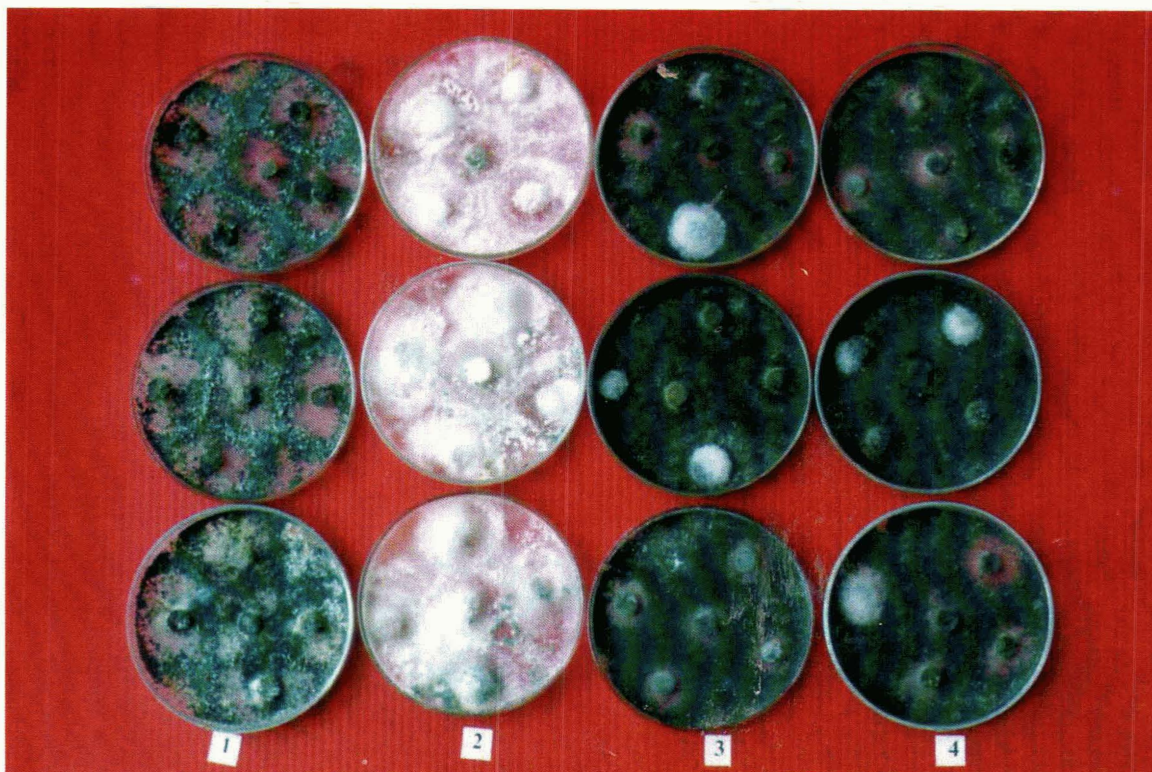
From the data obtained it was revealed that the percentage of colonization of the mutants was almost on par with the wild type. The treated fungi colonized the plates almost completely after 10 days of incubation. This was because the *Trichoderma* are fast growing. After 3 days of growth, the colonies started sporulating and they were assigned with the rating of 1, 3/4, 1/2 & 1/4 depending upon the area of colonization of the disc. The values obtained were added up together and multiplied by 10 in order to give the percentage figure and it was revealed that as the percentage of inoculum decreased, the percentage of colonization of the treated fungi also decreased. The mean percentage of all the fungi were almost on par with each other. The main aim of the present investigation was to determine whether the introduced isolates are competitively saprophytic with the native microflora of the soil. The experimental results obtained clearly revealed that more than 50% of the colonies obtained were that of the introduced fungi. Therefore it could be concluded that all the fungi ie *Trichoderma* and its mutants are vigourously competitively saprophytic (Table 41).

Table 41: CSA by Agar plate method:

% of inoculum	CFU / g	% of colonization of			
		T.h-7	M_1-25	M_1-10	M_2-3
98	$50X10^7$	95	90	97.5	95
90	$51X10^7$	90	90	95	75
75	$48X10^7$	70	70	60	70
50	$30X10^7$	60	45	52.5	60
25	$35X10^7$	55	47.5	60.0	62.5
10	$23X10^7$	42.5	27.5	42.5	52.5
Mean		68.5	61.6	67.6	69.1



25-Inoculum of different strains of *Trichoderma* in sorghum –sand mixture



26-Colonisation of the inoculated discs by the different strains of *Trichoderma*

1-*Trichoderma harzianum-7* 2-M_1-25 3-M_1-10 4-M_2-3

Plates 25-26: Test to determine the competitive saprophytic ability

5.10: Enzyme Assay (β-1, 4 & β-1, 3 endoglucanase)

The data on the β-1, 4 & β-1, 3 endoglucanase activity showed the inducible and non inducible nature of the enzyme and the variation among the strains in enzyme production in all the three carbon sources.

When the strains were grown in medium supplemented by sucrose as the carbon source all the biotypes produced β-1, 4 endoglucanase activity higher than the wild type (Table 37). In the sucrose amended media higher quantity of enzyme was produced by M_I-25 (124.2 EGU), followed by M_I-10 (89.75 EGU) and M_2-3 (55.05 EGU) than the wild type (35.35 EGU).

The data on the β-1, 3 endoglucanase production revealed that when the autoclaved mycelia was supplemented as the carbon source, the enzyme activity was significantly higher (4-6 fold) than when sucrose was used as the carbon source. In this experiment also the enzyme activity of the mutant M_I-25 (338.5 GU), followed by M_I-10 (216.1 GU) and M_2-3 (213.15 GU) was found to be superior than the wild type (153.8 GU) (Table 42).

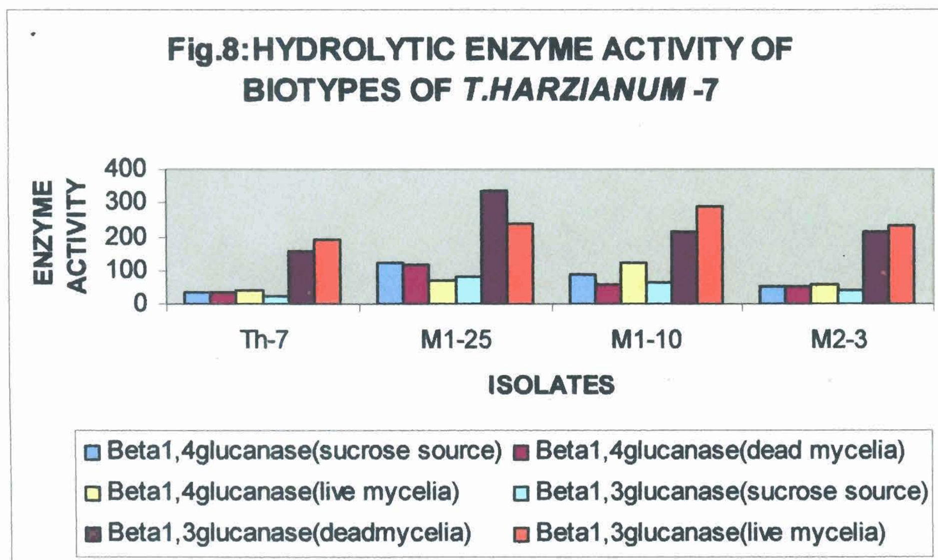
The enzyme activity of the biotypes in both the inducible and non inducible condition observed in the present study reveals the enzymatic variation among the biotypes. The variation in the enzyme activity clearly reflects the varying degree of the substrate utilization by the biotypes which in turn indicate their different mycoparasitic potential.

Table 42: Hydrolytic enzyme activities of biotypes of *T.harzianum*-7

Biotypes	β-1,4 endoglucanase*			β-1,3 endoglucanase**		
	Cabon source			Carbon source		
	Sucrose	Autoclaved mycelial mat	Live mycelial mat	Sucrose	Autoclaved mycelial mat	Live mycelial mat
Wild Type	35.35^{d}	30.25^{d}	40.4^{cd}	24.65^{c}	153.8^{cd}	192.4^{bd}
M_I-25	124.3^{a}	115.2^{ab}	67.31^{bcd}	83.6^{ab}	338.5^{a}	235.6^{abc}
M_I-10	89.75^{abc}	59.68^{cd}	119.8^{a}	60.95^{ab}	216.1^{bc}	292.7^{ab}
M_2-3	55.05^{cd}	53.70^{cd}	60.4^{cd}	38.1^{c}	213.1^{bc}	229.6^{bc}

*1EGU=Release of 1µmol glucose/ml of culture filtrate/min/mg protein

** 1GU=Release of 1µmol glucose/ml of culture filtrate/min/mg protein



6: Experiments conducted under *invivo* conditions:

6.1: Pot culture Experiments:

6.1.1: Integrated Management of rhizome rot of ginger:

The data obtained for the two consecutive years indicated that the isolate M_1-25 which was the mutant of *T.harzianum* –7 was found to be superior in its biocontrol efficacy since a reduced disease incidence as well as an increased yield was obtained when this isolate was tested in different combinations with the different fungicides as well as starch (Table 43).The same result was obtained in the next consecutive year also (Table 44). The pooled data obtained indicated that there was significant reduction in the disease incidence in the treated plots as compared to control where 50% disease incidence was recorded. An increased yield was also obtained in the different combinations of fungicides and starch with the biotype, M_1-25. Among the different treatments, the combination of the mutant with Ridomil- MZ was found to be superior as a reduced percentage of disease incidence (5.4%) and an increased yield (273.1 g/pot) was observe in these treatments. Similarly among the treatments there was increased bioefficacy in the different strains when compared to the wild type where a disease incidence ranging from 13-18.5 % was recorded while in the other strains only 5-17% disease incidence was recorded.Similarly a

comparison of the wild type with the mutants showed that the strain M_1-25 was superior in its bioefficacy when compared to the other strains as an increased germination percentage of 81.9, increased height of 34.9 cms, reduced disease incidence of 2.8% and increased yield of 177.6g / pot compared to a germination percentage of 75.7, height of 30.0 cms, disease incidence of 17.9 % and yield of 120.0g / pot in the wild type was obtained. These treatments were significantly superior over control where a germination percentage of 56.2, height of 24.9 cms disease incidence of 50% and a reduced yield of 37.5 g / pot was recorded (Table 45)(Fig.9)

Table 43: Integrated Management of rhizome rot of ginger- First Year

No.	Treatment	Germ %	Sprouts /pot	Heigh t(cms)	D.I (%)	Yield/po t(g)	Difference in yield over control
1	RMZ +M_1-25	91.7	10.2	40.6	2.4	283.0	+248.0
2	RMZ +M_1-10	91.7	8.8	46.5	6.5	240.3	+205.3
3	RMZ +M_2-3	95.8	7.0	33.9	6.7	186.3	+151.3
4	RMZ +*T.h*-7	83.3	10.8	41.2	8.9	209.3	+174.3
5	RMZ alone	91.7	9.3	33.5	10.7	165.0	+130.0
6	DM-45+ M_1-25	91.7	9.3	35.7	3.5	192.0	+157.0
7	DM-45+ M_1-10	83.3	7.8	33.5	12.4	187.3	+152.3
8	DM-45+ M_2-3	100.0	8.5	35.8	11.6	183.3	+148.3
9	DM-45+ *T.h*-7	87.5	8.3	35.6	14.8	178.7	+143.7
10	DM-45 alone	83.3	9.7	38.8	14.2	131.0	+96.0
11	Starch+ M_1-25	83.3	7.2	26.1	0.08	191.3	+156.3
12	Starch+ M_1-10	75.0	5.3	27.2	9.2	171.0	+136.0
13	Starch+ M_2-3	79.2	7.8	35.9	4.4	179.3	+144.3
14	Starch+ *T.h*-7	50.0	7.0	24.3	11.9	104.0	+69.0
15	Starch alone	37.5	5.7	24.3	21.3	91.7	+56.7
16	M_1-25 alone	75.0	9.0	34.7	1.5	147.0	+112.0
17	M_1-10alone	70.8	6.3	28.5	7.6	168.3	+133.3
18	M_2-3 alone	70.8	6.7	29.8	6.5	120.0	+85.0
19	*T.h*-7alone	62.5	7.2	30.2	8.1	96.7	+61.7
20	Control	45.8	6.8	24.8	39.1	35.0	
	CD at 5%	22.4	2.6	9.8	11.9	86.3	

+- increase in yield over control

Table 44: Integrated Management of rhizome rot of ginger –Second Year

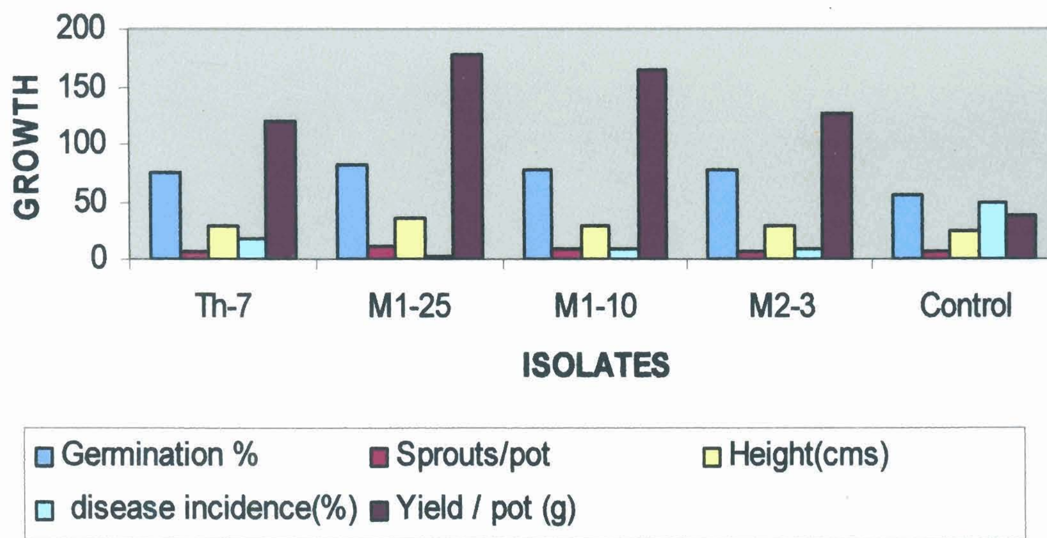
No	Treatment	Germ%	Sprouts /pot	Height (cms)	D.I (%)	Yield/pot(g)	Difference over control
1	RMZ +M_1-25	83.3	11.0	41.0	8.5	263.3	+223.3
2	RMZ +M_1-10	94.4	13.8	41.2	9.2	198.3	+158.3
3	RMZ +M_2-3	100.0	12.2	17.0	6.0	213.3	+173.3
4	RMZ +*T.h*-7	88.9	10.3	34.0	19.9	163.3	+123.3
5	RMZ alone	83.3	9.8	34.0	9.3	198.3	+158.3
6	DM-45+ M_1-25	94.5	10.0	36.	13.9	201.7	+161.7
7	DM-45+M_1-10	83.4	9.2	36.0	17.2	181.7	+147.7
8	DM-45+M_2-3	94.5	9.0	34.0	23.4	166.7	+126.7
9	DM-45+*T.h*-7	88.9	9.8	36.0	20.1	120.0	+80.0
10	DM-45 alone	72.2	7.8	39.0	27.6	83.3	+43.3
11	Starch+M_1-25	94.5	9.1	26.1	6.1	186.7	+146.7
12	Starch+M_1-10	100.0	10.3	24.3	14.5	198.3	+158.3
13	Starch+ M_2-3	94.5	10.3	27.2	15.8	176.7	+136.7
14	Starch+ *T.h*-7	88.9	12.7	36.0	25.0	166.7	+126.7
15	Starch alone	88.9	8.0	24.3	44.1	148.3	+108.3
16	M_1-25 alone	88.9	11.2	35.0	4.1	208.3	+168.3
17	M_1-10alone	83.4	10.0	31.0	10.9	161.7	+121.7
18	M_2-3 alone	83.3	7.8	29.0	10.9	133.3	+93.3
19	*T.h*-7alone	88.9	7.5	29.8	27.7	143.3	+103.3
20	Control	66.6	6.1	25.0	60.6	40.0	
CD at 5%		21.0	3.4	9.9	16.9	85.7	

+- increase in yield over control

Table 45: Pooled Data -Integrated Management of rhizome rot of ginger:

No.	Treatment	Germ%	Sprouts /pot	Height (cms)	D.I (%)	Yield/pot (g)	Difference over control
1	RMZ +M_1-25	87.5	10.5	40.8	5.45	273.1	+235.6
2	RMZ +M_1-10	93.05	11.3	43.85	7.85	219.3	+181.8
3	RMZ +M_2-3	97.9	9.6	35.45	13.3	199.8	+162.3
4	RMZ +*T.h*-7	86.1	10.55	37.6	8.35	186.3	+148.8
5	RMZ alone	87.5	9.55	33.75	9.1	181.6	+144.1
6	DM-45+M_1-25	93.1	9.65	35.85	8.7	196.9	+159.4
7	DM-45+M_1-10	83.35	8.5	34.75	14.8	184.5	+147.0
8	DM-45+ M_2-3	97.2	8.75	34.9	17.5	175.0	+137.5
9	DM-45+ *T.h*-7	88.2	9.05	35.8	17.45	149.4	+111.9
10	DM-45 alone	77.75	8.75	38.9	20.9	107.2	+69.7
11	Starch+ M_1-25	88.9	8.15	26.1	3.054	189.0	+151.5
12	Starch+ M_1-10	87.5	7.8	25.75	11.85	184.6	+147.1
13	Starch+ M_2-3	86.85	9.05	31.55	10.1	178.0	+140.5
14	Starch+ *T.h*-7	69.45	9.85	30.15	18.45	135.4	+97.9
15	Starch alone	63.2	6.85	24.3	32.7	120.0	+82.5
16	M_1-25 alone	81.95	10.1	34.85	2.8	177.6	+140.1
17	M_1-10alone	77.1	8.15	29.75	9.2	165.0	+127.5
18	M_2-3 alone	77.1	7.25	29.4	8.7	126.7	+89.2
19	*T.h*-7alone	75.7	7.35	30.0	17.9	120.0	+82.5
20	Control	56.2	6.45	24.9	49.85	37.5	
CD at 5%		27.87	4.1	8.1	17.9	53.5	

+- increase in yield over control



6.1.2: Combinations of bacteria and fungi on rhizome rot management:

The results obtained from this experiment revealed that the treatments with the combinations of the bacteria and fungi was found to be superior than the treatment of the biocontrol agents alone. The pooled data revealed that the various combinations of the different bacterial isolates with the strain, M_1-25 was found to show increased biocontrol efficacy in terms of increased yield (137.5-194.0 g / pot) and reduced disease incidence (2.5-8.1%) as against a yield of 26.9g / pot and disease incidence of 57.3% in the control. Although there was no significant differences among the other combinations, the pooled data revealed that the mutants were definitely superior to the wild type both in terms of increased yield as well as reduced disease incidence. This is clearly evident from the tables 46-48. When the different strains were treated alone, the superiority of the strains over the wild type was significantly established as an increased germination percentage of

81.25-93.95, height of 32.9-37.6 cms, reduced disease incidence of 10-16.5% and increased yield of 121-125.3 g / pot was obtained when compared to a germination percentage of 79.2%, height of 28.7cms, disease incidence of 31.4% and yield of 83.8 g/ pot in the wild type (Table 48).

Table 46: Combinations of bacteria and fungi on rhizome rot management-First year:

No.	Treatment	Germ%	Sprouts /pot	Height(cms)	D.I (%)	Yield/ pot(g)	Difference in yield over control
1	FP1+ M_1-25	87.5	8.3	35.7	1.7	176.3	+148.0
2	FP1+M_2-3	89.1	7.3	36.8	10.8	176.0	+147.7
3	FP1+M_1-10	79.2	7.6	32.9	11.0	133.7	+105.4
4	FP1+*T.h*-7	54.2	6.7	33.3	19.5	106.7	+78.4
5	FP1 alone	83.3	8.5	26.3	15.6	100.0	+71.7
6	FP43+ M_1-25	83.3	8.0	38.6	6.2	151.0	+122.7
7	FP43+M_2-3	75.0	8.0	35.7	9.4	143.3	+115.0
8	FP43+M_1-10	75.0	5.8	29.1	9.4	126.7	+98.4
9	FP43+*T.h*-7	41.7	7.0	26.9	11.1	61.7	+33.4
10	FP43 alone	79.2	7.2	23.6	30.8	132.0	+103.7
11	FP44+ M_1-25	75.0	4.7	30.9	3.3	113.3	+85.0
12	FP44+M_2-3	89.0	5.7	35.3	10.0	132.8	+104.5
13	FP44+M_1-10	65.8	8.0	32.2	13.5	159.8	+131.5
14	FP44+*T.h*-7	75.0	5.3	30.1	20.7	65.0	+36.7
15	FP44 alone	70.8	6.7	31.1	9.6	158.0	125.7
16	FP100+ M_1-25	91.7	8.8	41.5	0.0	185.0	+156.7
17	FP100+M_2-3	83.8	8.0	25.8	13.9	118.3	+90.0
18	FP100+M_1-10	87.9	9.0	30.2	9.3	126.0	+97.7
19	FP100+*T.h*-7	66.7	6.7	33.1	13.4	75.0	+46.7
20	FP100 alone	75.0	7.0	30.2	18.8	110.7	+82.4
21	FP113+ M_1-25	87.5	7.0	36.8	0.01	176.7	+148.4
22	FP113+M_2-3	82.2	8.0	36.3	6.6	146.5	+118.2
23	FP113+M_1-10	93.5	15.8	40.9	1.7	179.0	+150.7
24	FP113+*T.h*-7	62.5	9.3	29.0	11.9	78.3	+50.0
25	FP113 alone	54.2	6.0	24.6	20.1	103.7	+75.4
26	M_1-25alone	62.5	8.7	34.4	11.9	78.3	+50.0
27	M_2-3alone	64.6	8.2	38.0	11.6	101.2	+72.9
28	M_1-10alone	87.9	7.0	37.2	5.8	112.7	+84.4
29	*T.h*-7alone	58.3	7.2	28.6	26.8	81.7	+53.4
30	Control	41.7	5.8	28.7	54.7	28.3	
	CD at 5%	22.2	2.7	9.38	7.25	42.9	

+-increase in yield over control

Table 47: Combinations of bacteria and fungi on rhizome rot management-Second year:

No.	Treatment	Germ%	Sprouts /pot	Height (cms)	D.I (%)	Yield/ pot(g)	Difference in yield over control
1	FP1+ M_1-25	91.7	11.5	35.7	3.3	211.7	+186.2
2	FP1+M_2-3	91.7	8.0	32.9	12.2	133.3	+107.8
3	FP1+M_1-10	100.0	9.3	36.8	3.3	201.7	+176.5
4	FP1+*T.h*-7	83.3	7.3	33.3	7.8	153.3	+127.8
5	FP1 alone	100.0	9.5	29.0	3.8	141.5	+116.0
6	FP43+ M_1-25	91.6	8.0	38.7	9.9	216.7	+191.2
7	FP43+M_2-3	100.0	10.2	29.1	6.7	183.3	+157.8
8	FP43+M_1-10	91.7	11.3	35.7	7.6	220.0	+194.5
9	FP43+*T.h*-7	100.0	8.3	26.9	16.3	130.0	+104.5
10	FP43 alone	100.0	8.0	23.0	15.0	130.0	+104. 5
11	FP44+ M_1-25	83.3	10.0	30.9	2.9	161.7	+136.2
12	FP44+M_2-3	91.7	8.2	35.3	19.2	126.7	+101.2
13	FP44+M_1-10	100.0	6.3	30.1	7.1	163.3	+135.0
14	FP44+*T.h*-7	91.7	9.2	32.2	22.1	158.3	+132.8
15	FP44 alone	100.0	7.0	31.0	33.8	126.7	+101.2
16	FP100+ M_1-25	91.7	8.8	41.0	6.1	173.3	+147.8
17	FP100+M_2-3	83.3	6.3	28.0	7.7	126.3	+100.8
18	FP100+M_1-10	100.0	10.3	33.1	43.7	218.2	+192.7
19	FP100+*T.h*-7	75.0	5.5	30.2	79.7	126.0	+100.5
20	FP100 alone	100.0	5.6	36.8	19.6	80.0	+54.5
21	FP113+ M_1-25	91.7	5.6	36.2	4.1	175.0	+149.5
22	FP113+M_2-3	100.0	8.8	36.3	12.1	116.1	+90.6
23	FP113+M_1-10	91.7	7.8	40.0	6.7	113.2	+87.7
24	FP113+*T.h*-7	100.0	7.0	29.0	19.9	158.1	+132.6
25	FP113 alone	83.3	6.3	30.1	9.0	121.0	+95.5
26	M_1-25alone	100.0	7.8	38.0	0.3	166.0	+140.5
27	M_2-3alone	100.0	6.3	37.2	21.5	141.0	+115.5
28	M_1-10alone	100. 0	9.0	28.6	15.9	138.0	+112.5
29	*T.h*-7alone	100.0	6.8	28.7	35.9	85.2	+59.7
30	Control	83.3	7.2	28.7	60.0	25.5	
CD at 5%		18.4	2.9	7.24	29.9	83.0	

