

**MANAGEMENT OF WILT AND ROOT ROTS  
OF COTTON WITH SPECIAL REFERENCE  
TO BIO FUNGICIDES**

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**MANAGEMENT OF WILT AND ROOT ROTS  
OF COTTON WITH SPECIAL REFERENCE  
TO BIO FUNGICIDES**

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University of Agricultural Sciences, Dharwad  
in partial fulfilment of the requirements for the  
Degree of  
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in  
PLANT PATHOLOGY*

*By*

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**CERTIFICATE**

This is to certify that the thesis entitled “**MANAGEMENT OF WILT AND ROOT ROTS OF COTTON WITH SPECIAL REFERENCE TO BIO-FUNGICIDES**” submitted by **Mr. S.N. CHATTANAVAR** for the degree of **DOCTOR OF PHILOSOPHY** in **PLANT PATHOLOGY** to the University of Agricultural Sciences, Dharwad, is a record of research work carried out by him during the period of his study in this university, under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

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27 DECEMBER, 2002

  
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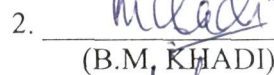
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27 December 2002



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# *Introduction*

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## INTRODUCTION

Cotton plays an important role in the economy of India. Besides meeting the total requirement of the country, it is exported as raw cotton, yarn and fabrics etc., earning a sizeable amount of foreign exchange that constitutes one third of the total foreign exchange earned through exports. The pests and diseases are the greatest enemies of cotton and account for huge losses varying from 30-70 per cent. Among these losses diseases have their own share. With the increasing commercial value of the crop, the limitations imposed by land use patterns, and the rising cost of seed and other inputs including labour wages, it is imperative that the maximum possible yield is realised from the crop and preventable losses through diseases to the minimum. Thus arises the need for understanding the nature of diseases, the losses caused by each of them and ways to control them.

It is essential to have precise statistics of potential and actual production as well as the loss in production attributable to different factors, especially diseases. The Cotton Diseases Council in the United States of America has designed techniques of estimating losses and prescribed guidelines for assessing yield reduction due to important diseases. Watkins (1981) gives figures of annual production in terms of number of bales of lint from 1953 to 1977. Production in the U.S.A. has varied from 7.60 to 16.40 million bales and diseases have taken a toll of 10.50 to 20.40 per cent of it. Thus losses, in terms of lint, averaged 1.90 million bales. Boll rot accounted for losses ranging from 0.55 per cent in 1953 to 5.10 per cent in 1970, seedling diseases 1.48 per cent in 1977 to 40.30 per cent in 1966, Verticillium wilt 1.01 per cent in 1954 to 4.40 per cent in 1967, Fusarium wilt 0.40 per cent in 1976 to 1.36 per cent in

1953, *Phymatotrichum* root rot 0.22 per cent in 1974 to 2.12 per cent in 1960 and bacterial blight from 0.38 per cent in 1974 to 3.42 per cent in 1958. In India, for understandable reasons, loss assessments have not been attempted on a national scale nor have any good techniques of assessment been developed to record the loss due to each major disease. However, a meager loss of one per cent due to disease will amount to a loss of 1.89 lakh bales of lint, keeping in view the total national production figure of 189 lakh bales.

Fungal, bacterial, viral and nematode pathogens affect the cotton crop. They affect all plant parts including foliage, bolls, stems and the root systems. Among the foliar diseases, bacterial blight is prevalent throughout the country. *Alternaria* blight has become endemic in Karnataka and appears every year in severe form affecting all the above ground parts of plant. Incidence of grey mildew is widespread almost every year on herbaceum and hirsutum varieties and hybrids. *Verticillium* wilt has been minimized with the cultivation of resistant varieties. But *Fusarium* wilt is becoming problem in command areas of Karnataka and the losses are up to 20 per cent and the disease is on increasing proportion. Recently, a new emerging problem, the cotton leaf curl disease has become a serious limitation to cotton cultivation in northern states of Rajasthan, Punjab and Haryana.

Several studies have been made for the management of cotton diseases. Most of the earlier researches and developed strategies employed chemicals for the control of diseases. Several chemicals effective against an individual pathogen are available, which in general can effectively control the specific disease caused by that pathogen. Quite often more than one disease could occur simultaneously as mixed

infection following one after other during the season. In such cases, application of chemical to control individual disease is neither feasible nor economical. A combination of fungicide like copper oxy Chloride and streptomycin sulphate based antibiotic has largely been found useful in the management of bacterial blight, most of the fungal foliar diseases and boll rot. Grey mildew however, needs specific fungicides, viz., Sulphur or Carbendazim for its management. No chemical provides protection from root rot. Seed treatment with Carbendazim is able to reduce incidence only at the seedling stage. The combination of various management components may reduce the incidence to some extent.

Scientists in both public and commercial institutions are investigating suppression of cotton pathogens by antagonists. To date, the search for disease control in cotton has been limited to those associated with soil and the rhizosphere. Control of foliar diseases such as *Alternaria* blight and anthracnose continues to rely on genetic resistance of the cultivar and chemical control strategies. Two fungal antagonists viz., *Trichoderma* and *Gliocladium* spp. and two bacteria viz., *Bacillus* and *Pseudomonas* spp. have been extensively exploited for the biocontrol of root rot and seedling diseases of cotton (Raj and Verma, 1988; Adams, 1990; Stewart, 1991; Jayarajan *et al.* 1994; Monga and Raj, 1996a; Monga and Raj 1996b).

In USA, two commercial preparations of bacteria as cotton seed treatment for control of seedling diseases are currently available. The first, *Bacillus subtilis* A-13, is manufactured by Abbott laboratory and sold as 'Quantum – 4000' by Gustafson Inc., USA for the suppression of *Rhizoctonia*. A new strain of *B.subtilis*, GB-03 was developed and was found to colonize cotton root more efficiently than the parental strain

(Stewart, 1991) In 1988, Ecogen Inc. in USA marketed a second seed treatment against seedling disease of cotton and named as 'Dagger G' – a formulation of *Pseudomonas fluorescens*.

A strain of the fungus *Trichoderma harzianum* (KRL-AG-2) was developed at the Cornell University, Geneva, New York as a bioprotectant against seedling diseases of cotton caused by species of *Pythium* and *Rhizoctonia* (Jin *et al.*, 1990). This improved strain was selected from the progeny that resulted from protoplast fusion of two parental strains. The strain performed better than either of the parents in its biological activity and grew faster than the parental strains at 11 and 25° C. The improved strain was also a competitive rhizosphere colonizer. In field trials, cotton yielded more when the seeds were treated with this bioprotectant than when treated with effective combinations of fungicides (Harman, 1989).

*Pseudomonas fluorescens* strain HV37A was also employed as seed treatment by DNA – Plant Technology Inc. New York, USA for suppression of seedling diseases of cotton, caused by species of *Pythium*, *Rhizoctonia* and *Thielaviopsis*. By developing and using a mutant deficient in antibiotic production, it was found that 60-70 per cent of the bio control was due to the antibiotic produced by the organism. With this knowledge, and by genetically engineering a stronger promoter into a gene critical for antibiotic synthesis, the antibiotic producing ability was increased by over 50 per cent. This resulted in additional suppression of diseases by 10-20 per cent.

Shapira *et al.* (1989) working in the Hebrew University in Israel obtained promising results with chitinase, in the control of *Rhizoctonia solani* on cotton. A chitinase gene was isolated from *Serratia marcescens*

and cloned into *Escherichia coli* with a strong promoter to obtain high expression of the gene. A crude preparation of the enzyme from the transformed *E. coli* reduced disease on cotton by 62 per cent under green house conditions. Similar control was also obtained when viable cells of genetically engineered *E. coli* were applied. These results indicate that the chitinase enzyme may have an important role for biological control of fungi in future.

The inhibitory effect of the microflora on the plant pathogens has been attributed to the production of either siderophores or antibiotics. Siderophores are compounds produced by bacteria and plant that bind iron. The basis of suppression of *Pythium ultimum* causing damping off, by a strain of *P. fluorescens* was due to siderophore production (Loper, 1988). Apparently, the scavenging of available iron in the rhizosphere will suppress some pathogens. Besides, siderophore compound itself may act as an inhibitor of some pathogens (Loper, 1990). Due to existence of large number of organisms producing siderophores and the variety of siderophore classes, this area of biological control appears to be fertile for genetic engineering/improvement of the efficacy of beneficial organisms.

In addition to finding and improving strains for greater effectivity, the research also needs to be strengthened to study the rhizosphere colonizing efficiency of the candidate antagonist. Studies conducted in US showed that the cotton genotypes varied widely with regard to ability of the antagonists for colonization in their rhizosphere (Stewart, 1991). Further the work showed that the multi-adversity resistant (MAR) cottons (Bird, 1982) were notable for supporting colonization by all the bacterial strains tested, viz. *Pseudomonas*, *Bacillus*, *Arthrobacter*. These

observations indicate that the factors affecting greater rhizosphere colonization should be studied and considered while breeding for MAR cottons. Both fungal and bacterial groups were also planned. Shelf life of selected antagonists was planned for enumeration in various effective carriers. Viability of *M. phaseolina* in the form of sclerotial bodies in infected stalks by preserving them under dry conditions at room temperature to understand the required cultural practices in its integrated management was planned.

In a nutshell, chemical control though necessary for many diseases at present, are undesirable and even inadequate as a long-term solution to crop and soil health. During the past several years some significant and successful disease control measures have been achieved through introduction of microorganisms in the laboratory, glasshouse and also fields. However, no single method would be sufficient and durable in disease control. A good disease management programme should incorporate various means together. In this context, investigations were undertaken to search for an effective (biofungicide)antagonist for management of fusarium wilt and root rots of cotton. With above points in view, the following objectives were formulated for the investigations.

1. Isolation and identification of native antagonistic organisms from cotton rhizosphere.
2. Mass production and their storage studies
3. Management of cotton wilt and root rots.

*Review of Literature*

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## REVIEW OF LITERATURE

India is one of the two centres of origin of cotton, the other being Peru. Its cultivation and use for making fabrics have been traced to the Mohen-jo-Daro age. Cotton plays a major role in India's economy, both in terms of providing employment directly and indirectly to about 60 million people and in terms of production of wealth and earning foreign exchange for the country. Losses suffered through diseases by the cotton crop in terms of lint have been estimated to vary between 10.50 and 20.40 per cent in the United States of America (USA) and with conditions of cotton culture prevailing in India the losses are certainly on a greater scale.

The present investigation included different aspects of cotton wilt and root rots caused by *Fusarium solani* (Mart.) Sacc., *Rhizoctonia solani* Kuhn., *Rhizoctonia bataticola* (Taub.) Butler and *Sclerotium rolfsii* Sacc. As the *Macrophomina phaseolina* (Tassi) Goid is a pycnidial stage of *R. bataticola*, the relevant literature reviewed here are partly pertaining to the *M. phaseolina* and more of *Fusarium oxysporum* f.sp. *vasinfectum* Snyder & Atkinson than *F. solani*.

### 2.1 Occurrence and Distribution

#### ***Fusarium* spp. on cotton:**

The genus *Fusarium* was erected by Link in 1809 for species with fusiform, nonseptate spores borne on a stroma and was based on *F. roseum* (Link) Sny. and Hans. (Booth, 1971).

Cotton seedlings are affected by *Fusarium solani*, *F. moniliforme*, *F. oxysporum* f.sp. *vasinfectum* and *F. roseum*.  
*Fusarium solani* (Mart.) Sacc., 1881.

= *Fusarium solani* (Mart.) Appel & Wollenw., 1910

= *Fusarium solani* Mart., 1842

and numerous other synonyms have been proposed by Subramanian (1952). Subramanian (1952) isolated this species from the cotton soils of Udumalpet (Tamil Nadu). It has occasionally been isolated from reddish brown cankers about 5 mm. in diameter that occurs on seedling roots and the hypocotyl of cotton seedlings. In bad cases, rotting and drying up of the seedlings is noticed. Bharathudu and Rao (1982) have reported a foot rot caused by *F. solani* from Andhra Pradesh.

Conidia scattered in false heads, sporodochia are formed in pionnotes, in mass brownish white, loam-yellow, golden yellow, pale brown and coffee-brown to greenish. Stroma are erumpent, sclerotial or leathery, blue, green-flecked, or olive-brown. Microconidia of variable size, dorsiventral, somewhat uniform in width, narrow and curved towards the ends, slightly pedicellate, blunt or rounded at the tip, 3-5-septate, hyaline, thickwalled. 0-septate 5-12.5 x 2-5 $\mu$ m; 3-septate 19-63 X 3.5-7 $\mu$ m; 5-septate 22-77 X 4-8 $\mu$ m. Chlamydospores present, 1-2 celled, in chains, clusters, formed on vegetative hyphae or on conidia, 6-11 to 8-22 X 6-12 $\mu$ m.

The vascular wilt caused by *Fusarium oxysporum* f.sp. *vasinfectum* is worldwide in distribution and occurs in all countries except Australia, West Africa and Turkey. It is important in the Turkmenistan Republic of the former U.S.S.R., as also in Egypt, where it affects *G. barbadense*. In China and Southeastern United States, it mostly affects *G. hirsutum* as it does also in the West Indies. It is known in Southern France, Yugoslavia, the Maceionian parts of Bulgaria and Greece as also in Israel. In recent decades, it has affected *G. hirsutum* in the former Soviet Union. In India,

it affects the oriental species, *Gossipium arboreum* and *Gossipium herbaceum*, grown in the states of Maharashtra, Gujarat. Madhya Pradesh. Punjab. Haryana, Andhra Pradesh, Karnataka and Tamil Nadu.

It has been known since the late 1800's in the United States and became the first Fusarial wilt to be described when Atkinson (1892) observed it in Alabama. It is believed that, the disease must have been prevalent in U.S.A. and India for many years before its discovery. Although there is a School of Thought which believes that the disease originated in Central America, considering the plasticity of the Fusaria. It is more probable that it arose independently in Central America, Egypt and India. This view is based on the picture presented by the distribution of the physiologic races of the pathogen. Indeed, India is believed to be the area of origin of several wilt causing *Fusaria* affecting different crops, for example, banana (Basu, 1911), lentil (Vasudeva and Srinivasan, 1952), chick pea and pigeon pea (Butler, 1910), in addition to several others. Snyder and Smith (1981) remarked that, the presence of a significant amount of Fusarial wilt in any area reflected the growing of the crop for a long time under intensive monoculture.

In India, the disease was first observed at Nagpur by Evans (1908). While, the disease is reported to cause severe losses in light, sandy and acidic soils in the U.S.A., it causes severe damage in the heavy black cotton soils of India, where the soil reaction is on the alkaline side. This difference is associated with the fact that in the U.S.A. and other countries, the severity of the disease is associated with a combination of the pathogen and nematodes.

## 2.1 Crop Losses:

The loss in terms of production potential attributable to *Fusarium* wilt in the U.S.A. has decreased from 1.36 per cent in 1953 0.53 percent in 1977 amounting to 72, 365 bales (Watkins, 1981). The loss in 1981 was 92,704 bales, (0.60 per cent) (Berggren, 1982). The combined loss from *Fusarium* wilt and nematode infestation in the U.S.A. used to be as high as 75 to 90 per cent in individual farms during the earlier years. This picture altered with the introduction of wilt-resistant cultivars and losses did not exceed 3-4 per cent (Smith, 1953). In India, losses used to be of the order of 5-47 per cent in Madhya Pradesh (Ajrekar and Bal, 1921) and 40-60 per cent in Khandesh and Karnataka (the present northern Karnataka) (Kulkarni and Mundkur, 1928) and 5 per cent of the total crop in the then presidency of Bombay (Kulkarni, 1934). Cultivars like *Maljari* (*G.arboreum*) have suffered loss of 25-40 per cent in Madhya Pradesh and the *Karunganni* cotton (*G. arboreum*) grown in the Palladam area of Tamil Nadu about 10-15 per cent. Production losses due to the disease have tended to decrease with the introduction into cultivation of resistant cultivars and selections on the one hand and with the replacement of oriental species with American cottons on the other. *Fusarium* wilt continues to be a problem in India in areas where the Asiatic species continue to be preferred as in northern Karnataka and also Gujarat.

The presence of nematodes, especially the root-knot nematode, *Meloidogyne incognita*, the sting nematode (*Belonolaimus gracilis*) and also *Pratyenchus brachyurus* predispose to attack even *Fusarium*-resistant varieties in the United States (Powell, 1971). However, such

destructive nematode – *Fusarium* associations have not been observed in India.

### 2.1.2 Symptoms:

Symptoms may appear at any stage of the crop growth. In the early seedling stage, death may be sudden and may be mistaken to be due to damping-off. Cotyledons may turn chlorotic, then yellow and brown, and may drop. Even the true leaves of young plants may show similar changes and drop off. At this stage, vascular browning may be observed but there is no discoloration or necrosis of the roots as in other root diseases like damping-off or root rot.

In older plants, while the disease may occur at any stage, it is prominent at square formation and flowering. The margins of the leaves shrink and may turn chlorotic and brown, followed by epinasty and they may ultimately be shed. The lowest leaves are the first to show symptoms, followed by epinasty and they may ultimately be shed. The entire plant or only one side may be affected. Often the bark of the stem turns dark brown either uniformly or on one side from the base upwards. Where only one side is affected, the leaves and branches on that side show signs of wilting, the other side remaining normal. Vascular browning can be traced continuously from the main stem into the branches and even into the leaves. The brown discoloration is confined to the vascular elements and does not spread to the intervascular tissue or cortex. While there is a marked discoloration of the bark and stem in young plants, the discoloration is seen only on peeling the bark in older plants. Microscopic examination of sections of the roots and stems show fungal hyphae inside the xylem, particularly in the lower part of the plant.

Foliar symptoms may appear even without the fungal hyphae being detected in the vascular tissue of the leaf. In badly affected fields, the plants may be heavily defoliated exposing bare stems. Many plants die and dry up. Under Indian conditions, it is common to see plants with most or all the leaves dried up and hanging by the petiole, but do not shed.

When the disease affects the plants early, there may be severe stunting of the main stem though there may be normal growth of some of the lower branches. There is a reduction in boll size, ginning percentage, fibre length, maturity and strength. Partial recovery of some wilted plants through the development of new short branches at the base has been noticed.

### **2.1.3 Causal Organism:**

Atkinson (1892) named the pathogen *Fusarium vasinfectum*. Smith (1898) thought that the perfect stage of this organism was *Neocosmospora vasinfecta* E.F. Smith., the type species of a new genus of the Hypocreales (Ascomycetes) erected by him. Following this Evans (1908) who identified cotton wilt at Nagpur attributed it to *N.vasinfecta*. However, Butler (1910) showed that *N.vasinfecta* was not the etiological agent of the wilt nor was it connected with *F.vasinfectum* as its perfect state. The misconception regarding the anamorph-teleomorph relationship between the two fungi was perhaps natural as the salmon-pink perithecia of *N.vasinfecta* can often be seen on the dead bark at the base of the stems of cotton plants affected with fusarial wilt. This ascomycetous fungus appears to be a favoured member of the saprophytic succession in the colonisation of the dead bark tissues of wilt-affected cotton plants. Butler

(1914) thought that cotton wilt was caused by a different species of *Fusarium*. Dastur (1924, 1929) put forth the theory that the disease was not caused by a pathogenic *Fusarium* but by a physiological factor the accumulation of iron and aluminium salts in the tissues. This view was refuted by Ajrekar (1926) and Bal (1926) who concurred with Butler's earlier view of a species of *Fusarium* being the incitant. Butler (1926) himself revised his opinion regarding the identity of the pathogen and thought that the disease was caused by a strain of *F.vasinflectum*. Kulkarni (1934) established that *F.vasinflectum* was the pathogen involved and that for successful production of the disease, the inoculum had to be in contact with the roots and that disease was most severe between temperature of 20° and 27° C.

The various species under the genus, *Fusarium* were organized into different sections by Appel and Wollen-Weber (1910) and finally in the classic work "*Die Fusariem*" by Wollenweber and Reinking (1935). *F.vasinflectum* (with its two *formae speciales*) was included in section *elegans*.

Thus, all *elegans* Fusaria came under

*Fusarium oxysporum* Schlecht., Flora berol, 2:139, 1824 emend. Snyder and Hansen *pro parte*, *Am. J. Bot.* 27:64-67, 1940.

*Fusarium arysporum* Schl. f.sp. *vasinflectum* (Atk.) Snyder and Hansen *Am. J. Bot.* 27:66.1940.

The description of the cultural characters given below is adapted mainly from Booth (1971).

Cultures pale, salmon, rosy buff vinaceous, violet to pale slate on media of pH 6.5-7; mycelium striate, felted to floccose; microconidia always present, unicellular or bicellular, ellipsoidal or allantoid and borne on lateral phialides, or on phialides produced from short lateral conidiophores; macroconidia falcate and of the *elegans* type, 3-5 septate when mature and are initially formed from branched lateral phialides but later formed from sporodochia. Chlamydo spores intercalary or terminal on short lateral branches, solitary or in chains, hyaline, smooth to rough-walled. Stromatic pustules occasionally develop resembling perithecia of *Gibberella* but no asci or ascospores have been reported.

In cultures, the spores are often formed on the surface in slimy layers called "pionnotes" which overlie minute sporodochia without a stroma. Microconidia measure 5-12 x 22-3.5  $\mu$ . Macroconidia 3-septate 27-46 X 3-5 $\mu$ , 5-septate 35-60 X 3-5 $\mu$ , 6-7 septate 50-66 X 3.5-5 $\mu$ , 3-septate conidia are the most common.

Host range: Besides cotton, the pathogen infects species of *Cajanus*, *Coffea*, *Hevea*, *Hibiscus*, *Medicago*, *Ricinus*, *Solanum* and *Vigna*. Besides, the fungus invades without producing symptoms, the rhizosphere of *Eleusine coracana*, *Pennisetum typhoides*, *Sorghum vulgare* and *Phaseolus mungo*, which are rotational crops and thus can act as carrier hosts (Charudattan and Kalyanasundaram, 1996).

The fungus has relatively poor competitive saprophytic ability in the soil (Subramanian, 1950). It does not grow through the soil which explains its failure to infect plants whose roots do not come in contact with the resting structures laying in the soil. It can preemptively colonise

living cotton stem bits buried in the soil and survive on debris of infected cotton plants as chlamydospores and sclerotia-like resting structures.

#### 2.1.4 Control :

Seed treatment has little advantage in the control of cotton *Fusarium* wilt. Hot water treatment is ruled out as temperatures are lethal to the seed-borne pathogen also damage the embryo. However, seed-borne infections are not frequent and not a significant means of dissemination of the pathogen. Asiatic cottons which alone are liable to attack by the disease in India are mainly rain-grown and raised under minimal management and inputs and hence there is little scope for adjusting the soil moisture or altering the reaction of the soil or its texture. Even deep ploughing to expose the propagules to the lethal action of the summer sun is unlikely to be effective as cotton roots penetrate deep into the soil down to 50 cm or more. In Israel, soil solarization has been found to effectively reduce the pathogen population in the soil with decreased incidence of wilt and improved plant growth. Increased yields were obtained for three years after the treatment (Katan *et al.*, 1983). Crop rotations are not effective as the pathogen persists in the soil for a number of years. The vegetable okra (*Abelmoschus esculentus*) is a collateral host and should be excluded from the rotation. Crops like barley, *ragi* (Finger millet, *Eleusine coracana*) and sorghum (*Sorghum vulgare*) enable the pathogen to multiply as the latter invades their rhizosphere and thrives as a saprophyte without causing any injury to these crops which then act as carriers.

Potassium fertilizers improve the resistance of the plants. Nitrogen and phosphorus are likely to increase the severity of the disease, unless

balanced with appropriate potash applications. A judicious combination of fertilizers needs to be worked out for each area. Micronutrients like zinc can mitigate the incidence and severity of the disease.

Resistant varieties are the most effective and economical means of control. Using infested soils held in soil temperature control tanks at favourable temperatures (24° - 28° C), as also by growing plants in wilt-sick plots, the Pune centre of the All India Coordinated Cotton Improvement Project has been able to select wilt resistant individuals from susceptible cultivars and from segregating populations of progenies of crosses involving resistant parents (Desai, 1958; Hande and Rane 1983). Similar work has also been undertaken at the Ludhiana centre in the Punjab (Sharma *et al.*, 1984). At Pune, it was found that resistance was better in *G. herbaceum* cultivars than in those of *G. arboreum* tested.

With the replacement of indigenous cotton with American cotton, the disease has ceased to be a problem of any significance in South India but Fusarium wilt continues to cause losses in Gujarat, Maharashtra and northern Karnataka where Asiatic cotton continue to be grown. With the development of resistant selections from old varieties and of new ones through hybridization, losses through the disease are well on the way to being reduced to the minimum.

Ibragimov *et al.* (1966) evaluated Dipropyl fumacide and fungifos and reported their effectiveness against chickpea wilt. They also observed that, seed treatment with Phenthiuram molybdate stimulated the growth of chickpea plants and resulted into increased seed yield. Khare *et al.* (1973) reported Benlate and Thiram to be better seed dressers in reducing chickpea wilt incidence and increasing seed yield without any

harmful effect on nodulation. Verma (1976) treated the chickpea seeds with Bavistin, Vitavax @ 0.25 per cent and reported better protection. Verma and Vyas (1977) found benomyl, carboxin, thiabendazole and carbendazim as the superior seed dressers against chickpea wilt. Haware *et al.* (1978) eradicated seed borne *F.oxysporum* f.sp.*ciceri* by treating the seeds with Benlate – T @ 0.15 per cent. Shukla *et al.* (1981) observed improved chickpea seed germination by 16.5 per cent with Bavistin as seed dresser @ 0.5 g/kg of seed and reduced wilt incidence. They found Bavistin + Thiram as best seed dresser. Benlate and Carbofuran gave the highest seedling emergence i.e., 68.82 and 63.58 per cent, respectively in the presence of *F.oxysporum* f.sp. *ciceri*, *F.solani* and *M.incognita* as compared to 46.20 per cent in control (Mani and Sethi, 1984).

### 2.2.1 ROOT ROT

Root rot of cotton is world-wide in occurrence and has been a major cause of loss in many countries. Different fungi as well as nematodes cause injury to the roots. Among the fungi, *Phymatotrichopsis omnivora* (Shear) Henneb. causes a serious root rot of cotton, sometimes called ‘the Texas root rot’, in Southern and Southeastern United States and Northern Mexico. Root rot is caused by *Macrophomina phaseolina* in Venezuela, where it is said to be favoured by waterlogged situations. Root rot occurs in several countries including U.S.A., Venezuela, Trinidad, Uganda, Zaire, Egypt, Sudan, Greece, Isreal. Pakistan and India. It is present in the Punjab, Rajasthan, Uttar Pradesh, Bihar. Gujarat, Andhra Pradesh and Tamil Nadu (Butler, 1918; Vasudeva, 1960;). It was noticed as early as 1904 at Dehra Dun, at Kanpur in 1911 and in Andhra Pradesh in 1913. It is prevalent in the

sandy soils, alluvial soils as also in the heavy clays constituting the black cotton soils.

Losses caused by the disease can be severe, but not much information is available on this aspect. Vasudeva (1942) reported a loss of about three per cent in the Punjab under normal conditions. In extreme cases, it could be as high as 90 per cent. Both American and Indian cottons are susceptible, but the former suffers greater losses than the latter. "Rozi" cotton (*G. arboreum* race *inaicum*) is reported to have a high degree of field resistance.

### **2.2.2 Symptoms:**

The disease occurs in circular patches, which enlarge year after year. It appears either in the seedling stage or after wood formation. In young seedlings when the stem is still soft, a yellow patch appears on the lower part of the stem and if the attack is severe the seedling may collapse. In older seedlings which have developed some wood, the patch becomes blackened at ground level and the seedlings dry up and wither. According to Butler (1918), some plants are able to survive the attack but a dry brown patch may persist on the stem. However, the attack is usually fatal, although rarely some plants may recover during cool, wet weather or following irrigation. Affected plants are easily pulled out of the ground. With the exception of the tap root and a few secondary roots, the others are all decayed. The tips of the roots are moist, discoloured yellow and sticky with soil adhering to them. The bark is shredded on the roots and basal part of the stem. The stele turns yellow or brown or in severe cases black. Black, dot-like sclerotia may be seen on the wood beneath the bark and between the shredded strands of the bark on the

subterranean parts of the plant. These symptoms are common in Gujarat and elsewhere in India, but rare in the Punjab.

### 2.2.3 Causal organism:

The etiological agent has been identified as *Rhizoctonia bataticola* in Gujarat by Likhite and Kulkarni (1934). Vasudeva has done considerable work on the disease in the Punjab and published a series of papers on various aspects of the disease during the thirties and forties. According to the earlier views held by him (Vasudeva, 1935, 1960), the disease was caused by two fungi in the Punjab, *Rhizoctonia solani* and *R. bataticola*. Vasudeva (1963) in a later publication mentions only *Macrophomina phaseoli* (*R. bataticola*,) as the cause of root rot which was “one of the serious diseases of cotton in the irrigated areas of the Punjab and the sandy, alluvial soils of Gujarat...” In the rest of the country, in Madhya Pradesh, Andhra Pradesh and in Tamil Nadu too, *R. bataticola* is the cause of root rot. *R. solani* is involved in causing seed decay and preemergence and postemergence damping-off of seedlings, as also in causing sore shin along with *R. bataticola*. In fact, the ‘root rot’ attributed to *R. solani* is considered to be not different from sore shin (Hunter *et al.*, 1960).

*Rhizoctonia bataticola* (Taub.) Butler 1925

= *Sclerotium bataticola* Taub. 1913

The hyphae are found in the rotted area, at first in the cortex, later, in bad cases, extending in as far as the pith. Hyphae colourless when young, abundantly branched and the branches arising almost at a right angle from the parent hypha, then often bending to lie nearly parallel to it. The branch is usually constricted at the base and a cross wall usually

arises about 15 $\mu$  away. The diameter of the main hyphae is 8-9 $\mu$  and the cells are 50-150 $\mu$  long; older hyphae are brown, composed of short, barrel-shaped cells. Sclerotia appear when the tissues dry up. They are jet black, minute, smooth externally, composed of anastomosed black hyphae; interior light or dark brown composed of free thick-walled cells. Sclerotia variable in shape, globose, oval, oblong, elliptical, curved or even forked, varying in size from 25 x 22 to 152 x 32 $\mu$ , abundant on host tissue and in culture (Butler, 1918; Subramanian, 1971).

The pycnidial stage was first encountered on bean. It has been observed on cotton at Pune in India and at Lyallpur in Pakistan. But it is not common in occurrence. The pycnidial stage is called *Macrophomina phaseolina*.

*Macrophomina phaseolina* (Tassi) Goid.

Syn. *Macrophomina phaseoli* (Maubl.) Ashby

*Macrophoma phaseoli* Maubl.

*Macrophomina phaseolina* Tassi

Goidanich found that the name given by Tassi was the earliest and suggested the combination *Macrophomina phaseolina* (Tassi) Goid.

Pycnidia separate, with a single ostiole, wall thin, with a pigmented surface, sometimes with a sclerotoid base. Conidiogenous cells undifferentiated from inner cell walls; conidium formation phialidic; ostiole truncate; spores 1-celled, hyaline, somewhat oval, elongate-cylindrical, 16-30 x 5-10 $\mu$ .

The fungus is common in the tropics and infects the roots of a variety of crops and causes damping-off in seedlings and root rot in older

plants. It is polyphagous and its hosts include a number of legumes, cotton, jute, potatoes, sweet potato etc., on these it causes charcoal rot. In fact, the disease occurring on the older cotton plants is often referred to as charcoal rot. Besides cotton, others affected include viz., castor, maize, cowpea, eggplant, peanut, sesame, sorghum, soybean, tobacco and tomato (Watkins, 1981).

#### 2.2.4 Control:

An abundance of resistance cannot be expected against a polyphagous pathogen like *R.bataticola*. However, six varieties with low percentage of disease were reported by Raju *et al.*(1981). Tiwari and Shroff (1982) also reported three resistant lines viz., KH-33-146, 15-KW-2 (MB) and 9-KW-2 (MB).

The greater resistance to *R.bataticola* in older seedlings than in younger ones has been attributed to higher contents of gossypol and phenols and low levels of sugars (Ramasamy and Shanmugam, 1977). Hunter *et al.* (1978) observed higher concentrations of the terpenoids, desoxyhemigossypol, desoxy-6-methoxy-hemigossypol, hemigossypol, 6-methoxy-hemigossypol and 6,6-dimethoxyhemigossypol in the healthy hypocotyls of 12-day-old seedlings than in 5-day-old seedlings. However, increase in their levels following infection was not significantly different. It was suggested that native terpenoids, rather than the induced increase in their content in response to infection, were involved in age-related resistance. Resistance was lowered by deficiencies of potassium, sulphur and phosphorus (Ramasamy and Shanmugam, 1976).

The disease is said to be controlled by the application of PCNB containing preparations like Brassicol to the soil (Mathur *et al.* 1971). Other successful soil applications reported are Brassicoll + Captan (Sharma and Sharma, 1976) and Bavistin slurry or powder seed dressing (Narayanaswamy *et al.*, 1979). Lowering the soil temperature by intercropping with moth (*Vigna aconitifolia*) was very effective in controlling the disease in the Punjab (Vasudeva, 1941). Sowing in April or June instead of May also reduced disease incidence in the Punjab (Vasudeva, 1960). Heavy applications of farmyard manure and cellulose containing composts, which increased microbial activity, reduced the occurrence of the disease. Pelleting the seeds with spores of *Trichoderma viride* and *T.harzianum* was an effective means of control. *T.harzianum* established itself in the soil and the protective effect was longer lasting while the effect of *T.viride* was only transient.

### **2.3.1 Dry rot or Sore shin :**

Sore shin also goes by the name of Dry rot. Sore shin is considered to be the most serious disease of cotton seedlings in the United States. Alternate light and brown bands observed on cotton were called 'Sore shin' by southern planters (Atkinson, 1892) and the name was extended to the stem-girdling disease. It causes heavy loss of seedlings in Egypt, but is unimportant in the Sudan. It is prevalent also in Morocco. In India it was reported from the former state of C.P. and Berar by Dastur (1931) and from Hyderabad by Sawhney and Narayanayya (1932). It is also prevalent in the Khandesh region of Maharashtra (Uppal, 1948). It has been observed commonly in Tamil Nadu in recent decades. It is a disease causing severe damage to seedlings and young plants often less than 12 weeks in age. Older plants have also been observed to be attacked.

Losses caused by *Rhizoctonia solani* alone in California during 1964 were estimated at one half of the collective loss (Anon., 1965).

### 2.3.2 Symptoms:

It is essentially a disease affecting the collar, roots and leaves. It is distinguished from damping-off by the affected young plants not falling over but standing erect. The diseased lesions are not water-soaked. The plants are killed when the collar is girdled and the roots are also damaged. The seedlings are easily pulled out without the taproot coming apart. The taproot in young seedlings at the point where it is affected is thinner than the healthy roots. Lesions occur in the collar near soil level, pale brown or reddish brown at first, turning dark brown. When *Rhizoctonia bataticola* is the pathogen involved, sclerotia may be seen in abundance imparting a smoky or black colour. But the lesions remain dark brown when they result from the attack of *R.solani*. Large brown sclerotia and dirty-white mycelial strands may be seen. The cortical tissues are invaded, the size and depth of the resulting cavity depending on environmental conditions. The bark at the basal part of the stem and upper part of the root is shredded in severely attacked plants. The wood is discoloured brown or black. The upper portion of the taproot is also discoloured but without shrinking. Preemergence infections affect germination of the seed.

The cotyledons, when lightly affected, look thin and pinched but in more severe manifestations they shrink and hang down. In such cases, infection remains restricted to the leaves and does not spread to the stem. The affected leaves may be shed later and the plant may develop new shoots from the buds. Infection may spread from leaf to leaf by contact

especially during the rains when the diseased and healthy leaves may stick together on account of the film of water. Spiders have been observed to transmit the pathogen from leaf to leaf.

Although, mature plants are not affected as a rule, three-to four-month-old plants may be affected, with only the leaves showing symptoms. In waterlogged situations, mature plants are frequently attacked.

### **2.3.3 Etiology:**

The pathogen attacks the collar and apical parts of the root, often following heavy rains. In the process of drying after the rains, the soil may form a hard crust pressing against the plant and cracking the bark. Some varieties like Dadiotico in Greece have a thin bark on the roots which cracks easily and permits entry. The pathogen is believed to enter through such injuries. *R.solani* is also capable of parasitizing the plants from infection cushions (Prillieux, 1891) pressing against the epidermal layers in the hypocotyl region (Khadga *et al.*, 1963). Wounds permitting entry may also be caused by insects and agricultural implements. The intensity of the infection is high, when the hyphae are thin and hyaline. Factors causing high densities of mycelium as also substances released by *R.solani* itself may inhibit infection (Sanford, 1941).