+- increase in yield over control

Table48: Combinations *of bacteria and fungi on rhizome rot management-Pooled data:

No.	Treatment	Germ%	Sprouts /pot	Height (cms)	D.I(%)	Yield/pot (g)	Difference in yield over control
1	FP1+ M_1-25	89.6	9.9	35.7	2.5	194.0	+167.1
2	FP1+M_2-3	90.4	7.65	34.85	11.5	154.6	+127.7
3	FP1+M_1-10	89.6	8.45	34.85	7.15	167.7	+140.8
4	FP1+*T.h*-7	68.75	7.0	33.3	13.65	130.0	+103.1
5	FP1 alone	91.65	9.6	29.15	9.7	120.8	+93.9
6	FP43+ M_1-25	87.45	8.0	38.65	8.05	183.9	+157.0
7	FP43+M_2-3	87.5	9.1	32.4	8.05	163.3	+136.4
8	FP43+M_1-10	83.35	8.55	32.4	8.5	171.9	+146.4
9	FP43+*T.h*-7	70.85	7.65	26.9	13.7	95.8	+68.9
10	FP43 alone	88.1	7.6	23.3	22.0	131.0	+104.1
11	FP44+ M_1-25	79.15	7.35	30.9	3.1	137.5	+110.6
12	FP44+M_2-3	90.35	6.95	35.3	14.6	129.8	+102.9
13	FP44+M_1-10	82.9	7.15	31.2	10.3	161.6	+134.7
14	FP44+*T.h*-7	83.35	7.25	31.15	21.4	111.7	+84.8
15	FP44 alone	85.4	6.85	31.0	21.7	140.4	+113.5
16	FP100+ M_1-25	91.7	8.8	41.25	3.05	179.1	+152.2
17	FP100+M_2-3	83.6	7.15	26.9	10.8	122.5	+95.6
18	FP100+M_1-10	93.95	9.65	31.6	26.5	172.1	+145.2
19	FP100+*T.h*-7	70.85	6.1	31.6	46.5	100.7	+73.8
20	FP100 alone	87.5	6.3	33.5	19.2	95.4	+68.5
21	FP113+ M_1-25	89.6	6.3	36.5	2.05	176.2	+149.3
22	FP113+M_2-3	91.1	8.4	36.3	9.35	131.3	+104.4
23	FP113+M_1-10	92.6	11.8	40.45	4.2	146.0	+119.1
24	FP113+*T.h*-7	81.25	8.15	29.0	15.9	118.2	+91.3
25	FP113 alone	68.75	6.15	27.35	14.5	112.3	+85.4
26	M_1-25alone	81.25	8.25	36.2	11.1	122.2	+95.3
27	M_2-3alone	82.3	7.25	37.6	16.5	121.2	+94.2
28	M_1-10alone	93.95	8.0	32.92	10.8	125.3	+98.4
29	*T.h*-7alone	79.15	7.0	28.65	31.35	83.8.9	+56.9
30	Control	62.5	6.5	28.7	57.3	26.9	
CD at 5%		35.96	3.7	4.64	23.1	73.4	

*-Seed treatment of bacteria and soil application of of the fungal antagonists

Plate 27: Pot Culture Experiment in the green house

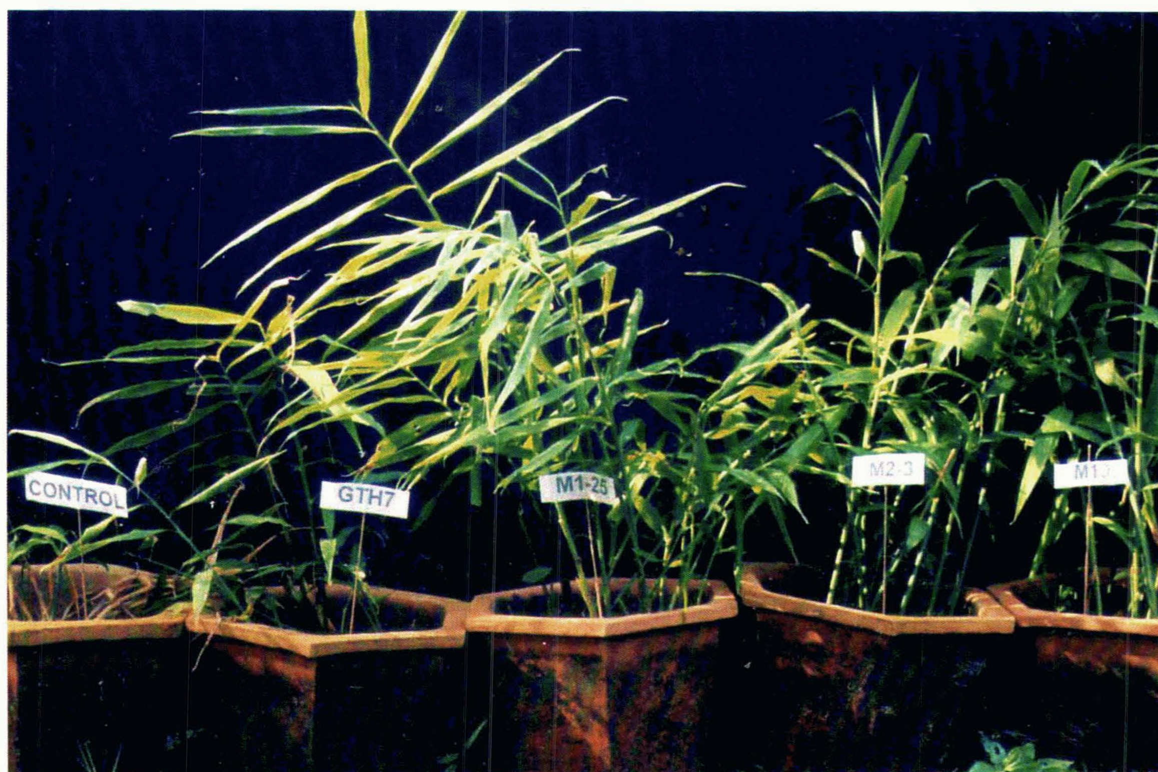


Plate 28: Bioefficacy of the mutants on growth of ginger

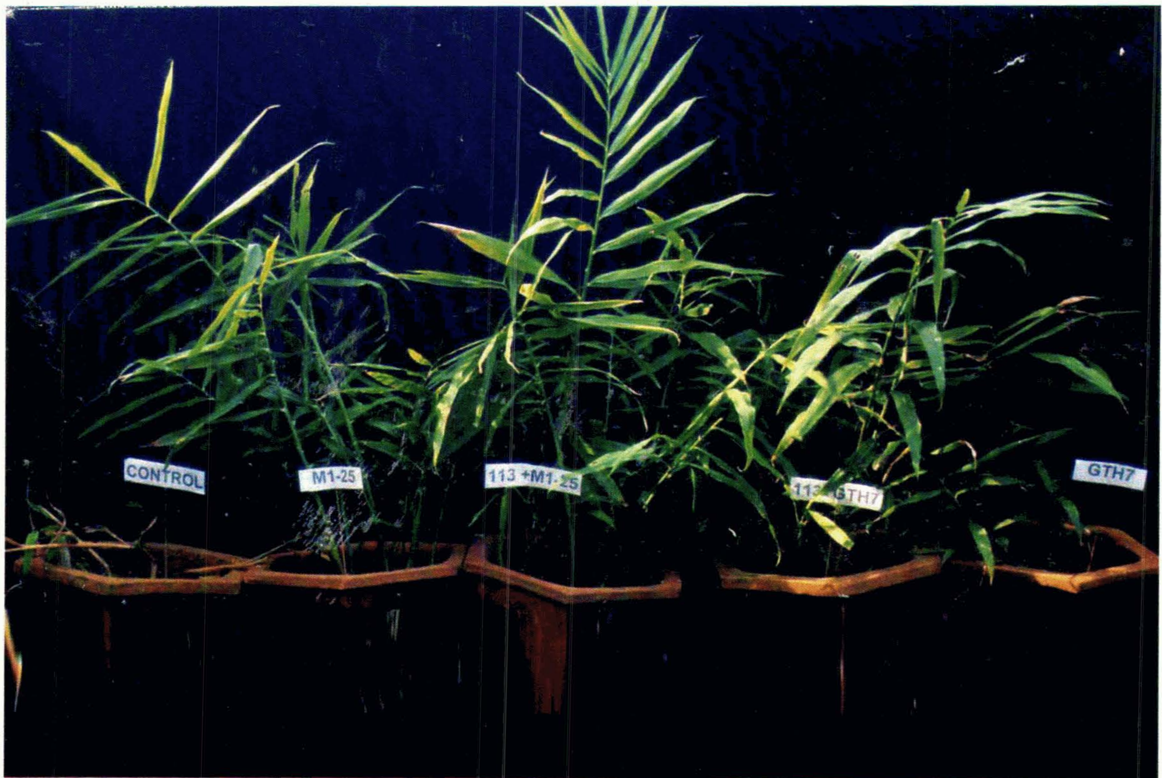


Plate 29:Effect of combinations of bacteria and fungi on the growth of ginger

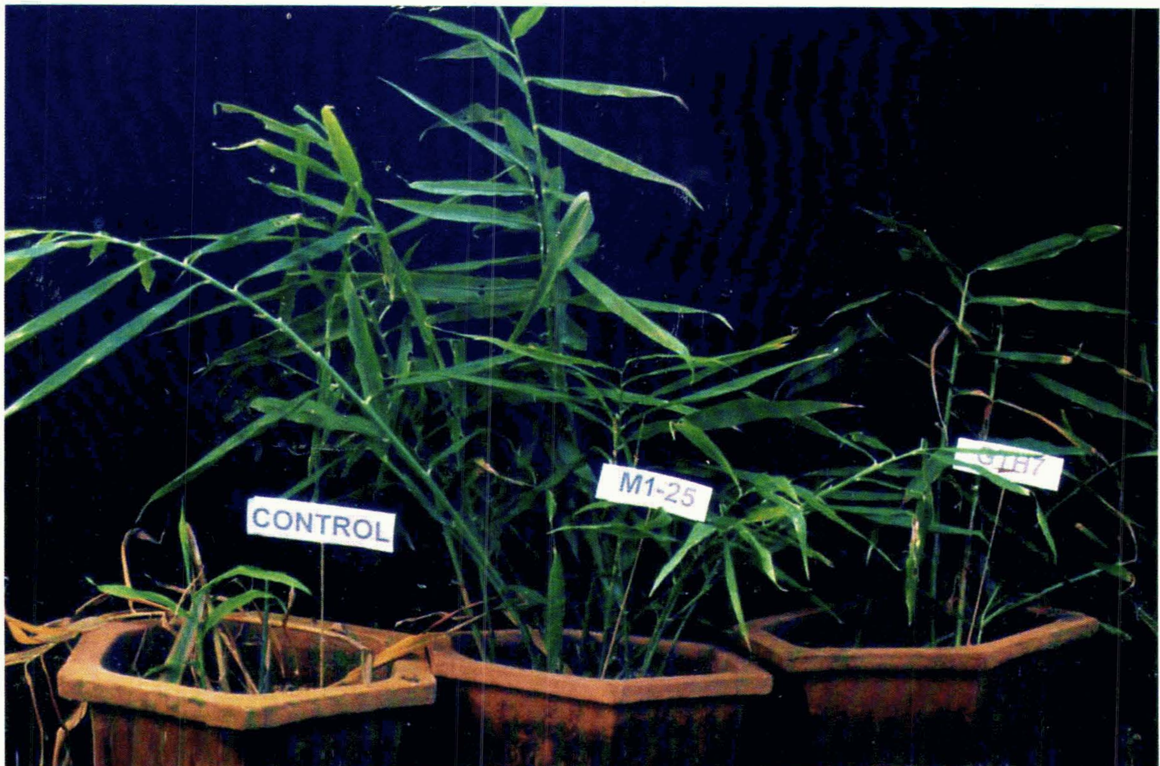


Plate 30: Comparison of the mutant M_1-25 and the wild type *(Trichoderma harzianum-7)* on the growth of ginger

6.2: Field Experiments:

6.2.1: Integrated management of rhizome rot disease:

All the experiments were conducted in *Pythium* sick soils. The data obtained from the field experiments also revealed that the combination with the fungicides Metalaxyl Mancozeb (RMZ) with the mutant M_1-25 was found to be promising as an increased yield (3.5 kg / bed) was obtained in this combination than when treated alone or with other combinations (Fig.10). The mutants M_2-3 and M_1-10 were also found to be superior to the wild type *T.h*-7. The disease incidence was comparatively less in the second year when compared to the first year. The combinations of the fungicides with M_1-25 was found to be superior than the wild type. The data obtained indicated that there was a significant reduction in the disease incidence (2-8.9%) in the plots in which the different strains of *T.harzianum–7* were treated when compared to the plots treated with the wild type, *T.harzianum-7* where a disease incidence of 6.6-12% was recorded. However there was a significant reduction in the disease incidence of the biocontrol plots when compared to the absolute control where 17.5% D.I was recorded. Similarly the yield in the BCA treated plots were higher (1-3.5kg / bed) when compared to the control (0.65 kg / bed). But the combination of the mutant with the fungicide Metalaxyl Mancozeb was found to be the best treatment since an increased yield of 3.5 kg / bed was obtained in this treatment.

Table 49: Integrated Management of rhizome rot of ginger-First Year:

No.	Treatments	Germ %	Sprouts /bed	D.I (%)	Yield/ bed (kg)	Difference in yield over control(kg)
1	RMZ +M_1-25	84.2	172.5	4.7	4.7	+4.1
2	RMZ +M_1-10	84.2	168.5	9.0	2.8	+2.2
3	RMZ +M_2-3	85.4	158.5	6.1	2.6	+2.0
4	RMZ +*T.h*-7	83.8	167.7	18.3	2.6	+2.0
5	RMZ alone	84.6	173.3	16.4	1.1	+0.5
6	DM-45+ M_1-25	77.5	161.2	10.3	2.4	+1.8
7	DM-45+ M_1-10	87.5	171.2	6.1	2.2	+1.6
8	DM-45+ M_2-3	82.9	166.3	9.2	2.9	+2.3
9	DM-45+ *T.h*-7	82.1	184.8	10.4	2.3	+1.7
10	DM-45+ M_1-25	80.4	180.3	12.4	1.4	+0.8
11	Starch+ M_1-25	74.2	159.2	13.3	2.5	+1.9
12	Starch+ M_1-10	75.8	175.5	14.6	1.5	+0.9
13	Starch+ M_2-3	70.4	143.2	15.6	2.1	+1.5
14	Starch+ *T.h*-7	78.3	166.0	20.6	1.9	+1.3
15	Starch alone	68.3	167.7	16.7	2.3	+1.7
16	M_1-25 alone	72.1	149.5	9.1	2.3	+1.7
17	M_1-10alone	80.1	155.2	11.6	1.3	+0.7
18	M_2-3 alone	78.8	177.0	10.8	1.5	+0.9
19	*T.h*-7alone	70.0	158.8	16.3	0.9	+0.3
20	Control	72.9	175.3	27.5	0.6	
CD at 5%		14.24	46.15	9.05	1.55	

+-increase in yield over control

Table 50: Integrated Management of rhizome rot of ginger-Second Year:

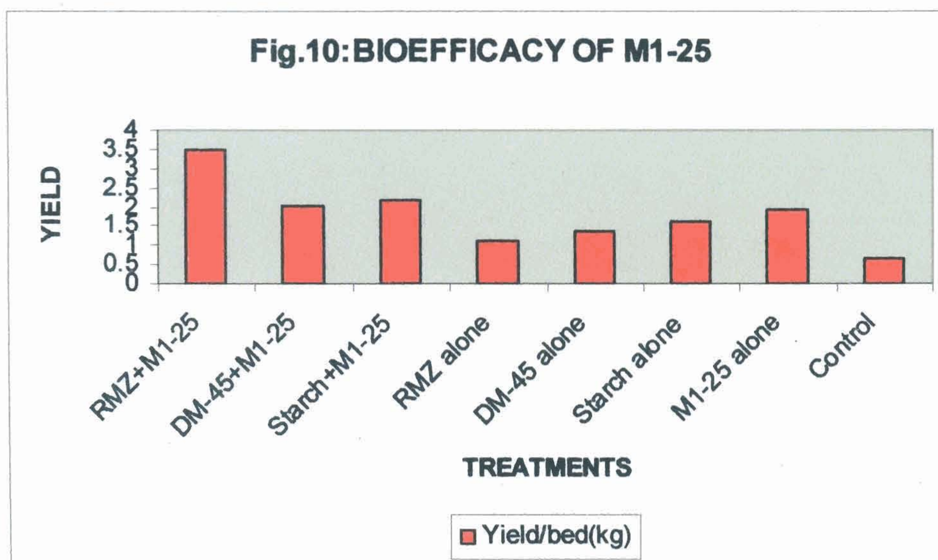
No.	Treatment	Ger m%	Sprouts /bed	D.I (%)	Yield/ bed kg)	Difference in yield over control(kg)
1	RMZ +M_1-25	88.5	232.6	1.2	2.3	+1.6
2	RMZ +M_1-10	86.0	224.6	1.7	1.6	+0.9
3	RMZ +M_2-3	79.5	205.0	3.1	1.5	+0.8
4	RMZ +*T.h*-7	74.0	161.2	5.8	1.0	+0.3
5	RMZ alone	74.5	135.0	5.7	1.1	+0.4
6	DM-45+ M_1-25	77.5	216.4	3.8	1.7	+1.0
7	DM-45+ M_1-10	76.0	151.4	5.9	1.3	+0.6
8	DM-45+ M_2-3	81.0	157.6	5.1	1.3	+0.6
9	DM-45+ *T.h*-7	76.5	146.6	2.8	1.1	+0.4
10	DM-45+ M_1-25	72.5	163.8	4.8	1.3	+0.6
11	Starch+ M_1-25	93.0	236.8	1.5	1.9	+1.2
12	Starch+ M_1-10	89.0	227.2	1.1	1.5	+0.8
13	Starch+ M_2-3	85.5	203.0	1.8	1.8	+1.1
14	Starch+ *T.h*-7	84.0	169.0	4.3	1.4	+0.7
15	Starch alone	75.5	146.8	3.9	0.9	+0.2
16	M_1-25 alone	86.5	242.6	1.2	1.5	+0.8
17	M_1-10alone	84.0	210.4	0.6	1.8	+1.1
18	M_2-3 alone	85.0	173.4	1.42	1.7	+1.0
19	*T.h*-7alone	76.0	160.8	4.7	1.2	+0.5
20	Control	76.0	162.0	7.4	0.7	
CD at 5%		10.03	45.07	2.05	0.55	

+- increase in yield over control

Table 51: Pooled Data -Integrated Management of rhizome rot of ginger:

No.	Treatment	Germ %	Sprouts /bed	D.I (%)	Yield/ bed kg)	Difference in yield over control (kg)
1	RMZ +M_1-25	86.3	202.6	2.95	3.5	+2.85
2	RMZ +M_1-10	85.1	196.6	5.35	2.2	+1.55
3	RMZ +M_2-3	8245	181.8	4.6	2.05	+1.4
4	RMZ +*T.h*-7	78.9	164.4	12.05	1.8	+0.95
5	RMZ alone	79.55	154.1	11.05	1.1	+0.45
6	DM-45+ M_1-25	77.5	188.8	7.0	2.05	+1.4
7	DM-45+ M_1-10	81.75	161.3	6.0	1.75	+1.1
8	DM-45+ M_2-3	81.95	162.0	7.15	2.1	+1.45
9	DM-45+ *T.h*-7	79.3	165.7	6.6	1.7	+1.05
10	DM-45+ M_1-25	76.45	172.1	8.6	1.35	+0.7
11	Starch+ M_1-25	83.6	198.0	7.4	2.2	+1.55
12	Starch+ M_1-10	82.4	201.9	7.85	1.5	+0.85
13	Starch+ M_2-3	77.95	173.1	8.7	1.95	+1.3
14	Starch+ *T.h*-7	81.15	167.5	12.4	1.65	+1.0
15	Starch alone	71.9	157.3	10.3	1.6	+0.95
16	M_1-25 alone	79.3	196.1	5.1	1.9	+1.25
17	M_1-10alone	82.05	182.8	6.1	1.55	+0.9
18	M_2-3 alone	81.9	175.2	6.1	1.6	+0.95
19	*T.h*-7alone	73.0	159.8	10.5	1.05	+0.4
20	Control	74.45	168.6	17.45	0.65	
CD at 5%		13.32	66.21	15.72	1.474	

+-increase in yield over control



6.2.2: Combinations of bacteria and fungi on rhizome rot of ginger:

The efficacy of the different bacterial isolates (Fluorescent pseudomonads), FP1, FP43, FP44, FP100 & FP113 were tested in combination with the mutants, M_1-25, M_1-10, M_2-3 and the wild type *T.h*-7. The biocontrol efficacy was already established in the pot culture studies and in the field experiments also the superiority of biocontrol efficacy of the mutant, M_1-25 in combination with the different bacterial isolates was established (Tables 52 & 53). Although there was a reduction in the disease incidence, when the isolates were tested alone, their superiority was established by the increased yield that was obtained when the different combinations were used (Fig 10). In the pooled data also (Table 54), it was found that the combinations of the different bacterial isolates with the mutant, M_1-25 was found to show an increased yield (2.3-2.7 kg / bed) when compared to control (0.64 kg / bed). Observations of the different strains of *Trichoderma* indicate that the strain M_1-25 showed an increased germination percentage (90.15%), reduced disease incidence (2.8%) and increased yield (1.87 kg / bed) when compared to its wild type where the readings were 89.7 %, 8.43% and 1.18 kg / bed respectively. Similarly an increased yield was obtained when the different strains were treated in combination with the different bacterial isolates than when treated alone (Fig 11). The superiority of the developed strains over the wild type was also established by the data obtained (Table 54)

Table 52:Combinations of bacteria and fungi on rhizome rot of ginger-First Year:

No.	Treatments	Germ%	Sprouts/bed	D.I%	Yield (kg/bed)	Difference in yield over control(kg)
1	FP1+ M_1-25	94.2	203.0	10.5	2.7	+2.1
2	FP1+M_2-3	86.3	239.7	18.2	3.1	+2.5
3	FP1+M_1-10	83.8	244.2	10.5	2.4	+1.8
4	FP1+*T.h*-7	96.3	114.3	19.3	1.8	+1.2
5	FP1 alone	91.6	182.8	10.9	1.7	+1.1
6	FP43+ M_1-25	96.7	183.0	9.4	2.6	+2.0
7	FP43+M_2-3	82.1	168.5	4.8	1.3	+0.7
8	FP43+M_1-10	84.6	197.8	7.7	1.8	+1.2
9	FP43+*T.h*-7	97.5	114.8	18.8	1.8	+1.2
10	FP43 alone	92.1	177.5	13.9	1.4	+0.8
11	FP44+ M_1-25	92.9	190.8	4.9	2.8	+2.2
12	FP44+M_2-3	72.1	146.8	16.5	0.8	+0.2
13	FP44+M_1-10	87.5	207.7	2.4	2.6	+2.0
14	FP44+*T.h*-7	95.0	170.7	11.1	1.7	+1.1
15	FP44 alone	97.1	171.5	11.2	1.6	+1.0
16	FP100+ M_1-25	93.8	144.2	8.2	2.9	+2.3
17	FP100+M_2-3	82.1	198.7	15.7	1.2	+0.6
18	FP100+M_1-10	88.8	228.0	9.6	1.7	+1.1
19	FP100+*T.h*-7	88.8	154.5	15.9	0.8	+0.2
20	FP100 alone	93.3	156.5	7.0	1.9	+1.3
21	FP113+ M_1-25	94.4	162.7	8.1	2.6	+2.0
22	FP113+M_2-3	82.1	175.3	13.0	1.7	+1.1
23	FP113+M_1-10	90.0	218.3	9.1	1.6	+1.0
24	FP113+*T.h*-7	95.8	180.2	13.2	1.7	+1.1
25	FP113 alone	90.8	127.3	21.9	1.4	+0.8
26	M_1-25alone	90.3	131.3	4.3	3.5	+2.9
27	M_2-3alone	75.4	156.0	11.7	1.3	+0.7
28	M_1-10alone	88.7	224.5	10.7	1.6	+1.0
29	*T.h*-7alone	95.4	124.2	16.0	1.4	+0.8
30	Control	87.9	156.5	31.3	0.6	
CD at 5%		6.332	73.5	8.09	1.336	