Infection of seedlings takes place only under a limited range of soil conditions. Any delay in emergence of the seedlings from the soil favours the pathogen. Attack by the pathogen is made easy when seedling emergence is delayed either by the poor quality of the seed itself or through the formation of a hard crust on the surface soil due to rapid

drying out after rains or by unfavourable temperature. Temperature plays an important role on the growth of the seedling as well as that of the pathogen. Cotton grows and can form defensive cork at an optimum temperature of 33° C and 37° C (Vasudeva and Ashraf, 1939). Thus, the date of sowing which determines soil temperature also influences the incidence of the disease. Disease occurs when the temperature and soil moisture conditions are favourable for the pathogen but unfavourable for the host (Neal, 1953). However, there are conflicting reports about sore shin being a high temperature disease (Vasudeva and Ashraf, 1939) or a low temperature disease. Hunter *et al.*, (1960) found this to be due to the occurrence of at least three strains of *R.solani* with optima at 24°, 27.5° and 32° C. Vasudeva (1960) stated that the disease is caused by *R.bataticola* in India and by *R.solani* in other countries. Later observations particularly in South India have shown that although *R.bataticola* is often involved in the disease, *R.solani* is more frequently encountered as the etiological agent. There appears to be a correlation between the intensity of the use of plant protection chemicals both against insect-pests and diseases in India and the upsurge of sore shin caused by *R.solani*. Areas which had only stray incidence in the early seventies had 5 to 6 per cent of the plants affected by the disease within a few years during which farmers resorted to heavy use of plant protection chemicals. In certain fields, the figures went up to 30-35 percent. This increased damage has followed the heavy and indiscriminate use of chemical sprays, as also their direct applications of plant protection chemicals. In arable soils not under cotton, the bacterial numbers were 6-8 million per gramme of soil and fungi 400,000 to 900,000. In cotton soils with comparable organic matter content, in the patches with high incidence of *R.solani*, the figures had fallen to 300,000 to 400,000 bacterial and 6,000 to 28,000 fungal numbers.

### 2.3.4 Causal organisms:

1) *Rhizoctonia bataticola* (Taub.) Butler. See description under 'Root rot'.

2) *Thanatephorus cucumeris* (Frank) Donk

This pathogen is usually encountered in its mycelial form (*R.solani*), but the basidiomycetous state has been encountered on cotton.

*Thanatephorus cucumeris* (Frank) Donk 1956

= *Hypochnus cucumeris* Frank 1883

= *Corticium solani* (Prill. & Delacr.) Bourd. & Galzin 1911.

= *Hypochnus solani* Prill. & Delacr. 1891

= *Pellicularia filamentosa* (Pat.) Rogers 1943

= *Hypochnus filamentosus* Pat. 1891

= *Ceratobasidium filamentosum* (Pat.) Olive 1957.

**Anamorph (mycelial form) of *Rhizoctonia solani* Kuhn 1858.**

Colonies fast growing, at first colourless, submerged or with some radiating aerial hyphae, rapidly becoming brown. Hyaline to pale brown, thick-walled cells about 100-220 x 7-12 $\mu$  often constricted near septa and where branching, with conspicuous dolipore septa, lacking clamp-connexions, branched at wide angles (nearly 90°) often anastomosing. Monilioid hyphae upto 30 $\mu$  wide commonly present. Hyphal cells contain 2-18 nuclei. Sclerotia developing from irregular agglomerations of uniform moniliform hyphae, irregular in outline, solitary, about 1 mm in diameter, sometimes confluent to form conspicuous crusts, soon turning brown.

Basidial hymenia appearing on stems or leaves just above the soil or on soil particles. Fructifications resupinate, creamy, effuse, loosely attached to the substratum. Basidia arising in asymmetrical cymes or

racemes from tufts of ascending hyphae, barrel-shaped to cylindrical, 10-25 x 6-12 $\mu$ , with (2-)4(-7) stout, straight sterigmata as long or longer than the metabasidia. Basidiospores oblong to broadly ellipsoidal, unilaterally flattened, apiculate at base, hyaline, smooth-walled, 6-14 x 4-8 $\mu$ , germinating by repetition with secondary spores (Domsch *et al.*, 1980).

### **2.3.5 Control:**

Control of sore shin involves (1) modification of cultural practices, (2) eradication or suppression, (3) biological control and (4) resistant varieties. Since the disease occurs in well defined patches, it will be useful to mark off infested spots and exercise care while working in them so that infested soil is not carried on implements and on the feet of workers and work animals. Improved drainage will help in maintaining a healthy soil structure. Crop rotations including nonsusceptible crops will keep down the level of inoculum present in the soil. Optimal temperatures for disease development appear to vary from a high of 33°C in the Punjab (Vasudeva, 1960) to a low of 27°-28°C in the winter cotton areas of South India. In the U.S.A. and Egypt, delayed sowing helps to warm up the soil and permit quick germination and growth of seedlings reducing the duration of the susceptible stage. Too early sowing in anticipation of rains will also be harmful as the partial availability of moisture may stimulate the germination process but not be sufficient to support rapid growth. Proper preparation of seedbeds is essential. Addition of fertilizers to enable vigorous early growth will be of help. Shallow sowing will reduce preemergence killings, as the seedlings will emerge quickly from the infested surface soil.

Acid-delinting of seed and application of chemical seed dressings are designed to eliminate the initial contamination of the seed. The chemicals will also protect the germinating seed in the infested soil environment. Among chemical applications, PCNB is specific against *R.solani* and can be applied as a seed dressing at 25 g per kg of seed or as a spot application to infested patches of the field soil at 25 kg per hectare mixed with the top layers of the soil. Seed dressing is relatively inexpensive. Caution has to be exercised in the application of PCNB to the soil for the control of *R.solani*. This can result in the outbreak of *Verticillium* wilt of cotton in infested fields of the winter cotton areas (Srinivasan and Kannan, 1975). This is an example of 'disease trading' or the 'boomrang phenomenon' (Kreutzer, 1960).

The pathogens are sensitive to antagonistic attacks. Antagonists include species of *Trichoderma*, *Talaromyces* (= *Penicillium*), *Streptomyces* and various bacteria. Pelleting the seeds with the spores of *Trichoderma viride* or *T.harzianum* has given good results, although adding cultures of these fungi, particularly *T.viride*, to the soil has not always met with the same degree of success. Antagonistic activity can be encouraged by the application of bulky organic manures and composts containing cellulosic materials, including straw. Addition of straw supplemented with ammonium nitrate and chitin has given good results. Rice bran and oak sawdust amendments have also been proved useful (Domsch *et al.*, 1980).

There is a paucity of resistance in the breeding stocks to these polyphagous pathogens. However, among the cultivars, which have shown promise, is CRH-71 (released as cotton cultivar MCU-10) evolved

at the Tamil Nadu Agricultural University. This cultivar combines in itself resistance to bacterial blight also.

#### **2.4.1 Collar Rot or *Sclerotium* Rot:**

The disease, also known sometimes as *Sclerotium* stem and root rot is soilborne and occurs in tropical and subtropical regions like the southern parts of the U.S.A., the island of St. Vincent in the Caribbean, Peru in South America, El Salvador in Central America, New South Wales in Australia, and in India. It has been reported from Maharashtra and Madhya Pradesh (Vasudeva, 1960). It has been observed near Tenali in Andhra Pradesh and Coimbatore in Tamil Nadu (Srinivasan, unpublished). It is primarily a disease of seedlings and young plants, but may also affect older plants as it does in Arizona in the U.S.A. Even bolls 1.5 to 2.0 feet (45-60 cm) above the ground are affected as reported by Lyle from Alabama (Watkins, 1981).

It occurs mostly in sandy soils or sandy loams of neutral or slightly acid reaction in scattered spots, which gradually enlarge into circular patches.

#### **2.4.2 Symptoms:**

Plants are attacked at or near the soil surface causing a typical girdling of the stem at soil level, which is followed by decay of the roots and wilting of the plants. A white mass of mycelium with numerous spherical, black sclerotia of the size of mustard seeds is seen at the base of the stem and top of the roots. Young plants are killed quickly but on

older plants the leaves turn yellow and then wilt. Affected plants rarely topple over and generally stand erect.

### **2.4.3 Etiology:**

The pathogen attacks the plant from the surface layers of the soil and is favoured by the presence of undecomposed or partially decomposed organic debris lying on the surface on which the mycelium grows and multiplies. When soil moisture is abundant, the mycelium attaches itself to the collar region, which is in contact with the soil. When the soil starts drying out, the mycelium appears to become parasitically active and the bark in the collar region is penetrated and infection takes place. The mycelium secretes considerable amounts of acid, which is toxic to the host cells and hydrolytic enzymes like polygalacturonases and cellulases, which break down the host-cell walls and macerate the tissues, and a canker develops. The collar is rapidly engirdled leading to further invasion of the apical portions of the root. In continued shade and persistent dampness, the stem is also affected for 2-3 cm above the ground level. The mycelium grows out of the plant as a cottony growth and produces sclerotia in concentric rings on the surrounding damp soil for distances upto 7.5-10 cm. Old sclerotia become dry and wrinkled, with which is associated enhanced rapidity and vigour in germination when moistened. Often, after a prolonged period of heat and drought, the occurrence of rains is followed by the incidence of severe wilting and girdling of the plant. Aycock (1966) has given a detailed account of the diseases caused by and the status of Rolf's fungus after 70 years.

### **2.4.4 Causal organism:**

*Sclerotium rolfsii* Sacc. 1911

Saccardo isolated the organism from infected material received from Florida. He described it as a nonsporulating soilborne fungus with hyaline mycelium conspicuous chiefly for its tendency to produce light tan to brown sclerotia in abundance.

Mycelium very floccose, not ropy, producing numerous sclerotia which are pinkish buff, olive-brown to clove-brown, globose, 0.8-2.5 mm in diameter.

The basidial stage is occasionally encountered.

*Corticium rolfsii* (Sacc.) Curzi 1931

= *Pellicularia rolfsii* (Sacc.) West.

Hymenium areolate, putty coloured 30-40 $\mu$  thick; basidia obovoid, 7-9 x 4-5 $\mu$ , each bearing 2 or 4 parallel or divergent sterigmata 2.5-4 (-6)  $\mu$  long. Basidiospores elliptical to obovate, hyaline, smooth, rounded above, rounded or apiculate at base, 6-7 x 3-5.5 $\mu$  (Subramanian, 1971). According to Mundkur (1934) who got the perfect stage on onion, asparagine and peptone agar, basidia are produced in crusts 6-12 mm in diameter, on white cushions of dense mycelium. Basidia clavate, hyaline, septate at the base and producing 2, 3 or 4 sterigmata. Most basidia are sterile. Basidiospores obovate, globose or slightly cylindrical 4.9-9.4 $\mu$  (in length).

The pathogen attacks numerous hosts, particularly cultivated crops and Indian records include besides cotton, *Solanum tuberosum*. *Arachis hypogea*, *Piper betle*. *Amorphophallus companulatus*, *Delphirium*, *Dianthus*, *Medicago sativa*, *Eleusine coracana*, *Sesbania grandiflora*.

*Cajanus cajan*, *Solanum melongena*, *Cyamopsis tetragonoloba*, *C.psoraloides*, *Zingiber officinale*, *Corchorus capsularis*, *C.olitorius*, *Capsicum annuum*, *Citrus marima*, *Saccherum officinarum*, and *Ipomoea batatas*.

There is a view, that the mycelial fungus now known as *Sclerotium rolfsii* may be a collection of geographic races differing in the characters of the perfect stage as also in the size of the sclerotia. Taubenhaus (1919) observed that the pathogen probably had no distinct physiologic races. Epps *et al.* (1951) reported finding four cultural variants and some minor differences in the degree of aggressiveness among the strains.

#### **2.4.5 Control:**

Application of ammonium sulphate at 4-5 cwt per acre (500-625 kg/ha) reduced the incidence of the disease (Magee, 1947). In some countries, rotation with wheat and barley in the winter and autumn months has been reported to reduce the carryover of the disease (Vasudeva, 1960).

#### **2.5 Organic amendments**

Organic amendments have a multicorner impact upon soil borne pathogens. Chauhan (1963) reported significant reduction in the incidence of *F.orthoceras* var.*ciceri* in soil amended with groundnut, sesamum or mustard cake. Buxton *et al.* (1965), under field conditions, observed that, chitin applied to soil naturally infested with *F.oxysporum* f.sp.*pisi* (Linford) Snyd. and Hans. greatly reduced the severity of wilt in garden peas, sown at the time the chitin was applied to the field. Further,

in the glass house, different pea varieties, sown in the field soil eight months after the chitin was applied, were protected equally against infection by any of the three races of the pathogen. The severity of wilt decreased with increasing amounts of chitin added to the soil. Adding chitin to soil decreased the population of *Fusarium* and increased the number of actinomycetes.

Srivastava and Sinha (1971) showed that oilcakes were more effective in reducing the disease in case of coriander wilt caused by *F.oxysporum* f.sp.*corianderii* Kul., Nikam and Joshi. Gurha and Singh (1981) reported groundnut, sesamum and mustard cakes to be effective against chickpea wilt. Among the six organic amendments tested, only chopped cabbage leaves effectively reduced the rate of chlamyospore germination, longevity in soil and disease incidence of radish caused by *F.oxysporum* f.sp.*raphani* (Huang and Hoes., 1976). In the laboratory and pots buried in the field, the severity of cabbage yellow and population of *F.oxysporum* f.sp.*conglutinans* (Wr.) Snyder and Hans. in soils were markedly reduced by amendments of nine cruciferous species viz., cabbage, mustard, cauliflower, broccoli, collard, burssels sprouts, turnip, radish and alfalfa hay (Viilapudua and Munnecke, 1988). Yellow disease of bean caused by *F.oxysporum* f.sp.*phaseoli* was effectively controlled by amendments of coffee hulls and farm yard manure but not by rice husks (Mutitu *et al.*, 1989).

Satischandra *et al.* (1979) observed the detrimental influence of soil amendments with paddy hull and wheat straw for the saprophytic activity of *R.bataticola*. Studies on volatile soil fungistasis showed that sclerotia of *M.phaseolina* were not sensitive to volatiles liberated by natural soils with varying pH value. Soil amended with alfalfa hay (1%

W/W) liberated volatiles fungistatic to sclerotia only when the assay was performed two weeks or less after amendments (Papavizas, 1976). The results of a study on the effect of soil amendments of *R.bataticola* causing dry root rot of chickpea revealed that crop residues of mature wheat and oats considerably reduced the population of pathogen and disease incidence when they were incorporated in the soil (Singh and Nema, 1987).

Chowdhury (1948) reported that mustard cake was very effective against *S.rolfsii*. Alexander (1961) has stated that soil amendments with plant residues increased the population and activities of actinomycetes. Mathur and Sinha (1970) found that the application of farm yard manure reduced the infection of *S.rolfsii* of chickpea. Of the organic amendments studied, groundnut cake was the most effective against saprophytic activity of *S.rolfsii* (Lingaraju, 1977).

Oat straw and castor cake amended soil showed reduction in sunflower wilt caused by *S.rolfsii*. Neem cake was inferior to castor cake (Mukesh – Gautam and Kolte, 1978). Among the six organic amendments tested in pot culture experiment against *S.rolfsii*, the groundnut cake and safflower cake were found superior to other organic amendments both in sterile and unsterile amended soils (Palakshappa *et al.*, 1989).

### **2.6.1 Mechanisms of Biocontrol**

The mechanisms proposed during last ten years or so to explain the biocontrol of plant pathogens by *Trichoderma* or *Gliocladium* are presumptive. Suggested mechanisms for biocontrol are antibiosis, lysis,

competition and mycoparasitism (Hadar *et al.*, 1984; Cook and Baker, 1983).

Several toxic metabolites are produced *in vitro* by *Trichoderma* spp. and there is some evidence that such metabolites are produced in bits of organic matter in soil (Wright, 1956). However, there is no direct evidence to unequivocally implicate such metabolites as a mechanism for biocontrol in soil. For instance, the addressed growth habit of *T.harzianum* around hyphae of *R.solani* (Lewis and Papavizas, 1980) could involve antibiotic production and direct parasitism as well as lysis. It is evident that the intricacies of soil and plant rhizosphere make it a difficult task to discover the precise mechanism.

Recent research has presented less conjectural evidence on the importance of antibiosis on *G.virens* (Howell, 1982). Howell (1982) showed that cotton seed treatment with *G.virens* protected seedlings from damping off caused by *R.solani* or *P.ultimum*. The antagonist parasitized hyphae of *R.solani* *in vitro* and reduced the number of viable sclerotia after three weeks in natural soil.

Following the discovery that *T.lignorum* has greater potential for biocontrol (Weindling, 1941). Many researchers dealing with *Trichoderma* and *Gliocladium* noticed that, hyphae of the natagonists parasitized hyphae of other fungi *in vitro* and brought about several morphological changes (coiling haustoria, disorganization of host cell contents penetration of the host). Durrell (1976), using phase contrast and electron microscopy, produced interesting photographs showing haustoria and hyphae of *T.viride* Pers.ex S.F.Gray within hyphae of phycomycetes followed by digestion of their contents.

Research on mycoparasitism as a mechanism of biocontrol raises two additional questions: is physical contact necessary for host destruction and how important are enzymes and other extracellular compounds in mycoparasitism. Pachenari and Dix (1980) concluded that *G.roseum* need not make intimate contact with *Botrytis allii* to cause severe internal disorganization of host cells, coagulation of cytoplasm, vacuolation and loss of contents from organelles. The capacity to produce cell wall degrading enzyme is common among saprophytic fungi. More than 10 per cent of 160 fungi examined (Chesters and Bull 1963) produced B (1-3) glucanase. In mycoparasitism, the host may first be softened simultaneously by toxic metabolic products, including enzymes, before disorganization and death occur. Barnett and Binder (1973) were the first to implicate toxic metabolites in necrotrophic mycoparasitism. Chet and Baker (1981) showed that *Trichoderma* produces cellulase, B (1-3) glucanase and chitinase and degrades the glucans in the walls of *Pythium* spp. and the chitin and glucans in the walls of *Rhizoctonia solani*.

The possibility that biological activity resulted from the product of an enzymatic reaction with a substrate was suggested when it was discovered that activity could be restored by recombining two fractions (Kim *et al.*, 1987). The discovery that the acetone precipitable fraction reacted specifically with glucose facilitated the identification of this compound as glucose oxidase (Kim *et al.*, 1987). A product of this reaction is hydrogen peroxide, which kills sclerotia of the pathogen.

#### **2.6.2.1. Antagonistic microorganisms and their utilization in management of soil borne plant pathogens**

*Trichoderma* species represent to most thoroughly and widely studied fungi that show antagonistic activities towards soil borne plant pathogens. Insite of all the research with these fungi, their use as biological control agents, for the most part, has been limited to laboratory, green house and experimental field plots. Certain species of *Trichoderma* show promise as biological agents. Some successful biological control of soil borne plant pathogens like *Sclerotium rolfsii*, a causal agent of root rot of several agricultural crops has been achieved (Chet *et al.*, 1979; Elad *et al.*, 1980), but only when applied at relatively high inoculum rates. *T.harzianum* Rifai.also has been shown to be an effective agent in suppressing *Rhizoctonia solani* (Chet and Baker, 1980, 1981; Chet *et al.*, 1979, 1981; Lewis and Papavizas, 1987; Lumsden and Locke, 1989; Strashnow *et al.*, 1985) and against *Pythium* spp. Chet and Baker, 1981; Chet *et al.*, 1981; Lifshitz *et al.*, 1984; Lumsdent and Locke, 1989) and also against *Fusarium oxysporum* (Sivan and Chet, 1986, 1989).

*Trichoderma harzianum* Rifai. has shown potential to control diseases caused by *Fusarium* spp. Thus, strain T-35 of *T.harzianum* controlled fusarium wilt of cotton and melons caused by *F.oxysporum* f.sp. *vasinfectum* and *F.oxysporum* f.sp. *melonis* respectively, fusarium seedling blight in wheat caused by *F. culmorum*, all under natural soil conditions (Kempf and Wolf, 1989; Sivan and Chet, 1986). Fusarium crown rot of tomatoes was reduced by upto 80%. 75 days after sowing when *T.harzianum* was applied as either a seed coating or a wheat bran-peat preparation. Sivan *et al.* (1987) also reported 26.2% increase in yield of tomatoes in *T.harzianum* treated plots over fusarium infected plots. Upadhyay and Rai (1988) reported that antagonists viz.,

*Penicillium citrinum*, *T.harzianum* and *T.viride* contributed to suppressiveness of soils against *F.udum*. Many other groups of microorganisms have been proposed as having a role in the suppression of fusarium wilt viz., actinomycetes (Komada and Ezuka, 1970; Arjunarao, 1971), *Arthrobacter* (Smith, 1977) and *Clostridium* (Tu *et al.*, 1975). Singh *et al.* (1965) observed *Bacillus subtilis* treatment to seeds of pigeonpea reduced incidence of *F.udum* seven weeks after sowing. Many other reports show reduction of *F.udum* wilt incidence by *B.subtilis* (Vasudeva, 1949; Vasudeva *et al.*, 1952; Vasudeva and Govindaswamy, 1953). Utkhede and Rahe (1980) used as seed treatment for the control of onion white rot and found significant level of protection comparable to that of chemical treatments.

#### 2.6.2.2. *Fusarium* spp.

The growing realization of importance of biological control of plant pathogens has been discussed in many reviews (Garrett, 1956; Garrett, 1970; Papavizas, 1973; Cook, 1977 and Lumsden, 1980).

Biological control has been shown to have possibilities both in the cotton growing regions of the former USSR and in the United States of America. Studies in India have revealed the presence of several antagonistic microorganisms, in particular, actinomycetes (streptomycetes) in cotton soils. Wilt-suppressive soils have been shown to be present at Kovilpatti in South India. Antagonistic actinomycetes were present in significant numbers in such soils (Arjunarao, 1971).

Kotasthane and Agrawal (1978) reported promising results obtained by use of *Trichoderma harzianum* Rifai as biocontrol agent against chickpea seedling mortality. Seedling emergence and post

emergence mortality were 91.00 and 31.70 per cent in *T.harzianum* inoculated soil as against 61.80 and 51.90 per cent in untreated soil, respectively.

### 2.6.2.3. *M.phaseolina*

Microorganisms antagonistic to *M.phaseolina* have been reported by several workers (Norton, 1954; Radha, 1960; Mathur, 1965 and Rao and Rao, 1966). The hyphae of *T.viride* Pers.ex Gray and *An.niger* Van Tiegh coiled around the hyphae of *M.phaseolina* and then hyphae of *M.phaseolina* disintegrated (Vasudeva and Sikka, 1941). Ghaffar (1968) observed that *M.phaseolina* was inhibited as well as overgrown by *T.viride*.

In field tests, treatment of seed pieces and whole tubers with *B.subtilis* (Ehrenberg) Cohn reduced the frequency of charcoal rot of potato (Thirumalachar and O'Brien, 1977). Biological control of *R.bataticola* on chickpea by coating seeds with antagonistic microorganisms was investigated by Singh and Mehrotra (1980). Results showed that *B.subtilis* and unidentified *Bacillus* spp. as well as *Streptomyces* spp. significantly reduced disease incidence of chickpea dry root rot. Alagarsamy and Sivaprakasan (1988) observed that pelleting cowpea seed with *T.viride*, either alone or in combination with carbendazim, inhibited the growth of *M.phaseolina*. The treatment increased germination and reduced disease.

Jalali *et al.* (1989) reported that mycorrhizal inoculated chickpea plants grown in phosphate deficient soil significantly restricted the

activity of *R.bataticola* from 77.9 per cent disease in non-mycorrhizal plants to 13.3 per cent in dual inoculated plants.

#### 2.6.2.4. *S.rolfsii*

Weindling (1932) was first to demonstrate the antagonistic effects of a soil fungus to *S.rolfsii*. He found that advancing hyphae of *T.viride* secreted some substances, which were lethal to *S.rolfsii*. Hino and Endo (1940) stated that *T.viride* was able to attack and destroy sclerotia and mycelium of *S.rolfsii*. Wells *et al.* (1972) reported the potentiality of *T.harzianum* in greenhouse test against *S.rolfsii* on tomato. They were the first to report field control of *S.rolfsii* by the infestation of soil with *T.harzianum* grown on an autoclaved mixture of rye grass seeds and soil. Three applications of *T.harzianum* on to the soil surface was highly effective in reducing *S.rolfsii* damage to tomato transplants. Backmen and Rodriguez-Kabana (1975) used molasses- enriched clay granules as a food base for growing the *T.harzianum*. They observed a significant decrease in *S.rolfsii* damage to peanut and an increase in yield during a three year test.

Singh and Reddy (1979) stated that seedling blight of chickpea caused by *S.rolfsii* was reduced by 86 per cent and 59 per cent in autoclaved and natural soils, respectively. Palakshappa *et al.* (1986) observed antagonistic effects of *Aspergillus flavus* Link ex Fries, *A.niger*, *B.subtilis*, *Pencillium* spp. and *Streptomyces* sp. on *S.rolfsii*. Neypes *et al.* (1988) reported T-8-108, an isolate of *Trichoderma glaucum* Abbot, as most effective against *S.rolfsii* both in culture and in glasshouse tests.

#### 2.6.2.5. Occurrence and distribution of *Trichoderma*

*Trichoderma* and *Gliocladium* are widely distributed all over the world (Domsch *et al.*, 1980) and occur in nearly all soils and other natural habitats, especially or consisting of organic matter. Individual species aggregates may be restricted in their geographic distribution (Danielson and Davey, 1973). *Trichoderma* is also found on root surfaces of various plants (Parkinson *et al.*, 1963); on decaying bark, especially when it is damaged by other fungi (Danielson and Davey, 1973); and on sclerotia or other propagules of other fungi (Davet, 1979; Wells *et al.*, 1972). The ecological preferences of *Trichoderma* are discussed in the comprehensive review of Danielson and Davey (1973). When dry conditions in soil are maintained for long periods of time, populations of *Trichoderma* and *Gliocladium* as a group decrease (Davet, 1979). Danielson and Davey (1973) also concluded that certain strains of *T.hamatum* (Bon.) Bain and *T.pseudokoningii* are adapted to conditions of excessive soil moisture and that *T.viride* and *T.polysporum* are restricted to areas where low temperatures prevail, whereas *T.harzianum* is most commonly found in warm-climate regions and *T.hamatum* and *T.koningii* are widely distributed in areas of diverse climatic conditions.

#### 2.6.2.6. Phytotoxicity

Some potential non-target effects have been associated with the use of antibiosis-mediated biocontrol, namely the possible deleterious effects of antibiotics and antibiotic like compounds on plants. Viridiol produced by *Gliocladium virens* is phytotoxic to crop plants and herbicidal to some weeds (Howell and Stipanovic, 1984). Recently, it has been found that viridin produced by *G.virens*, which is both fungistatic and bacteriostatic,

is easily converted to the phytotoxic viridiol (Jones and Hancock, 1987). There are conflicting reports concerning the phytotoxicity of metabolites of *Bacillus* sp. Baker *et al.* (1985) reported that autoclaved culture filtrates from isolates of *B.subtilis* limited severity of bean rust in the field, but filterates from one isolate was also injurious to plant growth and resulted in lower yields. Similarly, five of six *Bacillus* metabolites were toxic to rice seedlings at the concentrations tested (Loeffler *et al.*, 1986). In contrast, Gregory *et al.* (1952) reported that antibiotics from *Streptomyces* and *Bacillus* spp. caused no harm to alfalfa seedlings.

There are some reports of metabolites of fungal antagonists affecting seed germination. Either soluble fractions of culture filtrates of *Chaetomium capreum* inhibited growth of several fungal pathogens and also delayed germination of soybean seeds (Yeh and Sinclair, 1980). Metabolites of *Trichoderma viride* and *Aspergillus*, *Fusarium* and *Penicillium* spp. have been reported to decrease the capacity of wheat, pea, rape and lettuce seeds to germinate under test conditions (Czachor; 1985). Kamal and Verma (1979) reported 100 per cent inhibition of pigeonpea seed germination by culture filtrate of *T.viride*.

#### **2.6.2.7. Isolation, identification and multiplication of antagonists**

Anahosur (1998b) quoted that *P.fluorescens* CHAO inhibited soil borne pathogen (*Pythium ultimum* Trow). Turhan and Grossmann (1986) investigated the antagonistic effects of 300 isolates of actinomycetes isolated from soils in Turkey against *M. phaseolina* and recorded 52 per cent inhibition. Biological control of soil borne plant pathogens in rhizosphere with bacteria was reviewed by Weller (1988). Meshram

(1984) reported suppressive effect of *Azotobacter chroococcum* Beijerinck on *Rhizoctonia solani* Kuhn, a soil borne sclerotial pathogen. Significant inhibition of growth of *M. phaseolina* by *B.subtilis* under *in vitro* conditions was reported (Jharia and Khare, 1986; Mukherjee *et al.*, 1987; Krishnaveni, 1991). Jacob (1989) reported that, *B.subtilis* inhibited *M. phaseolin*. Inhibition of growth of *M. phaseolina* by *P.fluorescens* has been reported by several workers (Muthusamy, 1989; Selvarajan, 1990; Ray *et al.*, 1990; Hebber *et al.*, 1991). Anahosur (1998b) has listed 13 pathogens in different crops controlled by *P.fluorescens* and their strains including Pf I and CHAO. Similarly, Dinakaran *et al.*, (1995); Sridar *et al.* (1991) and Hornby (1990) reported use of bacterial antagonists to control diseases.

#### **2.6.2.8. Production and Delivery Systems of *Trichoderma***

The development of a stable, cost effective and easy to apply biocontrol formulation is critical for the biological control of plant pathogens with introduced antagonists (Lisanky, 1985).

#### **2.6.2.9. Mass production of *Trichoderma* spp.**

As for mass production, there is still a debate on whether liquid or semi solid fermentation is preferable (Papavizas *et al.*, 1984). Solid media used for mass multiplications of *Trichoderma* include bark pellets (Sundheim, 1977), wheat bran (Knudsen and Bin, 1990), wheat bran saw dust (Elad *et al.*, 1980), sorghum grain, peat bran (Roiger and Jeffers, 1991, barley grain (AbdEl Moity and Shatla, 1981), composted hardwood bark (Hoitink, 1980; Nelson and Hoitink, 1983; Nelson *et al.*, 1983) and pellet formulations (Ricard, 1981).

### 2.6.2.10 Screening of substrates:

Kousalya Gangadharan (1988) reported, several substrates along with procedure for mass multiplication of antagonistic fungi. She mentioned that, tapioca rind, well decomposed farm yard manure, well decomposed press mud, gobar gas slurry, mushroom spent bed, paddy husk, wheat bran and groundnut shell were found to be superior for mass multiplication of *T.viride* and *T.harzianum*. Peat soil and poultry manure were poor substrates for mass multiplication of *T.viride* and *T.harzianum*. Several workers have reported the effective substrates for mass multiplication of antagonists. Effective substrates for *T.viride* *T.harzianum*, Wheat bran saw dust; same with 2 per cent molasses wheat bran saw dust 1:1:6 wheat bran saw dust medium have been reported by Mathur and Sarbhoy (1978), Samiyappan, (1988), Hadar *et al.*, (1979), Singh *et al.*, (1980), Mukhopadhyay and Chandra (1986), Sharma (1994); *Trichoderma* spp. Shelled cob, wheat straw and FYM Bharadwaj *et al.* (1987) *T.viride* Groundnut shell, well decomposed press mud and various agricultural wastes Kousalya Gangadharan and Jeyarajan (1988); *Trichoderma* spp. Farm yard manure and ground shell Sangeetha and Jayarajan (1993); *T.viride* Wheat bran, Farm yard manure and Dung Jahagirdar *et al.* (1998); *Trichoderma* spp. Tapioca rind, Tapioca refuse and well decomposed FYM followed by press mud, gobar gas slurry, mushroom spent bed straw, paddy chaff and wheat bran Kousalya Gangadharan and Jeyarajan (1990); *T.viride*, *T.harzianum* FYM, Wheat bran and rice bran Panicker and Jeyarajan (1993); *T.viride* Ground shell, Coir Pith and Pressmud Raguchandher *et al.* (1993); *Trichoderma* sp. And other antagonists Vermiculture, Sanker (1994); *Trichoderma* spp. Pigeonpea husk was best followed by Tapioca waste and press mud,

Jayaraj and Ramabadrana (1996b); *T.viride* Talc based formulation, Ramakrishnan *et al.* (1994); *T.harzianum* Potato jaggery (2%) broth in 750 ml wine bottles; coffee wastes, Sawant and Sawant, (1996); *Trichoderma* spp., Liquid fermentation technology, Papavizas (1985) ; *T.harzianum*, Coffee berry skin and poultry manure, Sawant and Sawant, (1996).

#### **2.6.2.11 Effect of fungicides on biofungicides**

Bollen (1979) critically analyzed and presented possible effects on antagonistic relationships. The indirect effect of toxicants on disease incidence may be positive or negative. Pesticides sometimes selectively enhance the antagonistic microbes or antagonism, which may lead to indirect control or integrated control. Several reports were made on fungicidal effect under different concentrations on antagonists used in biological control and test pathogens. Papavizas (1980) reported induced tolerance of *T. harzianum* to fungicides.

Integration of chemical(s) and a bio agent (*Trichoderma* spp.) has been the subject of research during recent years. Integration of biological and chemical control seems to be a very promising way of controlling pathogens with a minimal interference with biological equilibrium (Papavizas, 1973). Langerak (1977) reported that after treatments of bulbs of narcissus with aretan, pimaricin and thiram, the newly developing roots were more densely colonized by *T.viride* and *Penicillium janthinellus* during the entire growing season. These fungi were less sensitive to these fungicides than the *Fusarium* spp. and then more or less selectively favoured by the treatment. Curl *et al.* (1976) observed that ineffective amounts (1 to 2 mg/g soil) of PCNB, applied together with

*T.harzianum*, controlled *Rhizoctonia solani* more effectively than did *T.harzianum* alone in cotton seedling disease in the green house. Similarly, Henis *et al.* (1978) obtained green house control of *R.solani* damping of radish by integration of PCNB (4 mg/g soil) and *T.harzianum*. Chandra (1984) reported that integration of both chemical and biological control measures has a synergistic effect on the control of damping off in sugar beet. Seed treatment with metalaxyl alone at 0.01% was not very effective, but when used with 10.5 g of *T.harzianum* preparation, it provided enhanced control of damping off of sugar beet. Mukhopadhyay and Chaturvedi (1986) also obtained successful control of damping off of tobacco and egg plants by the application of *Trichoderma* preparation to soil and integrating it with metalaxyl seed treatment.

Ramdoss and Sivaprakasan (1987) reported, inhibitory effect of carbendazim and quinterozone, carbendazim at 10 ppm and thiram at 500 ppm were fungicidal, while quinterozone and all insecticides were fungistatic. Reddy and Dharamaveer (1998) reported that carboxin at 300 ppm was tolerated by *M. phaseolina* and concluded that, tolerance of carboxin as temporary. Algarsamy and Sivaprakasan (1988) reported that carbendazim did not show any adverse effect on *T. viride* and *T. harzianum* in vitro and pot culture studies. Chaudhary and Sharma (1988) reported increased inhibition of *M. phaseolina* with increased concentration among 14 fungicides tested. Dubey (1991) reported that effective inhibition of *M. phaseolina* by carbendazim, mancozeb on PCNB was reported. Rai and Vijay (1992) reported that 5 to 10 ppm concentration of carbendazim was lethal to *T. viride* and inhibition mycelial growth.

### 2.6.2.12 Effect of Insecticides on biofungicide

Various insecticides have shown antifungal properties. Ramdoss and Sivaprakasam (1987) reported that carbofuran at 250, 500 and 1000 ppm concentration was on linear growth of *Macrophomina phaseolina*. inhibition of *M. phaseolina* by all insecticides tested was fungistatic in nature. Dubey (1991) reported effective inhibition of *M. phaseolina* by endosulfan and monocrotophos and further observed that insecticides concentration of 500 to 1000 µg/g was needed to kill sclerotia of *M. phaseolina*. Martinez – Toledo *et al.* (1992) reported strong inhibition of *T. harzianum* by chlorpyrifos in addition to other methyl pyrimifos. Sridar *et al.* (1995) reported that Carbofuran at 100 ppm did not inhibit the mycelial growth of *T. viride* but enhanced the sporulation compared to control. Carbofuran at 500 and 1000 ppm inhibition the growth of *T. viride*. In this connection, Tu (1972) reported that *Trichoderma* became dominant in soil treated with Carbofuran due to mycotonic effect (Sridar *et al.*, 1995)

Jayaraj and Ramabadran (1996) reported that, phorate and carbofuran caused maximum inhibition of mycelial growth of *T. harzianum*. Hyphal growth reported with increase in concentration of Carbofuran. At lower dosages (5, 10, 50, 100 and 500 ppm) increased the growth of *T. harzianum*.

### 2.6.2.13 Effect of Herbicides on biofungicide

Anahosur *et al.* (1984a) reported *in vitro* and *in vivo* in-effective nature of Atrazene and 2, 4D on the growth of *M. phaseolina* causing charcoal rot of sorghum and concluded that, herbicide used in sorghum

may not control charcoal rot disease. Dubey (1991) reported the reduction in survival of *M. phaseolina* by 2-4 D, Thiobenzocarb and Butachlor indicating antifungal property of herbicides. Gouvêla *et al.* (1993) reported reduction of charcoal rot in stay green B-35 and non stay green 7 x 700 with increasing concentration of Glyphosate (0.016 to 0.25%) thus inhibitory and antifungal property of Glyphosate on *M. phaseolina* causing sorghum charcoal rot was reported. The reduction was observed in fungal colony diameter of *M. phaseolina* in sorghum by Atrazine, Alachlor and Metolachlor. Alachlor and Metolachlor reduced sclerotial germination whereas, Atrazine did not affect sclerotia of *M. phaseolina*.

## **2.7 Production, formulation and application**

### **2.7.1 Seed treatment with fungal antagonists**

Seed treatment of antagonists is the cheapest delivery method to the rhizosphere of crop plants to protect against seed borne pathogens. Soybean seeds treated with *T.harzianum* improved plant stand and reduced the infection by *Rhizoctonia* sp. (Kommedahl *et al.* (1981) and Wu, 1982) Anahosur (1998a), mentioned that, seed treatment with talc formulation of *Trichoderma* species @ 4 g/kg seed was useful in controlling different disease caused by various soil borne pathogens. Commercial formulations of fungal antagonists viz., WRC-GV (*Gliocladium virens*); BINAB-T (*Trichoderma* sp.), MONITOR Wp (*Trichoderma* sp.) and *Trichoderma* (*Trichoderma* sp.) are in use as seed dressers. Mukhopadhyay *et al.*, (1986) reported that, treatment of seeds with spore suspension of antagonists is relatively recent and more feasible way of delivery for management of seed borne diseases. Seed pelleting with *T.viride* was significantly superior to soil application of the antagonist in checking infection due to *T.harzianum* protects bean (Elad

*et al.*, 1986) and sunflower and mungbean (Hussain *et al.*, 1990) from *M.phaseolina*. Several workers have reported usefulness of seed treatment in controlling seed and soil borne diseases. Bhaskaran and Seetharaman (1986), Jacob (1989), Jayarajan *et al.*, (1994); reported that, *T.viride*, *T.harzianum* seed treatment in urdbean against root rot (*M.phaseolina*). Similarity Root rot reduction in chickpea from 23.6 to 3 per cent by seed treatment with *T.viride*, Parkhia and Vaishnav (1986); Jeyarajan and Ramakrishnan, (1991), For root rot reduced by seed treatment by *T.harzianum* (19 to 8 per cent), Kehri and Chandra (1991), and for Mustard root rot reduced by seed treatment by *T.harzianum*, Haque and Gaffar (1991); Seed treatment by *T.viride*, *T.harzianum* and *T.longibrachiatum* Rafai in groundnut, soybean, pigeonpea, chickpea, sesamum and mungbean reduced root rot incidence; Maharishi and Kumavat (1992); Umamaheshwari (1991); Krishanaveni (1991); Nakkeeran (1992); Sankar (1994); Vidya (1995); Jayarajan *et al.* (1994) developed and used talc formulation as seed treatment to reduce root rot in urd bean, chickpea, peanut and seasamum by 66, 50, 77 and 67 per cent, respectively. Raguchander *et al.* (1993) reported, talc formulation @ 4 g/kg reduced root rot in blackgram caused by *M.phaseolina*.

### 2.7.2. Soil application method

Anahosur (1998a) enlisted successful control of various diseases by antagonists. Soil application 130-160 kg/ha soil formulation of antagonists was applied either in furrow, row, filling holes with inoculum. Both *Trichoderma* spp. and *P.fluorescens* can be applied to soil to manage soil borne diseases effectively. Wheat bran saw dust formulation for soil application was common. Molasses enriched clay (Kaolin) granules encapsulation in organic polymer are discussed.

Furrow, broadcast and root zone application are practiced for both fungi and bacterial antagonists.

Anahosur (1998b) explained that peat formulation of *P.fluorescens* is applied directly to soil furrows 5-10 g for 3 m row and concentration should be 10 cfu/g. It is also applied to soil through mixing of FYM or saw dust. Vermiculite granules applied to soil gave better results. Hiremath and Kulkarni (1992) reported that, peat based *P.fluorescens* can be applied to soil @ 2.5 kg/ha after mixing with 50 kg organic manure or sand, 30 days after sowing. They mentioned that, *Bacillus subtilis* is compatible with biofertilizers.

Biswas and Das (1999) reported, soil application of *Trichoderma* spp. through sand maize sorghum (10 g : 90 g 16 ml water) was better than seed pelleting. Krishnamoorthy and Bhaskaran (1990) reported that, control of damping off in tomato was effective by soil application with *T. harzianum*. Raguchander *et al.* (1997) reported, better control of mung bean root rot by seed pelleting with *T.viride*. This method was better than row application. Effective disease control by soil application method is reported by several workers as Wheat bran based *T.harzianum* Chickpea root rot, Parkhia and Vaishnav (1986); Wheat bran based *T.harzianum*, bean root rot (*M.phaseolina*) Elad *et al.* (1986); wheat bran based *T.harzianum* Root rot of urdbean (*M.phaseolina*) Samiyappan *et al.*, (1987); FYM based *T.harzianum* root rot of bean (*M.phaseolina*) Jacob (1989); Organic amendment with *T.viride* & *T.harzianum* soybean charcoal rot (*M.phaseolina*) Muthusamy, 1989; Soil application of *T.viride* + *T.harzianum* before *T.harzianum* Urdbean bean root rot (*M.phaseolina*), Kousalya Gangadharan and Jeyarajan (1990); soil application of *G.virens* collar rot of lentil (*S.rolfsii* and *R.solani*) Shreshta

and Mukhopadhyay, (1993) and Furrow application of *P.fluorescens* @ 50 g of peat mixed inoculum per 4m row black gram root rot (*M.phaseolina*) Samiyappan (1988). Production and application of bioagents in plant disease management (Kulkarni, 2000).

### **2.7.3. Carrier materials talc: FYM ratio**

The viability of the propagules of *G.virens* and *T.harzianum* was more than 90 per cent after four months of storage at 20° C (Jones *et al.*, 1984). Hader *et al.* (1979) reported that, spore retained 80 per cent viability when *T.harzianum* was stored in wheat bran carrier for six months at 4° C or at room temperature. Samiyappan *et al.* (1987) reported that application of wheat bran based *T.viride* inoculum to soil resulted in good survival of antagonists. Similarly, wheat bran was found good by Malti *et al* (1987) and Sivan *et al.* (1987). Mathivanan *et al.* (1998) reported that, four locally available materials viz., Farm Yard Manure, rice bran, saw dust and wheat bran were evaluated for their suitability as a carrier for talc based formulation of *T.viride*. FYM and talc mixture was best followed by saw dust and talc mixture.

### **2.7.4. Storage and shelf life of biocontrol agents**

#### **2.7.4.1 Storage bag colour for mass multiplication of biocontrol agents in storage and studied on storage conditions and shelf life of biocontrol agents**

Vidhyasekaran and Muthamilan (1995) found that, *P.fluorescens* in talc formulation and peat based formulation survived upto 240 days although population decline occurred after 30 days. Similarly, Sanker

and Jayaraj (1996) reported talc based powder formulation of *Trichoderma* and *Gliocladium* retained more than 50 per cent population upto 120 days. Initial population was  $224 - 297 \times 10^6 \text{ g}^{-1}$  in *Trichoderma* spp and *Gliocladium* sp. Population declined in *Trichoderma* (19-21%) and *G.virens* (29.9%) after 7 days. Sanker and Jayaraj (1996) observed that storage as treated seeds of sesamum was better than treating during sowing. Antagonists remained viable without affecting seed germination. Prasad *et al.*, 1997 found that Molasses yeast medium and potato dextrose broth were best for *in vitro* multiplication of antagonists. Talc formulation supported growth upto 60 days. Viability upto 90 days of *T.harzianum* was achieved in talc, gypsum, vermiculite and wheat bran, three pesta granule formulation with wheat flour, wheat flour kaolin, wheat flour bentonite stored at 4° C was the best. Nakkeeran *et al.* (1997) observed that storage of *T.viride* in talc formulation in milky white bags at room temperature (20-30° C) for upto 75 days was observed (cfu  $206 - 276 \times 10^6 \text{ g}^{-1}$ ). Temperature for storage was on par and optimum at 10-15° C but zero temp (°C) was better than 10° C. Talc formulation of *T.viride* can be safely stored in milky white bags at room temperature (20-30° C) for upto 75 days. Similaly Saha and Pan (1998) reported that, 40° C temperature was lethal to survival of *Glicoladium virens*.

Storage and stability with prolonged shelf life are major criteria for the success of biocontrol agent in the management of disease. Lignite and stillage carrier system retained 90 per cent viability of antagonists (*G.virens*, *T.harzianum*) upto 120 days at 20° C (Jones *et al.*, 1984). Hader *et al.* (1979) reported, wheat bran with *T.harzianum* retained 80 per cent viability at 4° C. Similarly, Convey (1983) stored the conidia of *T.harzianum* in fluid drilling gels at four different temperature levels viz.,

5, 16, 20 and 25° C for 10 days without affecting viability. Manmohan Das (1992) reported that, the nucleus inoculum of *T.harzianum* developed on FYM or gobar gas effluent retained, its viability even after 300 days of storage. Lower temperature of 20° C is better than 30° C to retain viability for longer periods.

## **2.8. Application**

### **2.8.1. Field evaluation of biocontrol agents**

Several workers have reported biological control and effective antagonistic potential of both fungal and bacterial antagonistic microorganisms (Parkhia and Vaishnav, (1986); Kehri and Chandra (1991). Turhan and Grossman (1986) reported that 52 per cent reduction of *M.phaseolina* in soil by 300 isolates, of achinomyces, Sridar *et al.* (1991) reported antagonistic action of *B.subtilis* against *M.phaseolina*. Reduction of *M.phaseolina* in various crops was reported Raguchander *et al.* (1993) and (1995) Dinakaran *et al.* (1995) and Ramakrishnan *et al.* (1994) (Sen and Bandyopadhyay, 1988) have enlisted 17 effective antagonists for *M.phaseolina* and 13 successful examples of biological control of *M.phaseolina* using antagonistic species of *Trichoderma*, *Bacillus* and *Aspergillus* etc.

#### **2.4.7.3. Methods of application of *Trichoderma* spp.**

There are four different techniques for the application of *Trichoderma* spp. as biocontrol agents, they are Broadcast : *Trichoderma* preparations are broadcasted on the surface and incorporated into infested soil, Furrow: preparation put into infested soil in the planting furrow, Root zone : *Trichoderma* inoculated into the root zone by mixing before

transplanting seedling in an infested field, seed coating: seeds coated with *Trichoderma* spores using an adhesive. Each method has been shown to be effective in the field. While the broadcast application is practiced rarely, if ever, economical, other three methods and especially those methods that introduce the antagonist with the planting material are economical.

Gel seedling or fluid drilling, a technique by which pregerminated seed are extruded into soil in a fluid matrix, also has been used as a delivery system; for biological control agents (Conway *et al.*, 1982; Fisher *et al.*, 1983; Hadar *et al.*, 1984).

#### **2.4.7.4. Factors involved in establishment of *Trichoderma* in rhizosphere soil**

The efficiency of biocontrol may be determined by various factors viz., the soil temperature and soil reaction (Harman *et al.*, 1980, 1981; Knudsen and Bin, 1990), the kind of soil and its microbiota (Hadar *et al.*, 1984), the nutritional status of the inoculant (Harman *et al.*, 1981), the inoculant density on the seed (Papavizas *et al.*, 1982), the inoculum potential of the pathogen in soil (Wu, 1982), the rate of application of the antagonist to soil (Elad *et al.*, 1980; Kommedahl *et al.*, 1981).

#### **2.5. Effect of oil cake amendments on soil pathogens**

Organic and inorganic amendments have shown profound effects on the soil microbes (Alexander, 1957). Organic amendments have been employed in different forms by the earlier workers (Adams *et al.*, 1968; Chattopadhyaya and Mustafee, 1980; Goyal and Mehrotra, 1979; Gupta,

1987; Huber and Watson, 1970; khanna and Singh, 1974; lakshmanan and Nair, 1984; Singh and Singh, 1982, 1985 and Sudhir Chandra *et al.*, 1981) to findout the quantitative and qualitative changes in mycoflora of amended and unamended soils besides evolving a suitable control method of soil borne diseases.

Srivastava (1961) reported a significant reduction in soil population of *Fusarium oxysporum* as well as incidence of wilt of coriander by amending soil with linseed, groundnut or sesamum oil cakes. Mahmood (1964) also reported reduction of pigeonpea wilt incidence in soil supplemented with groundnut cake, molases and sweet clover after inoculations with *B.subtilis* which produces bulbiformin. Groundnut oil cake suppressed the *Fusarium* population in soil when amended at more than one per cent (Bhalla, 1966). Assay of autoclaved or unautoclaved field soil, reinfested with *F.udum* and amended with 0.5 to 5.0 per cent of oil cakes of margosa, groundnut or mustard 15 days after amendment, revealed that these oil cakes either stimulate or inhibit vegetative growth or sporulation or both (Singh and Singh, 1970). Singh and Singh (1982, 1985) also noticed that Neem cake inhibited radial growth of *F.udum* when exposed to 1 or 2 week after amendment. Amendment with chitin, cellulose and starch at 0.1 per cent reduced populations of *Fusarium* spp. in sugarcane soils (Gupta, 1987).

The incidence of rhizome root rot (*Pythium* spp.) of ginger was 4.70 per cent and 8.50 per cent in neem and pongamea mixed soil respectively compared with control plots i.e., 16.70 per cent (Sadanandan and Iyer, 1986). Lakshmanan and Nair (1984) reported, neem, gingilly and groundnut cakes to be very effective in reducing the viability of sclerotia of *Rhizoctonia solani* in paddy field. Cent per cent control of

root rot of cotton (*R.bataticola*) was observed with sesamum, groundnut and mustard oil cakes, when added to soil in the ratio of 1:25 and 1:50 (Anon., 1970).

## *Material and Methods*

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### **III. MATERIAL AND METHODS**

The foregoing investigations were comprised of laboratory, green house and field experiments conducted during 1998-2002 at Agricultural Research Station, Dharwad Farm and Department of Plant Pathology, College of Agriculture, Dharwad, University of Agricultural Sciences, Dharwad. The details of the methodology employed in these investigations are presented in the following pages.

#### **3.1 Glassware and sterilization**

Corning glassware were used in for all the experiments conducted. The glassware were kept in the cleaning solution for a day containing 60 g of Potassium dichromate ( $K_2Cr_2O_7$ ) and 60 ml of concentrated Sulphuric acid ( $H_2SO_4$ ) in one litre of water. They were washed with vim powder followed by washing in running tap water and rinsing in distilled water.

All glassware were sterilized in a hot air oven at 60° C for two hours. Both solid and liquid culture media were sterilized at 1.0 kg pressure per square centimeter for 20 minutes. Soil used for experiments was sterilized for two hours at 1.33 kg pressure per square centimeter in an autoclave.

#### **3.2 Isolation and identification:**

A survey was conducted to collect the wilted and root rotted cotton plants from certain cotton growing areas of Karnataka during 1997-98 and 1998-99. Ten plants were collected from each location for isolation

purpose. In a majority cases of locations, crop was irrigated. Details of locations, isolates collected, etc., are furnished in pages to follow.

### **3.3 Isolation**

Affected roots and collar portions of cotton were collected from different locations and used for isolation purpose. Isolations were made by following standard tissue isolation method.

Roots as well as collar portions were cut with a scalpel into small pieces of 5 to 7 mm and were washed in tap water in order to remove the soil particles. Simultaneously, Potato Dextrose Agar (PDA) was prepared. Approximately 20 ml of molten warm PDA, amended with Streptomycin sulphate @ 25 ppm, was poured into sterilized Petridishes aseptically. Medium was allowed to solidify in the Petridishes. Pieces were treated with 0.1 per cent Mercuric Chloride solution for sixty seconds followed by washing in sterile distilled water thrice. Such five pieces were transferred to each of 20 Petridishes at equal distance. Thus, 100 pieces were kept into 20 Petridishes for each location. Petridishes were incubated at room temperature for four days. Fungal growth of each Petridish was examined under the microscope and then the hyphal bits of growth were transferred to PDA slants.

### **3.4 Single spore isolation**

Ten ml of two per cent molten water agar was poured into Petridishes and allowed to solidify. Dilute suspension of conidia of *Fusarium solani* was prepared in distilled sterile water from a week old culture. One ml of suspension was poured in each of the water agar

Petridishes and these Petridishes were tilted in different directions to have a uniform spread of conidia on the water agar surface. Microconidia were examined under the microscope. Isolated micro conidia were marked and transferred on to PDA slants under aseptic conditions.

Cultures of *Rhizocionia solani*, *Rhizoctonia bataticola* and *S.rolfsii* were purified by hyphal tip method. Single growing tip of hypha was cut and transferred to PDA slants.

### **3.5 Maintenance of the cultures**

The cultures were stored in a refrigerator at 5° C and subcultured periodically, once in 30 days.

### **3.6 Pathogenicity**

#### **3.6.1 Pathogenicity of *Fusarium* isolates**

Monomicroconidial cultures were the source of inoculum. Mass culture of each of the isolates was prepared using on ground cotton seeds medium. Twenty days old mass cultures so obtained were thoroughly mixed with autoclaved field soil in 1:4 W/W ratio. Earthen pots of 15 cm diameter, previously surface sterilized by 0.1 per cent mercuric chloride solution, were filled with infested soil and pots were kept under glass house conditions for five days. On sixth day, five seeds of DCH-32 cotton genotypes, treated with a 0.1 per cent mercuric chloride solution for two minutes, were seeded in each of the pots. Pots were watered regularly so as to maintain soil moisture to 50 per cent of water holding capacity (WHC). Seeds sown in pots with sterile soil served as control treatment. Plants showing above ground wilting symptoms were

critically observed. Final observations were recorded 45 days after sowing. Reisolations were made from the root portions of affected plants and the cultures were compared with original ones.

### **3.6.2 Pathogenicity of *Rhizoctonia solani* and *Rhizoctonia bataticola***

Pure cultures, obtained by hyphal tip method, were used for pathogenicity tests under glass house conditions. DCH-32 was used as test genotype. Five days after filling the pots with artificially infested autoclaved soil with *Rhizoctonia solani* and *Rhizoctonia bataticola* separately seven seeds of cotton were sown in each pot. Water stress condition is known to be congenial for disease development caused by *Rhizoctonia bataticola*. Therefore, such condition was maintained in each pot throughout the course of this study. Final observations of disease development were recorded 45 days after sowing. Reisolations were made and pathogenic isolates were compared with original ones. Pathogenic isolates were further maintained for other experiments.

### **3.6.3 Pathogenicity of *Sclerotium rolfsii***

Pure culture, obtained by hyphal tip method, was used for pathogenicity test under glass house conditions. DCH-32 was test genotype. Mass culture was multiplied on giant culture (sand maize 4:1) medium for 20 days in 500 ml flasks. Autoclaved soil (3:1, field soil and sand by weight) was amended with mass culture @ 200 g per kg soil. Earthen pots of 15 cm diameter were surface sterilized with 0.1 per cent mercuric chloride solution. Pots were filled with amended soil. About 20 per cent soil moisture was maintained to ensure good growth of *S.rolfsii*. On sixth day of filling the pots, seven seeds of DCH-32 treated with 0.1

per cent mercuric chloride solution were sown in each pot. *S.rolfsii* is a highly aerobic fungus. Therefore, excessive watering was avoided. Pots without inoculum of *S.rolfsii* served as control. Plants were observed daily for disease development. Plants showing collar-rotting symptoms were removed carefully. Reisolation was made by tissue isolation procedure from affected collar portion using PDA. Cultures obtained through reisolation were compared with original culture. The pathogenic culture was preserved at 5° C in a refrigerator for other experiments.

### **3.7 Identification**

#### **3.7.1 *Fusarium* spp.**

Identification of *Fusarium* spp. was made on the basis of morphological and cultural characters described by Booth (1971). Further, the identity of pathogenic isolates of *Fusarium* was confirmed by the Division of Mycology, Agharkar Research Institute, Pune, Maharashtra. Following pathogenic isolates of *F.solani* were used for various experiments.

#### **3.7.2 Identification of *M.phaseolina* and *R.solani***

The morphological, cultural and formation of sclerotia were the principal characters to identify pure culture of *M.phaseolina* and *R.solani*. These characters were compared as described by Ashby (1927) and Goidanich (1947) and identified as *M.phaseolina* and *R.solani*.

### 3.7.3 *S.rolfsii*

This fungus was identified as per the morphological and cultural characters described by Domsch *et al.* (1980).

### 3.8. Cultural and morphological characters of pathogens on PDA

Nine isolates of *F.solani* and each of *R.solani*, *M.phaseolina* and *S.rolfsii* were grown on PDA Petridishes for six days at room temperature under alternate light and darkness. Growth diameter of each isolate was measured. Growth of each isolate was critically observed for pigmentation and growth pattern. The pigmentation was recorded 10 days after inoculation. Fifty microconidia as well as macroconidia were measured with stage and ocular micrometers and average values were worked out. Sporulation was studied by means of haemocytometer under the microscope. Five discs of fungal growth of 5 mm diameter were dissolved in 20 ml of distilled water and shaken well to get spore suspension. One drop of spore suspension was placed on haemocytometer. After placing a glass cover slip, spores were counted microscopically from five blocks of haemocytometer. Spores/ml of suspension was calculated using following formula

$$\text{Spores/ml} = \text{Spores observed from 5 blocks} \times 2000$$

Table: List of pathogenic isolates collected from different parts of Karnataka used in the study

Sl. No.	Code used	Isolate/Spp	Place
1.	F <sub>1</sub>	<i>Fusarium solani</i>	ARS, Dharwad (Dharwad)
2.	F <sub>2</sub>	<i>Fusarium solani</i>	Kasaba Camp (Raichur)
3.	F <sub>3</sub>	<i>Fusarium solani</i>	Hirebagewadi (Dharwad)
4.	F <sub>4</sub>	<i>Fusarium solani</i>	Navalgund (Dharwad)
5.	F <sub>5</sub>	<i>Fusarium solani</i>	Naregal (Gadag)
6.	F <sub>6</sub>	<i>Fusarium solani</i>	Baganandi (Mysore)
7.	F <sub>7</sub>	<i>Fusarium solani</i>	Mosarkunthe (Tumkur)
8.	F <sub>8</sub>	<i>Fusarium solani</i>	Ron (Gadag)
9.	F <sub>9</sub>	<i>Fusarium solani</i>	Siruguppa (Bellary)
10.	Rs	<i>R. solani</i>	Navalgund (Dharwad)
11.	Rb	<i>R. bataticola</i>	ARS, Dharwad Farm
12.	Sr	<i>S. rolfsii</i>	ARS, Dharwad Farm

### 3.8.1 Multiplication of mass inoculum

The *F. solani*, *M. phaseolina*, *R. solani* and *S. rolfsii* were multiplied on cotton seed medium and maize and sand mixture (Giant culture) respectively. (Abeygunawardena and Wood, 1957) in a ratio of 95:5 W/W and watered to 20 per cent by volume. Each of the 500 ml conical flasks were filled with 200 g of the mixture and sterilized at 1.0 kg pressure per sq.cm for 20 minutes. A mycelial disc of five mm diameter cut by sterile cork borer from the margin of a actively growing culture was transferred aseptically to each flask. The flasks were incubated at 25

**Table:** List of pathogenic isolates collected from different parts of Karnataka used in the study

Sl. No.	Code used	Isolate/Spp	Place
1.	F <sub>1</sub>	<i>Fusarium solani</i>	ARS, Dharwad (Dharwad)
2.	F <sub>2</sub>	<i>Fusarium solani</i>	Kasaba Camp (Raichur)
3.	F <sub>3</sub>	<i>Fusarium solani</i>	Hirebagewadi (Dharwad)
4.	F <sub>4</sub>	<i>Fusarium solani</i>	Navalgund (Dharwad)
5.	F <sub>5</sub>	<i>Fusarium solani</i>	Naregal (Gadag)
6.	F <sub>6</sub>	<i>Fusarium solani</i>	Baganandi (Mysore)
7.	F <sub>7</sub>	<i>Fusarium solani</i>	Mosarkunthe (Tumkur)
8.	F <sub>8</sub>	<i>Fusarium solani</i>	Ron (Gadag)
9.	F <sub>9</sub>	<i>Fusarium solani</i>	Siruguppa (Bellary)
10.	Rs	<i>R. solani</i>	Navalgund (Dharwad)
11.	Rb	<i>R. bataticola</i>	ARS, Dharwad Farm
12.	Sr	<i>S. rolfsii</i>	ARS, Dharwad Farm

### 3.8.1 Multiplication of mass inoculum

The *F. solani*, *M. phaseolina*, *R. solani* and *S. rolfsii* were multiplied on cotton seed medium and maize and sand mixture (Giant culture) respectively. (Abeygunawardena and Wood, 1957) in a ratio of 95:5 W/W and watered to 20 per cent by volume. Each of the 500 ml conical flasks were filled with 200 g of the mixture and sterilized at 1.0 kg pressure per sq.cm for 20 minutes. A mycelial disc of five mm diameter cut by sterile cork borer from the margin of a actively growing culture was transferred aseptically to each flask. The flasks were incubated at 25

$\pm 1^{\circ}$  C for 20 days. The flasks were shaken periodically in order to permit uniform growth. Such inoculum was used for experiments.

The composition of *Fusarium* selective medium (Peptone PCNB medium) was that of Nash and Snyder (1962). The components were 15 g Peptone, 1 g Potassium dihydrogen phosphate, 0.5 g Magnesium sulphate, 1 g Pentachloronitro benzene (75% wp), 300 ppm of streptomycin sulphate and 150 ppm Tetracycline and 20 g Agar per litre. The composition of *Trichoderma* selective medium (TSM) was that given by Elad *et al.* (1981a). The components were 0.2 g Magnesium sulphate, 0.9 g Dipotassium monohydrogen phosphate, 0.15 g Potassium chloride, 1 g Ammonium nitrate, 3 g Glucose, 0.15 g Rose bengal, 0.2 g PCNB and 1.2 g Captan, 20 g Agar per litre.

### **3.8.2. Rhizosphere microflora of cotton grown in the field**

Different fungi were observed during isolation of *F.solani*, *R.solani*, *R.bataticola* and *S.rolfsii* in the cotton rhizosphere. These fungi appeared to be commonly occurring in the rhizosphere and hence they were isolated and got identified by the Mycology Division, Agharkar Research Institute, Pune, Maharashtra.

### **3.8.3. Preparation of dilution blanks**

Several test tubes containing nine ml of water were sterilized at 1 kg pressure per sq.cm for 20 minutes in an autoclave. Ten grams of soil sample was added to a conical flask containing 90 ml of sterilized water and shaken by hand for five minutes so as to obtain  $10^{-1}$  dilution. One ml of soil suspension from  $10^{-1}$  dilution was pipetted out to nine ml blank.

This established,  $10^{-2}$  dilution. Further dilutions were prepared by transferring one ml of soil suspension successively and shaking thoroughly.

### 3.9 Biological control

#### Isolation, identification and multiplication of antagonistic microorganisms

##### Isolation of native antagonists

Soil samples from Agricultural Research Station, Dharwad farm, were collected from rhizosphere of escape plants from characteristically affected plots of cotton. Procedures of cleaning glasswares and sterilization was done as explained earlier. The organisms were cultured on specific media as indicated below. Different *Trichoderma* cultures from various sources (eleven cultures) and two *Gliocladium* cultures were obtained to test for their efficacy against the soilborne pathogens of cotton.

Procedures of Ramakrishnana (1989) were used to isolate microorganisms from soil. The techniques of serial dilution and purification were done (Sankaram, 1961).

Sl. No.	Antagonists	Media	Reference/Source
A.	<b>Fungal antagonists</b>		
	<i>Trichoderma</i> spp.	<i>Trichoderma</i> Specific medium	Elad and Chet (1983)
	<i>Gliocladium</i> spp.	Specific medium and sclerotium bait	Park <i>et al.</i> (1992), Mukherjee <i>et al.</i> (1993)

All isolates were purified further by single spore method or hyphal tip method as per procedure (Rangaswami, 1972). Identity of isolates was confirmed with the assistance of Agharkar Research Institute, Pune, Maharashtra. Totally 12 isolates of were *Trichoderma* including one from cotton Rhizosphere and used in the present study.

Mass multiplication of *T.harzianum* was made using wheat bran medium and Talc powder. (Henis *et al.*, 1978). Procedure followed for mass multiplication was essentially similar to that of *Fusarium* spp. mass multiplication except the medium.

### **3.9 Interaction study**

#### **3.9.1. *In vitro* interaction between *Trichoderma* species and their isolates with *Fusarium solani*, *R. solani*, *R.bataticola* and *S.rolfsii***

Biofungicides are known to suppress the activities of soil borne pathogens and to have the best biofungicide, which would affect all the soil borne pathogens of cotton, had to be standardised. In this view, the laboratory experiment was conducted. For this purpose 12 isolates and species of *Trichoderma* and two isolates of *Gliocladium virens* and nine isolates of *F. solani*, *R.solani*, *R.bataticola* and *S.rolfsii* were multiplied on PDA Petridishes for a week to get fresh and active growth. Approximately 20 ml molten PDA was poured into each Petridish. Fungal growth discs of five mm diameter were cut by means of a sterilized corkborer. After solidification of PDA into Petridishes, one disc of respective bioagent growth and pathogens were placed to the centre of Petridish at a distance of five cm from each other. The dual cultural method (Huang and Hoes, 1976) was followed Petridishes were incubated at room temperature for a week. On the basis of growth using

the following formula, percent growth of each fungus was calculated by using Vincent (1947) formula.

### **3.9.2. Standardising the peak period for growth of *T. harzianum*:**

This experiment was undertaken to find out the peak period at which *T. harzianum* would have its maximum growth and it was required for next experiment. In the present investigation, *T. harzianum* was grown on potato dextrose broth. Corning conical flasks of 100 ml capacity were taken and in each flask 25 ml of potato dextrose broth was put and flasks were sterilised. Afterwards these sterilised broths were seeded with 5 mm *T. harzianum* disc. At every two days at an interval the mycelial growth was filtered on pre-dry weighed whatman No. 41 filter paper. The oven dried mycelial weight on this filter paper was recorded. The difference gave the actual dry mycelial weight of *T. harzianum*. The recordings were made till the peak period and the growth started declined. The data recorded were analysed statistically.

### **3.9.3. Evaluation of carbon sources for *T. harzianum***

As the type of carbon source does play an important role on *T. harzianum* while its absorption and utilisation varies from carbon source to carbon sources in the organic matter in the soil, the present investigation was made to study the effect of carbon source on growth of *T. harzianum*. Here six carbon sources glucose, galactose, maltose, sucrose, fructose, mannitol and dextrose (control) were tested against *T. harzianum*. The quantity of carbon source was calculated on the basis of molecular weight of each carbon source and replaced in the basal medium accordingly. After 12-day incubation, the mycelial mat and weight were

harvested (Via filtration and drying in the oven) for all the treatments of three replications each. The recorded observations were further analysed statistically.

### 3.9.3 Screening of substrates

Nine substrates were evaluated for mass multiplication *T.harzianum* using the procedure of Kousalya Gangadharn and Jeyarajan (1990). The substrates used were dung ,FYM, groundnut shell paddy straw, poultry manure, vermicompost and wheat bran saw tap water medium (WBSD) (3:1:4).

The bags were incubated under room temperature for 30 days. After incubation the samples were drawn and population of *T.harzianum* was enumerated in respective selective medium (Elad and Chet, 1983, Park *et al* 1992 and Jayaraj and Ramabadran, 1996b). Colony forming units (cfu 10 g<sup>-1</sup>) were estimated by dilution plate technique (Pramer and Schimidt, 1956) and the data were analysed statistically.

## 3.10. PRODUCTION FORMULATION AND APPLICATION

### 3.10.1 Seed treatment with *T.harzianum*

Based on antagonistic potential against all nine isolates of *F.solani*, *Rhizoctonia solani*, *Rhizoctonia bataticola* & *S.rolfsii*, *T.harzianum* were selected for further studies. *T.harzianum* was multiplied in talc formulation with prior growth on Molasses Yeast Medium (MYM) for two weeks (Nakkeeran *et al.*, 1997). The talc formulation of *T.harzianum* for seed treatment was prepared as per the procedure given by Jeyarajan *et al.* (1994). *T.harzianum* multiplied on molasses yeast medium and was

used to prepare talc formulation for seed dressing purpose. In surface moistening, 6 ml sterile distilled water was used to moisten one kg seeds of DCH 32 cultivar of cotton followed by seed treatment of talc formulation @ 4g, 6g, 8g and 10g/kg of seeds. Then pretreated seeds were used for sowing after shade drying for two hrs. Methods of application of antagonists for biological control were followed as explained by Anahosur (1998c) and Sankar and Jeyarajan (1996). Data on various parameters were analysed.

### 3.10.2. Soil application method

*T.harzianum* was selected based on efficiency of antagonism for their mass multiplication for further delivery to the field as soil formulation.

Soil formulation of *T.harzianum* was prepared in well-decomposed FYM. Molasses at two per cent solution in tap water was prepared and thoroughly mixed with substrate. Substrate was sterilized in polypropylene bags following procedure as explained earlier.

The formulation of *T.harzianum* was mixed in substrate mechanically under aseptic conditions and incubated for 30 days to induce multiplication. The soil formulation containing  $67.5 \text{ (cfu } 10^5 \text{ g}^{-1})$  was confirmed and applied to soil @ 1 per cent, 2 per cent, 3 per cent, 4 per cent w/w in pots (Raguchander *et al.*, 1993). Population of *T.harzianum* was assessed at 60 to 150 days after sowing on *Trichoderma* specific medium. The data were analysed.

### **3.11. STORAGE AND SHELF LIFE OF BIOCONTROL AGENTS**

#### **3.11.1. Effect of storage bag colour for mass multiplication of biocontrol agents in storage**

Studies were conducted to assess influence of storage bag colour on mass multiplication of biocontrol agents in storage on *T.harzianum*. Nine treatments having various coloured (black, blue, pink, red, transparent and yellow) polythene bags including brown paper packet and cloth bag were selected. Talc formulation was prepared as per the procedure suggested by Jeyarajan *et al.* (1994). They were filled in different bags under aseptic conditions and incubated at room temperature  $28 \pm 1^\circ$  C in open air condition to provide day light to the packets having talc based formulation of antagonists. Such packets were incubated for 75 days. Population was assessed after incubation in selective media by following serial dilution method (Sankaram, 1961). The population (cfu  $\times 10^6$  g<sup>-1</sup>) count was enumerated in each treatment. The data were analysed statistically.

#### **3.11.2 Studies on storage conditions and shelf life of biological control agents**

Freshly packed talc based formulation of native isolates of *T.viride* and *T. harzianum* was collected in milky white polythene bags and the mouth of the bag was closed by tying. Similarly, it was multiplied in wheat bran saw dust tap water (WBSD) (3:1:4 W/W/V) as per the method of Kousalya Gangadharan and Jeyarajan (1990). Medium was autoclaved at  $121.6^\circ$  C for 1 h for two successive days after packing 200 g medium in polypropylene bags. Inoculation of antagonist was done by injecting 25 ml spore suspension having  $2 \times 10^8$  cfu ml<sup>-1</sup> using sterile

syringe following the method suggested by Kousalya Gangadharan (1990). Packets containing native isolates of antagonists were stored as follows.