+-increase in yield over control

Table 53:Combinations of bacteria and fungi on rhizome rot – Second Year:

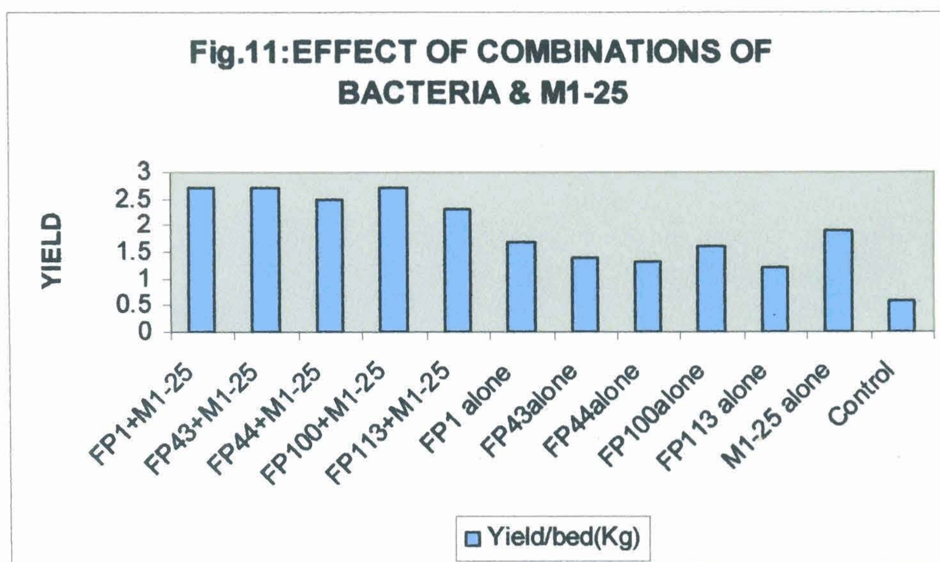
No	Treatments	Germ %	Sprouts/ bed	D.I (%)	Yield/bed (kg)	Difference in yield over control
1	FP1+ M_1-25	93.5	242.0	0.08	2.8	+2.1
2	FP1+M_2-3	85.5	246.8	1.42	1.3	+0.6
3	FP1+M_1-10	82.0	245.2	1.88	2.2	+1.5
4	FP1+*T.h*-7	75.5	144.2	3.68	1.9	+1.2
5	FP1 alone	72.0	148.0	3.16	1.7	+1.0
6	FP43+ M_1-25	85.0	242.6	0.96	2.8	+2.1
7	FP43+M_2-3	83.3	187.8	1.7	1.4	+0.7
8	FP43+M_1-10	83.5	211.0	1.26	1.9	+1.2
9	FP43+*T.h*-7	74.0	143.0	4.9	1.0	+0.3
10	FP43 alone	81.0	149.0	1.68	1.3	+0.6
11	FP44+ M_1-25	92.5	241.8	0.6	2.2	+1.5
12	FP44+M_2-3	74.5	156.2	2.68	1.1	+0.4
13	FP44+M_1-10	88.5	220.8	2.06	1.1	+0.4
14	FP44+*T.h*-7	65.5	143.8	3.18	0.9	+0.2
15	FP44 alone	76.5	147.0	1.48	0.9	+0.2
16	FP100+ M_1-25	89.0	244.3	0.50	2.4	+1.7
17	FP100+M_2-3	82.0	189.0	1.96	1.0	+0.3
18	FP100+M_1-10	89.0	233.0	1.04	1.8	+1.1
19	FP100+*T.h*-7	76.5	164.8	3.0	0.8	+0.1
20	FP100 alone	73.5	466.0	2.14	1.2	+0.5
21	FP113+ M_1-25	92.5	256.8	0.74	2.0	+1.3
22	FP113+M_2-3	83.0	180.8	1.48	1.2	+0.5
23	FP113+M_1-10	90.0	218.3	1.42	1.5	+0.8
24	FP113+*T.h*-7	80.5	182.2	2.04	0.9	+0.2
25	FP113 alone	81.0	163.6	0.78	0.9	+0.2
26	M_1-25alone	90.0	194.2	1.3	1.8	+1.1
27	M_2-3alone	75.0	161.2	1.2	1.4	+0.7
28	M_1-10alone	87.0	227.4	0.0	1.6	+0.9
29	*T.h*-7alone	84.0	161.6	0.86	0.9	+0.2
30	Control	76.5	156.8	4.87	0.7	
CD at 5%		8.642	43.0	2.278	0.55	

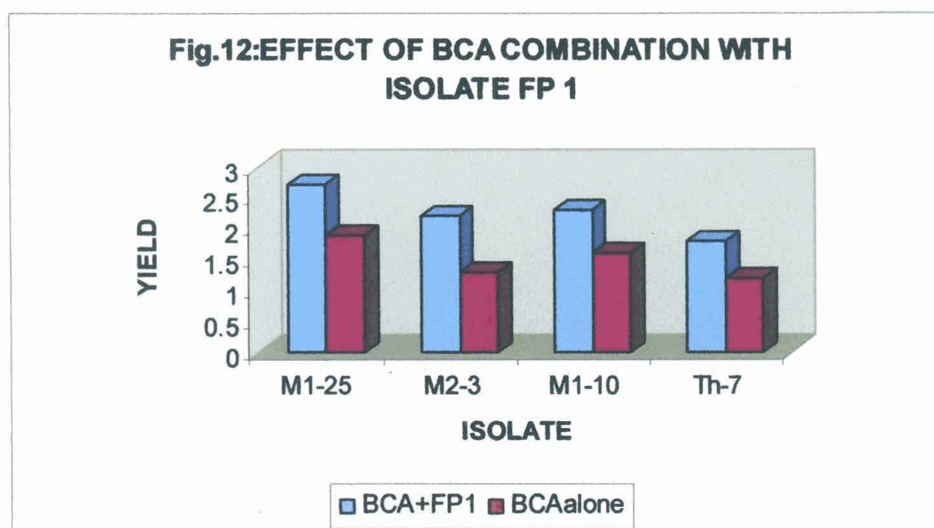
+-increase in yield over control

Table 54:Combinations of bacteria and fungi on rhizome rot of ginger-Pooled data:

No.	Treatments	Germ%	Sprouts	D.I (%)	Yield (kg/bed)	Difference in yield over control(kg)
1	FP1+ M_1-25	93.85	225.5		2.7	+2.1
2	FP1+M_2-3	85.9	243.3	9.81	2.2	+1.6
3	FP1+M_1-10	82.9	244.7	6.19	2.3	+1.7
4	FP1+*T.h*-7	85.9	129.3	11.49	1.9	+1.3
5	FP1 alone	81.8	165.4	7.03	1.7	+1.1
6	FP43+ M_1-25	90.85	212.8	5.18	2.7	+2.1
7	FP43+M_2-3	82.7	178.1	3.25	1.4	+0.8
8	FP43+M_1-10	84.05	204.4	4.48	1.9	+1.3
9	FP43+*T.h*-7	85.75	128.9	11.85	1.4	+0.8
10	FP43 alone	86.55	163.3	7.79	1.4	+0.8
11	FP44+ M_1-25	92.7	216.3	2.75	2.5	+1.9
12	FP44+M_2-3	73.3	151.5	9.59	1.0	+0.4
13	FP44+M_1-10	88.0	214.3	2.23	1.9	+1.3
14	FP44+*T.h*-7	80.25	157.3	7.14	1.3	+0.7
15	FP44 alone	86.8	159.3	6.34	1.3	+0.7
16	FP100+ M_1-25	91.4	194.3	4.35	2.7	+2.1
17	FP100+M_2-3	82.05	193.9	8.83	1.1	+0.5
18	FP100+M_1-10	88.9	230.5	5.32	1.8	+1.1
19	FP100+*T.h*-7	82.65	159.6	9.45	0.8	+0.2
20	FP100 alone	83.4	311.3	4.57	1.6	+1.0
21	FP113+ M_1-25	93.5	209.8	4.44	2.3	+1.7
22	FP113+M_2-3	82.55	178.1	7.24	1.4	+0.8
23	FP113+M_1-10	90.0	218.3	5.36	1.6	+1.0
24	FP113+*T.h*-7	88.15	181.2	7.62	1.3	+0.7
25	FP113 alone	85.9	145.5	11.34	1.2	+0.6
26	M_1-25alone	90.15	162.8	2.80	1.9	+1.3
27	M_2-3alone	75.2	158.6	6.45	1.3	+0.7
28	M_1-10alone	87.85	225.9	5.35	1.6	+1.0
29	*T.h*-7alone	89.7	142.9	8.43	1.2	+0.6
30	Control	82.2	156.6	18.06	0.6	
CD at 5%		16.89	96.95	16.86	0.95	

+-increase in yield over control





6.2.3:Comparison of the bioefficacy of the different strains of *Trichoderma:*

A comparison of the developed strains of *Trichoderma* showed that though all the mutants were found to exhibit an increased biocontrol efficiency, the albino mutant M_1-25 was superior to the other strains in all the growth parameters (Table 55, Fig.13). Similarly when the RC index, CSA index, enzyme activity and the yield of all the developed strains were compared to the wild type, it was found that all the strains exhibited superiority over the wild type. However the strain M_1-25 showed an increased efficiency of RC index, CSA

index, β 1,4 endoglucanase, β 1,3 endoglucanase activity and yield of 106.0, 17.6, 124.3, 83.6 and 150 g /pot when compared to 73.9, 8.5, 35.4, 24.7 and 102 g / pot respectively in the wild type. (Table 56, Fig.14).

Table 55: Bioefficacy of the developed strains of *Trichoderma*

Isolate	Germ %	Sprouts/pot	D.I(%)	Yield/ pot(g)
M_1-25	81.6[a]	9.2[a]	6.9[c]	150[a]
M_2-3	79.7[a]	7.3[a]	12.6[b]	124[b]
M_1-10	85.5[a]	8.1[a]	10.0[bc]	145[a]
T.h-7	77.4[a]	7.2[a]	25.0[a]	102[c]

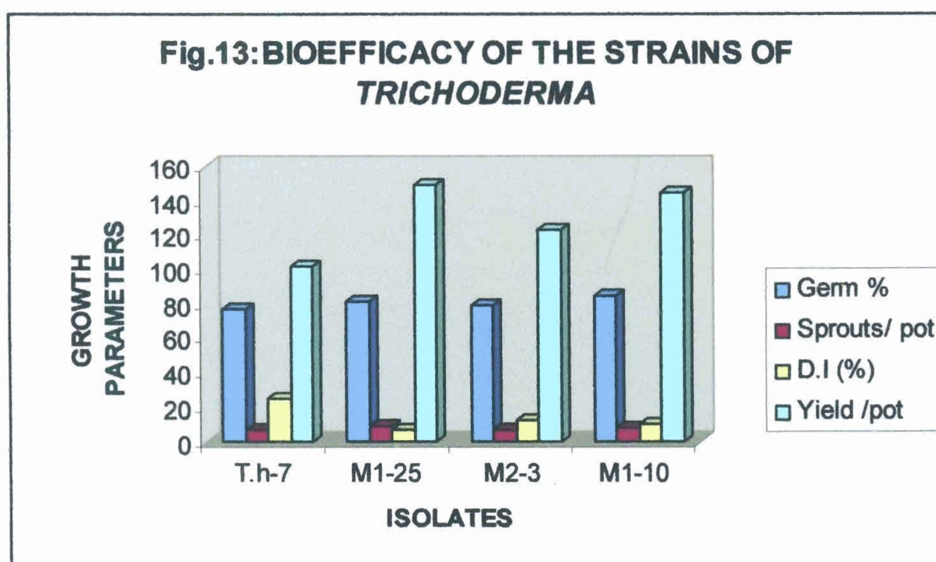


Table 56:Bioefficacy of the developed strains of *Trichoderma*

Isolate	RC index	CSA index	Enzyme Activity		Yield/ pot(g)
			β 1,4 endoglucanase	β 1,3 endoglucanase	
M_1-25	106.0	17.6	124.3	83.6	150
M_2-3	74.0	13.9	55.1	38.1	124
M_1-10	106.0	12.4	89.8	60.9	145
T.h-7	73.9	8.5	35.4	24.7	102

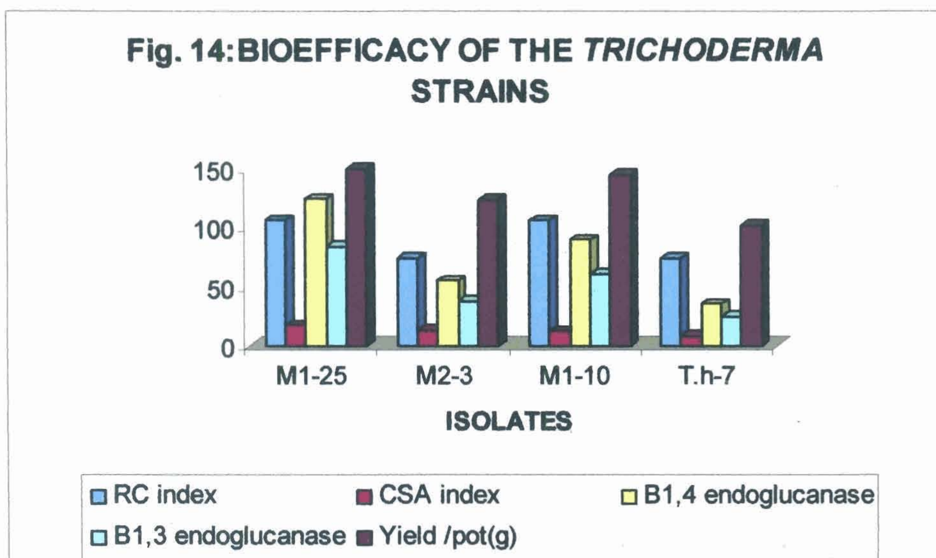


Fig. 14: BIOEFFICACY OF THE *TRICHODERMA* STRAINS

6.3: Monitoring of the population of the introduced biocontrol agents and the pathogen *Pythium aphanidermatum* in the soil:

When the soil samples obtained from the treated plots were monitored periodically, after 30 60, 90 and 120 days, it was found that the population of the biocontrol agents gradually increased from 30 days, reached the peak after 60 or 90 days and gradually reduced after 120 days. The population levels of the pathogen was almost stationary after the application of the biocontrol agents. However the initial population level of the pathogen was reduced after the application of the BCAs (Tables 57-59).

Table 57: Population levels of *Pythium* and the biocontrol agents-First Year:

Treatment	30 days (M.C-20%)		60days (M.C-20%)		90 days (M.C -20%)		120 days (M.C-10%)	
	BCA*	*Pythium***	BCA	*Pythium*		*Pythium*	BCA	*Pythium*
M_1-25	6.3	1.7	4.5	1.6	51.3	1.0	4.0	1.7
M_2-3	9.5	1.5	8.8	1.3	20.5	1.2	4.8	1.3
M_1-10	12.3	1.3	15.0	1.2	5.5	1.2	4.5	1.9
T.h-7	1.5	2.0	30	1.9	5.0	1.4	6.3	1.6
Control	1.0	2.17	1.5	2.0	1.5	2.17	1.8	2.17

Basal population of *Pythium*- $2X10^2$; M.C-Moisture Content
*CFU-$X10^3$/g of soil **CFU-$X10^2$/g of soil

Table 58: Population levels of *Pythium* and the biocontrol agents-Second Year:

Treatment	30 days (M.C-20%)		60 days (M.C-20%)		90 days (M.C -20%)		120 days (M.C-10%)	
	BCA	*Pythium*	BCA	*Pythium*	BCA	*Pythium*	BCA	*Pythium*
M_1-25	7.0	0.7	5.5	0.8	47.8	0.8	3.0	0.7
M_2-3	10.5	0.8	13.25	0.3	29.5	0.5	5.0	0.7
M_1-10	12.3	1.5	13.5	0.5	6.3	0.5	4.5	0.8
T.h-7	1.3	0.6	26.0	1.1	7.0	0.8	4.5	0.8
Control	1.1	2.0	1.2	2.1	1.3	2.0	1.2	2.2

Table 59: Population levels of *Pythium* and the biocontrol agents-Pooled data:

Treatment	30 days (M.C-20%)		60 days (M.C-20%)		90 days (M.C -15%)		120days (M.C-10%)	
	BCA	*Pythium*	BCA	*Pythium*	BCA	*Pythium*	BCA	*Pythium*
M_1-25	6.7	1.2	5.0	1.2	49.5	0.9	3.5	1.2
M_2-3	10.0	1.5	11.0	1.3	24.9	0.9	4.9	1.0
M_1-10	13.0	1.0	14.3	0.9	5.9	0.9	4.5	1.4
T.h-7	1.4	1.3	28	1.5	6	1.1	5.4	1.2
Control	1.1	2.1	1.4	2.1	1.4	2.1	1.5	2.2

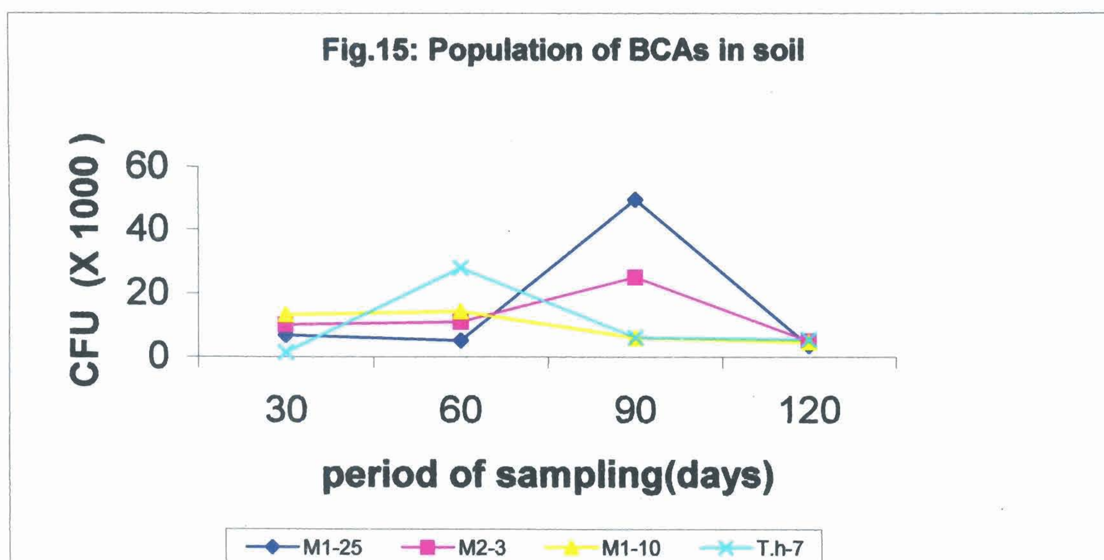


Plate 31- Field View

Plate 32-Metalaxyl Mancozeb + BCA treated bed

Plate 33-A rhizome rot infected bed

Plate 31 - 33: Integrated Management of rhizome rot of ginger

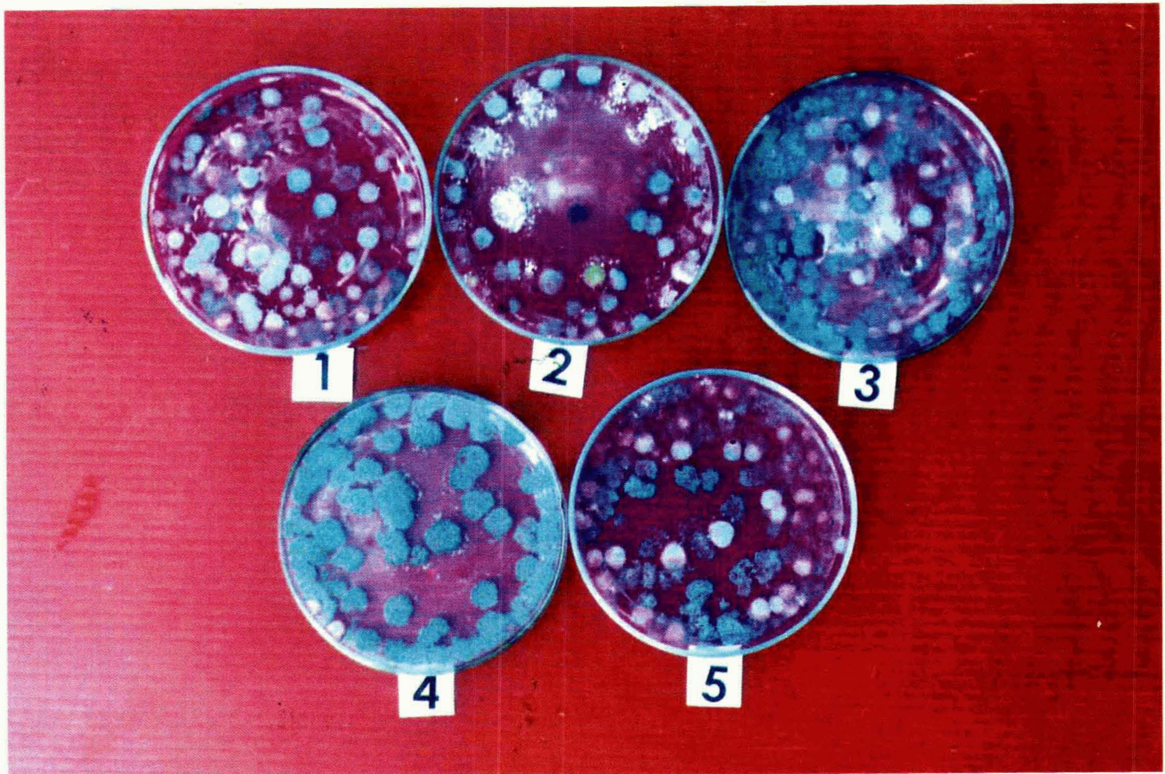


Plate 34 : Different strains of *Trichoderma* retrieved from the soil

1- *Trichoderma harzianum-7*
2- M_1-25
3- M_2-3
4- M_1-10
5- M_1-25 + T.harzianum-7

DISCUSSION

Introduction:

Root diseases of crop plants pose a serious threat to agricultural production. Their nature and mode of survival in the agricultural ecosystem make it very difficult to combat them by single plant protection approach. Rhizome rot, a serious disease affecting roots and rhizomes is the major constraint in the production of ginger. Causal agent of rhizome rot is the soilborne fungus, *Pythium aphanidermatum.*This in association with bacteria may affect the severity of the disease. Therefore the correct diagnosis of the disease problem is essential for planning effective disease management strategies.

Integrated disease management (IDM) / Integrated nutrient management (INM) are the practical strategies for effective disease management. Of the four major components of IDM viz. cultural, chemical, biological control coupled with host resistance, biological control is the sought after strategy for soil borne plant diseases.

Biological control provides an environmentally benign, economic and safe alternative for pesticides. Biological control of rhizome rot is especially preferred to fungicidal control because of its lack of health hazards. The potentiality of *Trichoderma* and *Gliocladium* on suppression of *Pythium* in general has been well established (Hadar *et.al.,* 1984 & Sivan *et.al.,* 1984). The bioefficacy of the different species of *Trichoderma* in the management of rhizome rot of ginger has been already established (Usman, 1997., Anon, 1994., Anon, 2000). The present investigation was undertaken to shortlist some of the effective fungal as well as bacterial biocontrol agents to increase their biocontrol efficacy through strainal improvement and eventually evaluate their potential for disease suppression.

Survey of the major ginger growing areas of Kerala:

Plant disease incidence is related to and is dependent on the climatic conditions prevalent in a particular area and its topographical parameters. In order to examine the severity of the disease incidence and for the collection of the biocontrol agents a roving survey of the major ginger growing districts of Kerala viz. Kozhikode, Ernakulam, Kottayam, Idukki and Wayanad was conducted and the incidence of the disease was recorded. Of the five districts surveyed, a greater incidence of rhizome rot was recorded from the fields of Ernakulam where 14.7% disease incidence was observed. This was followed by Wayanad with 12% incidence. Kozhikode and Idukki recorded disease

incidence of 10.7% while the least affected was the Kottayam area where only 1.0 % disease incidence was recorded (Table 6).

Diseased as well as healthy samples were collected from all the fields surveyed. The rhizome rot pathogen *Pythium aphanidermatum* was isolated from 101 samples. From the 149 healthy root samples 68 isolates of bacteria and 109 isolates of *Trichoderma* were isolated. Of these only 57 were found to be promising as antagonists and they were identified based on the classification of Rifai (1969). Of the 57 isolates, 34 (59.7%) were identified as *Trichoderma harzianum* Rifai, 19 (33.3%) as *Trichoderma virens*, Gidder & Foster and 4 (7%) were found to be *Trichoderma aureoviride.* Among the 68 bacterial isolates, 26 were found to pomising and they were identified as gram –ve rods belonging to the fluorescent pseudomonads group.