T1 – Storage at 0° C

T2 – Storage at 15° C

T3 – Storage in mist chamber at 15° C

T4 – Storage in earthen pot

T5 – Storage at room temperature

Storage in earthen pot was made by taking two earthen pots of one small and one big. Bigger pot was filled with boiled and cooled water upto quarter level and smaller earthen pot left floating in bigger pot and talc formulation and wheat bran saw dust formulation packets were kept inside the inner pot and covered with earthen lid. Water level was maintained. This treatment served as locally adoptable practice. It had temperature reduction by  $3 \pm 1^\circ$ . Gunny bags wrapped outside were kept wet by periodic sprinkling of boiled and cooled water. Population of antagonists was enumerated at an interval of 30, 60, 90 and 120 days after incubation in specified medium as explained earlier. The trial was laid out in three factorial completely randomized block design with three replications. The data on population ( $\text{cfu} \times 10^5 \text{ g}^{-1}$ ) were statistically analysed.

### **3.12. Evaluation of fungicides**

Eight fungicides were evaluated *in vitro* conditions at six concentrations (50, 100, 200, 500, 750 and 1000 ppm) against four test pathogens and test antagonist (*T. harzianum*). The trial was laid out in

two factorial CRD with three replications. The treatments were as follows. 72

#### Fungicides (Factor I)

Carbendazim, Carboxin, Thiram, Methoxy Ethyl Mercury Chloride, Captan, Copper-Oxy-Chloride, Benomyl, Mancozeb and Control.

Dosages (Factor 2): 50, 100, 200, 500, 750 and 1000 ppm. Poisoned food technique was adopted. Potato dextrose agar medium containing 50, 100, 200, 500, 750 and 1000 ppm was poured in sterile Petriplates and allowed to solidify. The plates were inoculated with 5 mm culture disc of 72 h old *T.harzianum*, *F.solani*, *R. solani*, *R.bataticola* and *S.rolfsii* in separate set of five experiments, was placed in the centre in all plates. Appropriate controls, were maintained in all the five sets of trials.

Percent inhibition was worked out for all main and sub treatments as per formula given by Vincent (1947). Per cent values pertaining to *T.harzianum*, *F.solani*, *R solani*, *R.bataticola* and *S.rolfsii* collected were analysed statistically after arc sin angular transformations.

#### 3.13. Evaluation of insecticides

Five insecticides were tested at six concentrations (50, 100, 200, 250, 500 and 750 ppm) on a.i. basis against *T. harzianum*, *F.solani*, *R solani*, *R.bataticola* and *S.rolfsii* using 'Poisoned food technique'.

Autoclaved and cooled potato dextrose agar medium was incorporated with (50, 100, 200, 250, 500 and 750 ppm of five insecticides (carbofuran, endosulfan, imidacloprid, chlorpyrifos and phorate) and allowed to solidify. Culture disc of 5 mm size of *T.harzianum* was inoculated in the centre of petriplates under aseptic conditions. The plates were incubated at room temperature  $28 \pm 1^\circ \text{C}$  for 72-96 h (till control plates achieved radial growth of 90 mm). Inhibition of mycelial growth of *T.harzianum*, *F.solani*, *R. solani*, *R.bataticola* and *S.rolfsii* over control was worked out using formula given by Vincent (1947). Per cent values were converted into angular arc sin transformations before statistical analysis.

### 3.14. Evaluation of herbicides

An experiment was conducted under *in vitro* conditions to know the effect of five weedicides on the growth of *T.harzianum*, *F.solani*, *R.solani*, *R.bataticola* and *S.rolfsii* in five separate sets of evaluations. Two factorial CRD with three replications was laid out with the following treatments.

#### Factor 1 Herbicides:

1. Alachlor, Roundup, Diuron, Stomp and Paraquat

**Factor 2** – Dosages : (50, 100, 200, 250, 500, 750 and 1000 ppm

Appropriate controls were used and PDA was a basal medium in these trials. Five herbicides were evaluated against *T.harzianum*, *F.solani*, *R.solani*, *R.bataticola* and *S.rolfsii* to assess the inhibition. Sterilized potato dextrose agar medium was poured in 90 mm sterile Petriplates to achieve the required concentration of test herbicides and

allowed to solidify. After solidification 5 mm culture disc *T.harzianum*, *F.solani*, *R. solani*, *R.bataticola* and *S.rolfsii* grown on PDA for 72 h was inoculated to respective sets of treatments in the centre of the Petriplate and incubated at room temperature  $28 \pm 1^\circ \text{C}$  for 72-96 h (till respective control growth reached 90 mm).

Observations were recorded on inhibition of hyphal growth in five separate trials. Percent values of inhibition were calculated. The data were analysed after conversion to arc sin angular transformations.

### **3.15. Evaluation of fungicides as seed dressers and soil drenching against pathogens.**

Since fungicides are recommended to manage the soil borne pathogens either as seed treatment or soil drenching and the present investigations are on four soil borne pathogens, eight fungicides viz. Carbendazim, Carboxin, Thiram, Methoxy Ethyl Mercury Chloride, Captan, Copper-oxy-chloride, Benomyl and Mancozeb were screened for their efficacy against *F.solani*, *R. solani*, *R.bataticola* and *S.rolfsii* as seed treatment @ 1g/kg and 2 g/kg for systemic and nonsystemic fungicides and soil drenching (@ 0.2%) separately for each pathogen DCH-32 was used as test genotype. Earthen pots of 4 inches diameter were filled with double sterilised soil and respective pathogen (grown on mass cultured medium) as presented earlier. After 48 h of inoculation in pots, fungicides treated seeds were sown in pots and for soil drenching seeds were not treated with fungicides was made (@0.2%) after sowing observations were recorded on pre-emergence and post emergence germination of seed. Three replications were maintained for each

treatment along with control. Per cent germination were converted into arc-sin values and analysed statistically.

### **3.16. Evaluation of organic amendments *T.harzianum*, and fungicides treatments for *F.solani*, *R. solani*, *R.bataticola* and *S.rolfsii***

The organic amendments play an important role in mechanism of biocontrol as biofungicides get required nutrients from these organic amendments. Further, some organic amendments also favour the pathogens. In this connection a pot culture experiment comprising of three separate trials for *F.solani*, *R. solani*, *R.bataticola* and *S.rolfsii* were conducted. Five commonly used organic amendments viz., FYM, groundnuts cake, neem cake, poultry manure, vermicompost were selected along with Captan seed treatment (@2g/kg) as a separate treatment and *T.harzianum*, as another treatment. Totally eight treatments were imposed for each of the pathogen. Per cent germination of cotton seeds in each treatment was recorded and was further converted into arc-sin values for statistical analysis.

#### **3.16.1 Evaluation of organic amendments *T.harzianum*, and fungicides treatments for *S.rolfsii***

The same five organic amendments tested for *F.solani*, *R.solani* and *R.bataticola*, were also tested for *S.rolfsii* and with also same other treatments but under field condition for two years i.e. during 1998-99 and 1999-2000. The field experiment was a randomized block design. Different parameters were considered like percent mortality, plant height, number of monopodia, sympodia, squares, fruiting points and yield

(kg/ha) were recorded for both years. The results were analysed statistically.

### **3.17. Effect of culture filtrates of *Trichoderma* and *Gliocladium* cultures on cotton seed germination.**

The culture filtrates of all twelve cultures of *Trichoderma* and two *Gliocladium* were obtained after 12 days of incubation. Seeds of two genotypes Jayadhar and Abhadita as fuzzed and delinted separately. They were immersed in culture filtrates of *Trichoderma* and *Gliocladium* separately for 24 hours and water blanks were used as control. The seeds were separated by decantation and were placed on sterilised moist chambers (Petridishes) with ten seeds for each moist chamber. After five days the percent germination was recorded.

### **3.18 Statistical analysis**

Statistical analyses were carried out wherever possible as per the procedure described by Panse and Sukhatme (1967) and presented in all the experimental results.

## *Experimental Results*

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## IV. EXPERIMENTAL RESULTS

### 4.1 Collection and isolation of fungi from wilted rotted cotton plants

A survey was conducted to collect the wilted or root rotting plants from cotton growing areas of Karnataka during post-rainy seasons of 1997 and 1998. Details of locations and genotypes are furnished under material and methods. Data are presented in Table 1 & Table 2 (Plate 1 & Plate 2).

It was found that mainly, *Fusarium solani* (Mart.) Sacc., *Rhizoctonia solani* Kuhn., *Rhizoctonia bataticola*(Taub.) Butler and *Sclerotium rolfsii* Sacc. were associated, while *Sclerotium rolfsii* was apparently negligible (maximum one per cent). Incidence of *F.solani*, *R.solani*, *R.bataticola* and *S.rolfsii* varied from location to location. The incidence of *F.solani* was higher (20%) than *R.solani* (6%). Specimens collected from different places had the mixtures of *Fusarium* and *Rhizoctonia* and *S.rolfsii* was not found occurring together with any fungi. Cotton crop in various locations under survey was 90 to 120 days old.

### 4.2 Identification of fungi isolated

*Fusarium solani*, *Rhizoctonia solani*, *Rhizoctonia bataticola* and *Sclerotium rolfsii* were identified based on their principal characters. Identity of *Fusarium solani* was further confirmed by Division of Mycology, Agharkar Research Institute, Pune, Maharashtra.

**Table 1: Percent Incidence of *Fusarium solani*, *Rhizoctonia solani*, *Rhizoctonia bataticola* and *Sclerotium rolfsii* at different cotton growing areas of Karnataka (Survey from 1997-98 to 1998-99)**

Sl. No.	Place & Districts	<i>F.solani</i>	<i>R.solani</i>	<i>R.bataticola</i>	<i>S.rolfsii</i>
1.	ARS, Dharwad (Dharwad)	2	1	0.5	Trace
2.	Bagalkot (Bagalkot)	3	-	-	-
3.	Baganandi (Mysore)	2	1	1	-
4.	Bailhongal (Belgaum)	2	Trace	-	-
5.	Dhanyal (Bijapur)	10	1	-	-
6.	Hirebagewadi (Dharwad)	5	2	1	-
7.	Kasaba Camp (Raichur)	20	5	2	-
8.	Mosarkunthe (Tumkur)	5	2	-	-
9.	Mundagod (Uttar Kannada)	5	1	-	-
10.	Naregal (Gadag)	5	2	1	-
11.	Navalgund (Dharwad)	10	2	1	1
12.	Ranebennur (Haveri)	5	1	-	-
13.	Ron (Gadag)	20	5	1	-
14.	Saundatti (Belgaum)	2	Trace	-	Trace
15.	Siruguppa (Bellary)	5	2	-	-
16.	Yelburga (Koppal)	20	6	1	-

**Table 2: List of pathogenic isolates collected from different parts of Karnataka.**

Sl. No.	Code used	Isolate/Spp	Locality
1.	F <sub>1</sub>	<i>Fusarium solani</i>	ARS, Dharwad (Dharwad)
2.	F <sub>2</sub>	<i>Fusarium solani</i>	Kasaba Camp (Raichur)
3.	F <sub>3</sub>	<i>Fusarium solani</i>	Hirebagewadi (Dharwad)
4.	F <sub>4</sub>	<i>Fusarium solani</i>	Navalgund (Dharwad)
5.	F <sub>5</sub>	<i>Fusarium solani</i>	Naregal (Gadag)
6.	F <sub>6</sub>	<i>Fusarium solani</i>	Baganandi (Mysore)
7.	F <sub>7</sub>	<i>Fusarium solani</i>	Mosarkunthe (Tumkur)
8.	F <sub>8</sub>	<i>Fusarium solani</i>	Ron (Gadag)
9.	F <sub>9</sub>	<i>Fusarium solani</i>	Siruguppa (Bellary)
10.	Rs	<i>Rhizoctonia solani</i>	Navalgund (Dharwad)
11.	Rb	<i>Rhizoctonia bataticola</i>	ARS, Dharwad Farm
12.	Sr	<i>Sclerotium rolfsii</i>	ARS, Dharwad Farm

## Fusarium wilt incidence of cotton : Field views



**Plate 1** : Kasabe camp, Raichur



**Plate 2** : ARS Dharwad Farm

#### 4.2.1 *Fusarium solani*

Aerial mycelium was sparse to dense, cottony white to pale white on potato dextrose agar (Table 3, Plate 3 & Plate 4). Microconidia were ostiole truncate; spores 1-celled, hyaline, somewhat oval, elongate-cylindrical, 16-30 x 5-10 $\mu$ .

#### 4.2.3 *Rhizoctonia solani*

Colonies were fast growing (Table 3 & Plate 5), at first colourless, submerged or with some radiating aerial hyphae, rapidly becoming brown. Hyaline to pale brown, thick-walled cells about 98-200 x 8-11 $\mu$ m often constricted near septa and where branching, with conspicuous dolipore septa, lacking clamp-connections, branched at wide angles (nearly 90°) often anastomosing. Monilioid hyphae upto 30 $\mu$ m wide commonly present. Sclerotia developing from irregular agglomerations of uniform moniliform hyphae, irregular in outline, solitary, about one mm in diameter, sometimes confluent to form conspicuous crusts and soon turning brown.

#### 4.2.4 *Rhizoctonia bataticola*

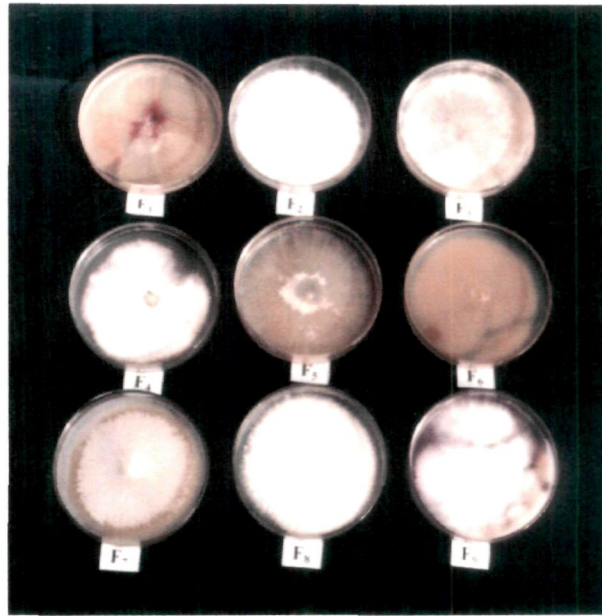
Pycnidia separate, with a single ostiole, wall thin, with a pigmented surface, sometimes with a sclerotoid base. Conidiogenous cells undifferentiated from inner cell walls; conidium formation phialidic; ostiole truncate; spores 1-celled, hyaline, somewhat oval, elongate-cylindrical, 16-30 x 5-10 $\mu$ .

**Table 3: Colony characters of *Fusarium solani* isolates, *Rhizoctonia solani*, *Rhizoctonia bataticola* and *Sclerotium rolfsii***

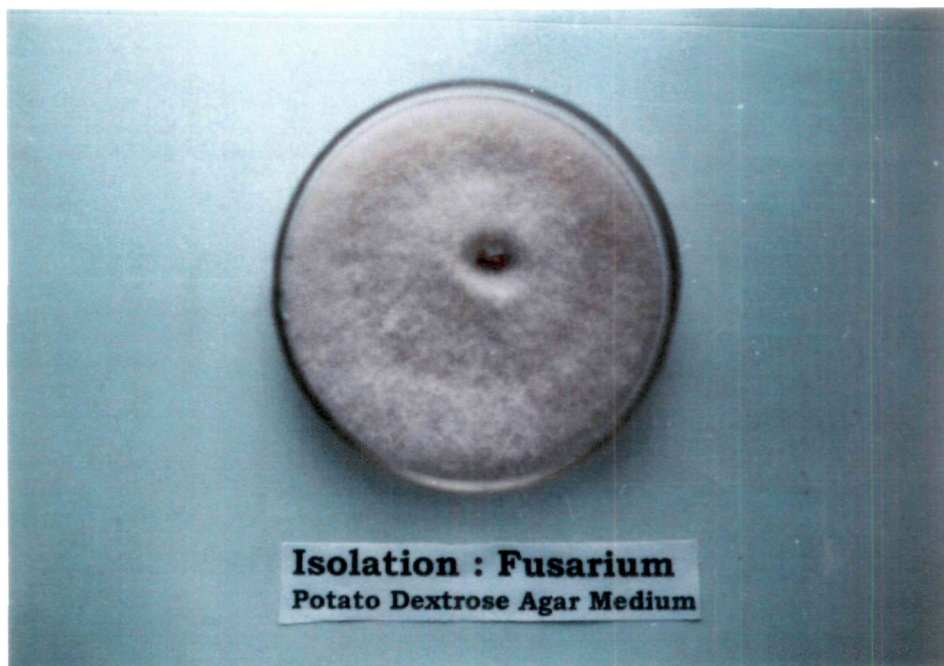
Sl. No.	Code	Genotypes	Colony characters	Sporulation
1.	F <sub>1</sub>	DCH - 32	Dark pinkish thin layer of growth moderate grower.	+++
2.	F <sub>2</sub>	DHH - 11	Light violet, profuse mycelial growth moderate grower.	+
3.	F <sub>3</sub>	DCH - 32	Creamy white, profuse mycelial growth fast grower.	++++
4.	F <sub>4</sub>	DHB - 105	Creamy white, profuse mycelial growth moderate grower .	+
5.	F <sub>5</sub>	DCH - 32	Creamy white, profuse growth in center layers in margin.	+
6.	F <sub>6</sub>	DCH - 32	Dull white, shining, fast grower.	++
7.	F <sub>7</sub>	DCH - 32	Grayish white, zigzag margin slow grower.	+++
8.	F <sub>8</sub>	DCH - 32	Creamy whitish colony, highly profuse growth, fast grower.	+
9.	F <sub>9</sub>	CPD - 423	Pinkish white, sparse but profuse growth .	++++
10.	Rs.	NHH - 44	Light brownish, fast grower.	--
11.	Rb.	NHH - 44	Dark coloured colonies, slow grower.	--
12.	Sr.	DHH -11	Fast grower, sclerotia are seen 4-5 days after inoculation.	--

Sporulation: + = Low < 10: ++ = Medium (10-25): +++ = Good (25-50): ++++ = Heavy sporulation (>50 spores per microscopic field).

## Morphological colony characters of Pathogens



**Plate 3 :** *Fusarium solani* cultures



**Plate 4 :** Colony character of *Fusarium solani*

Colonies were fast growing, at first colourless, submerged or with some radiating aerial hyphae, rapidly becoming brown. Hyaline to pale brown, thick-walled cells about 98-200 x 8-11  $\mu$ m often constricted near septa and where branching, with conspicuous dolipore septa, lacking clamp-connections, branched at wide angles (nearly 90°) often anastomosing. Monilioid hyphae upto 30  $\mu$ m wide commonly present. Sclerotia developing from irregular agglomerations of uniform moniliform hyphae, irregular in outline, solitary, about one mm in diameter, sometimes confluent to form conspicuous crusts, soon turning brown (Table 3 and Plate 4).

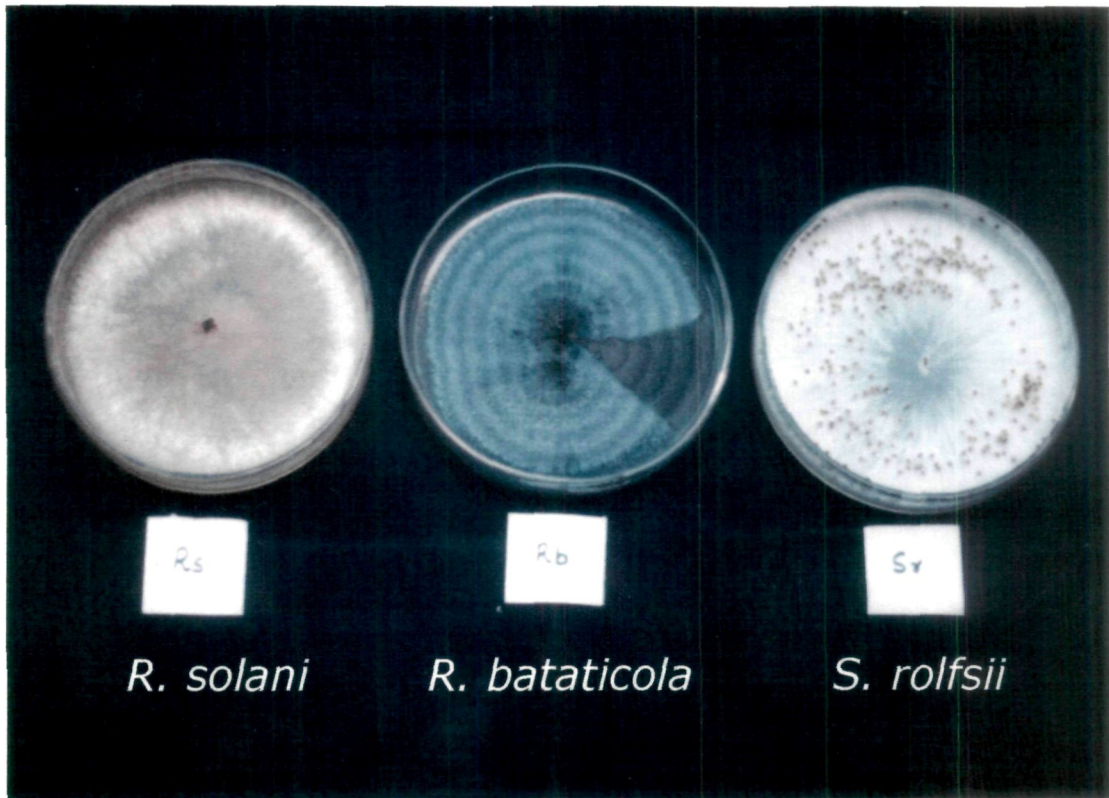
**4.2.5 *Sclerotium rolfsii***

Colonies were very fast growing showing white hyphal strand (Table 3 & Plate 4). Primary conductive hyphae were superficial and 4.5 to 9.0  $\mu$ m wide generally bearing clamp connection at widely spaced secondary and tertiary hyphae were narrower. Sclerotia were produced superficial but abundantly near the colony margin. They were globose, brown coloured and 1-1.2 mm diameter.

**4.3 Pathogenicity**

Pathogenicity tests of *Fusarium solani*, *Rhizoctonia solani*, *Rhizoctonia bataticola* and *Sclerotium rolfsii* were carried out under the glass house conditions (Plate 6 and Plate 7) as described in material and methods. Details of the above ground and underground symptoms

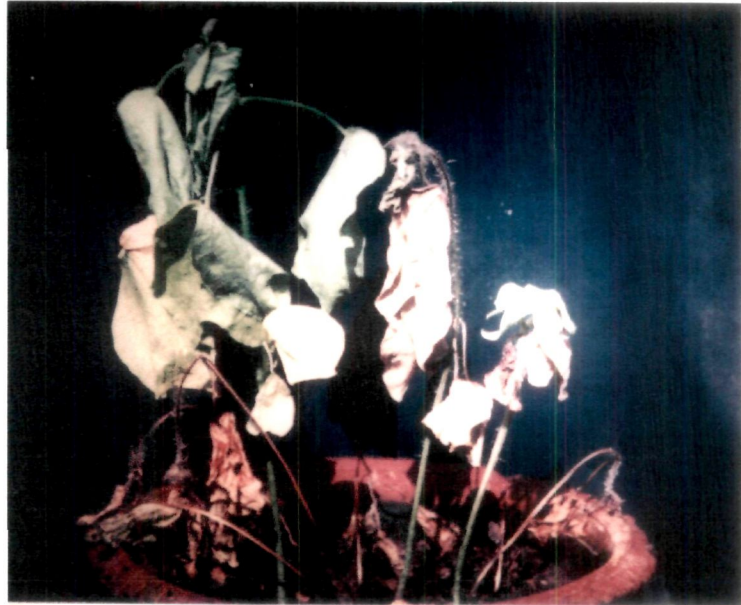
Morphological colony characters of  
*Rhizoctonia solani*, *Rhizoctonia*  
*bataticola* and *Sclerotium rolfsii*



**Plate 5**

# Pathogenicity Studies

**Plate 6 :** Pathogenicity of *Fusarium solani*



**Plate 7 :** Pathogenicity studies on *R. solani*, *R. bataticola* and *S. rolfsii*

expressed on plants were recorded. Reisolations from collar and root portions of plants were made and pathogenic cultures obtained were compared with original ones.

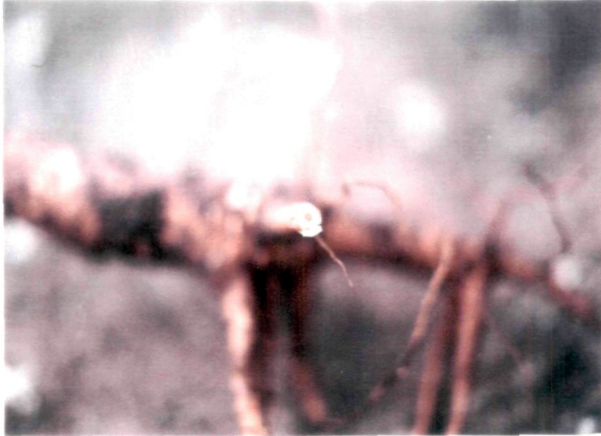
#### **4.4 Symptoms caused by various fungi**

Critical studies by visual observations of various wilting cotton plants and isolations were made during crop seasons of 1998 and 1999 at various stages of crop growth under irrigated and rainfed conditions. Studies revealed that, often wilting plants showed complex symptoms and association of more than one pathogen. However, it was observed that, generally plants expressed typical symptoms caused by a particular pathogen. Such typical symptoms, observed under the field conditions, were confirmed by the pathogenicity test under glass house conditions. The symptoms caused by different pathogens are described as under.

##### **4.4.1 *Fusarium solani***

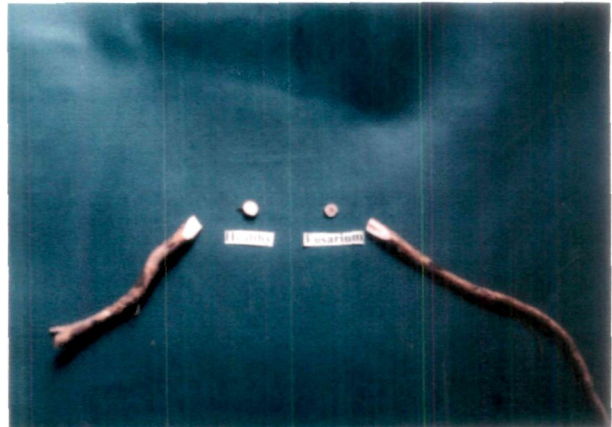
Initially, the distinctive sign of root invasion was dark brownish appearance over the entire surface of the root or in the form of localized streaks. Later, the taproot and underground part of stem base showed shriveling symptoms and became black (Plate 8, Plate 9 and Plate 10). Vascular wilt type of symptoms were observed 25 days after sowing. Initially, very few plants showed symptoms, but it was more evident after 45 days after sowing. Wilting plants expressed the drooping and flaccidity of leaves extending up to delicate rachis. Collar portion appeared somewhat narrow due to shrinkage. Generally no external rotting of root portion could be noticed, but affected plants showed internal browning of xylem and pith region invariably (Plate 9). As the

# Cross section views of cotton root with brownish discoloration by *Fusarium solani*



**Plate 8 :** Diseased root

**Plate 9 :** Diseased root in comparison with healthy root



**Plate 10 :** Longitudinal sectional view of roots affected by *Fusarium solani*

disease advanced, leaves turned to yellow and some what straw-coloured. Intensity of discolouration of internal portion decreased towards the stem portion. Fungus attacked root and base of the stem. There was extensive damage to lateral roots, which showed rotting symptoms (Plate 10).

#### **4.4.2 *Rhizoctonia solani* and *Rhizoctonia bataticola***

Root rot of cotton occurred 45 days after sowing and more towards flowering stage and its incidence increased with increasing growth stage of crop upto the harvesting stage. Whole plant dried without showing conspicuous flaccidity symptoms. Leaves, alongwith petioles, branches and stem became straw-coloured due to drying. Upon uprooting (Plate 11), affected plants indicated darkening of roots and shredding of bark. Roots showed dry root rot symptoms. Roots appeared brittle and could be broken easily. Minute black-coloured sclerotial bodies were present inside the shredded bark as well as in pith portion.

#### **4.4.3 *Sclerotium rolfsii***

The occurrence of collar rot was observed in the seedling stage. However, pre emergence attack on germinating seed was quite common. Initially, lower leaves showed yellowing and drying symptoms followed by death of entire plant. Rotting of collar portion progressed downwards (Plate 12) and leading to death of plant.

Plants attacked by *Sclerotium rolfsii* were characterized by a visible mat of white mycelium over the surface of the collar portion near the surface of the soil. Later on, mustard seed- like brown sclerotia were formed and intermingled with white mycelium, particularly at the

# Disease symptoms

**Plate 11 :** *Rhizoctonia bataticola*



**Plate 12 :** *Sclerotium rolfsii*

advanced stage of the disease. Roots did not show any darkening symptoms. The bark underneath the mycelium was decayed, but central portion remained undecayed and often plants remained erect after death.

#### **4.5 Association of fungi with cotton rhizosphere**

During isolation of soil borne pathogens from cotton rhizosphere different fungi were also found associated and very commonly occurring are presented in Table 4. These fungi were identified by Division of Mycology, Agharkar Research Institute, Pune, Maharashtra.

#### **4.6 Variability and Virulence studies on *Fusarium solani***

Variations were noticed among the nine isolates of *Fusarium solani* with respect of colony characters and sporulations (Plate 13). The morphological colony characters are presented in Table 3. These isolates also differed with respect to their virulence (Table 5 and Figure 1). Among the isolates F<sub>2</sub> (Raichur), F<sub>4</sub> (Navalagund) and F<sub>5</sub> (Gadag) with mean per cent death of seedlings of 90.47, 66.67 and 66.67 respectively and were more virulent than the remaining isolates were less virulent i.e., less than 66.67 per cent death of seedlings. In view of these, F<sub>2</sub> was selected for further studies.

Since there was only one isolate of each of *Rhizoctonia solani*, *Rhizoctonia bataticola* and *Sclerotium rolfsii*, hence the root rot causing fungi were selected for further studies. However, the percentage rotting of each of these was also taken into consideration before proceeding for further investigation (Table 5.)

**Table 4: List of commonly associated fungi in cotton *Rhizosphere***

Sl. No.	Name
1.	<i>Eurotium amstelodi</i>
2.	<i>Aspergillus versicolor</i>
3.	<i>Chaetomium globosum</i>
4.	<i>Aspergillus flavus</i>
5.	<i>Botrydiplodia theobromae</i>
6.	<i>Pencillium oxalicum</i>
7.	<i>Drechslera state of Cochliobolus spicifer</i>

**Table 5: Percent wilting and rotting of cotton plants by different isolates of *Fusarium.solani* *Rhizoctonia solani*, *Rhizoctonia bataticola* and *Sclerotium rolfsii* at different levels of inoculation**

Sl. No.	Isolate	Percent Inoculation levels			Mean
		1	2	4	
	<b>A. Wilting</b>				
1.	F <sub>1</sub>	57.14	57.14	71.43	60.90
2.	F <sub>2</sub>	85.71	85.71	100.00	90.47
3.	F <sub>3</sub>	42.86	57.14	71.43	57.14
4.	F <sub>4</sub>	57.14	57.14	100.00	66.67
5.	F <sub>5</sub>	71.43	57.14	71.43	66.67
6.	F <sub>6</sub>	57.14	57.14	71.43	60.90
7.	F <sub>7</sub>	42.86	71.43	71.43	61.90
8.	F <sub>8</sub>	42.86	42.86	71.43	52.38
9.	F <sub>9</sub>	57.14	57.14	71.43	60.90
	<b>B. Rotting</b>				
10.	<i>R. solani</i>	57.14	71.43	71.43	66.67
11.	<i>R.bataticola</i>	42.86	71.43	71.43	61.90
12.	<i>S.rolfsii</i>	71.43	100.00	100.00	90.47

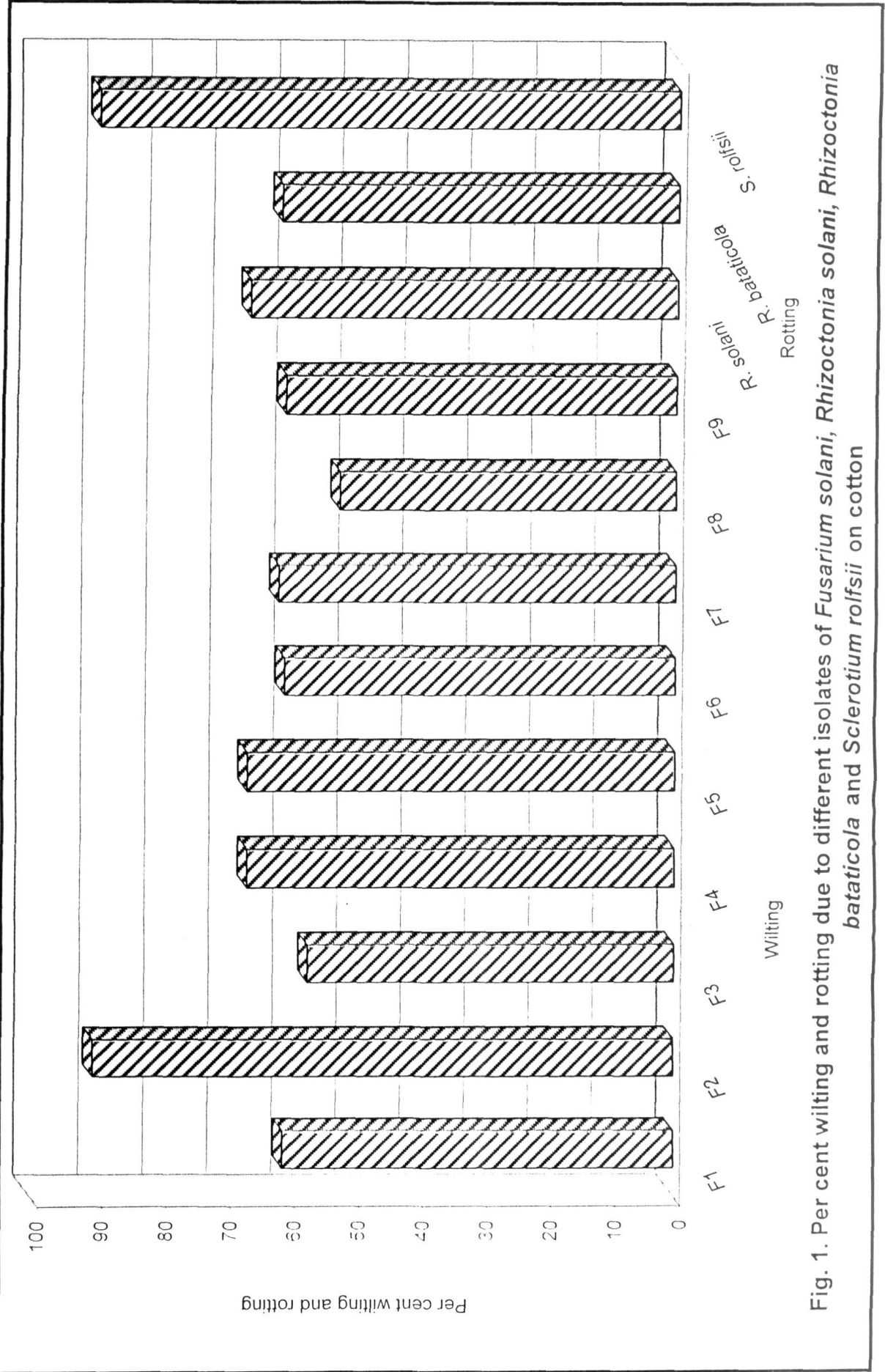


Fig. 1. Per cent wilting and rotting due to different isolates of *Fusarium solani*, *Rhizoctonia solani*, *Rhizoctonia bataticola* and *Sclerotium rolfsii* on cotton

## Virulence studies on *F. solani*



**Plate 13**

#### 4.7. Isolates of *Trichoderma* spp. and *Gliocladium virens*

Twelve *Trichoderma* cultures and two *Gliocladium virens* (Table 6, Table 6a, Plate 14, Plate 15 and Plate 16) were used in the study. These cultures were grown on Potato Dextrose Agar medium as mentioned in material and methods. The colony characters and sporulation were also recorded (Table 6a).

#### 4.8. Interaction between isolates of *Trichoderma* spp. *Gliocladium virens* and *Fusarium solani*, *Rhizoctonia solani*, *Rhizoctonia bataticola* and *Sclerotium rolfsii*:

This investigation was important to know the reaction between the isolates of *Trichoderma*, *Gliocladium* and soil borne pathogens viz., *Fusarium solani*, *Rhizoctonia solani*, *Rhizoctonia bataticola* and *Sclerotium rolfsii* in order to rank the best biofungicide for further studies. The per cent inhibition was recorded and presented in Table 7 and Plates No. 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 and 30. Treatment (T<sub>8</sub>), *Trichoderma harzianum* Rifai. of ARS, Dharwad was the best which could inhibit on an average of 51.77 per cent inhibition followed by T<sub>7</sub> (*Trichoderma longibrachteum* 49.31 per cent of TNAU, Coimbatore) and other isolates of *Trichoderma*. Among *Gliocladium* cultures G<sub>2</sub> (CICR, Sirsa) was the best with percent inhibition of 47.01 and G<sub>1</sub> (TNAU, Coimbatore) was on par (46.03%). Further among *F. solani*, F<sub>2</sub> (Raichur), F<sub>7</sub> (Tumkur), F<sub>4</sub> (Navalagund), F<sub>3</sub> (Hirebagewadi, Dharwad), F<sub>1</sub> (ARS, Dhawad farm) and F<sub>6</sub> (Baganandi, Mysore) had more than 45 per cent inhibition (mean), i.e., 48.91, 47.41, 46.38, 47.29, 45.97 and 45.11 (mean values) (Table 7) respectively. However F<sub>8</sub> (Ron, Gadag) and F<sub>9</sub> (Siruguppa, Bellary) were least in

**Table 6: List of isolates of *Trichoderma* species and *Gliocladium virens* used in the investigation.**

Sl. No.	Code used	Species/ Isolate	Sources
1.	T <sub>1</sub>	<i>T.harzianum</i>	TNAU, Coimbatore
2.	T <sub>2</sub>	<i>T.harzianum</i>	UAS, Bangalore
3.	T <sub>3</sub>	<i>T.viride</i>	UAS, Bangalore
4.	T <sub>4</sub>	<i>T.hamatum</i>	TNAU, Coimbatore
5.	T <sub>5</sub>	<i>T.koningi</i>	TNAU, Coimbatore
6.	T <sub>6</sub>	<i>T.pseudokoningi</i>	TNAU, Coimbatore
7.	T <sub>7</sub>	<i>T.longibrachetum</i>	TNAU, Coimbatore
8.	T <sub>8</sub>	<i>T.harzianum</i>	ARS, Dharwad Farm
9.	T <sub>9</sub>	<i>T.viride</i>	TNAU, Coimbatore
10.	T <sub>10</sub>	<i>T.pseudokoningi</i>	Agharkar Research Institute, Pune
11.	T <sub>11</sub>	<i>T.viride</i>	Agharkar Research Institute, Pune
12.	T <sub>12</sub>	<i>T.reesai</i>	NCIM, Pune
13.	G <sub>1</sub>	<i>Gliocladium virens</i>	TNAU, Coimbatore
14.	G <sub>2</sub>	<i>Gliocladium virens</i>	CICR, Sirsa

**Table 6a: Cultural and morphological characters of isolates of *Trichoderma* spp. Used in investigation**

Sl. No.	Species/Isolate	Colour	Colony growth	Sporulation
T <sub>1</sub>	<i>T.harzianum</i>	Yellow light greenish	Dark green spores with whitish mycelial growth	+++
T <sub>2</sub>	<i>T.harzianum</i>	White	Ash green with less mycelial growth like actinomycetes	+++
T <sub>3</sub>	<i>T.viride</i>	Medium yellow	Whitish mycelium with dark green growth	+++
T <sub>4</sub>	<i>T.hamatum</i>	Dark yellow	Dark green with almost slow mycelial growth	++++
T <sub>5</sub>	<i>T.koningi</i>	Whitish yellow	Fast grower with whitish mycelium followed by late greenish growth	++
T <sub>6</sub>	<i>T.pseudokoningi</i>	Dark yellow	Whitish mycelial growth with almost nil sporulation	+
T <sub>7</sub>	<i>T.longibrachetum</i>	Medium yellow	Sparse growth with dark green sporulation	++
T <sub>8</sub>	<i>T.harzianum</i>	Whitish yellow	Slow T <sub>5</sub> whitish mycelium yellow with more spores (greenish than T <sub>5</sub> )	+++
T <sub>9</sub>	<i>T.viride</i>	Dark yellow	Very dark greenish with whitish mycelium	++
T <sub>10</sub>	<i>T.pseudokoningi</i>	Medium yellow	Heavy sporulation with whitish mycelium growth	++++
T <sub>11</sub>	<i>T.viride</i>	White	Light yellow mycelial growth slow growth	++
T <sub>12</sub>	<i>T.reesai</i>	Light yellow	Light yellow mycelial growth.	++

Sporulation: + = Low (< 10): ++ = Medium (10-25): +++ = Good (25-50): ++++ = Heavy sporulation (>50 spores per microscopic field).

**Table 7: Percent inhibition of *Fusarium solani*, *Rhizoctonia solani*, *Rhizoctonia bataticola* and *Sclerotium rolfsii* by bioagents**

Sl. No.	Pathogens	Bioagents														Mean
		T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	G1	G2	
1.	F <sub>1</sub>	34.82 (32.58)	48.04 (55.33)	47.93 (55.08)	38.65 (38.97)	50.77 (59.99)	49.95 (58.58)	49.43 (57.57)	38.06 (38.02)	45.97 (51.70)	43.45 (47.34)	48.56 (56.18)	51.12 (60.62)	49.43 (57.71)	47.47 (54.29)	45.97 (51.71)
2.	F <sub>2</sub>	40.22 (55.62)	45.92 (51.63)	44.31 (48.78)	51.41 (61.08)	50.18 (58.94)	49.08 (57.08)	51.47 (61.24)	54.76 (66.72)	42.71 (45.99)	40.18 (55.35)	49.08 (57.14)	50.59 (59.65)	51.12 (60.63)	47.87 (55.05)	48.91 (56.77)
3.	F <sub>3</sub>	51.06 (60.51)	26.92 (20.52)	48.45 (55.91)	47.70 (54.68)	52.36 (62.73)	48.50 (56.08)	52.83 (63.49)	54.51 (66.29)	50.94 (60.33)	38.12 (38.05)	44.20 (48.63)	50.24 (59.06)	48.45 (56.01)	47.47 (54.27)	47.29 (54.04)
4.	F <sub>4</sub>	47.41 (54.24)	48.10 (55.39)	40.63 (42.40)	47.47 (54.32)	50.77 (59.93)	44.26 (48.70)	52.30 (62.64)	53.49 (64.59)	44.91 (49.88)	40.40 (42.03)	45.46 (50.81)	48.16 (55.49)	43.28 (47.01)	42.71 (46.02)	46.38 (52.39)
5.	F <sub>5</sub>	52.30 (62.54)	47.12 (53.69)	44.43 (48.90)	47.07 (44.85)	51.06 (60.49)	49.20 (57.32)	43.11 (46.73)	51.53 (61.27)	42.88 (46.31)	44.43 (48.98)	23.03 (15.27)	31.11 (26.72)	46.78 (43.07)	44.43 (49.03)	43.97 (48.23)
6.	F <sub>6</sub>	52.54 (62.91)	26.28 (19.59)	43.39 (47.24)	47.47 (54.30)	48.91 (56.81)	45.97 (51.67)	51.24 (60.80)	54.15 (59.68)	41.21 (43.36)	42.82 (46.16)	44.60 (49.30)	47.41 (44.21)	44.54 (49.21)	43.74 (47.78)	45.11 (50.22)
7.	F <sub>7</sub>	52.71 (63.26)	46.09 (51.86)	46.55 (52.68)	48.16 (55.51)	45.46 (50.78)	46.83 (53.16)	51.30 (60.89)	52.42 (65.68)	43.34 (47.11)	45.80 (51.37)	47.93 (55.11)	45.46 (50.77)	44.48 (49.11)	45.86 (51.54)	47.41 (54.20)
8.	F <sub>8</sub>	49.66 (58.12)	27.13 (20.82)	47.52 (54.36)	45.40 (50.69)	49.20 (57.29)	33.83 (31.02)	52.89 (63.63)	55.06 (62.84)	31.56 (27.39)	42.53 (45.65)	13.94 (5.82)	11.68 (4.12)	47.64 (54.59)	51.30 (60.87)	40.80 (42.66)
9.	F <sub>9</sub>	41.15 (43.30)	32.08 (28.18)	44.54 (49.20)	39.64 (40.74)	43.28 (46.90)	37.70 (37.40)	42.88 (46.27)	47.75 (50.11)	36.63 (35.55)	42.13 (45.01)	36.09 (34.72)	46.55 (51.68)	40.86 (42.81)	47.01 (53.47)	41.09 (43.24)
10.	R <sub>s</sub>	40.86 (42.78)	43.28 (46.98)	38.53 (38.78)	42.71 (46.03)	44.43 (49.04)	45.34 (50.64)	44.94 (49.85)	48.75 (54.83)	40.74 (42.62)	43.68 (47.73)	42.13 (45.00)	42.53 (45.72)	41.90 (44.64)	45.92 (51.63)	43.22 (46.88)
11.	R <sub>b</sub>	47.52 (54.39)	41.38 (43.72)	45.06 (50.11)	41.55 (44.00)	43.85 (48.04)	46.03 (51.83)	44.60 (49.34)	52.89 (63.55)	41.38 (43.65)	42.42 (45.50)	42.71 (45.97)	45.80 (51.39)	41.38 (43.74)	47.93 (55.12)	44.60 (49.31)
12.	<i>S.rolfsii</i>	26.57 (19.93)	34.63 (32.28)	40.86 (42.84)	25.47 (18.54)	56.30 (66.30)	36.27 (34.93)	55.24 (67.51)	68.61 (86.71)	48.33 (55.78)	36.63 (35.62)	41.55 (43.90)	48.91 (56.78)	52.18 (62.44)	52.54 (63.00)	44.43 (49.04)
	Mean	45.52 (50.85)	39.23 (40.00)	44.37 (48.85)	43.28 (46.97)	44.44 (56.44)	44.43 (49.03)	49.31 (57.50)	51.77 (61.69)	42.59 (45.81)	42.53 (45.73)	40.57 (42.32)	43.85 (48.02)	46.03 (51.75)	47.01 (53.51)	44.94 (49.89)

Note : Figures in the parenthesis are the arc-sin values

SEM ± : 0.577

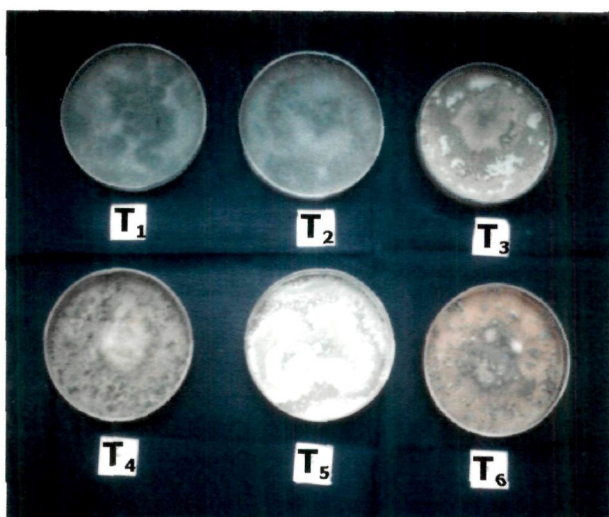
CD at 1% Bioagent : 1.91

CD at 1% Pathogen : 2.08

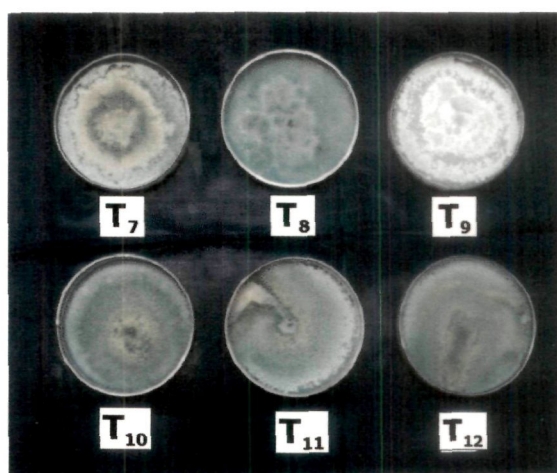
CD at 1% Bioagent V/s Pathogen : 1.60

CV % : 2.01

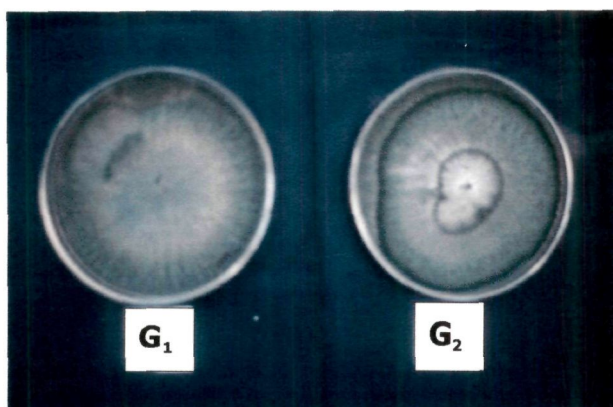
# Morphological colony characters of Biofungicides



**Plate 14 :** *Trichoderma* isolates



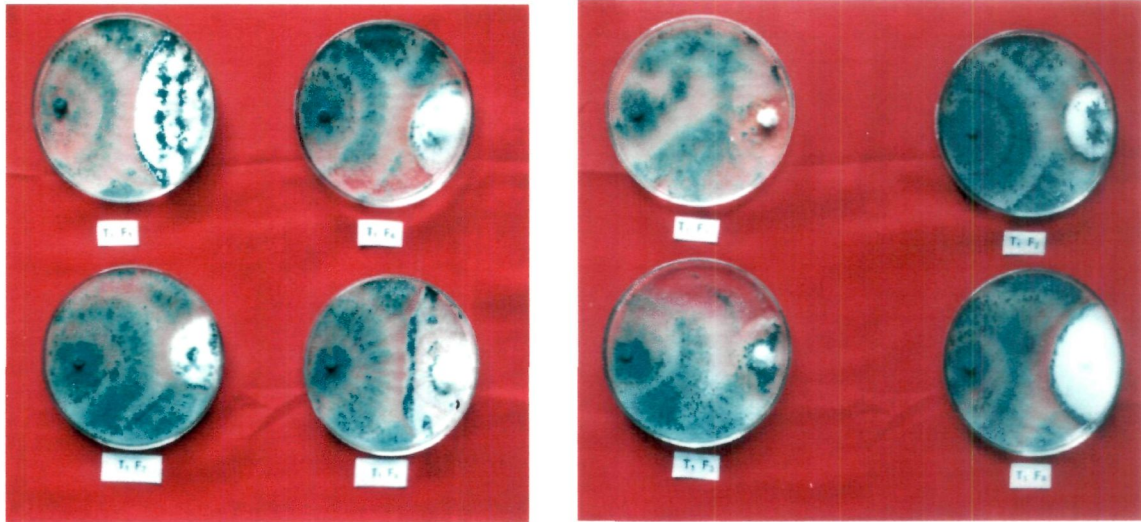
**Plate 15 :** *Trichoderma* isolates



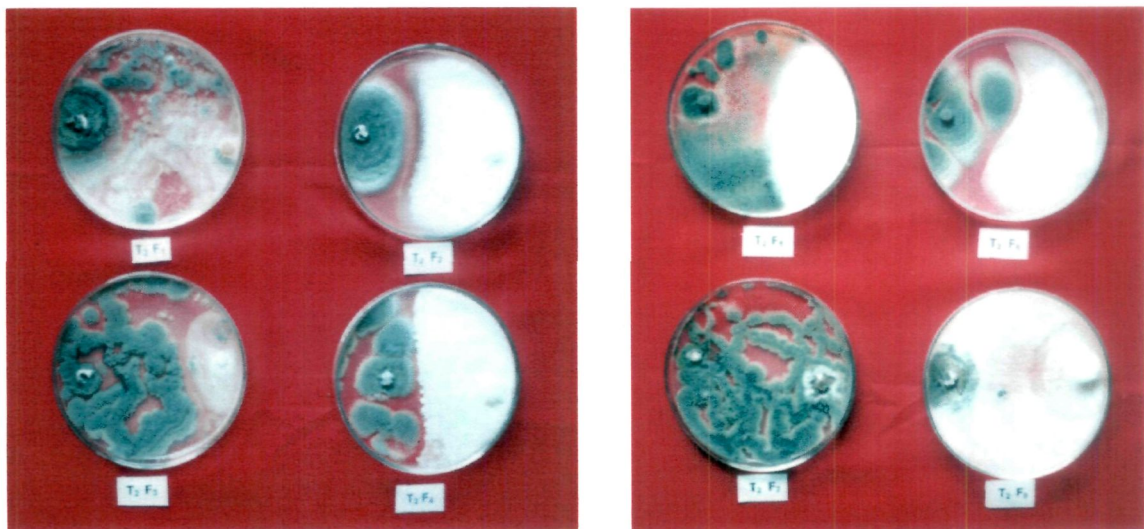
**Plate 16 :** *Gliocladium* isolates

# Interaction Studies of *Trichoderma* Isolates with Soil Borne Pathogens

*Trichoderma* Vs. *Fusarium* Isolates



**Plate 17 :** T1 with *Fusarium* Isolates



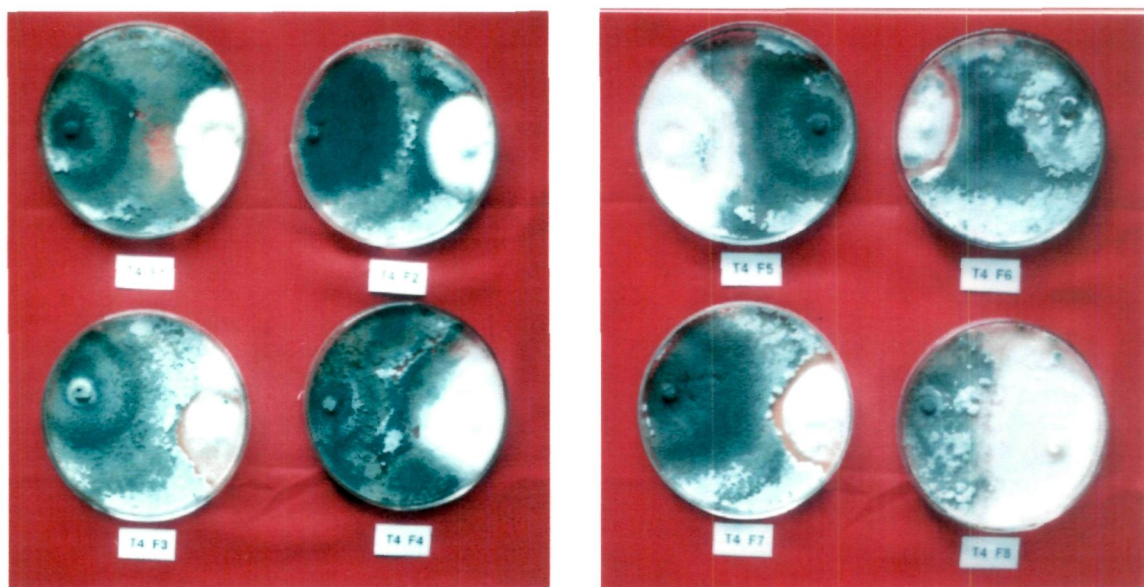
**Plate 18 :** T2 with *Fusarium* Isolates

# Interaction Studies of *Trichoderma* Isolates with Soil Borne Pathogens

*Trichoderma* Vs. *Fusarium* Isolates



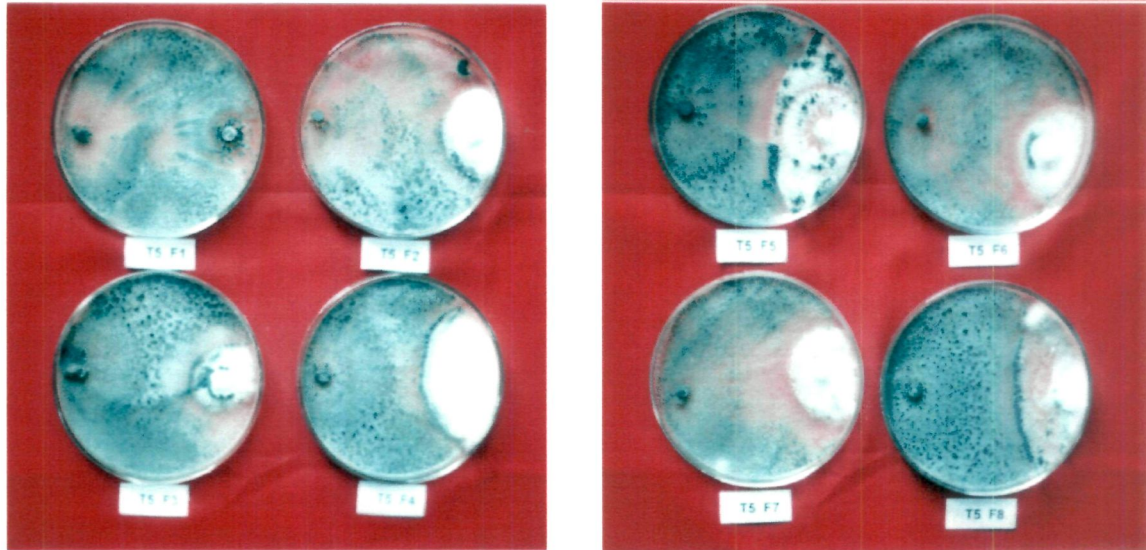
**Plate 19 :** T3 with *Fusarium* Isolates



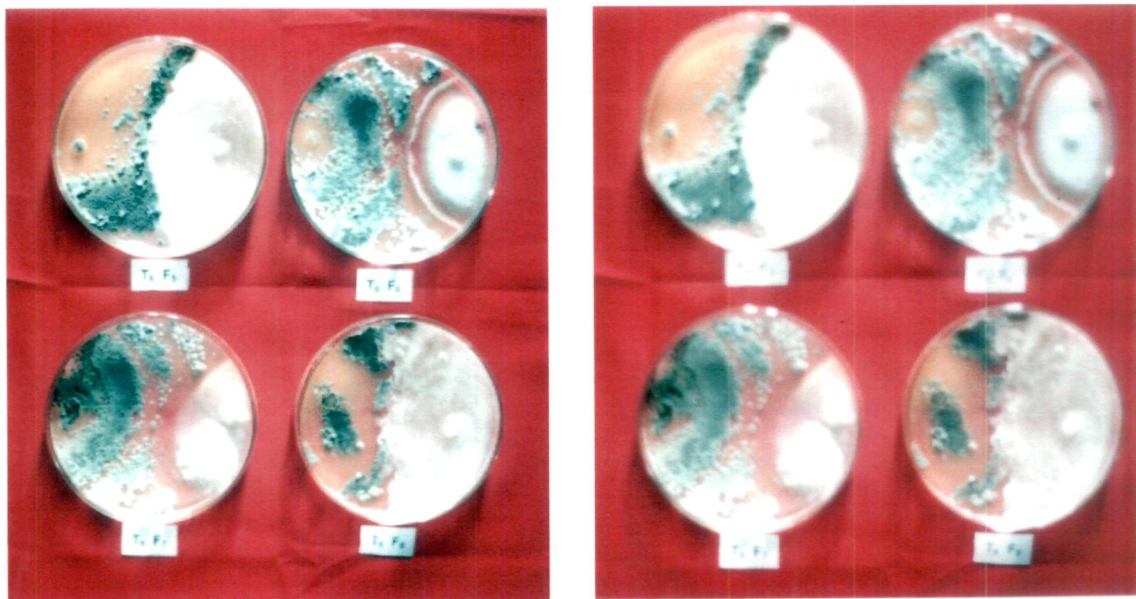
**Plate 20 :** T4 with *Fusarium* Isolates

# Interaction Studies of *Trichoderma* Isolates with Soil Borne Pathogens

*Trichoderma* Vs. *Fusarium* Isolates



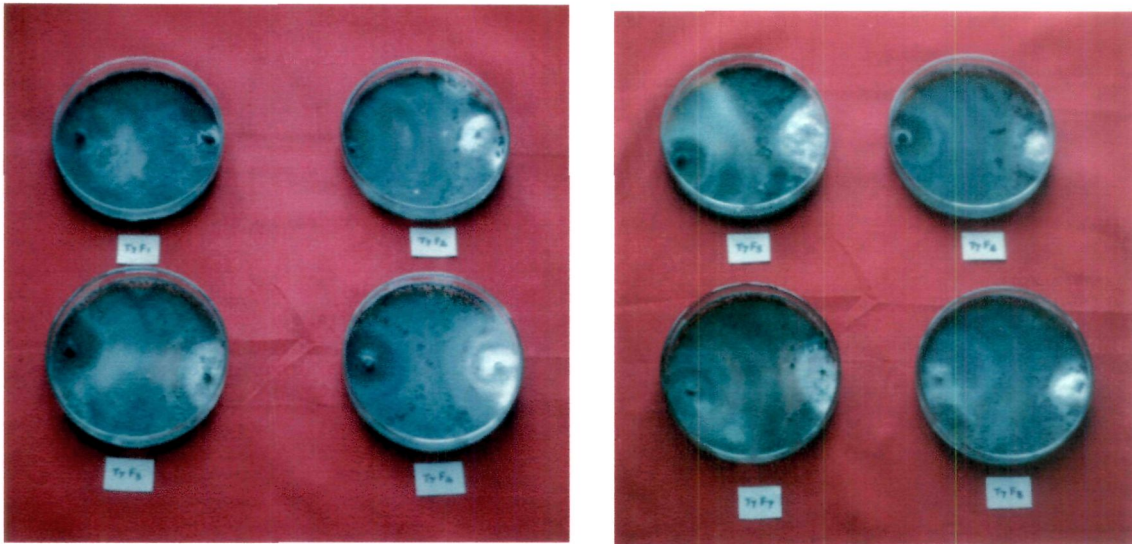
**Plate 21** : T5 with *Fusarium* Isolates



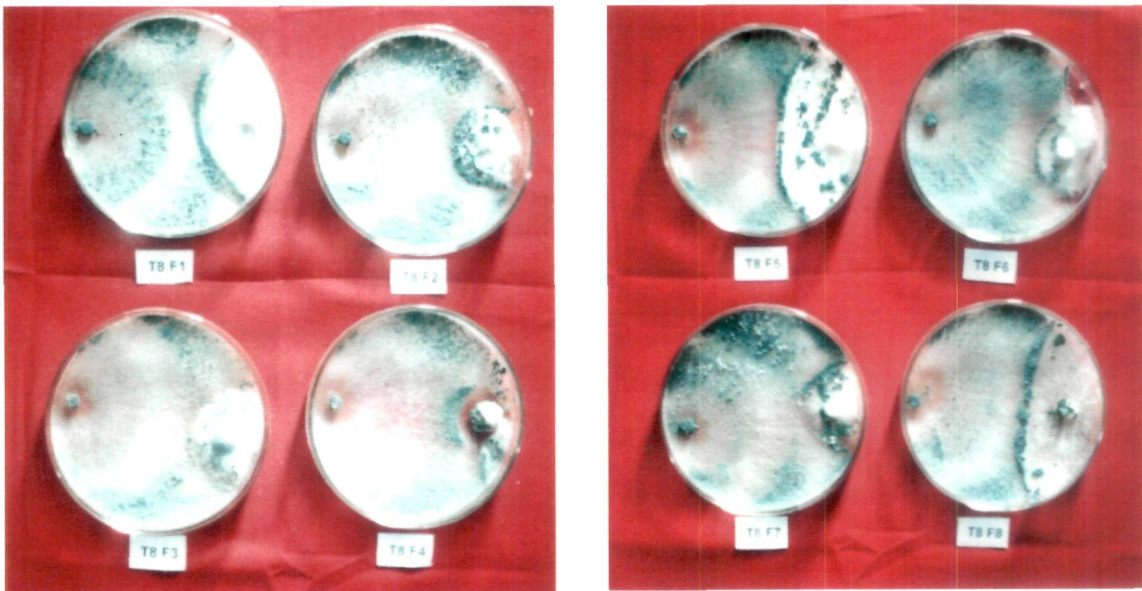
**Plate 22** : T6 with *Fusarium* Isolates

# Interaction Studies of *Trichoderma* Isolates with Soil Borne Pathogens

*Trichoderma* Vs. *Fusarium* Isolates



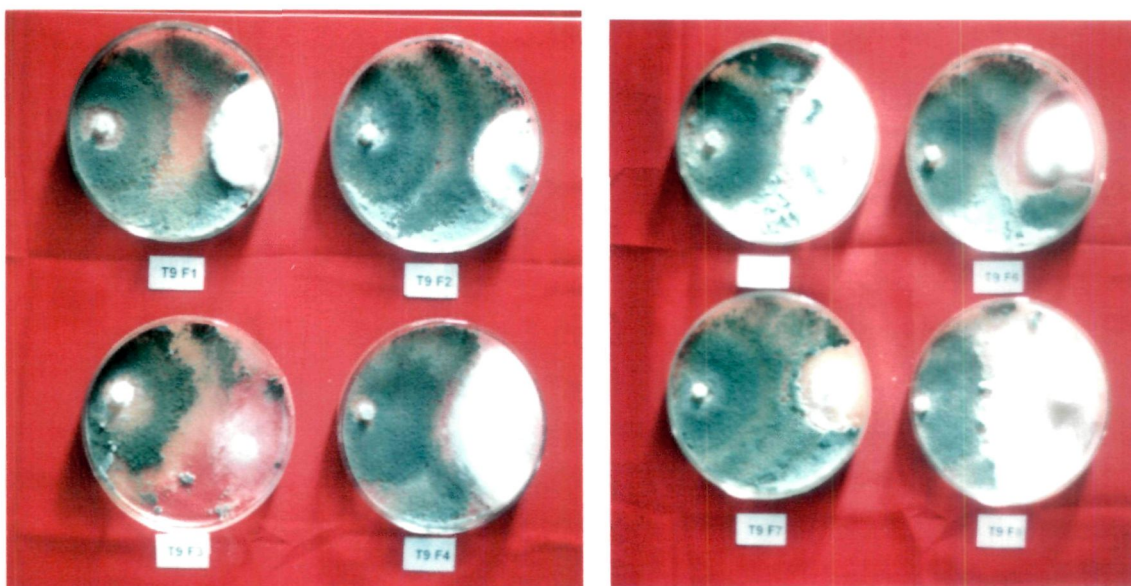
**Plate 23 :** T7 with *Fusarium* Isolates



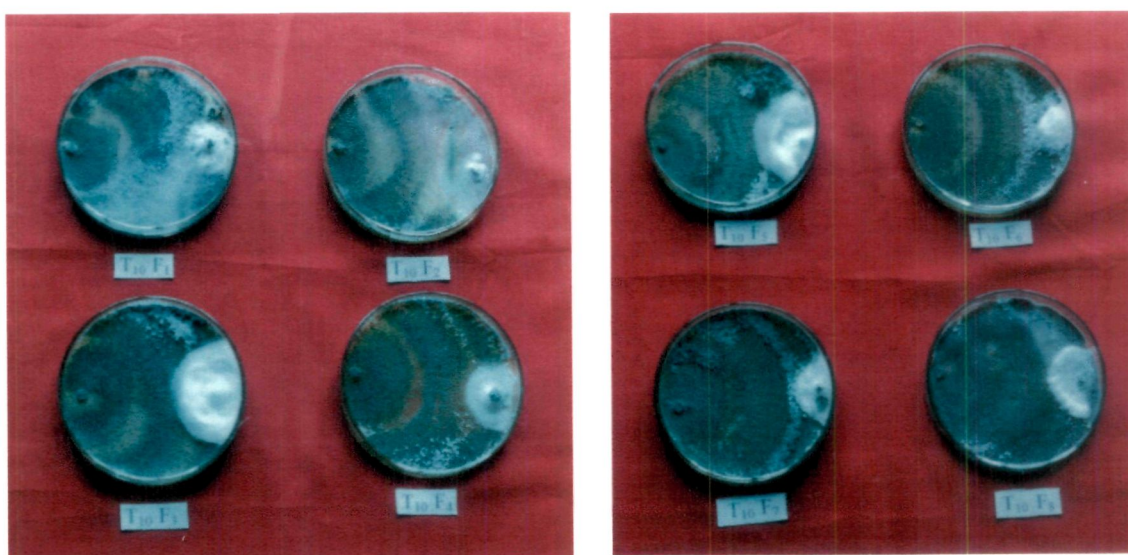
**Plate 24 :** T8 with *Fusarium* Isolates

# Interaction Studies of *Trichoderma* Isolates with Soil Borne Pathogens

*Trichoderma* Vs. *Fusarium* Isolates



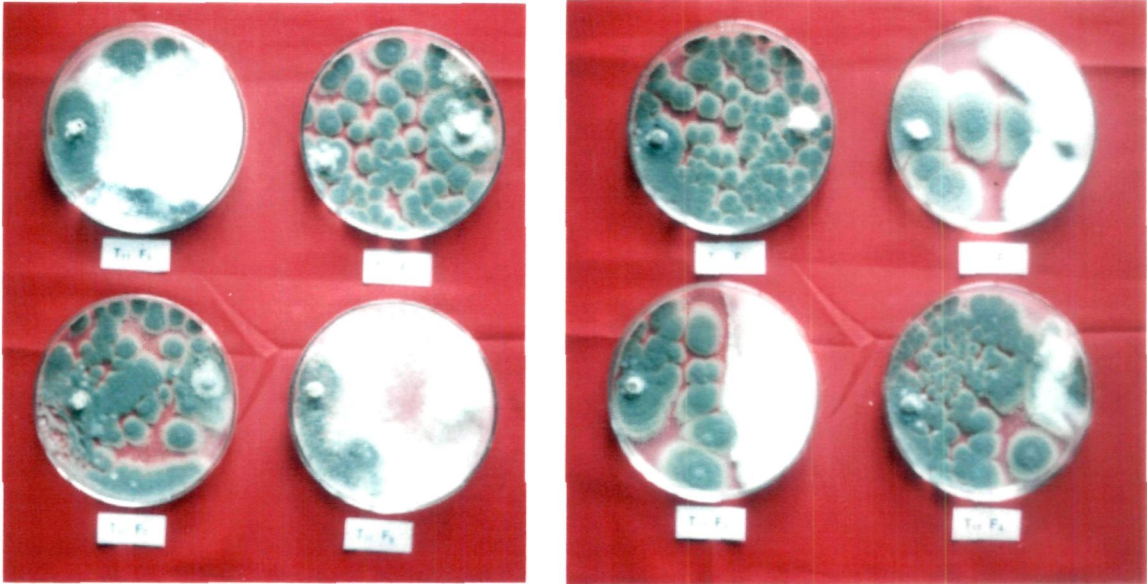
**Plate 25** : T9 with *Fusarium* Isolates



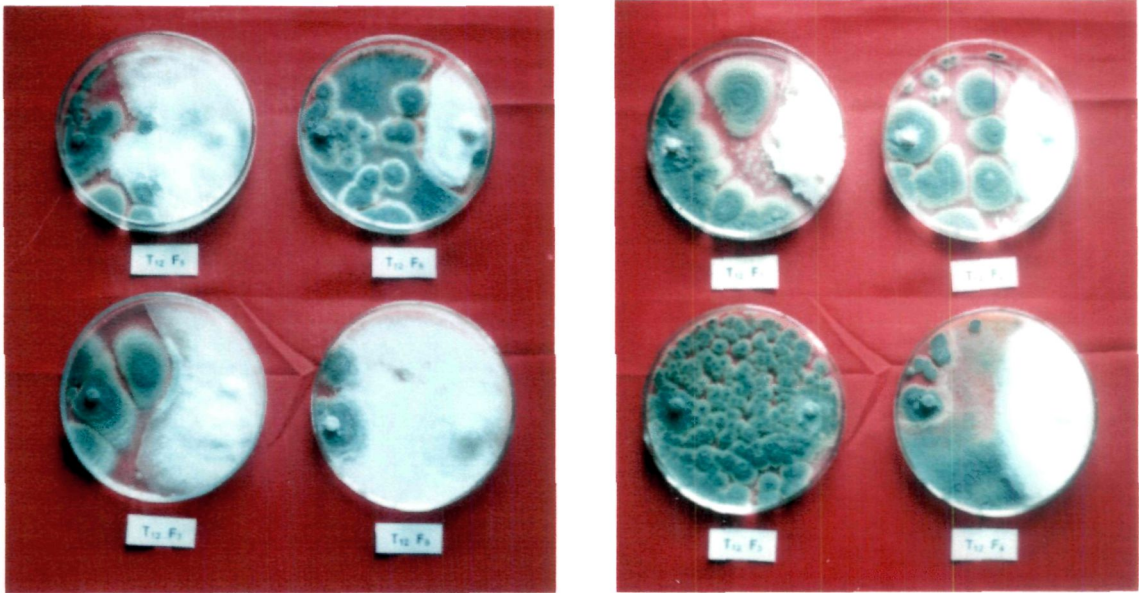
**Plate 26** : T10 with *Fusarium* Isolates

# Interaction Studies of *Trichoderma* Isolates with Soil Borne Pathogens

*Trichoderma* Vs. *Fusarium* Isolates



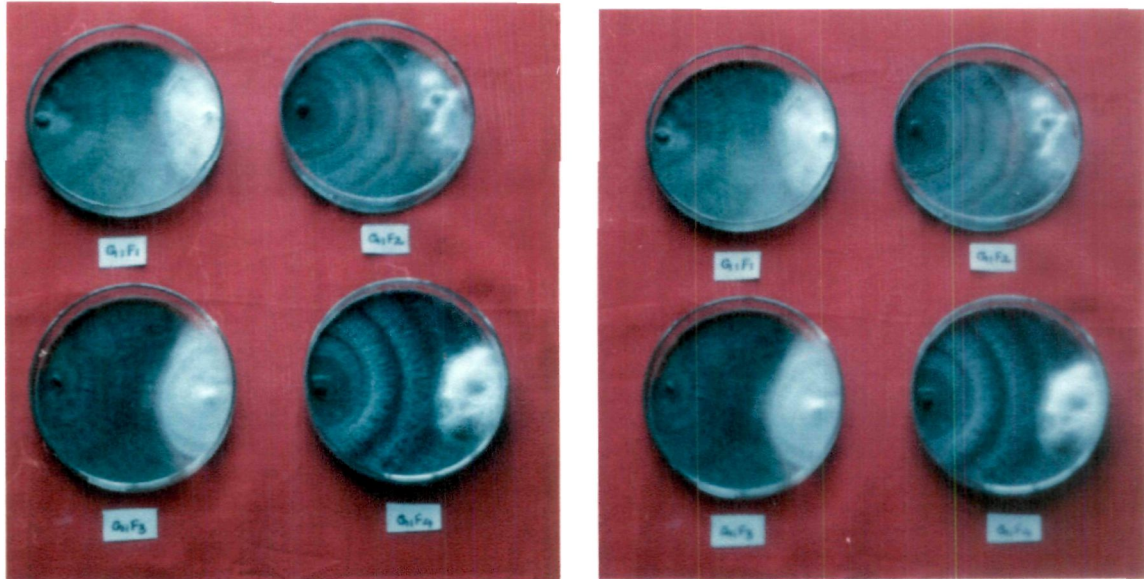
**Plate 27 :** T11 with *Fusarium* Isolates



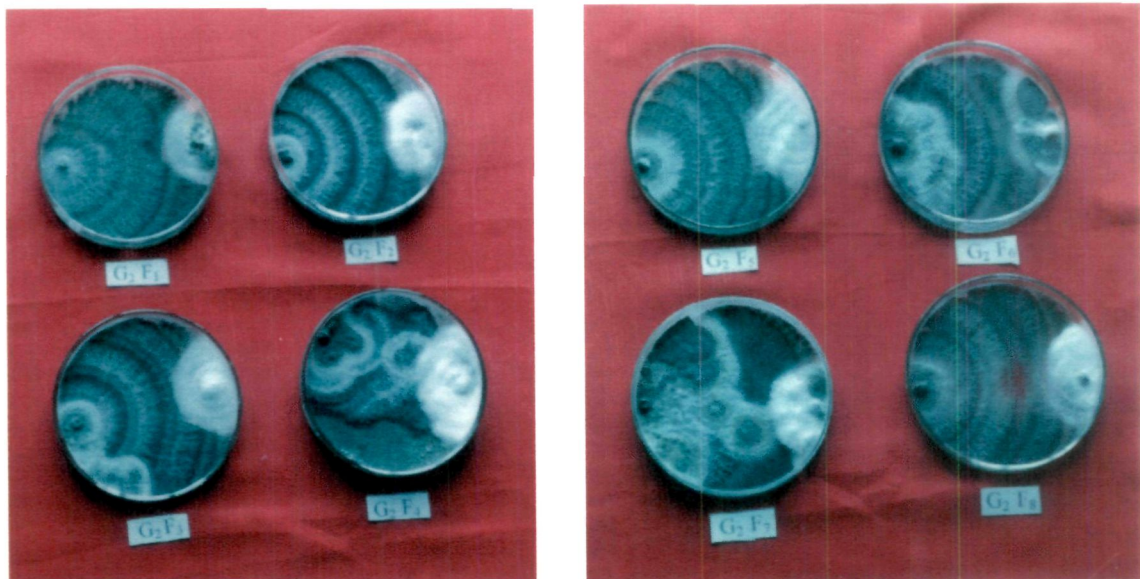
**Plate 28 :** T12 with *Fusarium* Isolates

# Interaction Studies of *Gliocladium* Isolates with Soil Borne Pathogens

*Gliocladium* Vs. *Fusarium* Isolates



**Plate 29** : G1 with *Fusarium* Isolates



**Plate 30** : G2 with *Fusarium* Isolates

ranking of inhibition of 40.80 and 41.09 respectively. Considering this interaction study T<sub>8</sub> (*T.harzianum*, Dharwad isolate) proved to be the efficient and hence was considered for further studies.