In the present investigation, it was observed that only three species of *Trichoderma* viz. ***Trichoderma harzianum, Trichoderma virens*** and ***Trichoderma aureoviride*** were isolated although there were about 33 different species known (Gams and Bisset, 1998). In a similar survey conducted earlier 8 different species of *Trichoderma* were isolated from the various ginger growing areas of Kerala and the frequency of association and isolation of *Trichoderma harzianum* (26%) was very high compared to all other species (Usman, 1997). The present study highlights the fact that the isolate that is predominantly found in the ginger rhizoplane is *Trichoderma harzianum* followed by *Trichoderma virens.*

Assessment of the bioefficacy of the biocontrol agents:

The main objective of the present investigation was to improve the biocontrol efficacy of the biocontrol agents. For this the most efficient isolate had to be shortlisted and for this a variety of screening tests were performed.

Trichoderma isolates possess many antagonistic mechanisms like competition, antibiosis and mycoparasitism (Dennis and Webster, 1971a, b & c, Tronsmo, 1986).

In the *in vitro* screening of the biocontrol agents, all the 68 isolates of bacteria and 109 isolates of *Trichoderma* that were isolated from the field survey were screened against the pathogen, *Pythium aphanidermatum* by adopting the dual culture technique (Webber and Hedger, 1986).

After the *in vitro* screening, the 26 bacterial isolates and 57 isolates of *Trichoderma* were shortlisted. These along with 116 *Trichoderma* isolates from the IISR repository of

biocontrol agents were further screened *in vivo* to shortlist the most efficient isolates. The evaluation of the biocontrol efficacy was made by comparing the different growth parameters. Five different isolates of bacteria viz. **FP 1, FP 43, FP 44, FP 100** and **FP 113** and five species of *Trichoderma* viz. ***T.harzianum-7, T.virens-6, T.aureoviride-24, T.pseudokoningii-6*** and ***T.hamatum-6*** were shortlisted for further studies.

Compatibility of the bacterial and fungal antagonists:

The *Trichoderma* isolates are distributed in almost all the soils of Kerala. Similarly the bacterial isolates are also distributed in the rhizoplane and it is possible that these two biocontrol agents are lying in close association with one another and there is a possibility of interaction among these different isolates. In the present investigation a test was conducted to study the cumulative or additive effect of the biocontrol agents on the disease suppression of rhizome rot of ginger. The tested BCAs included five isolates of *Trichoderma* viz *T. pseudokoningii*-6, *T. hamatum*-6, *T. virens*-6, *T. aureoviride*-21 and *T. harzianum*-7. The bacterial isolates were FP1, FP43, FP44, FP100 and FP113. These different biocontrol agents were tested in different combinations (Table 17 & 18).

Among the studies carried out to determine the synergistic interaction between the fungal or bacterial antagonists, Harman's group at Cornell University (Lorito *et.*al., 1993a, b; 1994 a, b) determined the inhibitory effects on the spore germination and germ tube elongation of *B. cinerea, F. solani* and *Uncinula necator* were synergistically increased by mixing fungal enzymes and bacterial cells of *Enterobacter cloaceae.*

The present study revealed that there was a significant reduction in the disease incidence (0-21%) and increased yield (108-220 g / pot) when the fungal antagonists i.e., *Trichoderma* isolates were combined with the bacterial isolates. When treated with the biocontrol agents alone, the disease incidence ranged between 11.5-32 % and yield obtained was between 100-200 g / pot. Among the different combinations of the bacterial isolates, FP No.113 was found to be superior to the rest of the combinations where a yield of 200-225g / pot was obtained when compared to 52g / pot in the control. (Table18). In a similar study conducted in ginger, Usman (1997) had observed a significant reduction in disease incidence, disease severity and yield when different combinations of the fungal isolates like *T. harzianum* + *T. virens*, *T. hamatum* + *T. virens*, *T. polysporum* + *T. virens* were used. An increased germination percentage, increased yield and reduction in disease

severity were obtained when the combination of *T. hamatum* + *T. virens* was used than when treated alone.

The study reveals that the chitinolytic enzymes released by the *Trichoderma* spp. probably help by releasing nutrients from hyphae of target fungi and the bacterial cells could utilize these nutrients for proliferation.The subsequent increase in the bacterial population would enhance the ability of bacteria to act as biocontrol agents.Thus according to Lorito *et. al.,* (1993b), synergism would most likely occur if the bacteria were in a perfect position to utilize nutrients released by the chitinolytic enzymes. Similar results were obtained in a study conducted with black pepper (Saju, 2004).

Potentiating biocontrol efficacy of biocontrol agents:

Identification of the efficient *Trichodermas* through intensive screening is the first step. Further improving the bioefficacy through various manipulations through mutagenesis and biotechnological means is the next step. The wild type of *Trichoderm harzianum* Rifai was mutated for the tolerance to benomyl (100μg) and such strains were rhizosphere competent (Ahmad and Baker, 1987). To induce the compatibility of the wild type *Trichoderma* spp. with agrochemicals, for enhancing the biological control potential, to increase the enzyme production for industrial application or to enhance their survival potential in the soil and other ecological niches, genetic improvement of the biocontrol agents are some of the options available.

The present investigation was thus aimed at improving the biocontrol efficacy of the wild type strain and for their use in integrated management of rhizome rot. Random mutation by UV, γ rays or chemical agents like ethyl methyl sulphonate are the most common methods adopted for genetic improvement (Ahmad and Baker, 1988; Papavizas *et. al.,* 1982). In another study conducted by Kumar (1997), tebuconazole tolerant mutants were obtained by irradiation using UV and γ rays.

In the present investigation random mutation by UV rays and Ethyl Methyl Sulphonate (EMS) at different doses (200ppm, 500ppm, 1000ppm and 2000ppm) was attempted. Although no success was attained on the treatment with EMS, the UV irradiation was found to be more effective. Three different strains were obtained after irradiation with UV rays. Of these one was an albino and this trait could be used as a marker for further studies.

Mutation by UV irradiation:

Chemical mutagenesis has given rise to several effective strains (Ahmad and Baker, 1988; Papavizas *et* al., 1982). Fungicidal resistance of Bavistin in *Trichoderma spp.* was induced through UV irradiation (Viji *et.al*, 1993). In the present study, the wild type *Trichoderma harzianum* was chosen for the strainal improvement. A few colonies were isolated after the first round of irradiation under the UV rays and they were irradiated for a second time and two more colonies were isolated. The colonies that were isolated were more or less similar to the wild type except that they were a little more dark green in colour and the colony had a bigger diameter than the parental strain (Plate 9).

The treatment with other mutagens like ethyl methyl sulphonate did not have any effect and the further experimentation was performed with the mutants thus obtained, through UV radiation.

***In vitro* and *in vivo* screening of the mutants :**

To ascertain the biocontrol potential of the mutagens obtained, in a preliminary test, the mutants were screened *in vitro* against the fungal pathogen, *Pythium aphanidermatum* by the dual culture technique to assess its biocontrol potential. The results obtained showed that only three different strains viz M_1-25, M_1-10 and M_2-3 were found to be superior than to the rest in the percentage of inhibition of the pathogen and they exhibited an inhibition of 44 to 51% as against 39.8% in control (Table 25). This was further justified by the *in vivo* screening tests also. When the mutants were screened *in vivo, the mutants* M_1-25, M_1-10 and M_2-3 showed a D.I of 0%, 10%, and 10% and yield of 60 g, 45 g, and 58 g / polybag respectively, while in the other mutants, the disease incidence ranged between 40-80 % and a yield of only 15-35 g/ polybag was obtained. In the control, the disease incidence recorded was 80% and the yield was only 10 g / polybag (Table 26).

In a similar study conducted, Kumar (1997) obtained four tebuconazole tolerant mutants that could inhibit the growth of the root rot pathogen of mung bean, *Macrophomina phaseolina* by dual culture technique. Papavizas and Lewis (1983) observed that 10 UV induced biotypes showed different abilities to inhibit the growth of 8 plant pathogens and only two of them produced a zone of inhibition against *Fusarium oxysporum* f.sp. *melonis.*

Characterisation of the mutants:

The enhanced biocontrol activities of all the mutants isolated were established by the earlier workers (Papavizas *et.al.*, 1982). In the present study, having identified the superiority of the mutants over the parental strain in their ability to inhibit the growth of the pathogen, *Pythium aphanidermatum,* further characterization of the mutants was carried out. The criteria for characterization of the mutants included the growth rate on different media, effect of light on sporulation on different media, rate of germination and the mass multiplication of *Trichoderma* and the mutants on sorghum grains and the bioassay of the culture filtrates.

In the present study, it was found that the mutants differed in their growth rate on different media. The rate of growth of the mutants was greater (7.1-8.7cms in diameter) than the wild type (5.9 cms in diameter) on potato dextrose agar medium after 48 hours. Though the rate of growth of these strains on corn meal agar and czapex dox agar were on par with the wild type, profuse sporulation and luxuriant growth of the strains was observed on the Potato dextrose agar and Corn meal agar which suggests that the mutants are better adapted to grow on natural media rather than a synthetic medium like Czapek Dox Agar.

The present studies showed that the rate of growth of the mutants was on par with the wild type. Though the percentage of germination of the spores of the mutant M_1-25, was comparatively lesser (55%) than the wild type (60%), the spores of the strain M_1-10 showed a higher percentage (65%) of germination. The length of the germ tube of the mutant M_2-3 was the biggest (20.71µm). Although the mutant M_1-25 took a longer time to put out its germ tube (19hrs), the rate of growth of M_1-10 was on par with the wild type (16 hrs). However the strain M_2-3 took 15 hrs to put out its germ tube. The survival of the wild type *Trichoderma harzianum*-7 was already established by Usman and Balakrishnan (1996). The present studies have revealed that the rate of growth of the mutants was on par with the wild type. From these observations it can be inferred that the mutants have the capacity to survive in the soil similar to the wild type.

The influence of light in the growth of the mutants was established by its rapid rate of sporulation in the mutant inoculated petri plates kept in the light. The influence of the abiotic factors in the growth of the biocontrol agents has been established Burpee (1990), reviewed the role of abiotic factors that influence the distribution, survival and functioning of the biocontrol agents in various climatic conditions.

Rate of growth of the mutants on Sorghum grains:

The mass multiplication of the biocontrol organisms becomes important for their field application. Fast growth and multiplication in organic wastes make *Trichoderma* an ideal candidate for biological control of plant pathogens (Prakash *et.al.,* 1999; Saju *et.al.*, 2000; Saju *et.al.*, 2002). Prakash *et.al.*, (1999) evaluated neemcake, farmyard manure, coffee husk and spent tea for mass multiplication of *Trichoderma harzianum* and *T. virens.* Backman and Rodrigues Kabana (1975) used molasses enriched clay granules as food base for *Trichoderma harzianum.* Sivan *et.al.,* (1984) used wheat bran peat to multiply the biocontrol agents. Kausalya and Jeyarajan (1990) multiplied *T. viride* and *T. hamatum* on agro wastes for mass multiplication. Suseela bhai *et.al.,* (1994) multiplied the biocontrol agents on spent tea wastes and coffee husks. Usman (1997) standardised the mass multiplication of *Trichoderma harzianum* and *Trichoderma viride* on sorghum grains.

In the present investigation an experiment was conducted to determine the rate of growth of the mutants on sorghum grains. The results obtained showed that the strains M_1-10 and M_2-3 grew profusely on the sorghum grains but the rate of growth of the wild type was higher (850 $X10^5$ cfu / g) than the mutants (515-801 $X10^5$ cfu /g) after 20 days. However the growth rate of the strains decreased after the 25th day and became steady there after. The rate of growth of the wild type decreased sharply after the 25th day (630 $X10^5$cfu / g). The albino mutant was slow in its growth on sorghum grains. This could be because of the longer time it takes for putting out its germ tube (Table 28). But the rate of growth of this mutant steadily increased from the 5th day, reached the peak on the 20th day (512 $X10^5$ cfu / g) and remained steady after the 25th day (500 $X10^5$ cfu / g) (Table 30). Since the rate of growth of the mutants remained almost steady after the 25th day it can be inferred that the mutants have a greater shelf life period and greater viability when compared to the wild type. The increased rate of growth of the different mutants on the natural media like PDA and CMA was recorded (Table 27). In PDA, CFU of 4.5 – 8 X 10^5 / 5mm disc, in CMA, CFU of 5 - 7 $X10^5$ / 5mm disc while in CDA, CFU of 3 - 4.5 $X10^5$ / 5mm disc was obtained (Table 27). This experiment further indicated that the mutants as well as the wild strains are capable of growing in any naturally available organic media. Thus any organic wastes could be used to mass multiply the biocontrol agents.

Bioassay of the culture filtrates:

It is well documented that the *Trichoderma spp.* produces a wide variety of antimicrobial or biologically active substances that are inhibitory to the plant pathogens (Ghisalberti and Sivasithamparan, 1991, Kumar, 1997). These metabolites play an important role in biological control of plant pathogens through antibiosis (Weindling and Emerson, 1936).

In the present investigation, the culture filtrates of *Trichoderma harzianum* and its mutants were extracted and the percentage of inhibition of the pathogen was determined. The results revealed that when 100 µl of the concentrated culture filtrate was amended with PDA, the percentage of inhibition of the pathogen was significantly higher (21-22%) when compared to the wild type where 17% inhibition was observed. This increased rate of inhibition could be attributed to the presence of antibiotics in the culture filtrates.

In a similar experiment conducted, Saju *et.al.,* 2000 observed that out of the 17 isolates of *Trichoderma* screened against *Phytophthora capsici,* nine were volatile producers and eight were non volatile producers. The reported interacting mechanisms of *Trichoderma spp.* and *Trichoderma virens* with soil borne plant pathogens are competition, antibiotic production, lysis and mycoparasitism (Henis and Chet, 1975, Papavizas and Lumsden, 1980; Cook and Baker, 1983). Antibiotics may be volatile and non volatile types (Dennis and Webster, 1971 b and c).

Hyphal interaction test:

The hyphal interaction tests revealed that the simultaneous inoculation of the pathogen and the test strain showed a higher percentage of inhibition when compared to the pre inoculation method. All the isolates except the strain M_1-25 over grew the pathogen. When the above strain was inoculated with the pathogen a clear inhibition zone was observed in the petriplate and it could be due to the disintegration of the cell walls and leakage of cytoplasm of the pathogen. Enzymatic decomposition of the host cell wall of *S. sclerotiorum* has been demonstrated with *G. virens* (Tu, 1980) and *Trichoderma* attacking hyphae of *R. solani* and *S. rolfsii* (Elad *et.al.*, 1983). Production of volatile compounds could also be another factor inhibitory to the growth of other fungi (Webber and Hedger, 1986). The production of non volatile antibiotics of antifungal nature by *Trichoderma spp.* and *Gliocladium spp.* has also been reported (Dennis and Webster, 1971a). *T. viride, T.*

pseudokoningii and *G. virens* produced antifungal metabolites which were effective in reducing the radial growth of *S. cepivorum* and ***Botrytis cinerea*** (Jackson *et.al.*, 1991).

In the present investigation on the role of the non volatile antibiotics it was found that all the mutant strains and the wild type exhibited the inhibition of the pathogen of more than 90% even after 48 hours. An inhibition percentage of 94.48% was recorded in all the developed strains as against 91.8% in the wild type.

All the isolates showed considerable inter and intra specific variation on the production of the volatile and non volatile antibiotics based on the inhibiting effects on *Pythium aphanidermatum*. In general the inhibiting effect of the nonvolatiles was more (96.8-98.4 % of inhibition) compared to the volatile tested (45-49% inhibition) and thus the study reveals that the wild type as well as its mutants are non volatile antibiotic producers. This is in agreement with the observations of Usman (1997) who observed that the non volatiles showed an increased percentage of inhibition of the pathogen compared to the volatiles.

Compatibility with agrochemicals:

Integrated disease management (IDM) involves using not only chemicals but also biocontrol agents which are compatible with the agrochemicals so that fungicide input can be reduced. Combining biocontrol and fungicides is a method to reduce the use of fungicides (Chet, 1987) and would result in enhanced disease suppression. Most genetic modifications of *Trichoderma* are aimed to induce fungicidal resistance in the antagonists to combine them with fungicides IDM.

An experiment was conducted to determine whether the mutants produced were compatible with the agrochemicals so that they could be used in integrated management trials. In the present study compatibility with the fungicides like Copper-Oxy-Chloride, Mancozeb and Metalaxyl Mancozeb were tested. In all the treatments, the mutants exhibited an increased percentage of growth over control (6-89.3%). When compared to the wild type, *T. harzianum-7*, the mutants showed an increased mycelial dry weight in the different concentrations of COC amended media (148.9-289.1g) while in the wild type only 40-214 g was recorded. However the mutant M_1-25 showed a decreased percentage of growth (10.9-41.5%) in some of the treatments with Mancozeb and Metalaxyl Mancozeb (Table 37). This observation suggests that these mutants could be used along with the different fungicides.

The tests showed that the different strains of *Trichoderma* were tolerant to the different fungicides as was reported by Sawant and Mukhopadhyay (1990). In this study it was found that these biotypes were tolerant to more than one fungicide and this multiple fungicide tolerant strains could be more beneficial for integrated disease management. The tolerant strains could be successfully used in association with minimal amounts of chemicals for disease management.

Some of the *Trichoderma* species are naturally resistant to pesticides. Stephen *et.al.*, 2000 found that *Trichoderma harzianum* used for the control of *Phytophthora* foot rot of black pepper is tolerant to chlorpyriphos and phorate at recommended concentrations for mealy bug and nematode control respectively. Rajan and Sarma (1997), found *T.harzianum* compatible with potassium phosphonate.

Kay and Stewart (1994) studied the sensitivity of *Chaetomium globosum, T. harzianum, T. viride* and *Trichoderma* spp. to mancozeb, procymidone, benomyl, iprodione and thiram. The fungicides were insensitive to captan, mancozeb but were sensitive to benomyl (EC_{50}< 0-3μg/ml) and the two dicarboxymides, iprodione and procymidone (EC_{50}<3.3μg/ml).

Rhizosphere Competence Assay and Competitive Saprophytic Ability:

Rhizosphere competence (RC) is the ability of an organism to establish itself and function in the rhizosphere of plants and is subject to influences of environment and competition with other organisms (Ahmad and Baker, 1987b., Harman, 1991).

In one of the tests conducted to determine the rhizosphere competence, it was observed that *Trichoderma* could be detected at any soil depth where the moisture content ranged between 15-20 %. However a maximum colonisation of the wild type and the mutants was detected in the upper 2-3 cms of the excised roots (RC index 9.3-9.4) (Table 38). Chao *et.al.*, (1986) reported that plants growing into soil free moist chambers were not colonized by *Trichoderma* spp. When the roots were grown in sterile soil *T. harzianum* was detected in the rhizosphere of the upper half of the roots. In the untreated soil none of the *Trichoderma* spp could be detected in the rhizosphere more than 3 cm below the planted seed. Peer *et.al.,* (1991) observed that soil inoculation resulted in effective colonization of the roots by the transformants of *T. harzianum.*

The rhizosphere competent mutants produce significantly higher biomass than the rhizosphere incompetent wild types (Ahmad and Baker, 1988a). The ability of the mutants

to grow more rapidly on complex carbon substrates could be of ecological significance and is indicative of their rhizosphere competence. In the present study, the mycelial dry weight of the mutants grown on the Czapek Dox broth amended with carboxy methyl cellulose was higher (168-215 mg) when compared to the mycelial weight of the wild type (150mg). Ahmad and Baker (1988b) observed that mutants tolerant to benomyl were rhizosphere competent in the presence and absence of benomyl. Their experiments indicated that a rhizosphere incompetent biological agent was induced by mutations to become rhizosphere competent. Rhizosphere competence has also been achieved by genetic manipulations (Sivan and Harman, 1991). In the present study, the rhizosphere competence of the mutants was established based on above cited parameters.The rhizosphere competence of the mutants further was shown by greater secretion of β 1.4 endoglucanase (60-120 EGU) compared to the wild type (40 EGU) .

This pattern of the hydrolytic enzymes used by strains of *Trichoderma spp.* for the hydrolysis of cellulose has been well studied (Ljungdahl and Eriksson, 1985).

The main aim of the present investigation was to determine whether the mutants are competitively saprophytic compared to the native microflora. The output obtained revealed that both the mutants and the wild type were competitively saprophytic because more than 50% of the colonies that were retrieved from the soil were that of the introduced fungal strains.The albino nature of the mutant M_1-25 and the sporulation patterns of the other mutants served as markers for their easy detection. The Competitive Saprophytic Ability index (CSA) of the mutants were 12-17.6 when compared to 8.5 in the wild type and the RC index of the mutants were higher (73-106.9) when compared to the wild type (73) and this ensures better survival capacity of the developed strains. However the RC index of the mutant M_2-3 was on par with the wild type (73.9). Ahmad and Baker (1987a) implicated that the CSA of a strain of *Trichoderma* spp. was directly correlated with rhizosphere competence. The amount of cellulase produced by these strains was directly correlated with CSA and RC. Isolation of the fungi from the baits of dead plant material buried in the field soil provides direct evidence that the recovered fungi can colonise the substrates as competitive saprophytes (Garret, 1970)

The CSA index measured the capacity of the different strains and wild type of *Trichoderma harzianum* to compete effectively in the colonization of the cellophane discs.

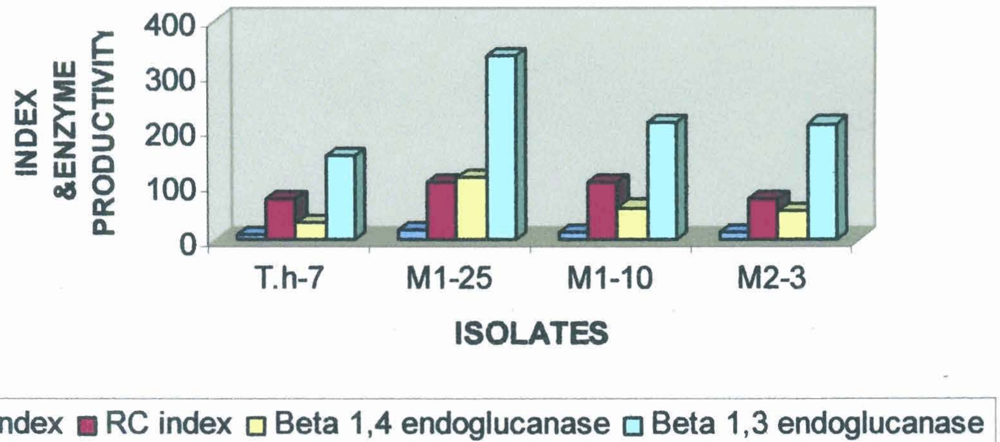
Enzyme activity of the developed strains:

One of the essential characteristics of the fungal biocontrol agents to act as mycoparasites of fungal pathogens is their ability to secrete hydrolytic enzymes such as glucanase, chitinase, cellulase, xylanase, alkaline phosphatase, esterase, α and ß glucosidase, N acetyl glucosaminidase and protease and are known to be produced by *Trichoderma* spp. (Aziz *et.al.,* 1993).

In the present investigation the β 1, 4 endoglucanase and β 1, 3 endoglucanase activity in the culture filtrate of *T. harzianum* and the mutants grown in both inducible and non inducible conditions were observed. The β 1, 4 endoglucanase activity of the mutants ranged between 53.7-115.2 GU when autoclaved mycelia was used as the carbon source and the β 1, 3 endoglucanase activity ranged between 213-338.5 GU as against 30.25 and 153.8 GU in the wild type respectively. Among the mutants also the albino mutant, M_1-25 showed a higher β 1, 4 endoglucanase enzyme activity of 115.2 GU and β 1, 3 endoglucanase activity of 338.5 GU. The CSA and RC index of this strain was higher (17.6 and 106 respectively). This study thus supports the findings that strains of *Trichoderma* spp. can be induced to be rhizosphere competent by appropriate mutations (Ahmad and Baker, 1987a).

Strains of *Trichoderma* produce cellulase and other cell wall degrading enzymes (Chet and Baker, 1980, 1981, Elad *et.al.*, 1982). In this study the C S A indices obtained were directly correlated with the production of glucanase enzyme. The mutants of *T harzainum* produced significantly greater amounts of glucanase units than the wild type when different materials where used as the carbon source (Table 42). The variation in the enzyme activity clearly reflects the varying degree of substrate utilization by the biotypes which in turn indicate their different mycoparasitic potential.