#### **4.9. Studies on growth period on *Trichoderma harzianum* at different intervals:**

As presented in Material and Methods, the peak period at which *Trichoderma harzianum* could put up maximum growth was studied by harvesting the dry mycelial weight of *Trichoderma harzianum* of Dharwad isolate at different intervals (Table 8 Figure 2). The study revealed that, *T.harzianum* slowly put on the weight and maximum mycelial growth on 12<sup>th</sup> day (304.21 mg) and then onwards it gradually declined.

##### **4.9.1. Effect of carbon sources on the growth of *Trichoderma harzianum***

Seven carbon sources were studied for growth of *Trichoderma harzianum* and the observations were recorded after 12 days (Table 9 and Figure 3). The results were statistically significant. Among these seven carbon sources, Maltose was recorded the highest dry mycelial weight (279.38 mg) followed by Dextrose i.e., the control with mycelial weight of 288.25 mg. Sucrose (225.22 mg) was the next best. Mannitol recorded the lowest (138.63 mg) than Galactose (153.52 mg) and Fructose (174.60 mg). Glucose (176.66 mg) and Fructose (174.60 mg) were intermediate.

**Table 8: Growth period of *Trichoderma harzianum* at different intervals, mycelial weight (in mg)**

Sl.No.	DAS	MEAN
1.	2	85.51
2.	4	138.93
3.	6	191.97
4.	8	256.87
5.	10	262.38
6.	12	304.21
7.	14	233.80
8.	16	229.15
9.	18	222.60
10.	20	216.67

**SEm ± : 0.931**

**CD at 1% level : 2.58**

**CV % : 0.75**

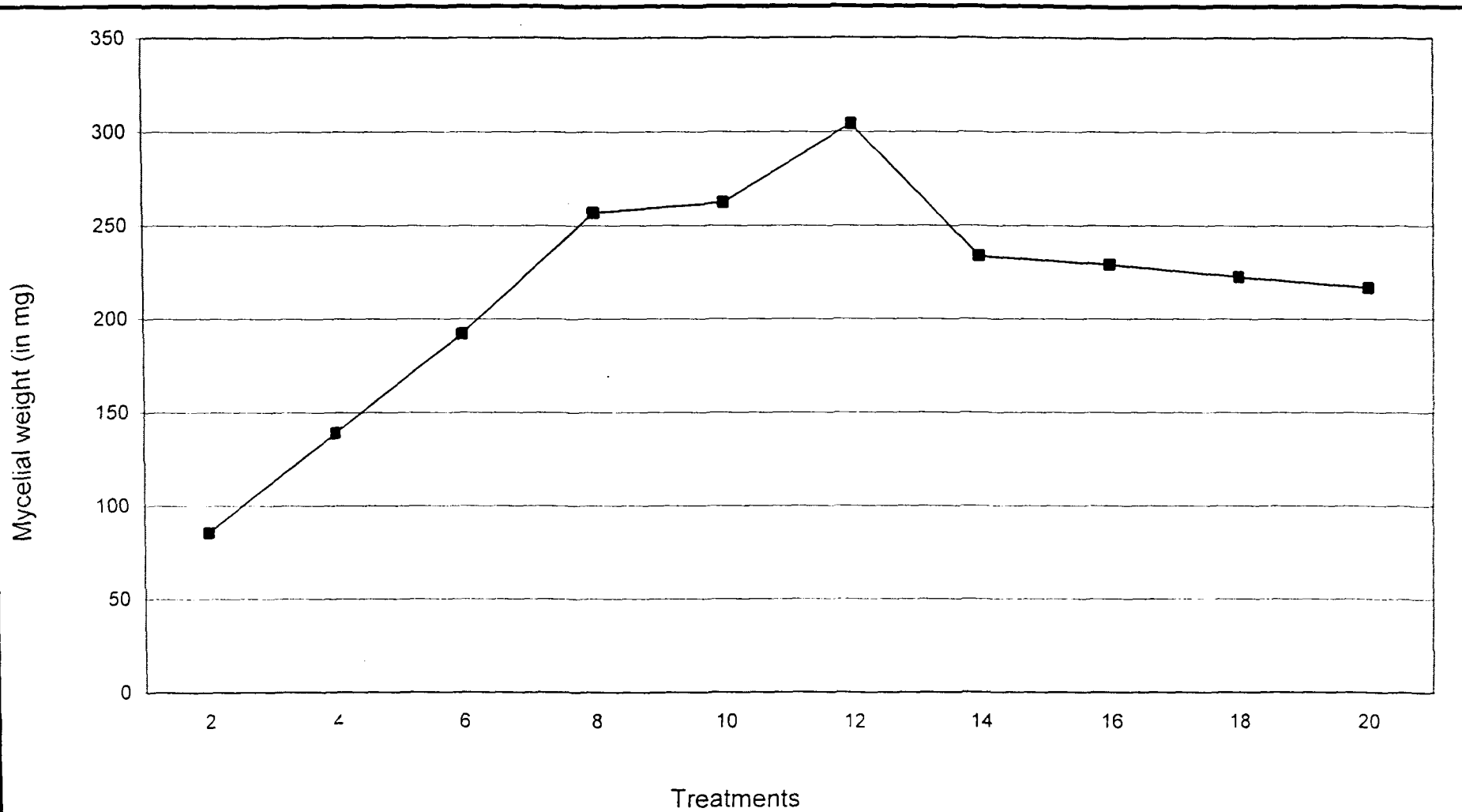


Fig. 2. Growth period studies on *Trichoderma harzianum* at different intervals

**Table 9: Effect of carbon sources on the growth of *Trichoderma harzianum*, mycelial weight (in mg)**

Sl. No.	Carbon Sources	MEAN
1.	Glucose	176.66
2.	Galactose	153.52
3.	Maltose	279.38
4.	Sucrose	225.22
5.	Fructose	174.60
6.	Mannitol	138.63
7.	Control	288.25

**SEm ±** : 1.335

**CD at 1% level** : 3.700

**CV %** : 1.13%

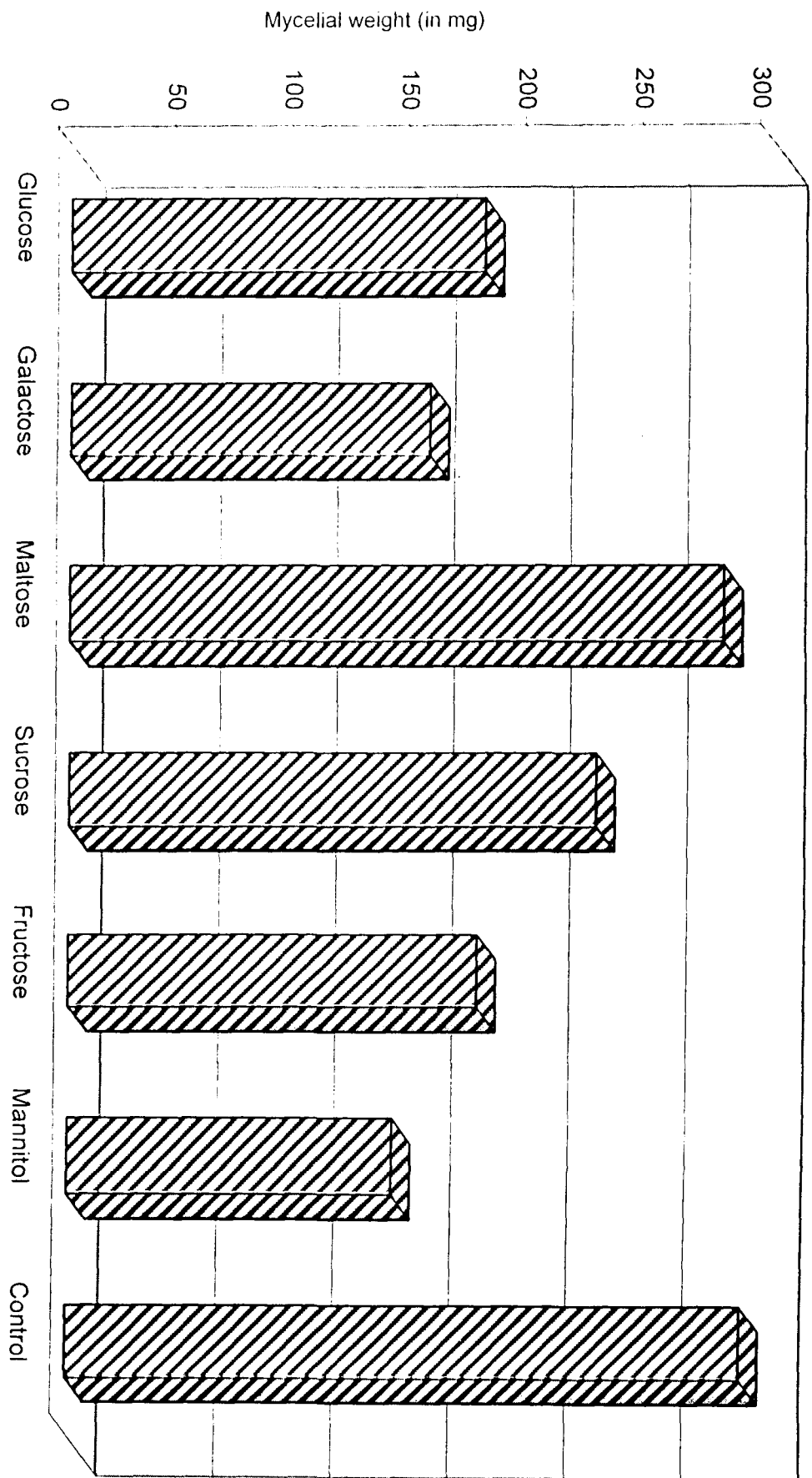


Fig. 3. Effect of carbon sources on growth of *Trichoderma harzianum*

#### 4.9.2 Screening of substrates for growth of *Trichoderma harzianum*

Nine substrates were screened for better growth (cfu value) of *Trichoderma harzianum*. The results are presented in Table 10 and Figure 4. Among these nine test substrates, talc formulation and wheat bran were recorded the highest cfu of 52.66 and 50.33 cfu respectively and followed by FYM (40.33 cfu). Groundnut cake (17.00 cfu) and Neem cake (17.66 cfu) have recorded very low cfu values. Paddy husk (33.66 cfu), Poultry manure (32.33 cfu) and Vermicompost (29.66 cfu) were intermediary.

#### 4.10. Standardization of seed treatment dosage and soil application

The quantity of *Trichoderma harzianum* required for seed treatment as well as soil application had to be standardised for further studies. In this connection, seed treatment @ 4g (T<sub>1</sub>), 6g (T<sub>2</sub>), 8g (T<sub>3</sub>) and 10g (T<sub>4</sub>) /kg of seed and soil application @ 1% (T<sub>5</sub>), 2% (T<sub>6</sub>), 3% (T<sub>7</sub>) and 4% (T<sub>8</sub>) and their combinations, comprising of 24 treatments against all four pathogens, viz., *Fusarium solani*, *Rhizoctonia solani*, *Rhizoctonia bataticola* and *Sclerotium rolfsii* were imposed (Table 11) as presented in material and methods. The observations were recorded on *T.harzianum*, cfu ( $10^{-5}$ ), g<sup>-1</sup> at two intervals i.e., 15 DAS (Table 11a) and 45 DAS (Table 11b). The results with respect to treatments, pathogens and their interactions were statistically significant. Among seed treatment dosages, there was increase in number of cfu as dosage increased at both 15 DAS and 45 DAS for all four pathogens under study. However, there was significant increase in treatments of 8 g/kg (T<sub>3</sub>) and 10 g/kg (T<sub>4</sub>) and were statistically superior over 4 g/kg (T<sub>1</sub>) and 6g/kg (T<sub>2</sub>). Among 8g/kg and 10 g/kg there was not significant increase in cfu

**Table 10: Evaluation of substrates for the growth of *Trichoderma harzianum*, cfu (10<sup>-5</sup>)/g**

<b>Sl. No.</b>	<b>Treatment</b>	<b>Mean</b>
1.	Vermicompost	29.66
2.	Poultry Manure	32.33
3.	Groundnut cake	17.00
4.	Neem cake	17.66
5.	Wheat bran	50.33
6.	Talc formulation	52.66
7.	FYM	40.33
8.	Dung	45.33
9.	Paddy husk	33.66
	<b>SEm±</b>	<b>0.333</b>
	<b>CD at 1% level</b>	<b>0.922</b>
	<b>CV %</b>	<b>1.68</b>

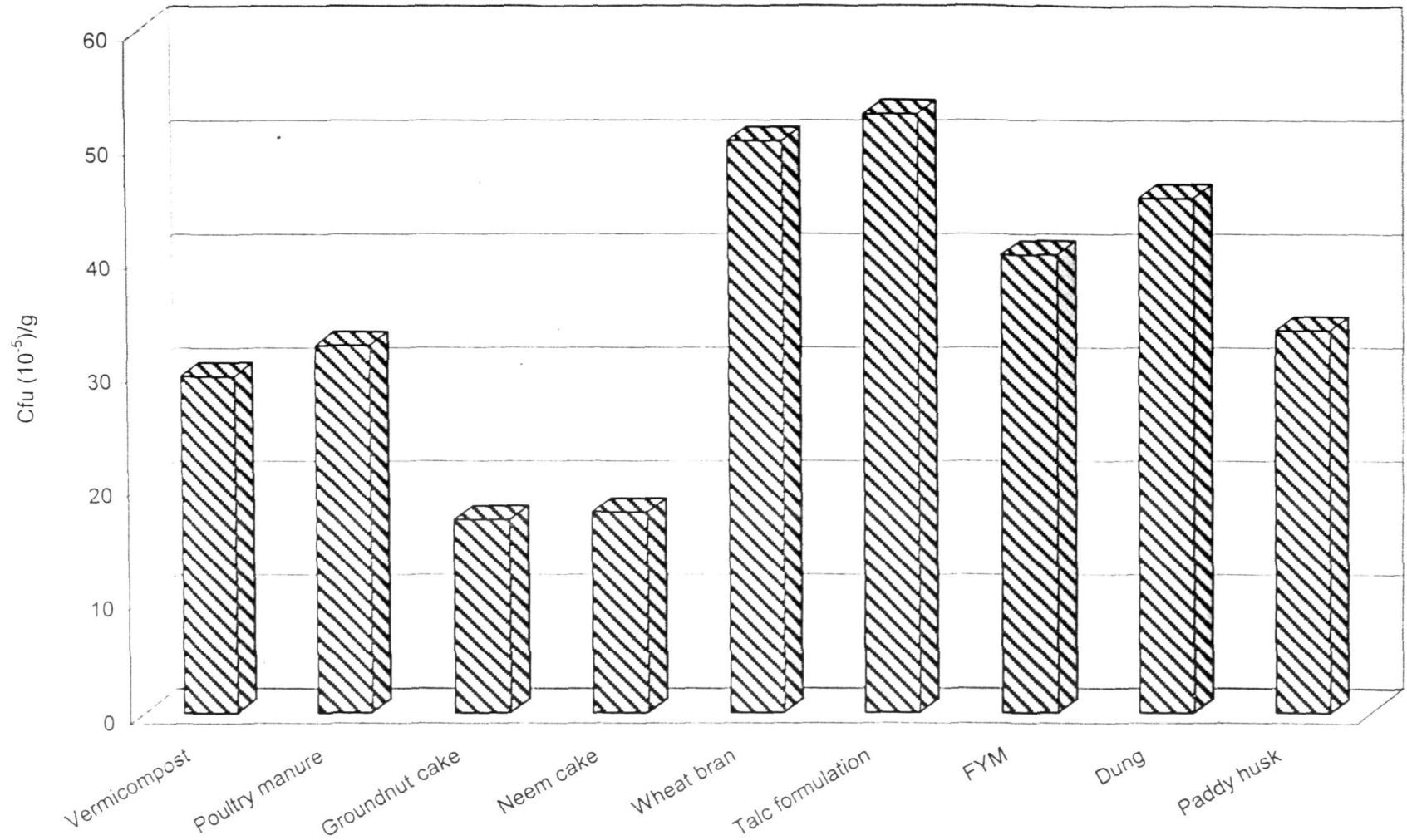


Fig. 4. Evaluation of substrates for growth of *Trichoderma harzianum*

**Table 11: Number of *Trichoderma harzianum* cfu ( $10^{-5}$ ) /g (pot culture experiment), treatment details**

Sl. No.	Treatment code	Treatments
1.	T <sub>1</sub>	Seed Treatment @ 4 g/kg
2.	T <sub>2</sub>	Seed Treatment @ 6 g/kg
3.	T <sub>3</sub>	Seed Treatment @ 8 g/kg
4.	T <sub>4</sub>	Seed Treatment @ 10 g/kg
5.	T <sub>5</sub>	Soil application 1%
6.	T <sub>6</sub>	Soil application 2%
7.	T <sub>7</sub>	Soil application 3%
8.	T <sub>8</sub>	Soil application 4%
9.	T <sub>1</sub> + T <sub>5</sub>	Seed Treatment @ 4 g/kg + Soil application 1%
10.	T <sub>1</sub> + T <sub>6</sub>	Seed Treatment @ 4 g/kg + Soil application 2%
11.	T <sub>1</sub> + T <sub>7</sub>	Seed Treatment @ 4 g/kg + Soil application 3%
12.	T <sub>1</sub> + T <sub>8</sub>	Seed Treatment @ 4 g/kg + Soil application 4%
13.	T <sub>2</sub> + T <sub>5</sub>	Seed Treatment @ 6 g/kg + Soil application 1%
14.	T <sub>2</sub> + T <sub>6</sub>	Seed Treatment @ 6 g/kg + Soil application 2%
15.	T <sub>2</sub> + T <sub>7</sub>	Seed Treatment @ 6 g/kg + Soil application 3%
16.	T <sub>2</sub> + T <sub>8</sub>	Seed Treatment @ 6 g/kg + Soil application 4%
17.	T <sub>3</sub> + T <sub>5</sub>	Seed Treatment @ 8 g/kg + Soil application 1%
18.	T <sub>3</sub> + T <sub>6</sub>	Seed Treatment @ 8 g/kg + Soil application 2%
19.	T <sub>3</sub> + T <sub>7</sub>	Seed Treatment @ 8 g/kg + Soil application 3%
20.	T <sub>3</sub> + T <sub>8</sub>	Seed Treatment @ 8 g/kg + Soil application 4%
21.	T <sub>4</sub> + T <sub>5</sub>	Seed Treatment @ 10 g/kg + Soil application 1%
22.	T <sub>4</sub> + T <sub>6</sub>	Seed Treatment @ 10 g/kg + Soil application 2%
23.	T <sub>4</sub> + T <sub>7</sub>	Seed Treatment @ 10 g/kg + Soil application 3%
24.	T <sub>4</sub> + T <sub>8</sub>	Seed Treatment @ 10 g/kg + Soil application 4%

**Table 11a: Number of *Trichoderma harzianum* cfu ( $10^{-5}$ )/g (pot culture experiment), 15 DAS**

Sl. No.	Treatments	<i>F.solani</i>	<i>R.solani</i>	<i>R.bataticola</i>	<i>S.rolfsii</i>
1.	T <sub>1</sub> : Seed Treatment @ 4 g/kg	72.33	71.67	64.33	98.33
2.	T <sub>2</sub> : Seed Treatment @ 6 g/kg	82.00	84.00	69.33	106.00
3.	T <sub>3</sub> : Seed Treatment @ 8 g/kg	85.33	86.67	80.67	130.00
4.	T <sub>4</sub> : Seed Treatment @ 10 g/kg	88.00	88.33	81.67	132.67
5.	T <sub>5</sub> : Soil application 1%	48.67	53.00	47.00	66.00
6.	T <sub>6</sub> : Soil application 2%	60.33	58.33	50.67	70.33
7.	T <sub>7</sub> : Soil application 3%	61.00	68.33	55.67	85.33
8.	T <sub>8</sub> : Soil application 4%	64.67	68.33	56.33	93.67
9.	T <sub>1</sub> + T <sub>5</sub>	90.00	92.67	75.67	113.33
10.	T <sub>1</sub> + T <sub>6</sub>	89.33	95.33	80.00	116.67
11.	T <sub>1</sub> + T <sub>7</sub>	98.67	100.33	84.00	124.0
12.	T <sub>1</sub> + T <sub>8</sub>	102.67	102.67	86.33	116.33
13.	T <sub>2</sub> + T <sub>5</sub>	113.33	117.00	90.33	135.67
14.	T <sub>2</sub> + T <sub>6</sub>	116.67	120.33	97.67	137.33
15.	T <sub>2</sub> + T <sub>7</sub>	122.67	122.67	99.67	142.67
16.	T <sub>2</sub> + T <sub>8</sub>	128.00	127.67	107.33	147.67
17.	T <sub>3</sub> + T <sub>5</sub>	121.00	122.00	108.67	143.67
18.	T <sub>3</sub> + T <sub>6</sub>	125.00	126.33	115.33	140.67
19.	T <sub>3</sub> + T <sub>7</sub>	122.00	125.33	115.33	141.33
20.	T <sub>3</sub> + T <sub>8</sub>	126.00	127.67	110.67	143.33
21.	T <sub>4</sub> + T <sub>5</sub>	116.67	119.33	106.00	136.33
22.	T <sub>4</sub> + T <sub>6</sub>	128.33	130.67	165.33	142.00
23.	T <sub>4</sub> + T <sub>7</sub>	127.33	132.00	105.33	135.33
24.	T <sub>4</sub> + T <sub>8</sub>	129.33	138.00	126.67	142.33
	<b>Mean</b>	<b>100.81</b>	<b>103.28</b>	<b>90.83</b>	<b>122.54</b>

SEm ± : 0.820  
 CD at 1% Treatment : 2.46  
 CD at 1% Pathogen : 7.52  
 CD at 1% Treatment V/s Pathogen : 2.27  
 CV % : 1.36

**Table 11: Number of *Trichoderma harzianum* cfu ( $10^5$ ) /g (pot culture experiment), treatment details**

Sl. No.	Treatment code	Treatments
1.	T <sub>1</sub>	Seed Treatment @ 4 g/kg
2.	T <sub>2</sub>	Seed Treatment @ 6 g/kg
3.	T <sub>3</sub>	Seed Treatment @ 8 g/kg
4.	T <sub>4</sub>	Seed Treatment @ 10 g/kg
5.	T <sub>5</sub>	Soil application 1%
6.	T <sub>6</sub>	Soil application 2%
7.	T <sub>7</sub>	Soil application 3%
8.	T <sub>8</sub>	Soil application 4%
9.	T <sub>1</sub> + T <sub>5</sub>	Seed Treatment @ 4 g/kg + Soil application 1%
10.	T <sub>1</sub> + T <sub>6</sub>	Seed Treatment @ 4 g/kg + Soil application 2%
11.	T <sub>1</sub> + T <sub>7</sub>	Seed Treatment @ 4 g/kg + Soil application 3%
12.	T <sub>1</sub> + T <sub>8</sub>	Seed Treatment @ 4 g/kg + Soil application 4%
13.	T <sub>2</sub> + T <sub>5</sub>	Seed Treatment @ 6 g/kg + Soil application 1%
14.	T <sub>2</sub> + T <sub>6</sub>	Seed Treatment @ 6 g/kg + Soil application 2%
15.	T <sub>2</sub> + T <sub>7</sub>	Seed Treatment @ 6 g/kg + Soil application 3%
16.	T <sub>2</sub> + T <sub>8</sub>	Seed Treatment @ 6 g/kg + Soil application 4%
17.	T <sub>3</sub> + T <sub>5</sub>	Seed Treatment @ 8 g/kg + Soil application 1%
18.	T <sub>3</sub> + T <sub>6</sub>	Seed Treatment @ 8 g/kg + Soil application 2%
19.	T <sub>3</sub> + T <sub>7</sub>	Seed Treatment @ 8 g/kg + Soil application 3%
20.	T <sub>3</sub> + T <sub>8</sub>	Seed Treatment @ 8 g/kg + Soil application 4%
21.	T <sub>4</sub> + T <sub>5</sub>	Seed Treatment @ 10 g/kg + Soil application 1%
22.	T <sub>4</sub> + T <sub>6</sub>	Seed Treatment @ 10 g/kg + Soil application 2%
23.	T <sub>4</sub> + T <sub>7</sub>	Seed Treatment @ 10 g/kg + Soil application 3%
24.	T <sub>4</sub> + T <sub>8</sub>	Seed Treatment @ 10 g/kg + Soil application 4%

Table 11b: Number of *T. harzianum* cfu ( $10^{-5}$ ) / g (pot culture experiment) , 45 DAS

Sl. No.	Treatments	<i>F.solani</i>	<i>R.solani</i>	<i>R.bataticola</i>	<i>S.rolfsii</i>
1.	T <sub>1</sub> : Seed Treatment @ 4 g/kg	91.33	105.67	81.33	102.33
2.	T <sub>2</sub> : Seed Treatment @ 6 g/kg	138.00	161.67	91.33	115.33
3.	T <sub>3</sub> : Seed Treatment @ 8 g/kg	190.67	197.33	137.67	145.33
4.	T <sub>4</sub> : Seed Treatment @ 10 g/kg	196.67	201.00	150.33	203.00
5.	T <sub>5</sub> : Soil application 1%	75.33	80.33	64.00	86.00
6.	T <sub>6</sub> : Soil application 2%	86.67	90.33	68.00	91.33
7.	T <sub>7</sub> : Soil application 3%	102.67	108.00	75.67	109.67
8.	T <sub>8</sub> : Soil application 4%	117.00	119.67	80.67	114.00
9.	T <sub>1</sub> + T <sub>5</sub>	137.33	145.00	96.67	48.00
10.	T <sub>1</sub> + T <sub>6</sub>	151.33	152.00	110.67	73.67
11.	T <sub>1</sub> + T <sub>7</sub>	161.00	170.33	120.00	184.33
12.	T <sub>1</sub> + T <sub>8</sub>	164.67	167.33	128.33	173.33
13.	T <sub>2</sub> + T <sub>5</sub>	139.67	142.33	131.33	180.33
14.	T <sub>2</sub> + T <sub>6</sub>	153.67	161.33	135.00	187.00
15.	T <sub>2</sub> + T <sub>7</sub>	162.67	167.33	139.00	170.67
16.	T <sub>2</sub> + T <sub>8</sub>	168.33	170.00	146.67	191.00
17.	T <sub>3</sub> + T <sub>5</sub>	197.67	203.33	171.33	206.67
18.	T <sub>3</sub> + T <sub>6</sub>	205.67	210.33	177.67	220.33
19.	T <sub>3</sub> + T <sub>7</sub>	216.33	218.33	177.67	224.67
20.	T <sub>3</sub> + T <sub>8</sub>	215.67	220.67	170.67	228.67
21.	T <sub>4</sub> + T <sub>5</sub>	199.33	199.33	169.67	211.33
22.	T <sub>4</sub> + T <sub>6</sub>	211.33	213.00	170.67	218.67
23.	T <sub>4</sub> + T <sub>7</sub>	218.33	217.33	198.33	219.33
24.	T <sub>4</sub> + T <sub>8</sub>	217.67	218.33	170.33	224.67
	<b>Mean</b>	<b>163.29</b>	<b>168.35</b>	<b>131.79</b>	<b>163.74</b>

SEM ± : 1.100

CD at 1% Treatment : 3.30

CD at 1% Pathogen : 4.80

CD at 1% Treatment V/s Pathogen: 3.04

CV % : 1.22

in all the fungi. But the cfu varied significantly among interaction of fungi. It was highest in *S.rolfsii* (203.00 cfu), followed by *R.solani* (201.00 cfu) (Table 11b) in the treatment of 10g/kg of seed (T<sub>4</sub>) at 45 DAS. Similar trend was observed at 15 DAS (Table 11a).

Among soil application method four percent (T<sub>8</sub>, *Trichoderma harzianum* Dharwad isolate) application recorded the highest mean cfu for all four pathogens under study, *Fusarium solani*, *Rhizoctonia solani*, *Rhizoctonia bataticola* and *Sclerotium rolfsii* i.e., 117.00, 119.67, 80.67 and 114.00 cfu (10<sup>-5</sup>) g<sup>-1</sup> respectively at 45 DAS and this treatment was found statistically superior to rest of the soil applications.

In the combinations tried, in the first seed treatment (T<sub>1</sub>)+ soil application (T<sub>2</sub> + T<sub>8</sub>), the eighth combination was superior for all fungi under study and was better than seed treatment (T<sub>1</sub>) alone and also the soil application methods under study at 15 DAS (Table 11a). But at 45 DAS T<sub>3</sub> + T<sub>7</sub> eleventh combination was exhibited higher cfu values for all pathogens under study (Table 11b). Further, although the results were statistically significant for seed treatments from T<sub>3</sub> to T<sub>4</sub>, soil application methods from T<sub>7</sub> to T<sub>8</sub> and among the combinations from T<sub>1</sub> + T<sub>7</sub> to T<sub>1</sub> + T<sub>8</sub>, T<sub>2</sub>+ T<sub>7</sub> to T<sub>2</sub> + T<sub>8</sub>, and T<sub>3</sub>+ T<sub>7</sub> to T<sub>3</sub> + T<sub>8</sub> and T<sub>4</sub>+ T<sub>7</sub> to T<sub>4</sub> + T<sub>8</sub> but variation among cfu values was not conspicuous as compared to single seed treatment (T<sub>1</sub>), soil application and their combinations studied (Table 11a and Table 11b) respectively i.e., at 15DAS and 45DAS. Considering 15 DAS and 45 DAS together, single seed treatment (T<sub>3</sub>) i.e., 8 g/kg was superior among the seed treatments, three per cent was the best among the soil application methods and T<sub>3</sub> + T<sub>5</sub> i.e., seed treatment @ 8 g/kg and soil application @ 1 per cent found to be better than rest of the combinations studied.

#### **4.11. Effect of bag colour on *Trichoderma harzianum***

Seven types of bags were used for studying better storage purpose of *Trichoderma harzianum*. The mean cfu values of *T.harzianum* are presented in Table 12 Figure 5. Results were statistically significant. Among the bags under study the transparent polythene bag recorded the highest cfu of 57.44 followed by cream (53.51 cfu), pink (51.05 cfu) and blue (50.47 cfu) colours. Black polythene (47.78 cfu) was least among polythene bags, however it was superior to paper cloth bag (46.06 cfu).

#### **4.12. Effect of fungicides on *Trichoderma harzianum* and pathogens**

##### **4.12.1. Effect of fungicides on *Trichoderma harzianum***

Eight fungicides at six concentrations viz., 50, 100, 200, 500, 750 and 1000 ppm were evaluated for their effect on *Trichoderma harzianum* under 'in vitro' conditions as presented in material and methods. Percent inhibition of growth was recorded. The results were highly significant. Both Carbendazim and Benomyl were inhibitory to *T.harzianum*, even at the lowest concentration (50 ppm) i.e., cent per cent inhibition (Table 13 and Figure 6). Captan was inhibitory (11.18) at 1000 ppm, otherwise no effect was seen at other concentrations. Copper-Oxy-Chloride and Methoxy Ethyl Mercury Chloride were low in inhibiting *T.harzianum* at lower dosages but Copper-Oxy-Chloride was inhibitory at 1000 ppm. Carboxin, Thiram and Mancozeb recorded similar trend of inhibition of *T.harzianum* but were more toxic than Copper-Oxy-Chloride and Methoxy Ethyl Mercury Chloride.

**Table 12: Effect of bag colour on multiplication of *Trichoderma harzianum* (cfu  $10^{-5}$ ) / g<sup>-1</sup>**

<b>Sl. No.</b>	<b>Treatments</b>	<b>Mean</b>
1.	Transparent polythene	57.44
2.	Black polythene	47.78
3.	Cloth Bag	48.23
4.	Blue polythene	50.47
5.	Cream polythene	53.51
6.	Paper cloth bag	46.06
7.	Pink polythene	51.05
	<b>Mean</b>	<b>0.516</b>
	<b>SEm ±</b>	<b>0.528</b>
	<b>CV%</b>	<b>1.77</b>
	<b>CD at 1%</b>	<b>1.43</b>

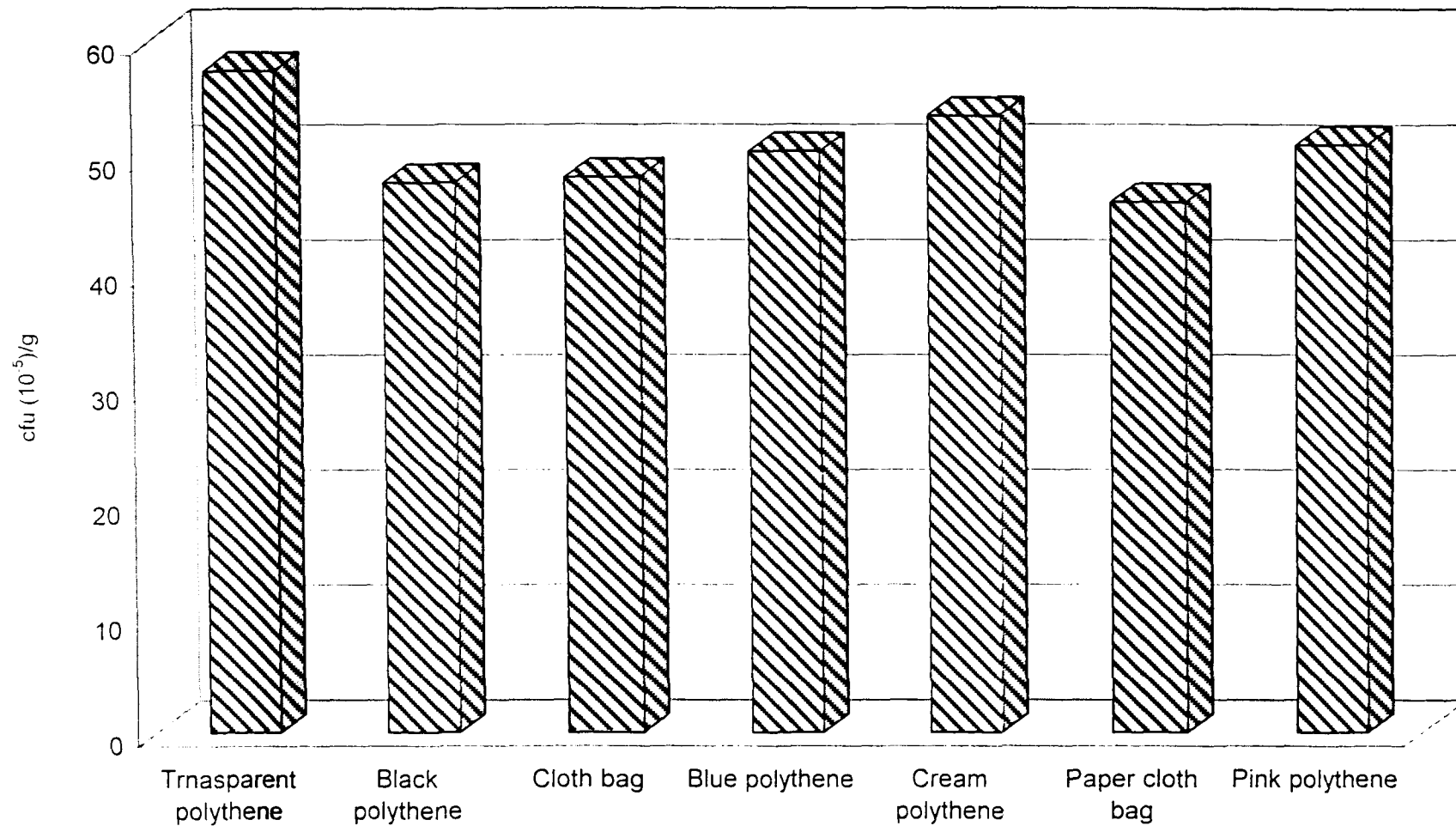


Fig. 5. Effect of bag colour on *Trichoderma harzianum*, cfu ( $10^{-5}$ )/g

**Table 13: Effect of fungicides against *Trichoderma harzianum***

Sl. No.	Concentration (ppm)	Carbendazim	Carboxin	Thiram	Methoxy Ethyl Mercury Chloride	Captan	Copper-oxy-chloride	Benomyl	Mancozeb	Control	Mean
1.	50	100.00 (90.00)	22.07 (19.86)	27.98 (25.18)	13.74 (12.37)	0.00 (0.00)	8.63 (7.77)	100.00 (90.00)	16.00 (14.40)	0.00 (0.00)	22.17 (29.96)
2.	100	100.00 (90.00)	27.20 (24.48)	36.58 (32.92)	15.21 (13.68)	0.00 (0.00)	17.86 (16.08)	100.00 (90.00)	21.98 (19.78)	0.00 (0.00)	35.42 (31.88)
3.	200	100.00 (90.00)	25.26 (22.74)	42.48 (38.23)	21.36 (19.22)	0.00 (0.00)	28.15 (25.33)	100.00 (90.00)	24.15 (21.74)	0.00 (0.00)	37.93 (34.14)
4.	500	100.00 (90.00)	40.00 (36.00)	79.43 (71.49)	34.28 (30.85)	0.00 (0.00)	36.72 (33.04)	100.00 (90.00)	30.08 (27.07)	0.00 (0.00)	46.72 (42.05)
5.	750	100.00 (90.00)	87.51 (78.76)	89.47 (80.52)	53.51 (48.16)	0.00 (0.00)	69.74 (62.76)	100.00 (90.00)	71.29 (64.16)	0.00 (0.00)	63.50 (57.15)
6.	1000	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	82.19 (73.97)	11.18 (10.06)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	0.00 (0.00)	97.45 (68.22)
	<b>Mean</b>	<b>100.00</b> <b>(90.00)</b>	<b>50.34</b> <b>(45.30)</b>	<b>62.65</b> <b>(56.39)</b>	<b>36.71</b> <b>(33.04)</b>	<b>1.86</b> <b>(1.67)</b>	<b>43.51</b> <b>(39.16)</b>	<b>100.00</b> <b>(90.00)</b>	<b>43.91</b> <b>(39.52)</b>	<b>0.00</b> <b>(0.00)</b>	<b>48.77</b> <b>(43.90)</b>

Note : Figures in the paranthesis are the arc-sin values

SEm ± : 0.686  
 CD at 1% Fungicide : 1.85  
 CD at 1% Concentration : 2.34  
 CD at 1% Fungicide V/s Concentration : 1.90  
 CV % : 2.71

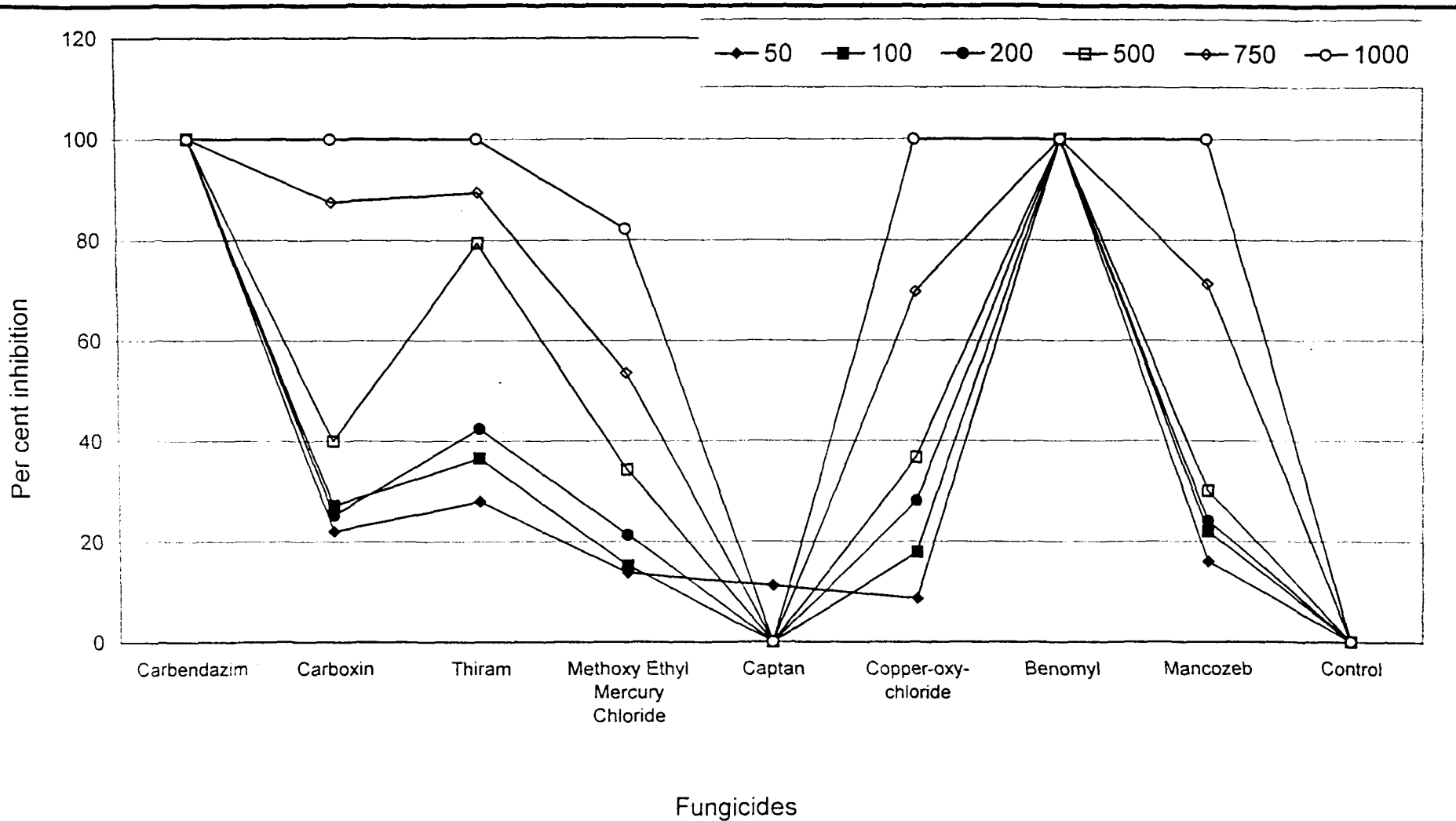


Fig. 6. Effect of fungicides against *Trichoderma harzianum*

#### 4.12.2. Effect of fungicides against *Fusarium solani*

Same nine fungicides and at same concentrations were tested for their efficacy against *Fusarium solani*. The per cent inhibition was recorded (Table 14 and Figure 7). The results were significant and three fungicides viz., Carbendazim, Carboxin and Benomyl completely inhibited the growth of *F.solani*. The next best fungicides were viz., Methoxy Ethyl Mercury Chloride and Captan which completely inhibited *F.solani* growth from 500 ppm. Thiram could inhibit the growth completely at 750 ppm. Although Mancozeb could inhibit the growth completely at 750 ppm but found least effective at lower concentrations as compared to other fungicides. Copper-Oxy-Chloride was also least effective at lower concentrations.

#### 4.12.3. Effect of fungicides against *Rhizoctonia solani*

For *Rhizoctonia solani*, nine fungicides and at same concentrations were tested for their efficacy under '*in vitro*' conditions. The percent inhibition of growth was recorded and the results were statistically significant and are presented in Table 15 and Figure 8. Here four fungicides viz., Carboxin, Thiram, Methoxy Ethyl Mercury Chloride and Benomyl found to be highly effective i.e., completely inhibiting growth even at the lowest concentration (50 ppm) tested, followed by Captan (500 ppm), Mancozeb and Copper-Oxy-Chloride at (750 ppm). However Carbendazim was least in inhibiting i.e., 73.42 per cent at 1000 ppm.

**Table 14: Effect of fungicides against *Fusarium solani***

Sl. No.	Concentration (ppm)	Carbendazim	Carboxin	Thiram	Methoxy Ethyl Mercury Chloride	Captan	Copper-oxy-chloride	Benamyl	Mancozeb	Control	Mean
1.	50	100.00 (90.00)	100.00 (90.00)	20.23 (18.21)	32.58 (29.32)	30.52 (27.47)	16.79 (15.11)	100.00 (90.00)	16.16 (14.54)	0.00 (0.00)	46.28 (41.62)
2.	100	100.00 (90.00)	100.00 (90.00)	36.33 (32.70)	50.41 (45.37)	52.02 (46.82)	34.42 (30.97)	100.00 (90.00)	21.21 (19.08)	0.00 (0.00)	54.93 (49.44)
3.	200	100.00 (90.00)	100.00 (90.00)	41.92 (37.73)	66.42 (59.78)	69.37 (62.43)	39.49 (35.54)	100.00 (90.00)	37.07 (33.36)	0.00 (0.00)	61.59 (55.42)
4.	500	100.00 (90.00)	100.00 (90.00)	81.90 (72.90)	100.00 (90.00)	100.00 (90.00)	76.44 (68.79)	100.00 (90.00)	64.44 (57.99)	0.00 (0.00)	80.31 (72.18)
5.	750	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	0.00 (0.00)	88.89 (80.00)
6.	1000	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	0.00 (0.00)	88.89 (80.00)
	<b>Mean</b>	<b>100.00</b> <b>(90.00)</b>	<b>100.00</b> <b>(90.00)</b>	<b>62.25</b> <b>(56.92)</b>	<b>74.96</b> <b>(67.41)</b>	<b>75.32</b> <b>(67.78)</b>	<b>61.19</b> <b>(55.07)</b>	<b>100.00</b> <b>(90.00)</b>	<b>56.48</b> <b>(50.83)</b>	<b>0.00</b> <b>(0.00)</b>	<b>70.02</b> <b>(63.11)</b>

Note : Figures in the paranthesis are the arc-sin values

SEm ± : 0.417

CD at 1% Fungicide : 0.93

CD at 1% Concentration : 1.18

CD at 1% Fungicide V/s Concentration : 1.15

CV % : 0.97

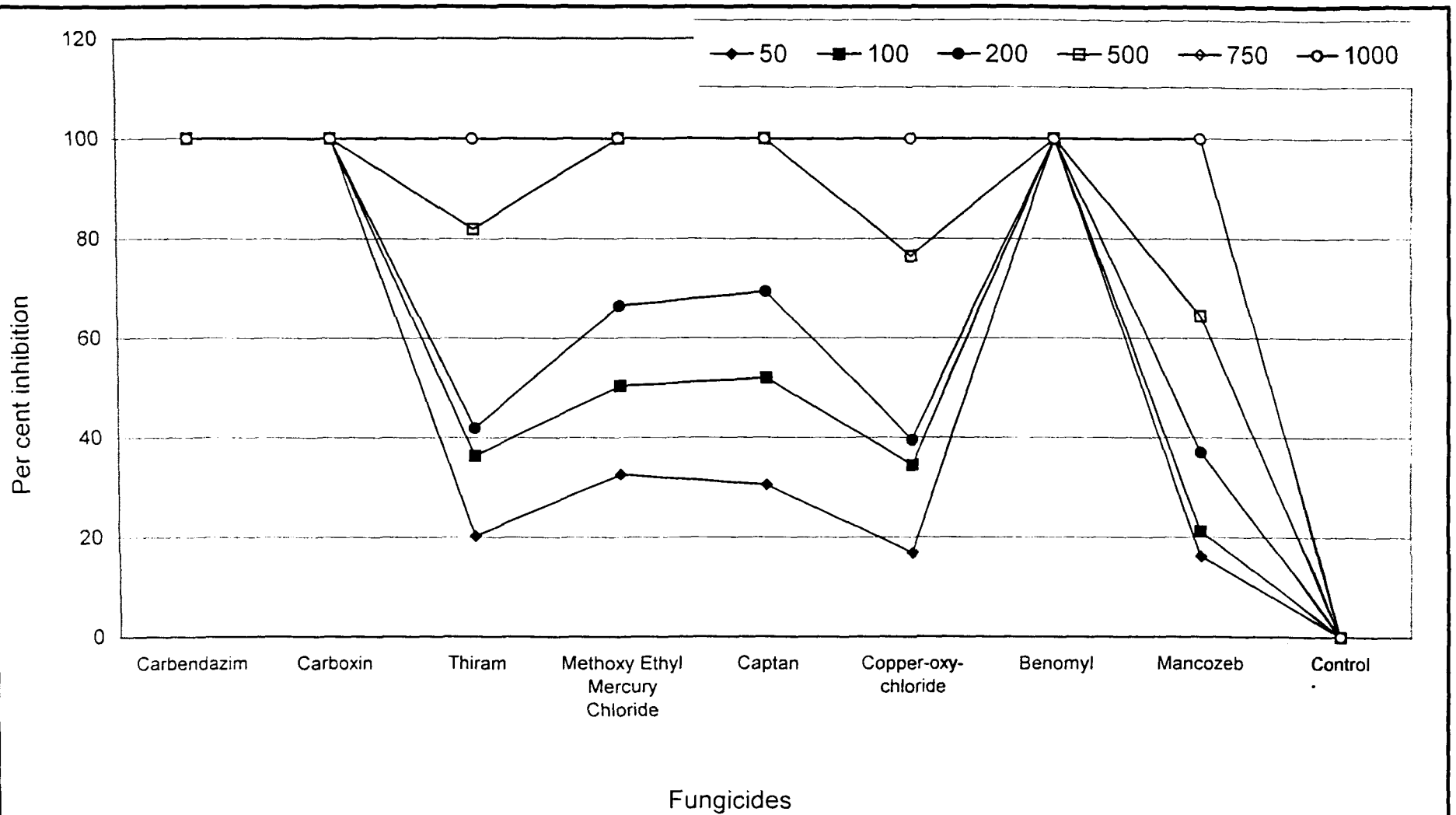


Fig. 7. Effect of fungicides against *Fusarium solani*

**Table 15: Effect of fungicides against *Rhizoctonia solani***

Sl. No.	Concentration (ppm)	Carbendazim	Carboxin	Thiram	Methoxy Ethyl Mercury Chloride	Captan	Copper-oxy-chloride	Benomyl	Mancozeb	Control	Mean
1.	50	12.76 (11.48)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	35.46 (31.91)	26.18 (23.56)	100.00 (90.00)	17.03 (15.33)	0.00 (0.00)	54.60 (49.14)
2.	100	12.88 (11.59)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	62.97 (56.67)	38.31 (34.47)	100.00 (90.00)	24.32 (21.88)	0.00 (0.00)	59.83 (53.84)
3.	200	15.73 (14.15)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	84.13 (75.72)	52.48 (47.23)	100.00 (90.00)	43.25 (38.93)	0.00 (0.00)	66.18 (59.56)
4.	500	38.80 (13.92)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	85.99 (77.39)	100.00 (90.00)	72.02 (64.82)	0.00 (0.00)	77.42 (69.68)
5.	750	57.34 (51.61)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	0.00 (0.00)	84.15 (75.73)
6.	1000	73.42 (66.08)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	0.00 (0.00)	85.94 (77.34)
	<b>Mean</b>	<b>35.15</b> <b>(31.64)</b>	<b>100.00</b> <b>(90.00)</b>	<b>100.00</b> <b>(90.00)</b>	<b>100.00</b> <b>(90.00)</b>	<b>80.42</b> <b>(72.38)</b>	<b>67.16</b> <b>(60.44)</b>	<b>100.00</b> <b>(90.00)</b>	<b>59.43</b> <b>(53.49)</b>	<b>0.00</b> <b>(0.00)</b>	<b>71.35</b> <b>(64.21)</b>

Note : Figures in the parenthesis are the arc-sin values

SEm ± : 0.501  
 CD at 1% Fungicide : 1.12  
 CD at 1% Concentration : 1.42  
 CD at 1% Fungicide V/s Concentration : 1.38  
 CV % : 1.36

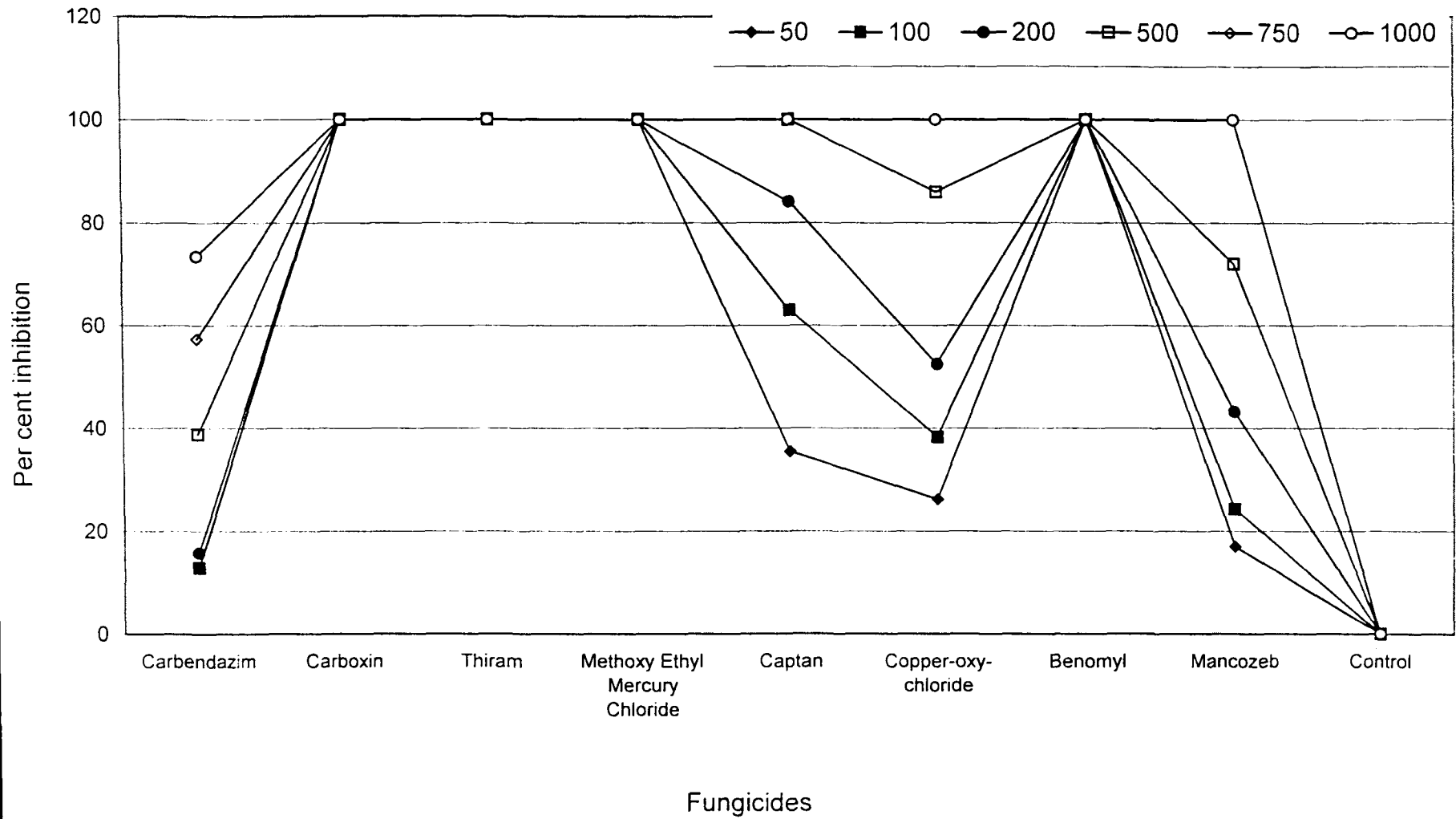


Fig. 8. Effect of fungicides against *Rhizoctonia solani*

#### 4.12.4. Effect of fungicides on *Rhizoctonia bataticola*

The nine fungicides at six concentrations were tested for their efficacy for *Rhizoctonia bataticola*. The per cent inhibition of growth was recorded and the results are presented in Table 16 and Figure 9. The results were statistically significant. Although the results were similar to *R.solani* i.e., complete inhibition of growth was seen in respect of Carboxin, Thiram, Methoxy Ethyl Mercury Chloride and Benomyl but, Mancozeb was inhibitory at 1000 ppm in *R.bataticola* and Captan at 200 ppm (Table 16). For Carbendazim same trend was observed and it was little more in *R.bataticola* than *R.solani*. Carbendazim @ 100 ppm was better *R.solani* than *R.bataticola*.

#### 4.12.5. Effect of fungicides against *Sclerotium rolfsii*

Fungicidal efficacy for *Sclerotium rolfsii* was also statistically significant. The results are presented in Table 17 and Figure 10. Three fungicides namely Carboxin, Methoxy Ethyl Mercury Chloride and Benomyl were highly effective at the lowest concentration (50 ppm) tested i.e., completely inhibiting the growth of *S.rolfsii*, and at 750 ppm, Copper-Oxy-Chloride was highly effective. Thiram and Mancozeb were least effective at low concentrations i.e., at 50, 100, 200 and 500 ppm. Carbendazim was least in inhibiting the growth of *S.rolfsii*.

#### 4.12.6. Evaluation of fungicides against *Fusarium solani* as seed treatment and soil drenching methods

Eight fungicides were used as seed treatment and soil drenching separately against *Fusarium solani* and were evaluated for pre emergence

**Table 16: Effect of fungicides against *Rhizoctonia bataticola***

Sl. No.	Concentration (ppm)	Carbendazim	Carboxin	Thiram	Methoxy Ethyl Mercury Chloride	Captan	Copper-oxy-chloride	Benomyl	Mancozeb	Control	Mean
1.	50	10.70 (9.63)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	41.41 (37.27)	20.14 (18.13)	100.00 (90.00)	13.27 (11.94)	0.00 (0.00)	53.95 (48.55)
2.	100	11.38 (10.24)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	69.06 (62.16)	25.94 (23.34)	100.00 (90.00)	21.11 (19.00)	0.00 (0.00)	58.61 (52.75)
3.	200	16.83 (15.15)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	46.06 (41.45)	100.00 (90.00)	35.56 (32.01)	0.00 (0.00)	66.49 (59.84)
4.	500	40.01 (36.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	71.67 (64.50)	100.00 (90.00)	62.89 (56.60)	0.00 (0.00)	74.95 (67.45)
5.	750	60.76 (54.69)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	85.79 (77.21)	100.00 (90.00)	73.90 (66.51)	0.00 (0.00)	80.05 (72.04)
6.	1000	81.72 (73.55)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	0.00 (0.00)	86.86 (78.17)
	<b>Mean</b>	<b>36.90</b> <b>(33.21)</b>	<b>100.00</b> <b>(90.00)</b>	<b>100.00</b> <b>(90.00)</b>	<b>100.00</b> <b>(90.00)</b>	<b>85.08</b> <b>(76.57)</b>	<b>58.27</b> <b>(52.44)</b>	<b>100.00</b> <b>(90.00)</b>	<b>51.12</b> <b>(46.01)</b>	<b>0.00</b> <b>(0.00)</b>	<b>70.15</b> <b>(63.13)</b>

Note : Figures in the parenthesis are the arc-sin values

SEm ± : 0.761  
 CD at 1% Fungicide : 1.70  
 CD at 1% Concentration : 2.15  
 CD at 1% Fungicide V/s Concentration : 2.11  
 CV % : 2.09

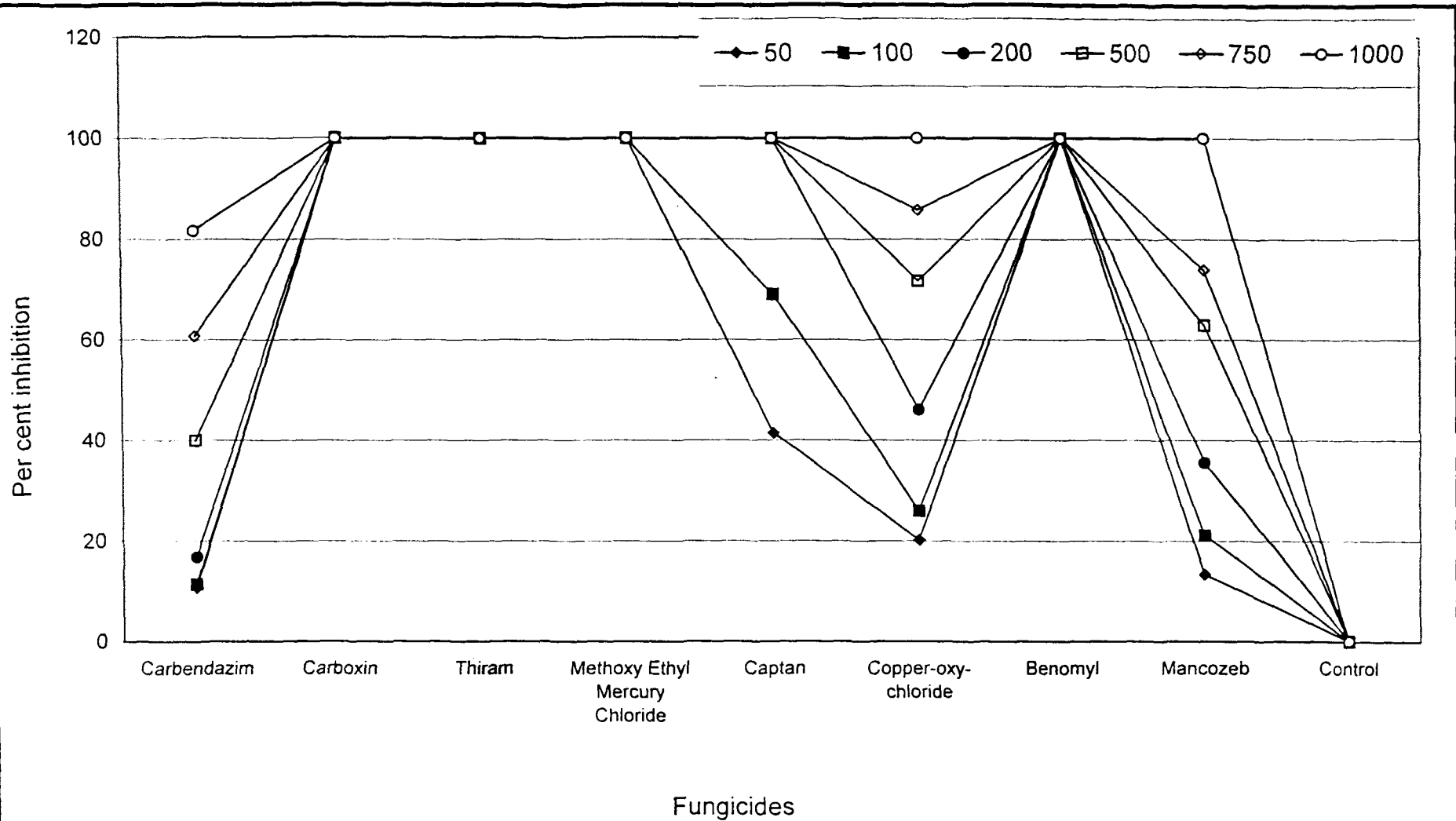


Fig. 9. Effect of fungicides against *Rhizoctonia bataticola*

**Table 17: Effect of fungicides against *Sclerotium rolfsii***

Sl. No.	Concentration (ppm)	Carbendazim	Carboxin	Thiram	Methoxy Ethy Mercury Chloride	Captan	Copper-oxy-chloride	Benamyl	Mancozeb	Control	Mean
1.	50	1.10 (0.99)	100.00 (90.00)	10.63 (9.57)	100.00 (90.00)	2.24 (2.01)	32.58 (29.32)	100.00 (90.00)	1.95 (1.76)	0.00 (0.00)	38.72 (34.85)
2.	100	15.32 (13.79)	100.00 (90.00)	16.25 (14.62)	100.00 (90.00)	9.58 (8.62)	36.74 (33.06)	100.00 (90.00)	3.35 (3.01)	0.00 (0.00)	42.36 (38.12)
3.	200	18.27 (16.44)	100.00 (90.00)	16.24 (14.62)	100.00 (90.00)	15.94 (14.34)	62.56 (56.31)	100.00 (90.00)	4.57 (4.11)	0.00 (0.00)	46.40 (41.76)
4.	500	26.97 (24.27)	100.00 (90.00)	38.34 (34.51)	100.00 (90.00)	22.43 (20.19)	64.94 (58.44)	100.00 (90.00)	18.38 (16.54)	0.00 (0.00)	52.34 (47.10)
5.	750	31.55 (28.39)	100.00 (90.00)	39.98 (35.98)	100.00 (90.00)	62.48 (56.23)	100.00 (90.00)	100.00 (90.00)	60.50 (54.45)	0.00 (0.00)	66.10 (59.45)
6.	1000	53.02 (47.72)	100.00 (90.00)	72.44 (65.19)	100.00 (90.00)	69.46 (62.51)	100.00 (90.00)	100.00 (90.00)	70.74 (63.66)	0.00 (0.00)	73.96 (66.56)
	<b>Mean</b>	<b>24.37</b> <b>(21.93)</b>	<b>100.00</b> <b>(90.00)</b>	<b>32.31</b> <b>(29.08)</b>	<b>100.00</b> <b>(90.00)</b>	<b>30.35</b> <b>(27.32)</b>	<b>66.13</b> <b>(59.52)</b>	<b>100.00</b> <b>(90.00)</b>	<b>26.58</b> <b>(23.92)</b>	<b>0.00</b> <b>(0.00)</b>	<b>53.30</b> <b>(47.97)</b>

Note : Figures in the parenthesis are the arc-sin values

SEm ± : 1.89

CD at 1% Fungicide : 4.23

CD at 1% Concentration : 5.35

CD at 1% Fungicide V/s Concentration: 5.25

CV % : 6.84

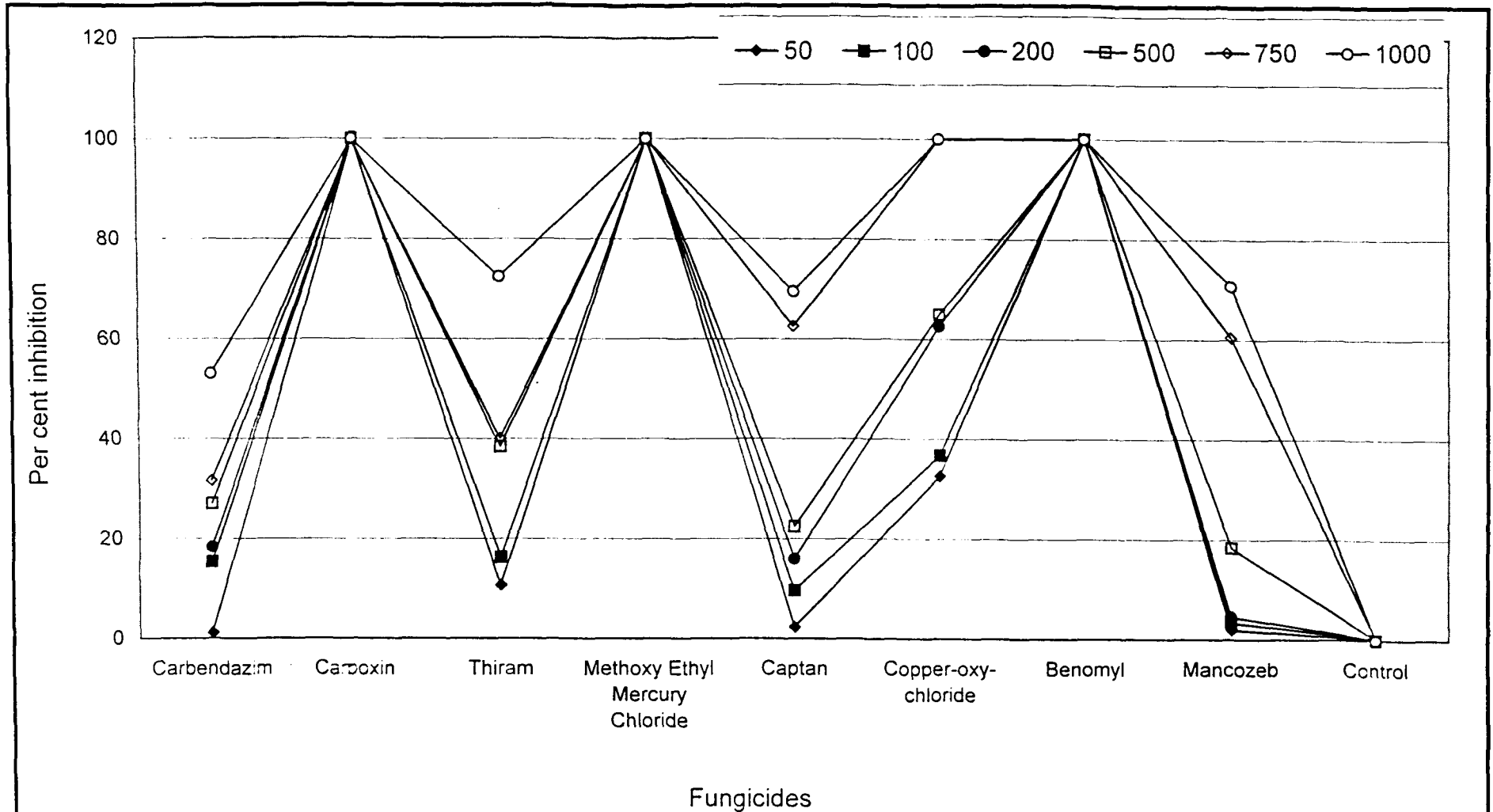


Fig. 10. Effect of fungicides against *Sclerotium rolfsii*

and post emergence germination. Results are presented in Table 18 and they were significant. In seed treatment Benomyl (73.80% mean per cent germination) recorded the highest germination followed by Carbendazim (71.42%) and Carboxin (71.42%) and were on par. Next best fungicides were Methoxy Ethyl Mercury Chloride (57.14%) and Captan (57.14%), followed by Thiram and Copper-Oxy-Chloride with same value of 45.23 percent germination. Mancozeb was least with 42.86 percent germination. While soil drenching, Carbendazim proved to be the best with 83.33 percent germination followed by Carboxin and Benomyl with 73.80 percent and 71.42 percent respectively (Table 18). Methoxy Ethyl Mercury Chloride and Thiram were next best 57.14 and 54.76 percent germination respectively, Copper-Oxy-Chloride (47.62%) and Mancozeb (42.62%) were low in their effectiveness. The pre emergence and post emergence when together, Carbendazim was the best (77.38%) followed by Carboxin and Benomyl with same value of 72.61 per cent. Mancozeb was least effective for *F.solani*.