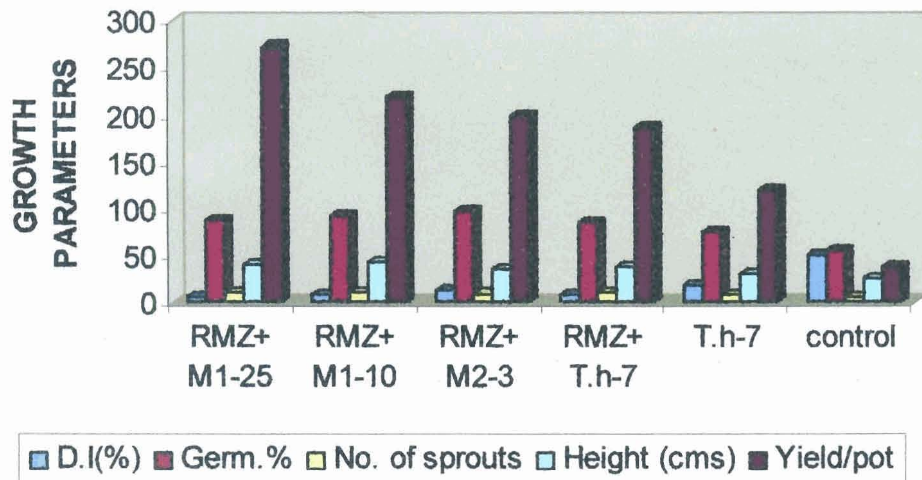
Similarly the RC index could also be correlated with the amounts of glucanase units produced by the mutants. These correlations suggest that the mutants with higher enzyme activity can utilize the substrates on or near the roots more effectively and are more rhizosphere competent. Thus this study suggests that the mutants of *T. harzianum* are rhizosphere competent because of increased enzyme activity that results in higher CSA for the colonisation of cellulose substrates on or near the root surface (Foster *et.al.,* 1983).



Pot Culture Studies:

The superiority of the developed strains over the wild type was established by different methods of characterization. Pot culture experiments were conducted to assess the *in vivo* efficiency of these strains.

The mutants were tested in combination with the different fungicides viz Metalaxyl Mancozeb, Mancozeb and organic amendments like starch antd the experiment were performed in pathogen challenged pots. The data obtained revealed the superiority of the mutants over the wild type in terms of reduced disease incidence (5.45-17%) and increased yield (175-273 g/pot) when compared to a D.I of 49.9% and yield of 37.5 g / pot in the control (Table 45). When treated in combination with Metalaxyl Mancozeb, all the mutants showed a reduced disease incidence and increased growth promotion in terms of increased germination percentage, increased number of sprouts, increased height and an increased yield (Table45, Fig. 17). The increased efficacy of the mutant, M_1-25 was noticed through a reduced percentage of disease incidence (2.8-8.7%) and increased yield of (177.6-273.1g/pot) as against 18% disease incidence and a yield of 120 g/pot in the wild type.



The effect of the synergistic action of the bacterial and fungal antagonists were also considered in the present investigation. For this the mutants and the wild type were treated in different combinations with some of the efficient biocontrol organisms. The data obtained revealed that the combinations of the bacterial isolates with the mutant M_1-25 was found to have a significant increase in the biocontrol efficacy in terms of increased yield and reduced disease incidence when compared to the wild type and control (Table 48). The disease incidence ranged from 2.5 to 8.05% when the strain M_1-25 was treated in different combinations of the bacterial isolates while the disease incidence varied between 13 to 46.5% when the wild type was treated in combinations of different bacterial isolates. The control pots recorded a disease incidence of 57.3%. Similarly an increased yield of 137.5 g/pot - 194 g/pot was obtained in these different treatments as against 26.9 g/pot in the control. Usman (1997) obtained similar results when *T. hamatum* was treated in combination with *G. virens.* In a similar study conducted against the foot rot pathogen, *Phytophthora capscici,* Saju (2004) got similar results in which an increased growth and suppressed root rot was observed when the combinations of *Trichoderma harzianum* and *Pseudomonas fluorescens* was used.

Field Experiments:

Integrated Management of rhizome rot of ginger:

Management of rhizome rot of ginger encounters certain limitations and difficulties.The soilborne nature of the disease, non availability of disease free seed rhizomes, leaching off of the fungicides applied due to heavy rainfall thus make the crop vulnerable to infection. Pesticide residue in the final product would also render it unfit for export consumption. Biological control, as a component of IDM opens up avenues for the reduction of the pesticide residues. The compatibility of the biocontrol agents with the agrochemicals would also ensure the reduction of pesticide inputs intt the environment. Under these circumstances an integrated approach, which consists of the rational use of all available management measures should be considered for effective management of rhizome rot. This point has been stressed by earlier investigators (Dake *et.al.*, 1988, Sarma *et.al.,* 1994). The integrated disease management trials conducted at the farmers' fields have clearly brought out the importance of integrated management for the control of rhizome rot of ginger (Anon, 1996., Anon, 2000).

In the present investigation attempts were made to incorporate the mutant strains of *Trichoderma harzianum* in the integrated disease management programmes. With this aim all the experiments conducted in the pot culture studies were repeated in the field conditions too. The experiments were performed in the *Pythium* infected soils to assess the disease suppressive potential of the strains.

When the mutants were treated in combination with fungicides, the mutant M_1-25 was found to be superior as there was a significant reduction in the disease incidence (2.8 - 9%) and an increased yield (2 - 3.5 kg / bed) when compared to 10.5% and 17.5% disease incidence and a yield of 1.0 and 0.65 kg / bed in the wild type treated and control beds respectively.

The biocontrol potential of the strain M_1-25 could be further established from the results obtained from its treatment in combination with other bacterial isolates and its comparison with the other developed strains when treated alone (Table 53). When treated in combination with the fluorescent pseudomonads, an increased yield of 2.3 - 2.7 kg / bed was obtained when the strain M_1-25 was treated in different combinations. This was significantly more than the yield obtained in the treatment with the wild type where 0.8 - 1.8 kg / bed was obtained and control where 0.64 kg / bed was obtained. Similarly a reduced disease incidence of 2.75-5.29% was obtained when M_1-25 was used in different

combinations with the bacterial isolates as against 7.6-11.5% disease incidence in the wild type and 18.06% in the control.These studies have clearly brought out the biefficacy of the developed strains over the wild type.The present investigation indicated the disease suppressive potential of the mutant strains. However in future large scale field demonstrations are essential.

The main aim of the present investigation was to assess the behaviour of the mutant strain in comparison with its wild type in the *in vivo* conditions. The efficacy of all the strains was established. However the strain M_I-25 proved to be more promising. A better protection in terms of reduced disease incidence and increased yield was provided to the crop when the strain M_I-25 was treated in different combinations. The present study also aimed at finding out the effects of different treatments along with biological control and chemical control. The leads obtained are directed towards integrated management practices involving the use of bicontrol and chemical control together.

Combining biocontrol and fungicides is a method for reducing the use of fungicides (Chet, 1987) and enhancing the effect of biocontrol agents. The studies conducted revealed that *Trichoderma* and its strains were highly tolerant to metalaxyl as also reported by Sawant and Mukhopadhyay (1990) and Balakrishnan (1997).

A comparison of the developed strains with the wild type indicated that the former are definitely superior over the wild type. In the potculture experiments conducted, an increased germination percentage ranging from 81-93%, increased height (32.9-37.2cms), reduced disease incidence (10.8-16.5%) and increased yield (121-125.3 g/pot) was obtained in the newly developed strains when compared to 79.15% germination, 28.65 cms height, 31.35% disease incidence and 83.75 g/pot yield in the wild type (Table 48).

Similarly in the enzyme study conducted, the β 1, 4 endoglucanase and β 1, 3 endoglucanase activities were significantly more than the wild type in both the inducible and non inducible conditions. When sucrose was used as the carbon source, the β 1, 4 endoglucanase activity of the developed strains ranged between 55-124.3GU when compared to 35.35GU in the wild type. When the autoclaved mycelia were used as the carbon source, it ranged from 53.7 to 115.2 GU compared to 30.25 in the wild type. Similarly when the live mycelia was used as the carbon source the enzyme activity ranged between 60-119.3GU when compared to 40.4 GU in the wild type. Similar results could be observed in the β 1, 3 endoglucanase activity also (Table 42).

The present investigation thus established the potential of the newly developed strains of *Trichoderma harzianum* in terms of better protection, with the growth mediated defence as one of the components of disease suppression.

Monitoring the population of *Pythium* and the biocontrol agents:

Periodic testing of the soil samples was done and when the populations of the introduced biocontrol agents were monitored. It was found that the populations of the introduced biocontrol agents increased from the 30th day, reached the peak after 60 or 90 days and then declined gradually. But the pathogen population remained unchanged (0.9-1.5 $X10^2$ CFU/g of soil) after the application of the BCAs (Table 56), thus indicating the biocontrol activity of *Trichoderma.*

The decline in the rate of growth of the BCAs after the 120th day could be attributed to the other abiotic factors which influence their rate of growth. Burpee (1990) has advocated that the abiotic factors influence the bioefficacy of biocontrol in the soil. Low pH (<7) favours disease suppression induced by *T. harzianum* (Chet and Baker, 1981). Acid conditions may also enhance the spore germination, mycelial growth and production of antibiotics (Dennis and Webster, 1971) or the activity of the lytic enzymes (Chet and Baker, 1980). Similarly moisture can be another factor which attributes to the reduction in the population. In the present study also, it was observed that the moisture content of the soil samples was reduced to 10% after 120 days (Table 56). The survival of *T.harzianum* under varying soil moisture levels was studied by Jeyaraj (1995).

The present study indicated the potential of the mutant strains of *Trichoderma harzianum* both in growth promotion and disease suppression of rhizome rot of ginger caused by *Pythium aphanidermatum.* The increased biological activity of the mutant strains was further substantiated based on the studies on hydrolytic enzymes of *T. harzianum.* It is important to study further the genetic stability of the mutants for all its bioefficacy and survival in the soil. Large scale field evaluations are needed to further strengthen the leads obtained in the present investigation.

SUMMARY AND CONCLUSION

Rhizome rot of ginger caused by *Pythium aphanidermatum* Edson, Fitz. is one of the major diseases affecting ginger production in Kerala. The epidemiology, chemical control and biological control of this disease have been studied in detail. Biological control as a major component of integrated disease management is the focus. In the present investigation, efforts were made to enhance the bioefficacy of the biocontrol agents used in the management of rhizome rot and integrating them with chemical control and other biocontrol agents to develop a blue print for further integrated disease management strategies.

1. A survey was conducted in five major ginger growing areas of Kerala viz. Ernakulam, Kottayam, Kozhikode, Idukki and Wayanad districts. About 150 fields were surveyed and healthy as well as diseasesd samples were collected from the fields visited.

2. One hundred and seventy seven (177) biocontrol agents which included 68 bacterial and 109 isolates of *Trichoderma* species were isolated by plating the healthy root samples on different culture media. The bioefficacy of the organisms isolated were tested rapidly by the dual culture plate technique.

3. Of the 109 isolates of *Trichoderma* only 57 were found promising in their potential to suppress the pathogen. Of these 19 were identified as *Trichoderma virens,* four as *Trichoderma aureoviride* and 34 were found to be *Trichoderma harzianum.*

4. Species of *Trichoderma* varied with geographical regions but the species that was predominantly found in the ginger rhizoplane was *Trichoderma harzianum.*

5. The variability of these organisms was tested by screening the different isolates *in vivo.* For this all the 57 *Trichoderma* isolates and 116 isolates from the IISR repository of biocontrol agents and the 26 bacterial isolates were screened *in vivo* in the green house conditions.

   Of the 173 isolates *of Trichoderma* screened five of the most promising isolates, viz. ***Trichoderma harzianum*-7, *Trichoderma virens*-6, *Trichoderma hamatum*-6, *Trichoderma aureoviride*-24** and ***Trichoderma pseudokoningii*-6** were

shortlisted for further studies. Of the 26 bacterial isolates screened, ten showed a reduced disease incidence of 9.5 to 30% when compared to 60% in control. Five among them, viz. **FP 1, FP 43, FP 44, FP 100** and **FP 113** were found to be superior in their disease suppressive effects and other growth parameters. These were shortlisted for further investigations.

6. The synergistic interactions between the fungal and bacterial antagonists were studied and a significant reduction in the disease incidence and an increased yield over control was observed in the results when the *Trichoderma* isolates were tested in combination with the bacterial isolates. The mutant M_1-25, when treated in combination with the different fluorescent pseudomonads gave an increased yield of 2.3-2.7 kg / bed, which was significantly more than that obtained in the different treatments with the wild type (0.8-1.8 kg/bed) and the control (0.64 kg/bed).

7. In the present investigation, improvement of the biocontrol agents by mutagenesis for enhanced enzyme activity was aimed at.

   For this both chemical mutagenesis using ethyl methyl sulphonate (EMS) and physical mutagenesis using UV radiations were tried. The former was ineffective, and hence physical mutagenesis with UV radiations were tried. The physical mutagenesis with UV rays yielded more than 25 colonies, of which one was an albino. Of the tests conducted for the strainal improvement using all the five different *Trichoderma* isolates, positive results could be obtained only with ***Trichoderma harzianum***-7.

8. The mutants obtained were screned *in vitro* and *in vivo* and only three of them were found to be promising as potential biocontrol agents. They were designated as **M_1-10, M_1-25** and **M_2-3** and they were selected for further studies.

9. The mutants obtained were characterized based on many parameters like growth on different media, effect of light on sporulation, growth on sorghum grains, bioassay of culture filtrates, enzyme activity, volatile and non volatile antibiotic tests, sensitivity to agrochemicals, rhizosphere competence assay, competitive saprophytic ability and rate of enzyme production.

10. Except the albino mutant, M_1-25 all the other mutants resembled the parental strain. The mutants, M_1-10 and M_2-3 were fast growers and showed an increased growth rate on different media like PDA and CMA. These mutants grew faster than the albino and sporulated profusely. The colony morphology of the albino mutant was different from the others. The morphological variation would serve as useful phenotypic markers for monitoring their population in soil after introduction.

    Although the rate of growth of the albino mutant on the sorghum grains was comparatively less than the others, the other mutants grew profusely on these grains and this indicated that they have greater scope for mass multiplication.

11. Bioassay of the culture filtrates of the different mutants was made and tested for the percentage of inhibition of the pathogen *Pythium aphanidermatum*. The results obtained indicated that the albino mutant, M_1-25 was found to exhibit an increased percentage of inhibition (22.4) against 16.9% exhibited in the wild type.

12. In the volatile antibiotic tests, all the isolates studied showed considerable inter and intra specific variation in the production of the volatiles and non volatiles based on their inhibitory effects of the pathogen *Pythium aphanidermatum*. The inhibitory effect of the nonvolatile antibiotics was more compared to the the volatile antibiotics tested. The study revealed that the wild type as well as the mutants are all non volatile producers.

13. Compatibility of the mutants with agrochemicals, viz Copper Oxy Chloride, Mancozeb and Metalaxyl Mancozeb was tested. All the mutants obtained were compatible with more than one fungicide and these multiple fungicide tolerant strains could be more beneficial because they could be used in the integrated management of rhizome rot of ginger. A comparison between the wild type, *Trichoderma harzianum* and the mutants showed that the mycelial dry weight in the latter was found to be more (148.9-289.1 g) when compared to the former which ranged between 40-214 g in the different treatments.

14. Tests were conducted to determine the rhizosphere competence as well as the competitive saprophytic ability of the mutants. The CSA index of the mutants ranged

between 12.4 - 17.6 and the RC index ranged between 73.9-106, while in the wild type CSA index and RC index were 8.5 and 73.9 respectively. The CSA and the RC index of the mutants were thus greater when compared to the wild type and this would ensure better survival capacity and its greater interaction with the native microflora.

15. The strainal improvement had a greater influence in the productivity of the enzymes because the mutants developed showed an enhanced β 1, 4 and β 1, 3 endo glucanase activity. Among the new strains developed, the mutant M_I-25 exhibited an increased activity of 115.2 and 338.5 GU of β 1, 4 and β 1, 3 endo glucanase units in the autoclaved mycelia when compared to 30.25 and 153.8 GU in the wild type respectively. This indicated the greater mycoparasitic potential of the mutants.

16. In the present study, the CSA indices that were obtained were directly correlated with the production of glucanase enzyme. The mutants of *T. harzianum* produced significantly greater amounts of hydrolytic enzymes (60.4 - 67.3 β 1, 4 endoglucanase and 229.6 - 292.7 β 1, 3 endoglucanase units) when compared to the wild type (40.4 β 1, 4 endoglucanase and 192.4 β 1, 3 endoglucanase units). The RC index was also higher (74 – 106 in the mutants and 73.9 in the wild type) and this could be attributed to the greater enzyme activity. Both these are indicative of the greater rhizosphere competence of the new strains developed.

17. When evaluated for their bioefficacy in the management of rhizome rot of ginger caused by *Pythium aphanidermatum,* in the field trials, the mutants of *T. harzianum* performed better than the wild type. The superiority of the mutants especially the mutant M_I-25 over the wild type was evident from its reduced disease incidence (2.8-9 %) and an increased yield (2-3.5kg / bed) when treated with the fungicides as against 10.5 % and 17.5% disease incidence and yield of 1 kg / bed and 0.65 g / bed in the wild type treated and control beds respectively. This was established both by the pot culture and the field experiments.

18. A comparison of the developed strains with the wild type *T. harzianum* indicated that the albino mutant, M_I-25 excelled all other strains in all the parameters like increased rhizosphere competence, competitive saprophytic ability, increased enzyme activity

and yield. This clearly showed the superiority of mutants in terms of an enhanced bioefficacy.

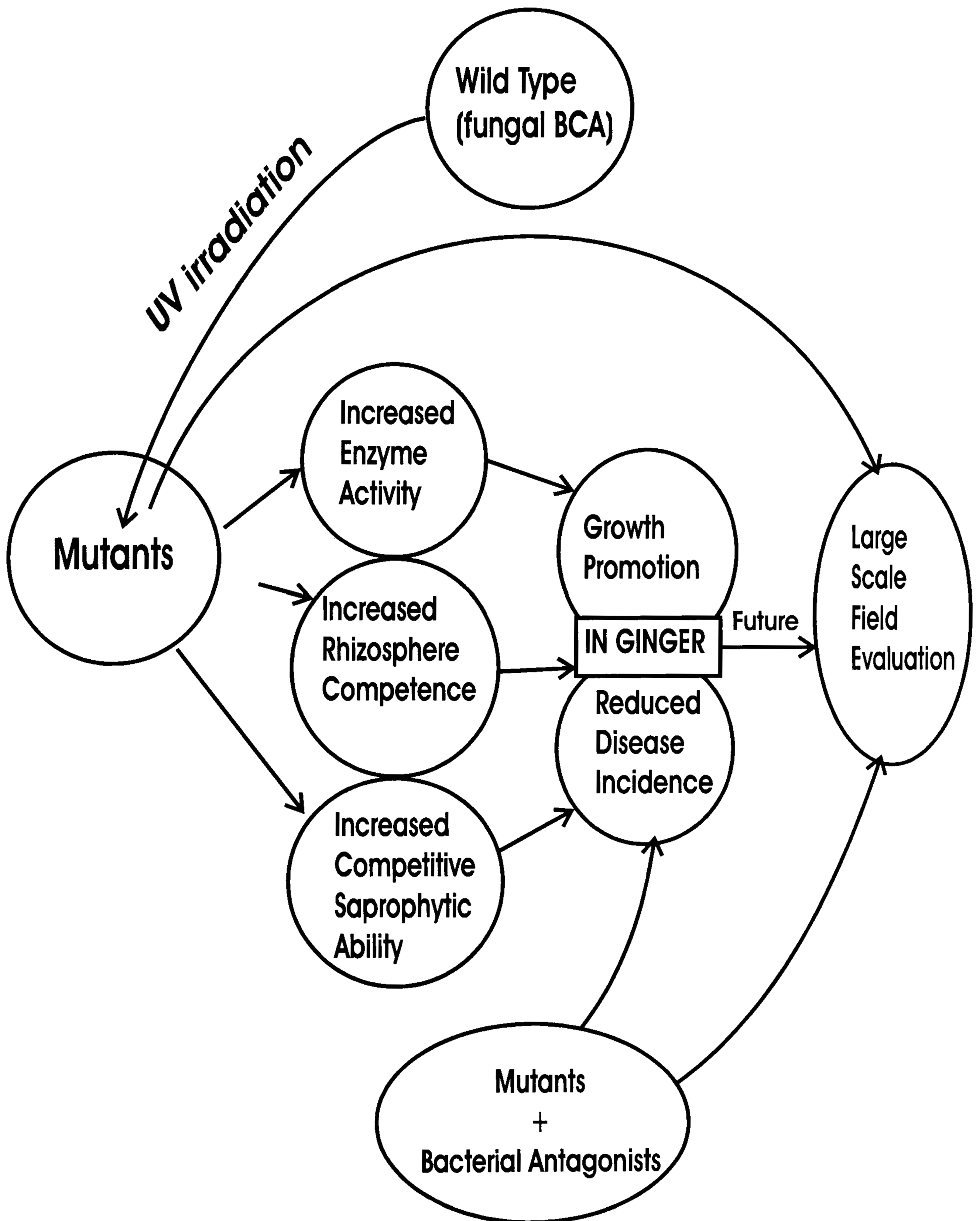
19. When the different strains were monitored after their introduction in the soil, there was a steady increase in the growth after the 30th day upto the 90th day and a decrease in the population after the 120th day. This decline in the growth rate could be attributed to other abiotic factors. However the pathogen population remained low.

20. The leads obtained in the present investigation justifies the biopotential of the strains developed by mutagenesis. There is a need to field evaluate these mutants for their disease suppressive potential.

CONCLUSION

The results obtained in the present study clearly opened up the avenues for strainal improvement. It highlighted the role played by the mutagens in enhancing the biopotential of the antagonists. This opens up new vistas in disease management of rhizome rot as the strainal improvement, like varietal improvement could be opted for as one of the disease management strategies (Fig 18).

Fig. 17: Strainal improvement and its future strategies



REFERENCES:

Abd-El Moity, T.H., Papavizas, G.C. and Shatla, M.N. (1982). Induction of new isolates of *Trichoderma harzianum* tolerant to fungicides and their experimental use for control of white rot of onion. *Phytopathology.* **72**: 396-400.

Abu-Blan, H.A. and Abu Gharbieh, W.I. (1994). Effect of solarisation on winter planting of potato, cauliflower and cucumber in Central Jordan valley, Dirasat Series B. *Pure & Applied Sci.* **21**: 203-213.

Adams, P.B. (1971). *Pythium aphanidermatum* oospore germination as affected by time, temperature and pH. *Phytopathology.* **61**: 1449-1450.

Agrios, G.N. (1997). *Plant pathology.* Fourth Edition. Academic Press, London, 635 pp

Ahmad, J.S. and Baker, R. (1987a). Rhizosphere competence of *Trichoderma harzianum. Phytopathology.* **77**: 182-189.

Ahmad, J.S. and Baker, R. (1987b). Competitive saprophytic ability and cellulolytic activity of rhizosphere competent mutant of *Trichoderma harzianum.Phytopathology.***77**: 358-362.

Ahmad, J.S. and Baker, R. (1988a). Implication of rhizosphere competence of *Trichoderma harzianum. Can. J. Microbiol.* **34**: 229-234.

Ahmad, J.S. and Baker, R. (1988b). Rhizosphere competence of benomyl tolerant mutants of *Trichoderma* spp. *Can. J. Microbiol.* **34**: 694-696.

Ainsworth, G.C., Sparrow, F.K. and Sussman, A.S. (1973). (Eds.) *The Fungi.* Vol IV.A. Academic Press, New York, pp. 621.

Al-Hamdani, A.M., Lutchmeah, R.S. and Cook, R.C. (1983). Biological control of *Pythium ultimum* induced damping off by treating cress seeds with mycoparasite with the mycoparasite *Pythium oligandrum. Plant Pathology.* **32**: 449-454.

Almassi, F., Ghisalberti, E.L., Narbey, M.J. and Sivasithamparan, K. (1991). New antibiotics from strains of *Trichoderma harzianum. J. Nat. Prod.* **54**: 396-402.

Anandaraj, M. and Sarma, Y. R. (1993). Role of root infection in rhizome rot of ginger (*Zingiber officinale* Rosc.). *J. Plantn. Crops:* **21** *(Suppl.)*: 140-143.

Anandaraj, M. and Sarma, Y. R. (1994). Biological control of black pepper diseases. *Indian Cocoa Arecanut Spices J.* **18**: 22-23.

Anandaraj, M. and Sarma, Y. R. (1997). Mature coconut water for mass culture of biocontrol agents . *J. Plantn. Crops.* **25**: 112-114

Anne, J. (1992). Methods and mechanisms of membrane fusion. In *Biotechnology*. pp. 95-133

Anonymous. (1984). Central Plantation Crops Research Institute. *Annual Report.* 1982. Kasaragod, India.

Anonymous. (1993). Compiled Annual Report of Multilocational project on rhizome rot of ginger (ICAR Cess fund Scheme). National Research Centre for Spices, Calicut., Rajasthan Agricultural University ,Udaipur,Rajasthan., Dr.Y.S.Parmar University of Horticulture and Forestry,Solan,H.P.

Anonymous. (1994). National Research Centre for Spices. *Annual Report.*1993. Calicut, India.

Anonymous. (1995). Ginger-A Spice crop grown across the country. *Spice India.***8**: 2-6.

Anonymous. (1996). Directorate of Economics and Statistics, New Delhi

Anonymous. 2000. National network project on *Phytophthora* diseases of horticultural crops (PHYTONET), Progress Report, Indian Institute of Spices Research, Kozhikode, 82pp

Arlorio, M., Ludwig, A., Boller, T. and Bonfante. P. (1992). Inhibition of fungal growth by plant chitinases and beta –I, 3-glucanases: A morphological study. *Protoplasma.* **171**: 34-43.

Aziz, A.Y., Foster, H.A and Fairhurst, C.D. (1993). Extracellular enzymes of *Trichoderma harzianum, T. polysporum and Scytalidium lignicola* in relation to biological control of Dutch elm disease. *Arboric. J.* **17**: 159-70.

Baby, U. I. (1998). Biocontrol potential of fungicide resistant mutants of *Trichoderma* spp. *Indian J. Microbiol.* **38:** 165-166.

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

Baker, K.F. and Cook, R.J. (1974). Biological control of plant pathogens. *J American Phytopathological Society.* pp. 433.

Baker, R., Elad, Y. and Chet, I. (1984). The controlled experiment in the scientific method with special emphasis on biological control. *Phytopathology.* **74:** 1019-1021.

Baker, R. and Dickman, M,B. (1993). Biocontrol with fungi. In: *Soil Microbial Ecology-Application in Agricultural and environmental management.* (Ed.F.Blaine Metting Jr.), Marvel Dekker Inc. New York. pp. 275-306.

Balakrishnan, P. (1997). Bio-ecology of rhizome rot pathogen(s) of ginger and disease management. Ph.D Thesis, University of Calicut, Calicut. 178 pp.

Balakrishnan, P., Usman, N,M. and Sarma, Y.R. (1996). Management of rhizome rot disease of ginger by soil solarisation. *J. Plantn.Crops.* **24** (Suppl.): 192-199.

Baldwin, J.E., Derome, A.E., Field, L., Gallagher, P.T., Taha, A.A., Thaller, V., Brewer, D. and A.Taylor. (1981). Biosynthesis of a cyclopentyl dienylisonitrile acid in cultures of the fungus *Trichoderma hamatum* (Bon.) Bain Aggr. *J. Chem. Soc. Chem. Commun.* 1227-1229.

Ball.C. (1973). The genetics of *Penicillium chrysogenum.Prog. Ind. Microbiol.* **12**: 47-72.

Ball.C. (1980). Genetic manipulation in filamentous fungi. In: *Fungal Biotechnology,* (Eds. J.E.Smith, D.R.Berry and B.Kristiansen). Academic Press, London. pp: 43-54.

Ball.C. (1984). Genetics and Breeding of Industrial micro organisms. CRC, Boca Raton, FL. pp. 203.

Barak. R., Elad,Y., Mirelman, D., and Chet, I. (1985). Lecithins: a possible basis for specific recognition in the interaction of *Trichoderma* and *Sclerotium rolfsii. Phytopathology.* **75**: 458-462.

Beena, N., Rajan, P.P, Sarma,Y.R, and Anandaraj, M. (1997). Control of ginger storage rot by agrochemicals and biocontrol. International Conference on Integrated Plant Disease Management for sustainable agriculture. 10-15 November, 1997.New Delhi. In: *Proceedings of the Indian Phytopathological Society.*Vol.1.pp.493-495.

Belanger, R.R., Dufour, N., Caron,J. and Benhamou, N. (1995). Chronological events associated with the antagonistic properties of *Trichoderma harzianum* against *Botrytis cinerea-* indirect evidence for sequential role of antibiosis and parasitism. *Biocontrol Sci.Technol.* **5**: 41-53.

Benitez, T., Limon, C., Delgado-Jerana, J and Rey, M. (1998). Glucanolytic and other enzymes and their genes. In: *Trichoderma* and *Gliocladium* volume II, (ed) G.E.Harman and C.P. Kubicek, Taylor and Francis Ltd, London. p 101-128

Benoni, H., Taraz, K., Korth, H. and Pulverer, G. (1990). Characterisation of 6- pentyl-α-pyrone from the soil fungus *Trichoderma koningii. Naturwissenchaften* **7**: 539-540.

Bensaci, M. and Newman, P. (1989). Selection of *Trichoderma* spp. strains as biocontrol agents resistant to fungicides. *Can. J. Pl. Pathol.* **11**: 185-186.

Bertus, L. S. (1942). Plant Pathology- *Adm. Rep. Dir. Agric.* Ceylon 1941. D 5.

Bhagawat, V.Y. (1960). Control of root rot of ginger. *Poona Agric. College Magazine,***51**:47-49.

Bharadwaj, S.S. and Gupta, P.K. (1987). *Invitro* antagonism of *Trichoderma* species against fungal pathogens associated with rhizome rot of ginger. *Indian J. Pl. Pathol.* **5**: 41-42.

Bharadwaj, S.S., Gupta, P.K., Dohroo, N.P. and Shyam, K.R. (1988). Biological control of rhizome rot of ginger in storage. *Indian J. Pl. Pathol.* **6**: 56-58.

Biely, P. and Tenkanen, M. (1998). Enzymology of hemicellulose degradation. In: *Trichoderma* and *Gliocladium* volume II, (ed) G.E.Harman and C.P. Kubicek, Taylor and Francis Ltd, London. p25-48

Bien, M. and Witkowska, D. (1991). Preparation of *Trichoderma viride* M4-4 mutants with enhanced cellulase activity using ethylenimine. *Zeszyty Naukowe Akademi Rolniczej we- Wroclawin Technologia Zywnosci(Poland).* No. 215, 219-227.

Bisset, J. (1991a). A revision of the genus *Trichoderma* 11. Infrageneric classification. *Can. J. Bot.* **69**: 2357-2372

Bisset, J. 1991b). A revision of the genus *Trichoderma* 111. Section Pachybasidium. *Can. J. Bot.* **69**: 2373-2417.

Bisset, J. (1991c). A revision of the genus *Trichoderma* IV. Additional notes on section *Longibracheatum*. *Can. J. Bot.* **69**: 2418-2420.

Bisset, J. (1992). A revision of the genus *Trichoderma atroviride.* Additional notes on section *Longibracheatum*. *Can. J. Bot.* **70**: 639-641.

Blanchette, R.A., Abad, A.R., Cease, K.R., Lorrien, R.E. and Leathers. T.D. (1989). Colloidal and cytochemistry of endo- 1 ,4- beta glucanase 1, 4 - beta –D- glucan cello-biohydrolase, and endo-1,4- beta xylanase ultra structure of sound and decayed birch wood. *Appl. Environ. Micribiol.* **55**: 2293-2301.

Bodo, B., Rebuffat, S., El Hajji, M. and Davoust, D. (1985). Structure of Trichoarzianines A 111c, an antifungal peptide from *Trichoderma harzianum. J. Am.Chem. Soc.***107**: 6011-6017.

Bolton, A.T. (1980). Control of *Pythium aphanidermatum* in *Poinsettia* in a soilless culture by *Trichoderma viride* and a *Streptomyces* spp. *Canadian J. Pl. Path.* **2:** 93-95.

Brian, P.W. and McGowan, J. C. (1945).Viridin: A highly fungistatic substance produced by *Trichoderma viride. Nature* (London) **156**: 144-145.

Brewer, D., Gabe, E.J., Hanson, A.W., Taylor. A., Keeping, J.W., Thaller, V. and Das, B.C. (1979). Isonitrile acid from cultures of the fungus *Trichoderma harzianum* (Bon.) Bain. aggr. X-ray Structure. *J. Chem. Soc. Chem. Commun.* 1061-1062.

Brewer, D., Mason, F.G. and Taylor, A. (1987). The production of alamethicins by *Trichoderma* spp. *Can.J. Microbiol.* **33**: 619-625.

Broadbent, P., Baker, R. F. and Waterworth, Y. (1971). Bacteria and actinomycetes antagonistic to fungal root pathogens in Australian soils. *Australian J. Biol. Sci.* **24:** 925-944.

Bruehl, G.W. (1989). Integrated control of soil borne plant pathogens: an overview. *Can. J. Plant Pathol.* **11**: 153-157.

Bruckner, H., Konig, W. A., Aydin, M. and Jung, G. (1985). Trichotoxin A40. Purification by counter current distribution and sequencing of isolated fragments. *Biochem. Biophys. Acta* **827**: 51-62.

Bruckner, H., Reinecke, C., Kripp, T. and Kieb, M. (1990). Screening, isolation and sequence determination of a unique group of polypeptide antibiotics from filamentous fungi. *Proc. Int. Mycol. Cong.,* Regensburg FRG, pp. 224.

Burpee, L.L. (1990). The influence of abiotic factors on biological control of soil borne plant pathogenic fungi. *Canadian J. of Pl. Path.* **12**: 308-317.

Butler, E.J. (1907). An account of the genus *Pythium* and some Chytridiaceae. *Mem.Dept.Agric.* India (Bot.Ser.), **1**: 70

Butler, E.J. (1918). *Fungi and Diseases in Plants.* Thacker, Spink & Co., Calcutta.

Butler, E. J. and Bisby, G.R. (1931). *The Fungi of India.* Sci.Monogr.1. ICAR New Delhi Indian Govt.Cent. Publ. Br. Calcutta. P.6., 153.

Carsolio, C., Gutierrez, A., Jimenez. B., Van Montagu, M. and Herera- Estrella, A. 1994. Characterisation of ech-42, a *Trichoderma harzianum* endo chitinase gene expressed during mycoparasitism. *Proc. Natl. Acad. Sci.* USA **91:** 10903-10907.

Cassiolato, A.M.R., Baker, R., Melo I.S – de., De- Melo-I.S. (1997). Effect of *Trichoderma harzianum* mutants on formulation and carpogenic germination of *Sclerotinia sclerotiorum* sclerotia and lettuce plant survival. *Fitopatologia-Brasileira.* **22**: 34-38.

Chandel, D.S. and Choudhary, S.R. (1995). Antagonism of *Trichoderma longibracheatum* Rifai against microfungi isolated from the phylloplane of soybean. *Indian J. Mycol.Pl. Pathol.* **25:** 124-125.

Chang, Y.C., Baker, R., Kleifeld, O. and Chet, I. (1986). Increased growth of plants in the presence of the biological control agent *Trichoderma harzianum. Plant Dis.* **70**:145-148.

Chao, W.L., Nelson, E.B., Harman, G. E. and Hoch, H.C. (1986). Colonisation of the rhizosphere by biological control agents applied to seeds. *Phytopathology.* **76**: 60-65.

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

Chesters, C.G.C. and Bull, A.T. (1963).The enzymatic degradation of laminarin 1.The distribution of laminarinase among micro-organisms. *Biochem. J.* **86**: 28-31.

Chet, I. and Henis, Y. (1969). Effect of catechol and disodium EDTA on melanin content of hyphal and sclerotial walls of *Sclerotium rolfsii* Sacc. and the role of melanin in the susceptibility of these walls to β- 1,3- glucanase and chitinase. *Soil Biol. Biochem.* **1**: 131-138.

Chet, I., Hadar, Y., Elad, Y., Katan, J and Henis,Y. (1979). Biological control of soil borne plant pathogens by *Trichoderma harzianum.* In : *Soil borne plant pathogens* (B.Schippers and W.Gams.eds.) Academic Press, London. pp. 585-592.

Chet. I. and Baker, R. (1980). Induction of suppressiveness to *Rhizoctonia solani* in soil. *Phytopathology.* **70:** 994-998.

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

Chet, I., Harman, G.E. and Baker, R. (1981). *Trichoderma hamatum*: Its hyphal interaction with *Rhizoctonia solani* and *Pythium* spp. *Microbial. Ecol.* **7**: 29-38.

Chet, I., Elad, Y., Kalfon, A., Hadar, Y. and Katan, J. (1982). Integrated control of soilborne and bulbborne pathogens in iris. *Phytoparasitica.* **10**: 229-236.

Chet, I. (1987).*Trichoderma* – application, mode of action and potential as a biocontrol agent of soilborne plant pathogenic fungi. In: *Innovative approaches to plant disease control.* (Ed. I.Chet). John Wiley & Sons, New York. pp: 137-160.

Chet, I. (1990). Biological control of soilborne plant pathogens with fungal antagonist in combination with soil treatments. In: *Biological control of soil borne plant pathogens.* (Ed.) D.Hornby, CAB international pp: 15-25.

Chet, I., Barak, Z. and Oppenheim, A. (1993). Genetic engineering of micro-organisms for improved biocontrol activity. In; *Biotechnology in Plant Disease Control.* (Ed.) I. Chet, John Wiley-Liss, Inc., New York. Pp: 211-236.

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

Claydon, N., Hanson, J.R., Trunch, A. and Avent, A.G. (1991). Harzianolides-a butenolide metabolite from cultures of *Trichoderma harzianum. Phytochemistry.* **30**: 3802-3803.

Cole, J.S. and Zvenyika, Z. (1988). Integrated control of *Rhizoctonia solani* and *Fusarium solani* in tobacco transplants with *Trichoderma harzianum* and triadimenol. *Plant Pathol.* **37**: 271-277.

Cook, R.J. and Baker, K.F. (1983). *The Nature and Practice of Biological Control of plant pathogens.* American Phytopathological Society. St.Paul, MN. pp.539.

Cotes, A.M., Lepoivre, P. and Semal, J. (1996). Correlation between hydrolytic enzyme activities measured in bean seedlings after *Trichoderma koningii* treatment combined

with pregermination and the protective effect against *Pythium splendens. Eur. J. Pl. Pathol.* **102**: 497-506.

Curl E.E., Wiggins, E.A. and Anders, J.C. (1977). Interactions of *Rhizoctonia solani* and *Trichoderma* spp. with PCNB and herbicides affecting cotton seedling disease. *Proc. American Phytopath. Soc.* 3:221.

Czajkowska, D., Ilnicka Olenjniczak, O. and Witkowska Gwiazdowska, A. (1992). Obtainment of *Trichoderma reesei* mutants over producers of cellulase. *Pr. Inst. Lab.* Badaw. Przem. Spozyw (Poland). **46**: 5-19.

Dake, G.N. and Edison, S. (1988). Survey for disease incidence in major ginger growing areas of Kerala during 1984 & 1985. *J. Plantn. Crops*. **16**: 55-57.

Dake, G.N., Ramachandran, N. and Sarma, Y.R. (1988). Strategies to control rhizome rot (*Pythium* spp.) and bacterial wilt (*Pseudomonas solanacearum)* of ginger. *J. Coffee Research.* **18**: 61-67 (Suppln).

Dake, G.N. and Edison, S. (1989). Association of pathogens with rhizome rot of ginger in Kerala. *Indian Phytopath*. **42**: 16-19.

Dake, G.N., Anandaraj, M., Raju, C.A. and Iyer, R. (1989). Storage method of ginger seed rhizomes. National Research Centre for Spices, Calicut 673012. Kerala, India.

Dataram. (1988). Studies on the management of Rhizome rot. MSc. Thesis. Rajasthan College of Agric. Udaipur.

Davet, P., Martin, C. and Artigues, M. (1981). Aspects of biological control with *Trichoderma harzianum. Agronomic* 1: 933-936.

Davet. P. (1987). Criteria for selecting *Trichoderma* clones antagonistic to sclerotial fungi in soil. *Bull. OEPP.* **17**: 535-540.

Davis, B. (1985). Factors influencing protoplast fusion. In: Fungal protoplasts: applications in biochemistry and Genetics. (Ed) J.F. Peberdy and L. Ferency. Marcel Dekker, New York. Pp. 45-71.

Dean, J.F. and Anderson, J.D. (1991). Ethylene biosynthesis –inducing xylanase. Purification and physical characterization of the enzyme produced by *Trichoderma viride.Plant Physiol.* **85**: 316-323.

De La Cruz, J., Hidalgo Gallego, A., Lora, J.M., Benitez, T., Pinto Toro, J.A. and Llobell, A. (1992). Isolation and characterization of three chitinases from *Trichoderma harzianum. Eur. J. Biochem.* **206**: 859-867.

Dennis, C. and Webster, J. (1971a). Antagonistic properties of species groups of *Trichoderma*-1. Production of non volatile antibiotics. *Trans. Br. Mycol. Soc.* **57:** 25-39.

Dennis, C. and Webster, J. (1971b). Antagonistic properties of species groups of *Trichoderma*-11. Production of volatile antibiotics. *Trans. Br. Mycol. Soc.* **57:** 41-48.

Dennis, C. and Webster, J. (1971c). Antagonistic properties of species groups of *Trichoderma*-111. Hyphal interraction. *Trans. Br. Mycol. Soc.* **57:** 363-369.

Dharmaputra, O.S. and Retnowati, I. (1994). The possibility of controlling *Sclerotium rolfsii* on soyabean *(Glycine max)* using *Trichoderma* and tebuconazole. *Biotropia.* **7:** 18-29.

Dinakaran, D. and Marimuthu, T. (1997). Inhibition of *Macrophomina phaseolina (* Tassi.) Goid. by mutants of *Trichoderma viride* Pers. ex. Fr.. *J. Biological Control.* **11**: 43-47.

Doceae, E., Geaman, I. and Beratlief, Z. (1974). Research on the antagonism between *Trichoderma lignorum, Trichoderma viride* and *Pythium* spp. and practical prospects for biological control of plant diseases.*Lucrari Stintifice. Institutal Agronomic Nbalcesec.* **17:**128-134.

Dodd, S.L., and Steward, A. (1992). Biological Control of *Pythium* induced damping off of beet root (*Beta vulgaris*) in the glass house. *New Zealand Journal of Crop and Horticultural Science.* **20:** 421-426.

Dohroo, N.P. and Sharma S.L. (1985). *Pythium pleroticum* on *Zingiber officinale. Indian Phytopath.* **38:** 391

Dohroo, N.P., Shyam, K.R. and Bharadwaj, S.S. (1987). Distribution, diagnosis and incidence of rhizome rot complex of ginger in Himachal Pradesh. *Indian J. Plant. Pathol.* **5**: 4-25.

Domsch, K.H., Gams, W. and Anderson, T. H. (1980). Compendium of Soil Fungi. Vol. 1. London Academic Press. Pp. 859.

Don-Jenson, J. (1992). Influence of nitrogen. pH and high concentrations of nutrient solutions on the ability of *Trichoderma harzianum* and *Gliocladium virens* to control *Pythium* spp. which cause damping off in cucumber seedlings. *Kongelige veterinaer-og Landbohojskole.* Pp. 121.

Dunn, R.T., Lewis, S. A. and Papavizas, G.C. (1983). Production and formulation of two biocontrol agents from liquid fermentation. *Phytopathology.* **73:** 165.

Dunlop, R.W., Simon, A. and Sivasithamparam, K. (1989). An antibiotic from *Trichoderma koningii,* active against soil borne plant pathogens. *J. Nat. Prod.* **52:** 67-74

Edenborough, M.S. and Hebert, R.B. (1988). Naturally occurring isocyanides. *Nat. Prod. Rep.* **5:**229-245.

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

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

Elad, Y., Chet, I., Boyle, P. and Henis, Y. (1983). Parasitism of *Trichoderma* spp. on *Rhizoctonia solani* and *Sclerotium rolfsii.* Scanning electron microscopy and fluorescence microscopy. *Phytopathology.* **73:** 85-88.

Elad, Y., Zvieli, Y. and Chet, I. (1986). Biological control of *Macrophomina phaseolina* by *Trichoderma harzianum. Crop. Prot.* **5:** 288-292.

Elad, Y. and Chet, I. (1987). Possible role of competition for nutrients in biocontrol of *Pythium* damping off by bacteria. *Phytopathology.* **77:** 190-195.

Endo, A., Hasumi. K., Sakai, K. and Kanbe, T. (1985). Specific inhibition of glyceraldehydes-3-phoshate dehydrogenase by koningic acid (heptelidic acid). *Journal Antibio T* (Tokyo) **38:** 920-925

El-Hajji, M., Rebuffat, S., Lecommaneteur, D. and Bodo, B. (1987). Isolation and sequence determination of trichorzianines: An antifungal peptide from *Trichoderma harzianum. Int. J. Protein Peptide Res.* **29:** 207-215.

Faull, J. L., Graeme Cook, K.A. and Pilkington, B.L. (1994). Induction of an isonitrile antibiotic by an UV induced mutant of *Trichoderma harzianum. Phytochemistry.***36:**1273-1276.

Flori, P. and Roberti, R. (1993). Treatment of onion bulbs with antagonistic fungi for the control of *Fusarium oxysporum* sp.*cepea. Difesa dille Diante.***16:** 5-12.

Foster, R.C., Rovira, A.D. and Cook, T.W. (1983). Ultra structure of the Root – Soil Interface. *American Phytopathological Society.*St.Paul, Minnesota, U S A

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

Fravel, D.R., Marois, J.J., Lumsden, R.D. and Connick, W.J. Jr. (1985). Encapsulation of potential biocontrol agents in an alginate clay matrix. *Phytopathology.* **75:** 774-777.

Fridlender, M., Inbar, J., Chet, I. (1993). Biological control of soil borne plant pathogens by a β-1, 3 glucanase producing *Pseudomonas cepacia. Soil Biology and Biochemistry.***25:** 1211-1222.

Fujiwara, A., Okuda, T., Masuda, S., Shiomi, Y., Miyamato, L., Sekene, Y., Tazoe, M. and Fujiwara, M. (1982). Fermentation, isolation and characterization of isonitrile antibiotics. *Agric. Biol.Chem.***46:**1803-1809.

Gams, W. and Bisset, J. (1998). Morphology and identification of *Trichoderma*. In: *Trichoderma* and *Gliocladium*.Volume 2, (Ed) G.E.Harman and C.P. Kubicek, Taylor and Francis Ltd. London. Pp. 3-34.

Garret, S.D. (1970). Pathogenic root infecting fungi. Cambridge University Press. Cambridge.England. pp. 294.

Geremia, R.A., Goldman, G.H., Jacobs, D., Ardiles, W., Vila, S.B., Van Monteagn, M. and Herera Estrella, A. (1993). .Molecular characterization of the proteinase encoding gene prb1, related to mycoparasitism by *Trichoderma harzianum. Mol. Microbiol.* **8:** 603-613.

Ghisalberti, E.L., Narbey, M.J., Dewan, M.M. and Sivasithamparam, K. (1990). Variability among strains of *Trichoderma harzianum* in their ability to reduce take all and to produce pyrones. *Plant Soil.* **121:** 287-291.

Ghisalberti, E.L. and Sivasithamparam, K. (1991). Antifungal antibiotics produced by *Trichoderma* spp. *Soil Biol. Biochem.* **23:** 1011-1020.

Ghisalberti, E.L., Hockless, D.C.R., Rowland, C. and White, A.H. (1992). Harziandione, a new class of diterpene from *Trichoderma harzianum. J. Nat. Prod.* **55:**1690-1694.

Ghisalberti, E.L. and Rowland, G.Y. (1993). Antifungal metabolites from *Trichoderma harzianum. J. Nat. Prod.* **56** :1799-1804.

Gomes ,I., Gomes, J., Steines,W. and Esterbauer, H. (1992). Production of cellulase and xylanase by a wild strain of *Trichoderma viride. Appl. Microbiol. Biotechnol.* **36:**701-707.

Godtfredson, W.O. and Vangedal, S. (1965). Trichodermin- A new sesquiterpene antibiotics.*Acta Chem. Scand.***19:**1088-1102.

Graeme Cook, K.A. and Faull, J.L. (1991). Effect of ultraviolet induced mutants of *Trichoderma harzianum* with altered antibiotic production on selected pathogens *invitro.Can. J. Microbiol.* **37:** 659-664.

Gracheck, S.J. and Emert, G.H. (1984). Protoplast formation and fusion using *Trichoderma reesei* mutants. In: Developments in Industrial Microbiology. (Ed) G.H. Nash and L.A. Underkofler.

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

Hadar, E., Elad, Y., Ovadia, S., Hadar, Y. and Chet, I. (1979b). Biological and chemical control of *Rhizoctonia solani* in carnation. *Phytoparasitica.***7:** 55. (Abstr.)

Hadar, Y., Harman, G. E., Taylor, A.G. and Norton, J.M. (1983). Effects of pre-germination of pea and cucumber seeds and of seed treatment with *Enterobacter cloacae* on rots caused by *Pythium* spp. *Phytopathology.* **73:** 1322-1325.

Hadar, Y., Harman, G.E. and Taylor, A.G. (1984). Evaluation of *Trichoderma koningii* and *Trichoderma harzianum* from New York soil for biological control of seed rot caused by *Pythium* spp. *Phytopathology.* **74:** 106-110.

Haran, S., Schickler, H., Oppenheim, A. and Chet, I. (1995). New components of the chitinolytic systems of *Trichoderma harzianum. Mycol.Res.***99:** 441-446.

Harman, G.E., Eckenrode, C.J. and Webb, D.R. (1978). Alteration of spermosphere ecosystems affecting oviposition by the bean seed fly and attack by soilborne fungi on germinating seeds. *Ann. Appln. Biol.* **90:** 1-6.

Harman, G.E., Chet. I. and Baker, R. (1981). Factors affecting *Trichoderma hamatum* applied to seeds as a biocontrol agent. *Phytopathology.* **71:** 569-572.

Harman, G.E. (1991). Seed treatments for biological control of plant diseases.*Crop Prot.***10:**166-171.

Harman G.E. and Tronsmo, A. (1992). Methods of genetic manipulation for the production of improved bioprotectant fungi. *Biological Control of Soil borne Diseases.* pp.181-187.

Harman, G.E., Hayes, C.K., Lorito, M., Broadway, R.M., Di Pietro, A., Peterbauer, C., and Tronsmo, A. (1993). Chitinolytic enzymes of *Trichoderma harzianum*: Purification of chitobiosidase and endochitinase. *Phytopathology.* **83:** 313-318.

Harman, G.E. and Stasz, T.E. (1991). Protoplast fusion for the production of superior biocontrol fungi. In: Microbial control of weeds. (Ed) D.O.Tebeest, Chapman and Hall, New York. Pp. 171-186

Hasegawa, S., Norden, J.H. and Kirk Wood, S. (1969). Enzyme that hydrolyse fungal cell wall polysaccharides 1, purification and properties of endo - α- 91-3) Glucanase from *Trichoderma viride. J. Bio. Chem.* **244:** 5460-5470.

Haware, M.P., Joshi, L.K and Sharma, N.D. (1973). Effect of post harvest treatment of aureofungin on Rhizome for viability of ginger seed rhizomes. H.A. Bull.**15**:84-85

Haware, M.P. and Joshi, L.K. (1974). Studies on soft rot of ginger from Madhya Pradesh. *Indian Phytopath.* **27**:158-161.

Hendrix, F.F. and Campbell, W.A. (1973). *Pythiums* as plant pathogens. *Annu. Rev.Phytopath.*11:77-78.

Henis, Y. and Chet, I. (1975). Microbiological control of plant pathogens. *Adv. Appl. Microbiol.***19**: 85-111

Henis, Y., Gaffar, A. and Baker, K. (1978). Integrated control of *Rhizoctonia solani* damping off of radish: effect of successive plantings. PCNB and *Trichoderma harzianum* on pathogen and disease. *Phytopathology.* **68:** 900-907.

Hjeljord, L. and Tronsmo, A. (1998). *Trichoderma* and *Gliocladium* in biological control: an overview. In: *Trichoderma* and *Gliocladium* volume II. (Ed) G.E Harman and C.P Kubicek, Taylor and Francis Ltd, London.pp.131-152.

Horowitz, M., Regev, Y. and Herzlinger, G. (1983). Solarisation for weed control. *Weed Sci.* **31:**170-179.

Horvath, E.M., Burgel. J.L. and Messner, K. (1995). The production of soluble antifungal metabolites by the biocontrol fungus, *Trichoderma harzianum* in connection with the formation of conidiospores. *Mater: Org.* **29:** 1-14.

Howell, C.R. and Stipanovic, R.D. (1983). Gliovirin, a new antibiotic from *Gliocladium virens* and its role in the biological control of *Pythium ultimum. Can. J. Microbiol.* **29:** 321-324.

Howell, C.R. and Stipanovic, R.D. (1995). Mechanisms in the biocontrol of *Rhizoctonia solani* induced cotton seedling disease by *Gliocladium virens*: antibiosis. *Phytopathology* **85:**469-472.

Howell, C.R. (1987). Relevance of mycoparasitism in the biological control of *Rhizoctonia solani* by *Gliocladium virens. Phytopathology* **77:** 992-994.

Howell, C.R. (1998). The role of antibiosis in biocontrol. In:*Trichoderma* and *Gliocladium* volume II. (Ed) G.E.Harman and C.P. Kubicek. Taylor and Francis Ltd, London. Pp. 173-184.

Huang, C.S., Tenente, R.C.V., Da Silva, F.C.C. and Lasa, I.A.R. (1981). Effect of *Crotolaria spectabilis* and two nematicides on numbers of *Meloidogyne incognita* and *Helicotylenchus dihystera. Nematologia.***27:** 1-5.

Hwang, S.F., Chakravarthy. P. and Prevorst, D. (1993). Effects of Rhizobia, metalaxyl and V A Mycorrhizal fungi on growth , nitrogen fixation and development of *Pythium* root rot of Saifonin. *Plant. Dis.* **77:** 1093-1098.

Inbar, J. and Chet, I. (1992). Biomimics of fungal cell wall recognition by use of lectin coated nylon fibers. *J. Bacteriol.***174:**1055-1059.

Inbar, J. and Chet, I. (1994). A newly isolated lectin from the plant pathogenic fungus *Sclerotium rolfsii*, purification, characterization and role in mycoparasitism. *Microbiology.***140:** 651-657.

Inbar, J., Abramsky, M., Cohen, D. and Chet, I. (1994). Plant growth enhancement and disease control by *Trichoderma harzianum* in vegetable seedlings grown under commercial conditions. *Eur. J. Path.* **100:** 337-346.

Inbar, J. and Chet, I. (1995). The role of recognition in the induction of specific chitinases during mycoparasitism by *Trichoderma harzianum. Microbiology.* **141:** 2823-2829.

Indu, S. and Sawant, S.D. (1990). Coffee fruit skin and cherry husk as substrates for mass multiplication of *Trichoderma harzianum* an antagonist on citrus *Phytophthora. Indian Phytopath.* **42:** 336 (Abs).

Iyer, R., Koya, K.M.A. and Banerjee, S.K. (1981). Relevance of soil and insects in the epidemiology of rhizome rot of ginger. *Abstracts of Third international symposium on Plant Pathology.* New Delhi 14-18 December. Pp.126.

Iyer, R. (1987. Diseases of ginger. *Rev.Trop.Plant Path.* **4**:2 51-88

Iyer, R. and Sunderraju, P. (1993). Interraction of V A Mychorrhiza with *Meloidogyne incognita* and *Pythium aphanidermatum* affecting ginger (*Zingiber officinale* Rosc.). *J. Plantn. Crops.* **21:** 30-34.

Iwasaki, T., Hayashi, K. and Funatsu, M. (1964). Purification and characterization of two types of cellulases from *Trichoderma koningii. J. Biochem.* (Tokyo).**55:** 209.

Jackson, G.V.H. (1995). Diseases of ginger in Sikkim and their control. A synthesis paper produced for the Indo Swiss Project, Sikkim.

Jackson, A.N., Whipps, J.M., Lynch, J.M. and Bazin, M.J. (1991). Effect of some carbon and nitrogen sources on spore germination, production of biomass and antifungal metabolites by species. of *Trichoderma* and *Gliocladium virens* antagonistic to *Sclerotium cepivorum. Biocontrol science and Technology.***1**: 43-51

Janardhan, K.K. and Hussain,A. (1974). Production of toxic metabolite and pectolytic enzyme by *Pythium butleri. Phytopath. Mic. Appl.* **52**: 305-330.

Jayaraj, J. (1995). Studies on the biological control potential of *Trichoderma harzianum* Rifai. Ph.D.Thesis, Annamalai University, Chennai, 312 pp

Jeyarajan, R. and Ramakrishnan, G. (1995). Mass production of *Trichoderma viride* for biological control. *Indian J. Mycol. and Pl. Pathol.* **25:**125

Jeyarajan, R., Ramakrishnan, G., Dinakaran, D. and Sridhar, R. (1993). Development of products of *Trichoderma viride* and *Bacillus subtilis* for biocontrol of root rot disease. In: *Biotechnology in India.*(Ed) R.Dwivedi. Bioved Research Society, Allahabad.

Johnson, L.F. and Curl, E.A. (1972). *Methods for research on the ecology of soilborne plant pathogens.* Burges Publishing Company. Pp. 247

Jones, D., Gordon, A.H. and Bacon, J.S.D. (1974). Co-operative action by endo and exo - β- (1-3) glucanase from parasitic fungi in the degradation of cell wall glucans of *Sclerotinia sclerotiorum .Biochem. J.* **140:** 47-55

Jones, D. and Watson, D. (1969). Parasitism and lysis by soil fungi of *Sclerotinia sclerotiorum*, a phytopathogenic fungus. *Nature* (London) **224:** 287.

Joshi, L.K. and Sharma, N.D. (1982). Diseases of ginger and turmeric. In: *Proc. of National Seminar on Ginger and Turmeric* (M.K.Nair, T. Premkumar, P. N. Ravindran and Y. R. Sarma. Eds). Calicut 8-9 April 1980. Central Plantation Crops Research Institute, Kasaragod. 670124,Kerala. India. pp.104 -119.

Katan, J., Greenberger. A., Alan, H. and Grinstein, A. (1976). Solar heating by polyethylene mulching for the control of diseases caused by soilborne pathogens. *Phytopathology*. **66:** 683-688.

Katan, J. (1987). Soil Solarisation. In: *Innovative approaches to Plant Disease control.* (Ed) Chet, I. John Wiley & Sons, New York. pp: 77-105.

Katan, J., Fisher, G. and Grinstein, A. (1983). Short and long term effects of soil solarisation and crop sequence on *Fusarium* wilt and yield of cotton in Israel. *Phyopathology*. **73:**1215.

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

Kay, S.J. and Stewart, A. (1994). The effect of fungicides on fungal antagonism of onion white rot and selection of dicarboximide – resistant biotypes. *Pl. Pathol.* **43:** 863-867.

Khara , H.S. and Hadwan, H.A. (1990). *Invitro* studies on antagonism of *Trichoderma* spp. against *Rhizoctonia solani,* the causal agent of damping off of tomato. *Plant Dis. Res.* **5:**142-144

King, E.O., Ward, M.K. and Raney, D.E. (1954). Two simple media for the demonstration of pyocyanin and fluorescein, *J. Lab. Clin. Med.* **44:** 301-307.

Kleinfeld. O. and Chet. I. (1992). *Trichoderma harzianum* – interaction with plants and effect on growth response. *Plant Soil.* **144:** 267-272.

Koivula, A., Linder, M. and Teeri, T.T. (1998). Structure – function relationships in *Trichoderma* cellulolytic enzymes. In: *Trichoderma* and *Gliocladium* volume II. (ed) G.E. Harman and C.P. Kubicek. Taylor and Francis Ltd, London. Pp.3-24.

Kommedahl, T., Windels, C.E., Sarbini, G. and Wiley, H.B, (1981). Viability in performance of biological and fungicidal seed treatment in corn, peas and soyabeans. *Prot. Ecol.* **3:** 55-61.

Koshy Abraham., Valsala, P.A., Maicekutty, P., Mathew and Abhicheeran. (1988a). Management of soft rot disease of ginger. In: *Proc. of the National Seminar on Chillies, Ginger and Turmeric.*11-12 January.(Sathyanarayana,G., Sukumara Reddy, M., Rama Rao, M., Azam,K.M and Naidu,R.eds.) Hyderabad. India.pp.156-161.

Koshy Abraham., Valsala, P.A., Maicekutty, P., Mathew and Abhicheeran. (1988b). Effect of seed treatment on the soft rot disease of ginger. In: *Proc. of the National Seminar on Chillies, Ginger and Turmeric.11-12 January.*(Sathyanarayana,G., Sukumara Reddy, M., Rama Rao, M., Azam, K. M and Naidu, R.eds.) Hyderabad. India.pp.160-161.

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

Koya, K.M.A. (1988). Distribution of dipteran maggots associated with ginger (*Zingiber officinale.*Rosc.) in Kerala. *J. Plantn. Crops.* **16:**137-140.

Kraft, J.M. and Papavizas, G.C. (1983). Use of host resistance, *Trichoderma* and fungicides to control soil borne disease and increased yields of pea. *Plant Disease.***67:**1234-1237.

Krishnamoorthy, A. S. and Bhaskaran, K. (1991a). Screening of fungal antagonists against *Pythium indicum* causing damping off of tomato. *Madras Agric. J.* **78:** pp.127-128.

Krishnamoorthy, A.S. and Bhaskaran, K. (1991b). Effect of organic amendments and the antagonist *Trichoderma viride* on the biological control of damping off disease of tomato caused by *Pythium indicum. J. Biol. Cont.* **5:** 61-62.

Kubicek, C.P. (1993). From cellulose to cellulose inducers. Facts and Fiction. In: *Trichoderma reesei* cellulases and other hydrolases. Enzyme structure biochemistry, genetics and application. (Eds.Souminen, P and Reinikainen, T.) Foundation of biotechnical and Industrial Fermentation Research, Helsinki, Finland.pp:181-188.

Kumar, A. and Gupta, J.P. (1999). Variations in enzyme activity of tebuconazole tolerant biotypes of *Trichoderma viride. Indian Phytopath.* **52:**263-266.

Kumar, A. and Marimuthu, T. (1997). Decomposed coconut coir pith – A conducive medium for colonization of *Trichoderma viride. Acta Phytopathologica et Entomologica Hungarica.* **32:** 51-58.

Kumar, A. (1997). Management of mungbean root rot by systemic fungicide and biocontrol agents. Ph.D. thesis. IARI, New Delhi.

Lalithakumari, D. (1996). Protoplasts-A biotechnological tool for plant pathological studies. *Indian Phytopath.***49:**199-212.

Lalithakumari, D., Mrinalini, S., Chandra, A.B., Annamalai, P. (1995). Strain improvement by protoplast fusion for enhancement of biocontrol potential integrated with fungicide tolerance in *Trichoderma* spp. *J.Plant Dis. Protect.* **103:** 206-212.

Lewis. J.A. and Papavizas, G.C. (1985). Effect of mycelial preparation of *Trichoderma* and *Gliocladium* on population of *Rhizoctonia solani* and the incidence of damping off. *Phytopathology.***75**: 812-817.

Lifshitz, R., Lifshitz, S. and Baker, R. (1985). Decrease in incidence of *Rhizoctonia* pre-emergence damping off by the use of integrated and chemical controls. *Plant Disease* **69:**4341-4344.

Lin, A., Lee, T.M. and Rern, J.C. (1994). Tricholin, a new antifungal agent from *Trichoderma viride* and its action in biological control of *Rhizoctonia solani. J. Antibiol.* (Tokyo) **47:** 720-725.

Lindow, S.E., Arny, D.C. and Upper, C.D. (1983). Biological control of frost injury: an isolate of *Erwinia herbicola* antagonistic to ice nucleation active bacteria. *Phytopathology.* **73:** 1097-1102.

Lisansky, S.G. (1985). Production and commercialization of pathogens. In: Biological Pest Control. (Eds.) Hussey, N.W. and Scopes, N. Blandford Press. Poole, UK. pp. 210-218.

Liu, S. and Vaughan, E.K. (1965). Control of *Pythium* infection in table beed seedlings by antagonistic microorganisms. *Phytopathology.*55: 986-989.

Locke, J.C., Marois, J.J. and Papavizas, G.C. (1985). Biological control of *Fusarium* wilt of green house grown Chrysanthemums.*Plant Dis.* **69:**167-169.

Lodha, B.C. and Webster, J. (1990). *Pythium acanthophoron* a mycoparasite rediscovered in India and Britain. *Mycol.Res.* **94:** 1006-1008.

Lorito, M., Di Pietro, A., Hayes, C.K., Woo, S.L. and Harman, G.E. (1993a). Antifungal, synergistic interaction between chitinolytic enzymes from *Trichoderma harzianum* and *Enterobacter cloacae. Phytopathology.* **83:** 721-728.

Lorito, M., Harman, G.E., Hayes, C.K., Broadway, P.M., Tronsmo, A., Woo, S.L. and Pietro, A.D. (1993b). Chitinolytic enzymes produced by *Trichoderma harzianum:* Antifungal activity of purified endochitinase and chitobiosidase. *Phytopathology.* **83:** 302-307.

Lorito, M., Hayes, C.K., Di. Pietro, A., Woo, S.L. and Harman, G.E. (1994a). Purification, characterization and synergistic activity of a glucan 1,3- β- glucisidase and an N- acetyl β- glucosaminidase from *Trichoderma harzianum. Phytopathology.* 84: 398-405.

Lorito, M., Hayes, C.K., Peterbauer, C., and Harman, G.E. (1994b). Synergistic interaction between fungal cell wall degrading enzymes and different antifungal compounds enhances spore germination. *Microbiology.***140:** 623-629.

Lorito, M. (1998). Chitinolytic enzymes and their genes. In *Trichoderma* and *Gliocladium* volume II. (Ed) G.E.Harman and C.P.Kubicek. Taylor and Francis Ltd. London. Pp.73-100.

Ljungdahl, L.G. and Eriksson, K.E. (1985). Ecology of microbial cellulose degradation. *Adv. Microb. Ecol.* **8:** 237-299.

Lumsden, R.D. and Locke, J.C. (1989). Biological control of damping off caused by *Pythium ultimum* and *Rhizoctonia solani* with *Gliocladium virens* in soilless mix. *Phytopathology.***79**: 361-366.

Lynch, J.M. (1990). Microbial metabolites. In: *The Rhizosphere* (Lynch, J.M. ed.) John Wiley & Sons. Pp.177-206.

Mach, R.L., Butterweck, A., Schindler, M., Messner, R., Herzug, P. and Kubieck. (1993). Molecular regulation of formation of xylanase (XYN) 1 and 2 by *Trichoderma reesei.* In: *Trichoderma reesei* cellulases and other hydrolases. Enzyme structure, biochemistry, genetics and applications (Eds. Suominen, P. and Reinkainen, T.) Foundation of Biotechnical and Industrial Fermentation Research, Helsinki, Finland. pp. 211-216.

Manmohandas, T.P., Devadas, V.S. and Pillai, G.R. (1990). Efficiency of fungicides for seed treatment against pre-emergence rhizome rot of ginger. *Indian Cocoa Arecanut and Spices Journal.***14:** 13-15.

Mandels, M., Weber, J. and Parizek, R. (1971). Enhanced cellulase production by a mutant of *Trichoderma viride. Appl. Microbiol.* **21:** 152-154.

Martin J,P. (1950). Use of acid, Rose Bengal and streptomycine in the plate method for estimating soil fungi. *Soil Science.***69**:215-232

Mathur, S., Thakore, B.L. and Singh, R.B. (1984). Effect of different fungicides on ginger *rhizome rot pathogen and their effect on germination and rotting of rhizomes.* Indian *J..Mycol. Plant Path.***14:**155-157.

Mc Rae, W. (1911). Soft rot of ginger in Rangpur district of East Bengal (E.Pakisthan). *Agric. J. India.* **6:** 139-146.

Merivuori, H., Sands, J.A. and Montenecourt, B.S. (1985). Effect of tunicamycin on secretion and enzymatic activities of cellulase from *Trichoderma reesei. Appl. Microbiol. Biotechnol.* **23:** 60-66.

Michalikova, A. and Michrina, J. (1997). Biological contol of ***Fusarium*** foot rot in wheat seedlings by *Trichoderma harzianum. Biologia Bratislava.* **52**: 591-598.

Migheli, Q., Whips, J.M., Budge, S.P. and Lynch, J.M. (1994). Production of inter and intra strain hybrids of *Trichoderma* spp. by protoplast fusion and evaluation of their biocontrol activity against soil borne foliar pathogens. *J. Pathology.* **143:** 91-97.

Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for the determination of reducing sugars. *Anal.Chem.* **31**:426

Mishra, A. and Iyer, R. (1981). Control of rhizome rot of seed ginger during storage. *Abst.Third Int.Symp. on Plant pathology.*New Delhi 14-18 December. pp:221.

Mitra, M. and Subramanian, L.S. (1928). Fruit rot diseases of cultivated cucurbitaceae caused by *Pythium aphanidermatum* fitz. *Mem.Dept. Agric. India Bot.***15:** 79-84.

Moffatt, J.S., Bu'lock, J.D. and Yuen, J.H. (1969). Viridiol, a steroid like product from *Trichoderma viride. Chem. Commun.* 139: pp. 839.

Mohammed, M.S. and Fahmy, F.G. (1988). Improvement of biological control of *Trichoderma harzianum* Rifai. to onion white rot. *Egyptian J. Phytopath.* **20:** 117-125.

Montenecourt, B.S. and Eveleigh, D.E. (1977). Semiquantitative plate assay for determination of cellulose production by *Trichoderma viride. Appl.Microbiol.***33:**178-183.

Mukherjee, P.K. (1997). *Trichoderma* species as microbial suppressive agents of plant pathogens. In:*Compendium of Agrimicrobes.* (Eds) Jha M. N and Venkataraman G. S. Today and Tomorrow Publishers, New Delhi.

Mukherjee, P. K. and Mukhopadhyay, A.N. (1993). Induction of stable mutants of *Gliocladium virens* by gamma irradiation. *Indian Phytopath.* **46**: 393-397.

Mukherjee, P.K. and Mukhopadhyay, A.D. (1995). *In situ* mycoparasitism of *Gliocladium virens* on *Rhizoctonia solani.Indian Phytopath.***48**:101-102.

Mukherjee, P.K., Haware, M.P. and Raghu, K. (1997). Induction and evaluation of benomyl tolerant mutants of *Trichoderma viride* for biological control of *Botrytis* grey mold of chick pea. *Indian Phytopathology.* **50**: 485-489.

Mukherjee, P.K., Sherkhane, P.D. and Murthy, N.B.K. (1999). Induction of stable benomyl tolerant phenotypic mutants of *Trichoderma pseudokoningii* MTCC 3011, and their evaluation for antagonistic and biocontrol potential. *Indian J. Experimental Biology.* 37:710-712.

Mukhopadhyay, A.N., Brahmbhatt, A,B., Patel, G.J. (1986).*Trichoderma harzianum*–A potential biocontrol agent for tobacco damping off. *Tobacco Res.***12:** 26-35.

Mukhopadhyay, A.N. and Kaur, N.P. (1990). Biological control of chickpea wilt complex by *Trichoderma harzianum. Proc.111 Int. Conf. on Plant Protection in the Tropics.* Malaysia. March, 20-23.

Mukhopadhyay, , A.N., Shreshtha, S.M. and Mukherjee, P.K. (1992). Biological seed treatment for the control of soil borne plant pathogens. *FAO Plant Prot. Bull.* 40:21-30.

Mukhopadhyay, A.N and Chandra, I. (1986). Biocontrol of sugarbeet and tobacco damping off by *Trichoderma harzianum* (Abs.) In: Seminar on management of soilborne diseases of crop plants.TNAU, CBE.

Mundkar, B.B. (1949). *Fungii and Plant Diseases.* Mac. Millan & Co. Ltd., London pp.246.

Muthumilan, M. and Jeyarajan, R. (1996). Integrated management of *Sclerotium* root rot of groundnut involving *Trichoderma harzianum, Rhizobium* and carbendazim. *Indian J. Mycol. Pl. Pathol.* **26:** 204-208.

National Academy of Sciences. (1987). *Report of the Research Briefing Panel on Biological Control in Managed Ecosystems.* National Academy Press.Washington, D.C.

Nelson, E.B. and Craft, C.M. (1992). A miniaturized and rapid bioassay for the selection of soil bacteria suppressive to *Pythium* blight of turf grass. *Phytopathology.* **82:** 206-210

Ollis, W.D., Rey, M., Gotfredsen, W.O., Rastrup-Andersen, N., Vangedal, S. and King, T. (1980). The constitution of the antibiotic trichoviridin.*Tetrahedron.***36:**515-520.

Okuda, T., Fujiwara, A and Fujiwara, M. (1982). Correlation between species of *Trichoderma* and production patterns of isonitrile antibiotics. *Agric.Biol.Chem.* **46:** 1811-1822.

Ordentlich, A., Wiesman, Z., Gottlieb, H.E., Cojocaru, M. and Chet, I. (1992). Inhibitory furanone produced by the biocontrol agent *Trichoderma harzianum.Phytochemistry.* **31:**. 485-486.

Osando, J,M. and Wando, S.W. (1994). Interraction between *Trichoderma* species and *Armillaria* root rot fungus of tea in Kenya. *Int. J.Pest Management.* **40**:69-79.

Panicker, S. and Jeyarajan, R. (1993). Mass application of biocontrol agent *Trichoderma* spp. *Indian J. Mycol. and Pl.Pathol.* **23**: 328-330.

Papavizas, G.C. (1973). Status of applied biological control of soil borne plant pathogens. *Soil Biol. Biochem.* **5**: 709-720.

Papavizas, G.C. (1980). Induced tolerance of *Trichoderma harzianum* to fungicides. *Phytopathology.* **70:** 691-692 (abstract).

Papavizas, G.C. (1985). *Trichoderma* and *Gliocladium*: Biology, Ecology and Potential for Biocontrol. *Annu. Rev. Phytopath.***23:** 23-54.

Papavizas, G.C. and Lumsden, R.D. (1980). Biological control of soil borne fungal propagules. *Annu.Rev.Phytopathol.***18:** 398-413.

Papavizas, G.C., Lewis, J.A., Abd-El Moity, T.H. (1982). Evaluation of new biotypes of *Trichoderma harzianum* for tolerance to benomyl and enhanced biocontrol capabilities. *Phytopathology.* **72:** 126-132.

Papavizas, G.C. and Lumsden, R.D. (1982). Improved medium for isolates of *Trichoderma* spp.from soil. *Plant Dis.* **66:**1019-1020.

Papavizas, G.C and Lewis, J.A. (1983). Physiological and biocontrol characteristics of stable mutants of *Trichoderma viride* resistant to MBC fungicides. *Phytopathology.***73:** 407-411.

Papavizas, G.C., Dunn, M.T., Lewis, J.A. and Beagle Ristaino. J. (1984). Liquid fermentation technology for experimental production of biocontrol fungi. *Phytopathology.* **74:**1171-1175.

Papavizas, G.C., Roberts, D.P. and Kim, K.K. (1990). Development of mutants of *Gliocladium virens* tolerant to benomyl. *Can. J. Microbiol.* **36:** 484-489.

Park, M. (1941). Report of the work of the Division of Plant Pathology.*Adm.Rep.Dir.Agric.,* Ceylon,1939. D20-D22.

Park, D. (1960). *The ecology of soil fungi.* (D.Parkinson and J.S.Waid eds.) Liverpool University Press, Liverpool. pp. 148-159.

Paul, B., Bhatnagar, T., Djennane, A. and Bennounane, L. M. (1992). Two species of *Pythium* causing damping off of pine seedlings and their possible biological control by *Pseudomonas fluorescens. International J. Mycol. Lichenol.* **4:** 347-360.

Peechia.S. (1994). Selection of fungicide resistant mutants from an antagonistic *Trichoderma harzianum* strain. *Agricoltura Mediterranea.* **124:** 159-169.

Pe'er, S., Barak, Z., Yarden, O. and Chet, I. (1991). Stability of *Trichoderma harzianum* amdS transformants in soil and rhizosphere. *Soil Biol. Biochem.* **23:** 1043-1046

Perseglove, J.W., Brown, E.G., Green, C. L. and Robbins, S.R.J. (1981). Spices.Vol.2.Longman, London.

Piccatiogo, S.K. (1983). .Maintenance and expression of cloned *Trichoderma reesei* DNA in yeast *Saccharomyces cerevisiae.* Ph.D thesis. Restgers. The State University of New Jersey. .pp. 258.

Poderes, D.E., Hockenhull, J., Jensen, D.F. and Mathur, S.B. (1992). *Invivo* screening of *Trichoderma* isolates for antagonism against *Sclerotium rolfsii* using rice seedlings. *Bulletin* OIL B/SROP **15:**33-35.

Prakash, M.G., Vinayagopal, K., Anandaraj, M. and Sarma, Y.R. (1999). Evaluation of substrates for mass multiplication of fungal biocontrol agents *Trichoderma harzianum* and *Trichoderma virens. Journal of Spices and Aromatic Crops* **8:**207-210.

Premkumar, T., Sarma, Y.R. and Gautam, S.S.S. (1982). Association of dipteran maggots in rhizome rot of ginger.*Proc. of National Seminar on Ginger and Turmeric.* April 8-9,1980,Calicut,India. pp.128-130.

Pyke, T.H. and Dietz, A. (1996). U-21, 9263, a new antibiotic - Discovery and biological activity. *Appln. Microbiol.* **14:** 506-510.

Rajan, K.M. and Singh, R.S. (1973). Proceedings of the first National Symposium on Plantation Crops, 8-9 December, 1972, Trivandrum Kerala, India. *J. Plant. Crops (Supplement):* 102-106.

Rajan, K.M. and Agnihoitri,V.P. (1989). *Pythium* induced rhizome rot of ginger, problems and progress. In: *Perspectives in Phytopathology* (Agnihotri, V.P., Singh,N., Chaube,H.S., Singh,V.S., Dwivedi,T.S. eds.) Today and Tomorrow Printers and Publishers, New Delhi. pp:189-198.

Rajan, P.P. and Sarma, Y.R. (1997). Compatibility of potassium phosphonate (Akomin-40) with different species of *Trichoderma* and *Gliocladium virens.* In: *Proc.of National Seminar on Biotechnology of Aromatic plants.*24-25 April.1996. Calicut, India. pp.15-155.

Rajan, P.P. (2000). Approaches towards integrated disease management of *Phytophthora* in black pepper *(Piper nigrum).* Ph.D thesis. University of Calicut. 198.pp.

Rajan P.P., Sarma, Y.R. and Ananadaraj, M. (2002). Management of foot rot disease of black pepper with *Trichoderma* spp. *Indian Phytopathology.***55:** 34-38.

Rajappan, K. (1997). Induction of mutants in *Trichoderma viride* by UV irradiation . *Plant Dis. Research.* **12:** 1-5.

Ramachandran, N., Dake, G.N. and Sarma, Y.R. (1989). Evaluation of systemic fungicides for efficacy against rhizome rot of ginger. *Indian Phytopath.* **42:**530-33.

Ramakrishnan, T.S. (1949). The occurrence of *Pythium vexans* de Bary in South India. *Indian Phytopath.* **2:** 27-30.

Rankin, L. and Paulitz, T.C. (1994). Evaluation of rhizosphere bacteria for biological control of *Pythium* root rot of greenhouse cucumbers in hydroponic culture. *Plant.Dis* **78:**447-451.

Rao, A.S. (1959). A comparative study of competitive saprophytic ability in twelve root infecting fungi by an agar plate method. *Trans Brit Mycol. Soc* **42:** 97-111.

Rathiah, Y. (1987). Control of soft rot of ginger with Ridomil. *Pesticides.* **21:**29-30.

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

Reese, E.T. and Mandels, M. (1959). β-D-1,3- glucanases in fungi. *Can. J. Microbiol.* **5:** 173-185.

Ricker, A.J. and Ricker, R.F. (1936). *Introduction to research of Plant diseases.* John. S. Swift Co. St. Louis, Mo.

Rifai, M.A. and Webster, J. (1966). Culture studies on *Hypocrea* and *Trichoderma 11, H. lactea (=H. citrina)* and *H. pulvinata. Trans. Br. Mycol. Soc.***49:** 297-310.

Rifai, M.A. (1969). A revision of the genus *Trichoderma. Mycol. Pap.***116**:1-56.

Rubin, B. and Benjamin, A. (1984). Solar heating of the soil - Involvement of environmental factors in the weed control process. *Weed Sci.* **32:** 138-142.

Rudawska, M. and Kamoen, O. (1992). Regulation of β-1, 3- glucanase synthesis in *Trichoderma harzianum. Arbor. Kornickie.* **37:** 51-59.

Rukumani, S. and Mariappan, V. (1994). Effect of *Trichoderma* spp. on root rot of black gram caused by *Macrophomina phaseolina.* In:*Crop Diseases Innovative techniques and Management.* (Eds)K.Sivaprakasan and K. Seetharam. Kalyani Pub. New Delhi. pp.217-220.

Sadanandan, A.K. and Iyer, R. (1986). Effect of organic amendments on rhizome rot of ginger. *Indian Cocoa Arecanut and Spices J.* **9:** 94-95.

Sadanandan, A.K., Raju, C.A. and Anandaraj, M. (1988). Effect of culture practices and organic amendments on the incidence of soft rot of ginger. In: Proc. of the National Seminar on chillies, Ginger and Turmeric, 11-12 January 1988.(Satyanarayana, G., Sukumara Reddy M., Rama Rao,M., Azam,K.M and Naidu,R eds.)Hyderabad, India.pp.149-152.

Sahare, K.C. and Asthana, R.P. (1962). Rhizome rot of ginger and its control. *Indian Phytopath.* **15:**77-78.

Saju , K.A., Anandaraj, M. and Sarma, Y.R. (2000). Mass production of *Trichoderma harzianum* in the farm using organic matter. Indian Phytopathological Society, southern zone annual meeting and symposium on emerging trends in plant disease management, December 7-8,Bangalore,p61 (Abstract).

Saju, K.A., Anandaraj, M. and Sarma, Y.R. (2002). On farm production of *Trichoderma harzianum* using organic matter. *Indian Phytopathology* **55:**177-183.

Saju, K.A. (2004). Factors affecting the biological control of *Phytophthora capsici* infections in black pepper (*Piper nigrum* L.) Ph.D. Thesis.University of Calicut.232 p.

Samuels.G.J. (1996). *Trichoderma*: a review of biology and systematics of the genus. *Mycol. Res.* **100:** 923-935.

Sankar, P. and Jeyarajan, R. (1996). Biological control of sesamum root rot by seed treatment with *Trichoderma* spp. and *Bacillus subtilis. Indian.J. Mycol. Pl. Pathol.***26:**217-220.

Saratchandra, V., Duganzich, D., Burch, G. (1993). Occurrence of antifungal fluorescent *Pseudomonas* spp. on some horticultural and pastural plants, *New Zealand J.Crop and Horticultural Sci.,* **21:** 267-272.

Sarma, Y.R., Nambiar, K.K.N and Brahma, R.N. (1979). Studies on rhizome rot of ginger and its control. In Proc. of PLACROSYM 11.Indian Soc.Platn.Crops., Central Plantation Crops Res. Institute.Kasaragod.670124.India.pp.386-397.

Sarma, Y.R. (1994). Rhizome rot diseases of ginger and turmeric.In: *Advances in Horticulture Vol.10-Plantation and Spice Crops*-Part 2 (1994). Eds. Chadha,K.L. and Rethinam,P. Malhotra Publishing House,New delhi-110064, India. p. 113-138.

Sarma, Y.R., Anandaraj, M. and Venugopal, M.N. (1994). Diseases of spice crops. In: *Advances in Horticulture Vol. 10 .Plantation and Spice Crops Part-2.* (Eds). K.L.Chadha and P.Rethinam. Malhotra Pub. House , Delhi. pp 1015-1057.

Sarma, Y.R., Anandaraj, M. and Venugopal, M.N. (1996). Biological control of diseases of spices. In; *Biological control in Spices.* (Eds) M.Anandaraj and K.V.Peter. Indian Institute of Spices Research, Calicut,Kerala. pp:1-19.

Sawant, I.S and Mukhopadhyay, A.N. (1990). Integration of Metalaxyl with *Trichoderma harzianum* for the control of *Pythium damping off* in sugarbeat. *Indian Phytopath.***43:** 535-541.

Scarselletti, R. and Faull, J.L. (1994). *In vitro* activity of 6-pentyl α-pyrone, a metabolite of *Trichoderma harzianum* in the inhibition of *Rhizoctonia solani* and *Fusarium oxysporum* f. sp. *Lycopersici. Mycol. Res.* **98:** 1207-1209.

Schirmbock, M., Lorito, M., Wang, Y.L., Hay, C.K., Arisan Atac, I., Scala, F., Harman, G.E. and Kubicek. (1994). Parallel formation and synergism of hydrolytic enzymes and peptabiol antibiotics, molecular mechanisms involved in the antagonistic action of *Trichoderma harzianum* Rifai. against phytopathogenic fungi. *Appl. Environ. Microbiol.* **60:**4364-4370.

Schippers, B., Lugtenberg, B. and Weisbeck, P.J. (1987). Plant growth control by fluorescent pseudomonads. In: *Innovative Approaches to Plant Disease Control.* (Chet, I. ed.) John Wiley & Sons, New York. p.19-39.

Schroth, M.N. and Hancock, J.G. (1982). Disease suppressive and root colonizing bacteria. *Science*: **216:**1376-1381.

Selvakumar, R., Srivastava, K.D., Rashmi-Aggarwal., Singh, D.V., Prem-Dureja., Aggarwal, R. and Dureja. P. (2000). Studies on development of *Trichoderma viride* mutants and their effect on *Ustilago segetum tritici. Indian Phytopath.***53**: 185-189.

Sethuraman, K. (1991). Biological control of sesamum root rot caused by *Macrophomina phaseolina* (Tessi.) Gold. *MSc. (Agr.) Thesis.* Tamilnadu Agric. University, Madurai Campus, India.

Shapira, R., Ordenltich, A., Chet, I. and Oppenheim, A. B. (1989). Control of plant diseases by chitinase expressed from cloned DNA in *Escherichia coli. Phytopathology.***79**: 1246-1249.

Sharif, F.N., Okasha, A. and Kazem, K.T. (1988). *Penicillium stipitatum* and *Trichoderma harzianum* in the biological control of cucumber damping off caused by *Pythium aphanidermatum. Journal of the University of Kuwait.Science.* **15:**107-113.

Sharma, B.K. (1998). Antifungal properties of biocontrol agents and plant extracts against causal fungi of yellows and rhizome rot of ginger. *J. Biol. Control.* **12:**77-80.

Sharma, S. L. and Dohroo, N. P. (1982). Efficacy of chemicals in controlling rhizome rot of ginger (*Zingiber officinale.* Rosc.). *Proc.of the National Seminar on Ginger and Turmeric.*April 8-9, calicut, India. pp. 120-122.

Shivanna, M.B., Meera, M.S. and Hyakumachi, M. (1994). Sterile fungi from zoysia grass rhizosphere as plant growth promoters in spring wheat. *Can. J. Microbiol.* **40:** 637-644.

Shivanna, M.B., Meera, M.S. Kageyama. K. and Hyakumachi, M. (1996). Growth promotion ability from zoysia grass rhizosphere fungi in consecutive plantings of wheat and soyabean. *Myco. Science.* **37:**163-168.

Simon, A., Dunlop, R.W., Ghisalberti, E.L and Sivasithamparam. (1987). *Trichoderma koningii* produces a pyrone compound with antibiotic properties. *Soil Biol.Biochem.* **20:** 263-264.

Sinha, A.P. and Mukhopadhyay, A.N. (1988). Foot rot of papaya and rhizome rot of ginger incited by *Pythium* spp. In: Perspectives in Mycology and Plant pathology (Agnihotri, V.P., Sarbhoy, A.K., Kumar, D eds.) Malhotra Publishing House, New Delhi.pp.282-291.

Sinha, I. and Upadhyay, R.S. (1993). Modification of biocontrol potential of *Trichoderma viride, Acta. Phytopatholol. Entomol. Hung.***28**: 209-214.

Singh, M. and Majumdar, U.L. (1995) Antagonistic activity of *Trichoderma* spp. to *Macrophomina phaseolina* (Tessi) Gold *in vltro. Environ. and Ecol.* **13:** 481-482.

Sivan, A. and Chet, I. (1982). Degradation of fungal cell wall by lytic enzymes of *Trichoderma harzianum. J. Gen. Microbiol.***135:** 675-682.

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

Sivan, A. and Chet, I. (1989). The possible role of competition between *Trichoderma harzianum* and *Fusarium oxysporum* on rhizosphere colonization. *Phytopathology.***79:**198-203.

Sivan,A. and Harman, G.E. (1991). Improved rhizosphere competence in a protoplast fusion progeny of *Trichoderma harzianum. J. Gen.Microbiol.***137**:23-29.

Sivasithamparam, K. and Ghisalberti, E.L (1998). Secondary metabolism in *Trichoderma* and *Gliocladium.* In :*Trichoderma* and *Gliocladium* volume I, (ed) G.E.Harman and C.P.Kubicek, Taylor and Francis Ltd. London. p 35-41.

Stanghellini, M.E. and Burr,T.J. (1973). Germination *in vivo* of *Pythium aphanidermatum* oospores and sporangia. *Phytopathology.***63:**1493-1496.

Stephen Jebakumar, R., Anandaraj, M. and Sarma, Y.R. (2000). Compatibility of phorate and chlorpyriphos with *Trichoderma harzianum* Rifai applied for integrated disease management in black pepper (*Piper nigrum* L.). *Journal of Spices and Aromatic Crops* **9:**111-115

Suseela, Bhai. R, Thomas, J. and Naidu, R. (1994). Evaluation of an easier media for field application of *Trichoderma* sp. in cardamom growing soils. *J.Plant.Crops.* 22:50-52.

Tamura, A., Kotani, H. and Naruto, S. (1975). Trichoviridin and dermadin from *Trichoderma* spp. Tk-1. *J. AntibioT.* (Tokyo). **28:** 161-162.

Thakore, B.L., Sneh Mathur and Singh, R.B. (1988). Effect of rhizome treatment with fungicides for economic control of rot. *J.Phytopathol. Res.***1**:83-84.

Thomas, K.M. (1938). *Detailed Administration report of the Government of Mycologist, Madras for the year* 1937-38.

Thomas, (1940). *Detailed administration report of the Government of Mycologist, Madras for the year* 1939-40.pp.18.

Thomas, K.M. (1941). *Detailed administration report of the Government of Mycologist, Madras for the year* 1940-41. *Rept. Dept. Agric Madras.* pp 53-74.

Thomashow, L.S. and Weller, D.M. (1996). Current concepts in the use of introduced bacteria for biological disease control: Mechanisms and antifungal metabolites.In: Plant Microbe Interaction (Eds) Gary Staecy, Univ. of Tennusse and Noel. T.Keen, Univ. of California, Riverside.p 187-235.

Tribe, H.T. and Ahmad, A.H.M. (1975). Use of autoclavable plastic bags in fungal culture work. *Trans. Br. Mycol. Soc.* **64:** 362-363.

Tronsmo, A. (1986). Use of *Trichoderma* spp. in biological control of necrotrophic pathogens. In: *Microbiology of the phyllosphere* (Fokkema,N.J and Heuvel, J.V.D Eds.) Cambridge University Press, Cambridge. Pp.348-362.

Tronsmo, A. (1989a). Effect of fungicides and insecticides on growth of *Botrytis cinerea, Trichoderma viride* and *Trichoderma harzianum.Norw. J.Agric.Sci.*3:151-156.

Tronsmo, A. (1989b) *Trichoderma harzianum* used for biological control of storage rot on carrots. *Norw J .Agric .Sci.* **3:**157-161.

Tronsmo, A. and Dennis, C. (1978). Effect of temperature on the antagonistic properties of *Trichoderma* spp. *Trans. Br. Mycol. Soc.***71:** 469-474.

Tronsmo, A. (1991). Biological and integrated control of *Botrytis cinerea* on apple with *Trichoderma harzianum. Biol.Control.* **1:** 59-62.

Tronsmo, A. and Harman, G.E. (1992). Coproduction of chitinases and biomass for biological control by *Trichoderma harzianum* on media containing chitin. *.Biol.Control.* **2:**272-277.

Tronsmo, A. and Hjeljord, C.G. (1992). *Plant Microbe interaction and Biological control* (Eds.Greg. J. Boland, L Dard Key Kendall) Marcell Dekker Inc. New York Basal Hong Kong. Pp.111-121

Tsao. and Ocana, G. (1969). Selective isolation of species of *Phytophthora* from natural soils on an improved antibiotic medium. *Nature.* **223:** 636-638.

Tu, J.C. (1980). *Gliocladium virens* a destructive mycoparasite of *Sclerotium sclerotorium. Phytopathology.* **70:** 670-674.

Ulhoa, C.J. and Peberdy, J.F. (1991). Regulation of chitinase synthesis in *Trichoderma harzianum. J.Gen.Microbiol.***137:**2163-2169.

Ulhoa, C.J. and Peberdy, J.F. (1992). Purification and some properties of the extracellular chitinase produced by *Trichoderma harzianum. Enzyme and Microb. Technol.* **14:** 236-240.

Ulhoa, C.J. and Peberdy, J.F. (1993). Effect of carbon source on chitobiose production by *Trichoderma harzianum. Mycol. Res.* 97: 45-48.

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

Uppal, N.N. (1940). Appendix 1C. Summary of the work done under plant pathologists to the Government of Bombay, Poona for the year 1938-39. *Rep. Dept. Agric.* Bombay.1938-39.
pp :2-3-211.

Usman, N. M. (1997). Biological control of rhizome rot of ginger. Ph.D. thesis, University of Calicut.

Usman, N.M., Balakrishnan. P. and Sarma, Y.R. (1996). Biocontrol of rhizome rot of ginger. *J. Plantn. Crops.* **24** (Suppl.) 184 -191.

Vaheduddin ,S. (1955). Pathological Survey of the Hyderabad state. *Indian Phytopath.***8:**166-170.

van der Plaats Niterink, A.J. (1981). Monograph of the genus *Pythium.* In: *Studies in Mycology.* Vol. 21. (Gams, W and Jacobs RPWM eds.).Central bureau voor schimmelcultures, Baarn, Netherlands.

Viji, G., Baby, U.I. and Manibhushan Rao, K. (1993). Induction of fungicidal resistance in *Trichoderma* spp.through UV irradiation. *Indian J. Microbiol.* **32:**125-129.

Vyas, S.C. (1994). Integrated biological and chemical control of dry root rot of soyabean. *Indian J. Mycol. Pl. Pathol.* **24:** 132-134.

Watts. R., Dahiya, J., Chaudhary, K. and Tauro, P. (1988). Isolation and characterization of a new antifungal metabolite of *Trichoderma reesei. Plant Soil.* **107:** 81-84.

Webber, J.F. and Hedger, J.N. (1985). Comparison of the interaction between *Ceratocystis ulmi* and elm bark saprobes *in vitro* and *in vivo. Trans. Br. Mycol. Soc.* **81:** 93-101.

Weidling, R. (1932). *Trichoderma lignorum* as a parasite of other fungi. *Phytopathology.***22:** 837-845.

Weindling, R. and Emerson, O.H. (1936). The isolation of toxic substance from the culture of a *Trichoderma.Phytopathology.* **26:** 1068-1070.

Whipps. and Lumsden, R. D. (1991). Biological control of *Pythium* species. *Biocontrol Sci. Technol.* **1:**75-90.

Winstead, N.N. and Mc Combi, C.L. (1961). Pectinolytic and cellulolytic enzyme production of *Pythium aphanidermatum.Phytopathology.***51:**270-273.

Witkowska, D. (1993). Study on biosynthesis and practical utilization of *Trichoderma viride* extracellular hydrolases. Wroclaw (Poland) Wydawnictwo *Akademii Rolniczej.* WC wroclawicc. pp:72.

Witkowska, D. and Bien, M. (1991). Activity in biosynthesis of extracellular hydrolases of *Trichoderma viride* mutants obtained in two stage mutation. *Acta Aliment. Pol* (Poland). **41:** 127-135.

Wolk, M. and Sarkar, S. (1994a) Growth promoting effect of some isolates of fluorescent pseudomonads on cucumber *(Cucumis sativus), Anzeiger fur Schadlingskunda, Planzenschutz, Umweltschutz* **67:**101-103.

Wolk, M. and Sarkar, S. (1994 b). Antagonism *in vivo* of *Bacillus* spp. against *Rhizoctonia solanii* and *Pythium* spp, *Anzeiger fur Schadlingskunda, Planzenschutz, Umweltschutz* **67**:1-5.

Worasatit, N., Sivasithamparam, K., Ghisalberti, E.L. and Rowland, C. (1994). Variation in pyrone production, lytic enzymes and control of *Rhizoctonia* root rot of wheat among single spore isolates of *Trichoderma koningii. Mycol.Res.* **98:** 1357-1363

Wright, J, M. (1956). The production of antibiotics in soil 111.Production of gliotoxin in wheat straw buried in soil. *Ann. Appln. Biol.* **44:** 461-466.

Xu, T., Zhong, J.P. and Li, D.B. (1993). Antagonism of *Trichoderma harzianum* 82 and *Trichoderma* spp. UF9 against soilborne fungal pathogens. *Acta Phytopathologica Sinica.* **23:** 63-67.

Zeppa, G., Allegrone, G., Barbeni, M. and Garuda, P. (1990). Variation in the production of volatile metabolites by *Trichoderma viride. Annali di Microbiologia ed Enzimologia.* **90:** 171-176.

Zhou, T. and Paultz, T.C. (1993). *In vitro* and *in vivo* effect of *Pseudomonas* spp. on *Pythium aphanidermatum* zoospore behaviour in exudates and on rhizoplane of bacteria treated cucumber roots. *Phytopathology.* **83:** 872-876.

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