#### **4.12.7. Evaluation of fungicides against *Rhizoctonia solani* as seed treatment and soil drenching methods**

Eight fungicides were tested in the pot culture experiment for efficacy as seed dressers and soil drenching against *Rhizoctonia solani*. The observations on per cent germination (Table 19) of cotton were recorded. The results were statistically significant. In case of seed treatment highest germination was recorded in Benomyl with 78.57 percent germination followed by Captan (71.42%). Thiram and Methoxy Ethyl Mercury Chloride were on par with 66.66 percent germination. Mancozeb (40.48%) and Copper Oxy Chloride were least (40.48%) followed by Carboxin 40.49 percent germination. In soil drenching

**Table 18: Effect of fungicides against *Fusarium solani***

Sl. No.	Chemicals	Percent germination								
		Seed treatment			Soil drenching			Mean		Grand Mean
		Pre Emergence	Post Emergence	Mean	Pre Emergence	Post Emergence	Mean	Pre Emergence	Post Emergence	
1.	Carbendazim	76.18 (68.57)	66.66 (59.99)	71.42 (64.28)	85.71 (77.14)	80.95 (72.85)	83.33 (74.99)	80.95 (72.85)	73.80 (66.42)	77.38 (69.63)
2.	Carboxin	76.18 (68.57)	66.66 (59.99)	71.42 (44.28)	80.95 (72.85)	66.66 (59.99)	73.80 (46.19)	78.57 (64.28)	66.66 (59.99)	72.61 (65.35)
3.	Thiram	52.38 (47.14)	38.08 (34.27)	45.23 (40.70)	61.90 (55.71)	47.62 (42.86)	54.76 (49.28)	57.14 (51.42)	42.85 (38.56)	49.99 (44.99)
4.	Methoxy Ethyl Mercury Chloride	61.90 (55.71)	52.38 (47.14)	57.14 (51.43)	76.18 (68.57)	57.14 (51.43)	66.66 (60.00)	69.04 (62.14)	54.76 (49.28)	61.90 (55.71)
5.	Captan	61.90 (55.71)	52.38 (47.14)	57.14 (51.43)	66.66 (59.99)	47.62 (42.86)	57.14 (51.43)	64.28 (57.85)	50.00 (45.00)	57.14 (51.43)
6.	Copper Oxy Chloride	47.62 (42.86)	42.84 (38.55)	45.23 (40.71)	52.38 (47.14)	42.86 (38.57)	47.62 (42.86)	50.00 (45.00)	42.85 (38.56)	46.42 (41.78)
7.	Benomyl	76.18 (68.56)	71.42 (64.28)	73.80 (66.42)	80.95 (72.85)	61.90 (55.71)	71.42 (64.23)	78.57 (70.71)	66.66 (59.99)	72.61 (65.35)
8.	Mancozeb	52.38 (47.14)	33.33 (29.99)	42.86 (38.57)	57.14 (51.43)	38.09 (34.28)	42.62 (42.86)	54.76 (49.28)	35.71 (32.14)	19.04 (40.71)
9.	Control	33.33 (30.00)	19.04 (17.14)	26.19 (23.57)	33.33 (30.00)	19.04 (17.14)	26.19 (23.57)	33.33 (30.00)	19.04 (17.14)	26.19 (23.57)

Note: Figures in the paranthesis are the arc-sin values

SEm ± : 4.031  
 CD at 1% Application : 19.75  
 CD at 1% Emergence : 19.75  
 CD at 1% Fungicide : 6.98  
 CD at 1% Application V/s Emergence : NS  
 CD at 1% Application V/s Fungicide : NS  
 CD at 1% Emergence V/s Fungicide : NS  
 CD at 1% Application V/s Emergence V/s Fungicide : NS  
 CV % : 13.70

**Table 19: Effect of fungicides against *Rhizoctonia solani***

Sl. No.	Chemicals	Percent germination								
		Seed treatment			Soil drenching			Mean		Grand Mean
		Pre Emergence	Post Emergence	Mean	Pre emergence	Post Emergence	Mean	Pre Emergence	Post emergence	
1.	Carbendazim	42.86 (38.57)	38.09 (34.29)	40.49 (36.43)	61.90 (55.71)	47.62 (42.86)	54.76 (49.28)	52.38 (47.14)	42.86 (38.57)	47.62 (42.86)
2.	Carboxin	90.47 (81.43)	66.66 (59.99)	78.57 (70.71)	90.47 (81.43)	71.42 (64.28)	80.95 (72.85)	90.47 (81.43)	69.04 (62.14)	79.76 (71.78)
3.	Thiram	80.95 (72.85)	52.38 (47.14)	66.66 (59.99)	95.24 (85.71)	66.66 (59.99)	80.95 (47.99)	88.09 (79.28)	59.52 (53.57)	73.80 (66.43)
4.	Methoxy Ethyl Mercury Chloride	80.95 (72.85)	52.38 (47.14)	66.66 (59.99)	95.24 (85.71)	71.42 (64.28)	83.33 (74.99)	88.09 (79.28)	61.90 (55.71)	74.99 (67.49)
5.	Captan	76.18 (68.57)	66.66 (59.99)	71.42 (64.28)	90.47 (81.43)	66.66 (59.99)	78.57 (70.71)	83.33 (74.99)	66.66 (59.99)	74.99 (67.49)
6.	Copper Oxy Chloride	42.86 (38.57)	38.09 (34.29)	40.48 (36.43)	52.38 (47.14)	47.62 (42.86)	50.00 (45.00)	47.62 (42.86)	42.86 (38.57)	55.24 (40.72)
7.	Benomyl	90.47 (81.43)	66.66 (59.99)	78.57 (70.71)	100.00 (90.00)	76.18 (68.57)	88.09 (79.28)	95.24 (85.71)	71.42 (64.28)	83.33 (74.99)
8.	Mancozeb	47.62 (42.86)	33.33 (30.00)	40.48 (36.43)	52.38 (47.14)	38.09 (34.29)	45.24 (40.72)	50.00 (45.00)	35.72 (32.14)	42.86 (38.57)
9.	Control	38.09 (34.29)	28.57 (25.71)	33.33 (30.00)	38.09 (34.29)	28.57 (25.71)	33.33 (30.00)	38.09 (34.29)	28.57 (25.71)	33.33 (30.00)

Note: Figures in the parenthesis are the arc-sin values

SEm ± : 3.679  
 CD at 1% Application : 28.84  
 CD at 1% Emergence : 28.84  
 CD at 1% Fungicide : NS  
 CD at 1% Application V/s Emergence : NS  
 CD at 1% Application V/s Fungicide : 26.98  
 CD at 1% Emergence V/s Fungicide : NS  
 CD at 1% Application V/s Emergence V/s Fungicide : NS  
 CV % : 11.46

similar trend was observed i.e., Benomyl ranking highest (88.09%), followed but followed by Methoxy Ethyl Mercury Chloride (83.33%) and then with fungicides of Carboxin and Thiram with 80.95% germination. Captan (78.57%) was also good. Mancozeb (45.24%) was least followed by Carbendazim (54.76%). Over all Benomyl was the best (83.33%), followed by Carboxin (79.76%).

#### **4.12.8. Evaluation of seed dressing and soil drenching of fungicides against *Rhizoctonia bataticola***

Same eight fungicides as seed treatment and soil drenching were evaluated as seed treatment and soil drenching separately against *Rhizoctonia bataticola* as presented in material and methods. Observations on percent germination of cotton were recorded and results were statistically significant (Table 20). In seed treatment experiment Carboxin (80.95%) was the highest followed by Thiram (73.81%) and Benomyl (73.81%). Captan was the next best (69.04%) followed by Methoxy Ethyl Mercury Chloride (66.66%). The least rankings were observed in Mancozeb (38.10%), Copper-Oxy-Chloride (40.47%) followed by Carbendazim (40.48%).

In soil drenching of fungicides Carboxin (85.71%) recorded the highest germination percentage followed by Methoxy Ethyl Mercury Chloride (83.33%), Benomyl (80.95%), Thiram (73.81%) and Captan (71.42%). Least germination was observed in Mancozeb (42.86%) followed by Copper-Oxy-Chloride (47.62%) and Carbendazim (57.14%). Over all (Table 20), Carboxin was the most effective fungicide (83.33%) followed by Benomyl (77.38%), Methoxy Ethyl Mercury Chloride

**Table 20: Effect of fungicides against *Rhizoctonia bataticola***

Sl. No.	Chemicals	Percent germination								
		Seed treatment			Soil drenching			Mean		Grand Mean
		Pre Emergence	Post Emergence	Mean	Pre Emergence	Post Emergence	Mean	Pre Emergence	Post Emergence	
1.	Carbendazim	47.62 (42.86)	33.33 (30.00)	40.48 (36.43)	66.66 (59.99)	47.62 (42.86)	57.14 (51.43)	57.14 (51.43)	40.48 (36.43)	48.81 (43.93)
2.	Carboxin	90.47 (81.43)	71.42 (64.28)	80.95 (72.85)	95.24 (85.71)	76.18 (68.57)	85.71 (77.14)	92.86 (83.57)	73.80 (66.42)	83.33 (74.99)
3.	Thiram	85.71 (77.14)	61.90 (55.71)	73.81 (66.43)	90.47 (81.43)	57.14 (51.43)	73.81 (66.43)	88.09 (79.28)	59.52 (53.57)	73.81 (66.43)
4.	Methoxy Ethyl Mercury Chloride	80.95 (72.85)	52.38 (47.14)	66.66 (59.99)	90.47 (81.43)	76.18 (68.57)	83.33 (74.99)	85.71 (78.57)	64.28 (57.85)	74.99 (67.49)
5.	Captan	76.18 (68.57)	61.90 (55.71)	69.04 (62.14)	80.95 (72.83)	61.90 (55.71)	71.42 (64.28)	78.57 (70.70)	61.90 (55.71)	70.23 (63.21)
6.	Copper Oxy chloride	47.62 (42.86)	33.32 (29.99)	40.47 (36.43)	57.14 (51.43)	38.09 (34.29)	47.62 (42.86)	52.38 (47.14)	35.71 (32.14)	44.05 (39.64)
7.	Benomyl	85.71 (77.14)	61.90 (55.71)	73.81 (66.43)	90.47 (81.43)	71.42 (64.29)	80.95 (72.85)	88.09 (79.28)	66.66 (59.99)	77.38 (69.64)
8.	Mancozeb	47.62 (42.86)	28.57 (25.71)	38.10 (34.29)	52.38 (47.12)	33.33 (30.00)	42.86 (38.57)	50.00 (45.00)	30.95 (27.86)	40.48 (36.43)
9.	Control	33.33 (30.00)	19.04 (17.14)	26.19 (23.57)	33.33 (30.00)	19.04 (17.14)	26.19 (23.57)	33.33 (30.00)	19.04 (17.14)	26.19 (23.57)

Note: Figures in the parenthesis are the arc-sin values

SEM ± : 3.349  
 CD at 1% Application : 26.26  
 CD at 1% Emergence : 26.27  
 CD at 1% Fungicide : 16.10  
 CD at 1% Application V/s Emergence : NS  
 CD at 1% Application V/s Fungicide : 24.56  
 CD at 1% Emergence V/s Fungicide : NS  
 CD at 1% Application V/s Emergence V/s Fungicide : NS  
 CV % : 10.76

(74.99%), Thiram (73.81%) and Captan (70.23%). Mancozeb was least effective (40.48%).

#### **4.12.9. Evaluation of fungicides against *Sclerotium rolfii* as seed treatment and soil drenching methods**

Eight fungicides were screened for their efficacy against *Sclerotium rolfii* in pots as seed treatment and soil application separately. Observations were recorded on percent germination of cotton and the results were analysed statistically and found significant (Table 21). In seed treatment Carboxin (90.47%) followed by Methoxy Ethyl Mercury Chloride (85.71%). Copper-Oxy-Chloride (78.56%), Benomyl (78.56%) and Captan (73.80%) were the next superior fungicides. Carbendazim (50.00%) and Mancozeb (47.62%) although found effective but less superior. Thiram (45.24%) was the least among all test fungicides against *S.rolfsii*.

In soil drenching Carboxin (92.96%) recorded the highest germination followed by Methoxy Ethyl Mercury Chloride (88.09%), Benomyl (83.33%), Copper-Oxy-Chloride (83.33%) and Captan (78.56%). Carbendazim, Thiram and Mancozeb recorded 57.14, 50.00 and 50.00 percent respectively.

### **4.13. Effect of insecticides on biofungicide and pathogens**

#### **4.13.1. Effect of insecticides on *Trichoderma harzianum***

Five commonly used insecticides in cotton were evaluated under 'in vitro' conditions at six concentrations of 50, 100, 200, 250, 500 and 750 ppm as presented in material and methods against *Trichoderma*

**Table 21: Effect of fungicides against *Sclerotium rolfsii***

Sl. No.	Chemicals	Percent germination							
		Seed treatment			Soil drenching			Grand Mean	
		Pre Emergence	Post emergence	Mean	Pre Emergence	Post emergence	Mean	Soil drenching	Post emergence
1.	Carbendazim	52.38 (47.14)	47.62 (42.86)	50.00 (47.14)	61.90 (55.71)	47.62 (42.86)	57.14 (51.43)	54.76 (49.30)	47.62 (42.90)
2.	Carboxin	90.47 (81.43)	90.47 (81.43)	90.47 (81.43)	95.24 (85.71)	90.50 (81.43)	92.96 (83.60)	92.86 (83.60)	90.47 (81.43)
3.	Thiram	47.62 (42.85)	42.86 (38.60)	45.24 (42.86)	52.40 (47.14)	47.62 (42.86)	50.00 (45.00)	50.00 (45.00)	45.24 (40.72)
4.	Methoxy Ethyl Mercury Chloride	85.71 (77.14)	85.71 (77.14)	85.71 (77.14)	90.50 (81.43)	80.95 (72.85)	88.09 (79.30)	85.71 (77.14)	83.33 (75.00)
5.	Captan	76.18 (68.57)	71.42 (64.30)	73.80 (68.57)	80.95 (72.85)	76.18 (68.57)	78.56 (70.71)	78.56 (70.71)	73.08 (66.42)
6.	Copper Oxy chloride	80.95 (72.85)	76.18 (68.57)	78.56 (72.85)	85.71 (77.14)	80.95 (72.85)	83.33 (75.00)	83.33 (75.00)	78.56 (70.71)
7.	Benomyl	80.95 (72.85)	76.18 (68.57)	78.56 (72.85)	85.71 (77.14)	80.95 (72.85)	83.33 (75.00)	83.33 (75.00)	78.56 (70.71)
8.	Mancozeb	47.62 (42.86)	38.09 (34.30)	47.62 (42.86)	52.40 (47.14)	42.86 (38.57)	50.00 (45.00)	47.62 (42.90)	40.47 (36.43)
9.	Control	33.33 (30.00)	19.04 (17.14)	26.19 (30.00)	33.33 (30.00)	19.04 (17.14)	33.33 (30.00)	26.19 (23.60)	19.04 (17.14)
	Mean	66.13 (59.52)	60.84 (54.77)	63.48 (57.14)	71.00 (63.81)	63.00 (56.70)	68.52 (61.70)	66.93 (60.24)	61.90 (55.71)

Note: Figures in the parenthesis are the arc-sin values

SEm ±	: 3.872
CD at 1% Application	: 6.71
CD at 1% Emergence	: 9.64
CD at 1% Fungicide	: 9.64
CD at 1% Application V/s Emergence	: 11.62
CD at 1% Application V/s Fungicide	: 11.62
CD at 1% Emergence V/s Fungicide	: 11.62
CD at 1% Application V/s Emergence V/s Fungicide	: NS
CV %	: 10.17

*harzianum*. The per cent inhibition of growth was recorded (Table 22 and Figure 11). The results were statistically significant. Chlorpyrifos was found more inhibitive to *T.harzianum* i.e., from 200 ppm completely inhibiting the growth, followed by Imidachloprid (500 ppm). Phorate and Carbofuran were also inhibitory to *T.harzianum* but Phorate was superior to Carbofuran (Table 22) at all concentrations. Phorate recorded 71.42 percent inhibition (mean of all concentrations) and Carbofuran with 50.36 percent. Endosulfan although better than rest of the other insecticides, but it was also inhibitory (51.44%).

#### **4.13.2. Effect of insecticides against *Fusarium solani***

Among five insecticides tested against *Fusarium solani*. Chlorpyrifos (61.28% mean of all concentrations) and Imidachloprid (59.55%) were highly suppressive for *F.solani* (Table 23 and Figure 12) and were on par. Endosulfan (17.42%), Carbofuran (23.74%) and Phorate (27.40%) were least in inhibiting the growth of *F.solani*.

#### **4.13.3. Effect of insecticides against *Rhizoctonia solani***

As presented in material and methods, five insecticides at six concentrations were tested against *Rhizoctonia solani*. The percent inhibition of *R.solani* was recorded (Table 24 and Figure 13). The results were statistically significant. Chlorpyrifos was the most effective against *R.solani*, completely inhibiting the growth even at 50 ppm. The next one was Imidacloprid had 42.92 percent inhibition at 50 ppm and a gradual increase in inhibition as the concentration increased and at 750 ppm, there was 71.69 percent inhibition. Endosulfan, Carbofuran and Phorate were not effective upto 250 ppm. But all three recorded more

**Table 22: Effect of insecticides against *Trichoderma harzianum***

Sl. No.	Concentration (ppm)	Phorate	Carbofuran	Endosulfan	Imidacloprid	Chlorpyrifos	Control	Mean
1.	50	54.95 (49.46)	10.00 (9.00)	18.74 (16.86)	27.77 (24.99)	47.34 (42.61)	0.00 (0.00)	26.47 (23.82)
2.	100	58.85 (52.96)	11.76 (10.58)	27.92 (25.12)	34.97 (31.47)	56.63 (50.97)	0.00 (0.00)	31.69 (28.52)
3.	200	71.04 (63.93)	34.08 (30.67)	33.33 (30.00)	58.59 (52.73)	100.00 (90.00)	0.00 (0.00)	49.41 (44.56)
4.	250	76.53 (68.87)	61.66 (55.50)	51.03 (45.93)	68.72 (61.85)	100.00 (90.00)	0.00 (0.00)	59.65 (53.69)
5.	500	79.68 (71.71)	84.66 (76.20)	77.66 (69.90)	100.00 (90.00)	100.00 (90.00)	0.00 (0.00)	73.66 (66.30)
6.	750	87.45 (78.71)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	0.00 (0.00)	81.24 (73.11)
	<b>Mean</b>	<b>71.42</b> <b>(64.27)</b>	<b>50.36</b> <b>(45.32)</b>	<b>51.44</b> <b>(46.30)</b>	<b>65.01</b> <b>(58.51)</b>	<b>83.99</b> <b>(75.59)</b>	<b>0.00</b> <b>(0.00)</b>	<b>53.70</b> <b>(48.33)</b>

Note: Figures in the parenthesis are the arc-sin values

SEm ± : 1.339  
 CD at 1% Insecticide : 2.99  
 CD at 1% Concentration : 2.99  
 CD at 1% Insecticide V/s Concentration : 3.77  
 CV % : 4.79

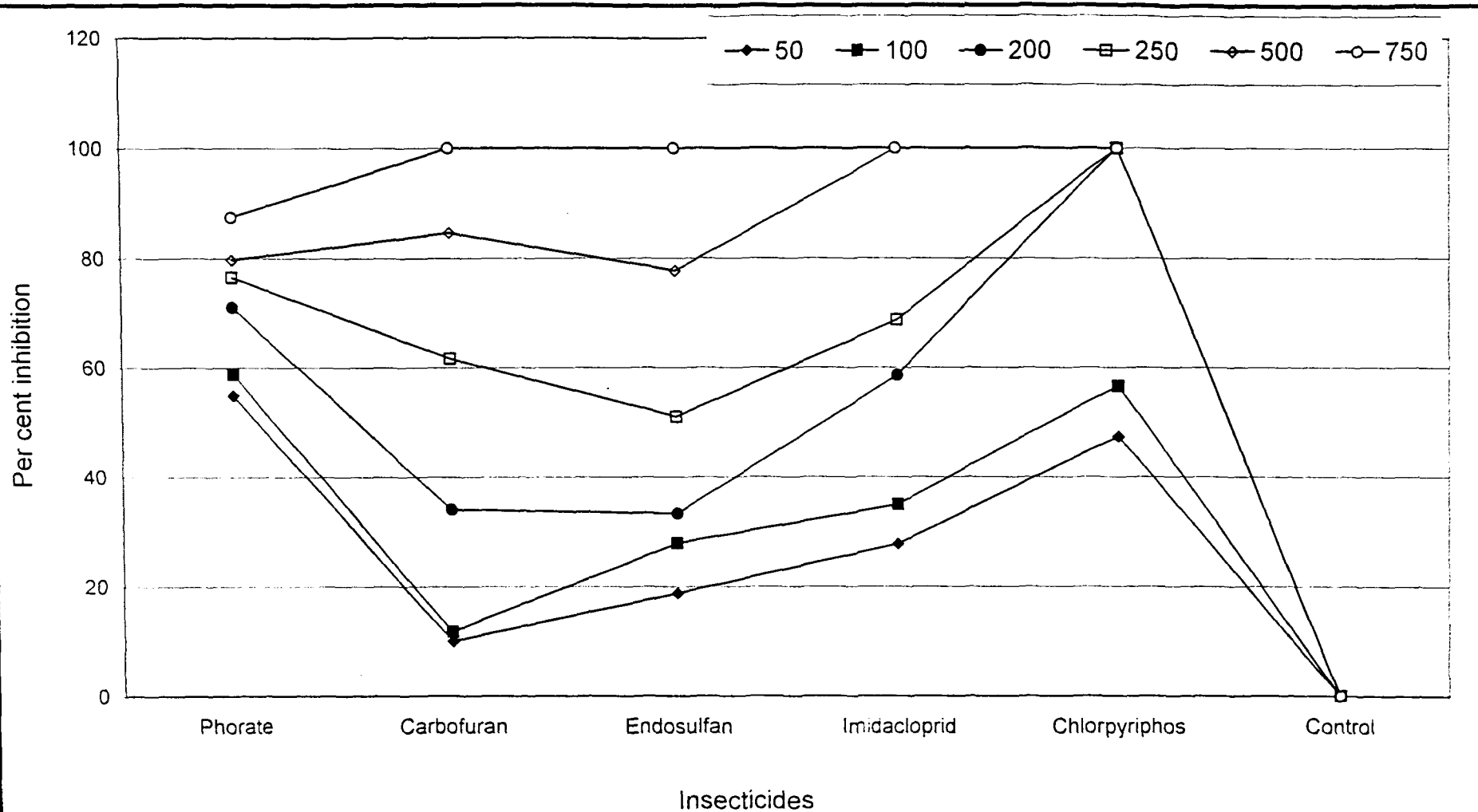


Fig. 11. Effect of insecticides against *Trichoderma harzianum*

**Table 23: Effect of insecticides against *Fusarium solani***

Sl. No.	Concentration (ppm)	Phorate	Carbofuran	Endosulfan	Imidacloprid	Chlorpyriphos	Control	Mean
1.	50	3.38 (3.04)	2.31 (2.08)	1.67 (1.50)	38.67 (34.80)	39.29 (35.36)	0.00 (0.00)	14.22 (12.80)
2.	100	3.74 (3.37)	2.05 (1.85)	1.79 (1.61)	39.69 (35.72)	42.60 (38.34)	0.00 (0.00)	14.98 (13.48)
3.	200	8.24 (7.41)	6.85 (6.16)	4.20 (3.78)	59.92 (53.93)	59.35 (53.41)	0.00 (0.00)	23.09 (20.78)
4.	250	23.04 (20.74)	18.89 (17.00)	16.33 (14.70)	61.39 (55.25)	63.44 (57.09)	0.00 (0.00)	30.51 (27.46)
5.	500	48.59 (34.73)	42.61 (38.35)	32.91 (29.62)	75.82 (68.24)	76.52 (68.87)	0.00 (0.00)	46.07 (41.46)
6.	750	77.42 (69.68)	69.73 (62.76)	47.62 (42.86)	81.82 (73.63)	86.47 (77.82)	0.00 (0.00)	60.51 (54.46)
	<b>Mean</b>	<b>27.40</b> <b>(24.66)</b>	<b>23.74</b> <b>(21.37)</b>	<b>17.42</b> <b>(15.68)</b>	<b>59.55</b> <b>(35.36)</b>	<b>61.28</b> <b>(55.15)</b>	<b>0.00</b> <b>(0.00)</b>	<b>31.56</b> <b>(28.41)</b>

Note: Figures in the parenthesis are the arc-sin values

SEm ± : 0.552  
 CD at 1% Insecticide : 1.23  
 CD at 1% Concentration : 1.23  
 CD at 1% Insecticide V/s Concentration : 1.53.  
 CV % : 3.36

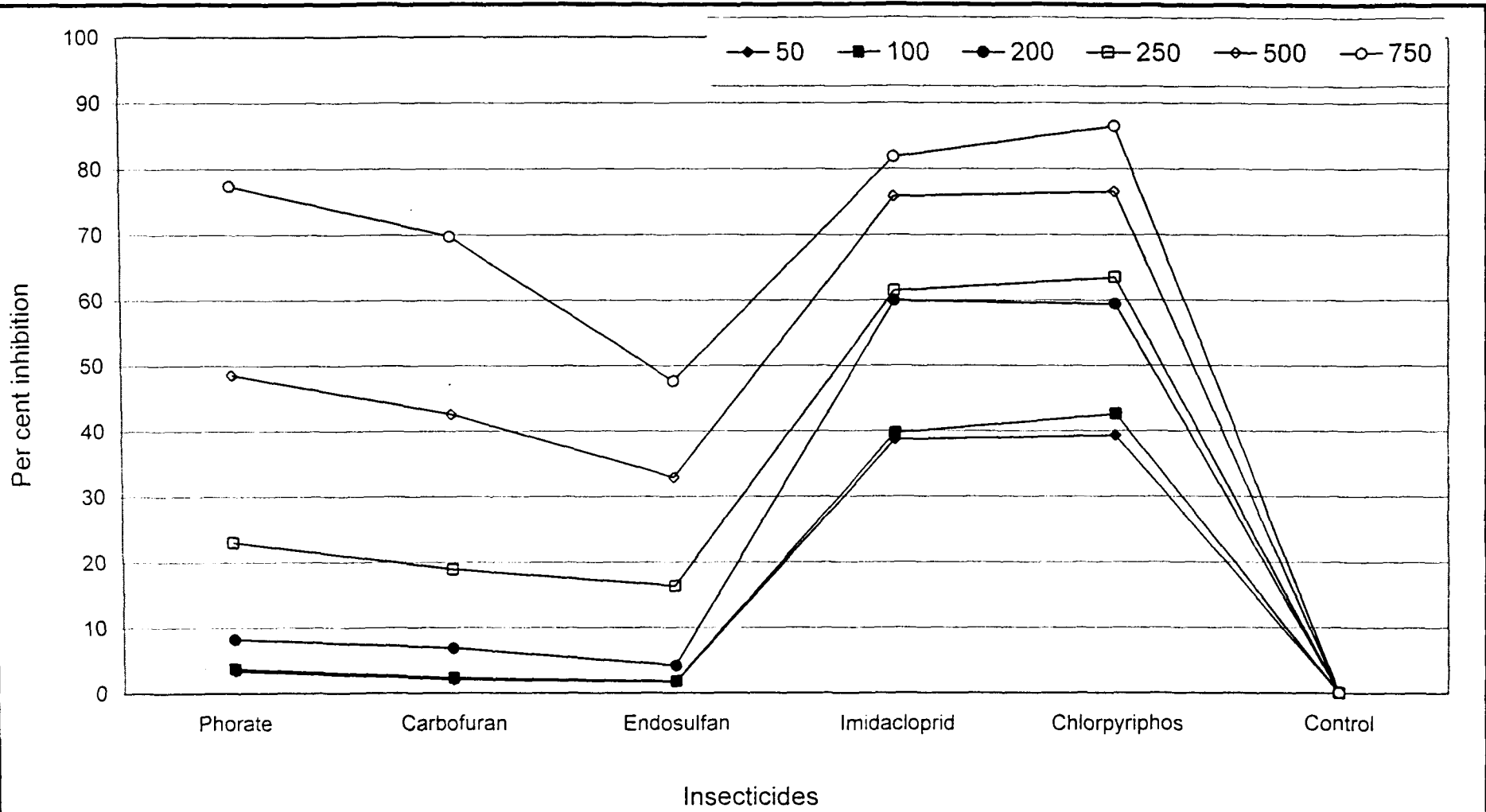


Fig. 12. Effect of insecticides against *Fusarium solani*

**Table 24: Effect of insecticides against *Rhizoctonia solani***

Sl. No.	Concentration (ppm)	Phorate	Carbofuran	Endosulfan	Imidacloprid	Chlorpyriphos	Control	Mean
1.	50	4.28 (3.85)	3.41 (3.07)	1.29 (1.16)	42.92 (38.62)	100.00 (90.00)	0.00 (0.00)	25.31 (22.78)
2.	100	4.43 (3.99)	3.74 (3.36)	2.26 (2.04)	44.12 (39.70)	100.00 (90.00)	0.00 (0.00)	25.76 (23.18)
3.	200	6.56 (5.90)	5.35 (4.82)	3.41 (3.07)	54.88 (49.39)	100.00 (90.00)	0.00 (0.00)	28.37 (25.53)
4.	250	16.91 (15.22)	15.27 (13.74)	12.00 (10.80)	55.84 (50.25)	100.00 (90.00)	0.00 (0.00)	33.33 (30.00)
5.	500	40.81 (36.72)	35.46 (31.92)	26.98 (24.28)	62.40 (56.16)	100.00 (90.00)	0.00 (0.00)	44.27 (39.84)
6.	750	61.07 (54.96)	56.66 (51.00)	50.75 (45.67)	71.69 (64.52)	100.00 (90.00)	0.00 (0.00)	56.69 (51.02)
	<b>Mean</b>	<b>22.34</b> <b>(20.11)</b>	<b>19.98</b> <b>(17.98)</b>	<b>16.11</b> <b>(14.50)</b>	<b>55.31</b> <b>(49.77)</b>	<b>100.00</b> <b>(90.00)</b>	<b>0.00</b> <b>(0.00)</b>	<b>35.62</b> <b>(32.06)</b>

Note: Figures in the parenthesis are the arc-sin values

SEm ± : 1.277  
 CD at 1% Insecticide : 2.86  
 CD at 1% Concentration : 2.86  
 CD at 1% Insecticide V/s Concentration : 3.54  
 CV % : 6.89

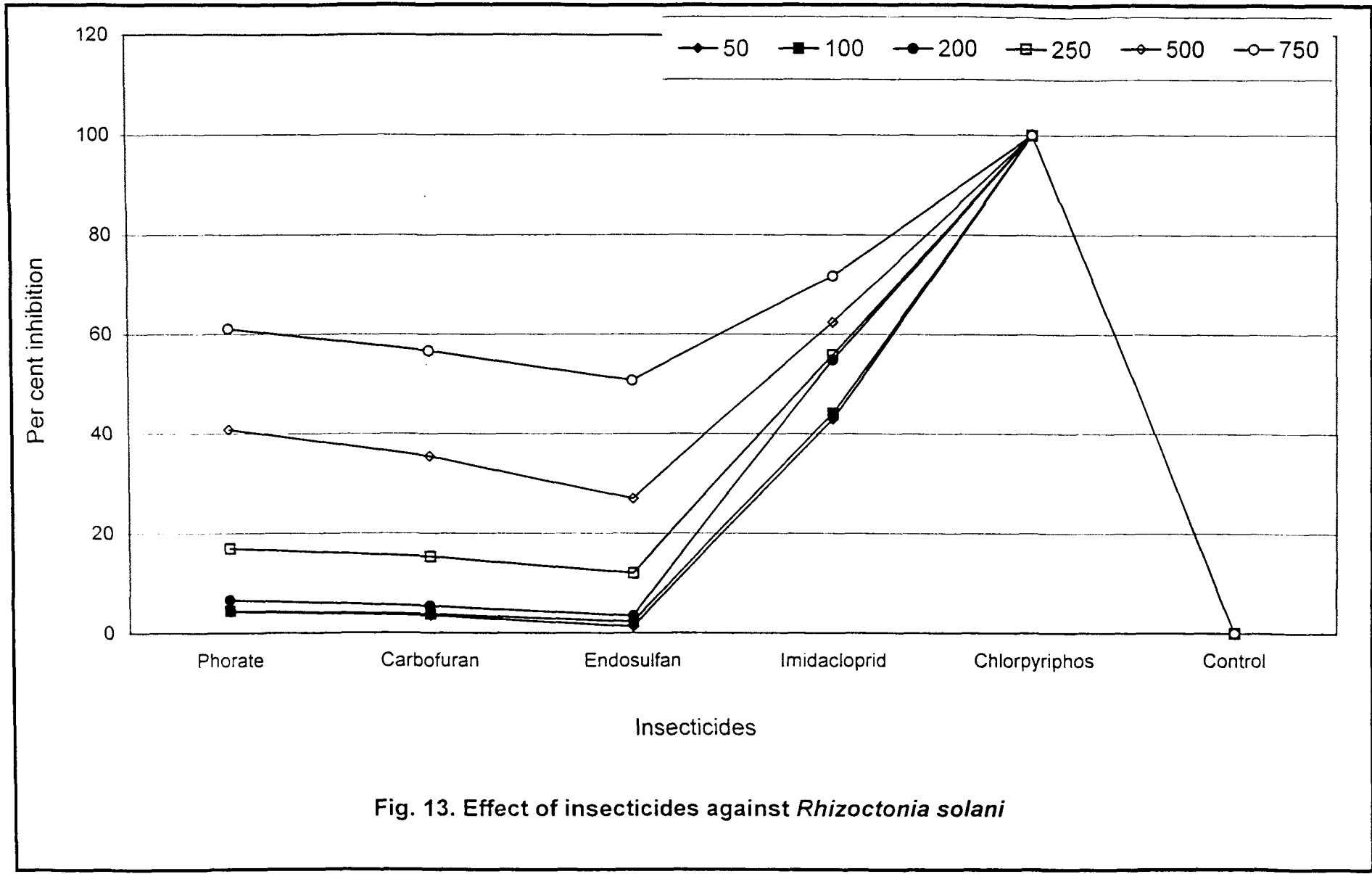


Fig. 13. Effect of insecticides against *Rhizoctonia solani*

than 50 percent inhibition at 750 ppm i.e., Endosulfan, Carbofuran and Phorate with 50.75 percent, 56.66 percent and 61.07 percent respectively.

#### **4.13.4. Effect of insecticides against *Rhizoctonia bataticola***

Observations on effect of six insecticides at six concentrations were recorded for *Rhizoctonia bataticola* and the results (Table 25 and Figure 14) were statistically significant. Chlorpyrifos had same result on *R.bataticola* too, i.e., complete inhibition of growth at 50 ppm., followed by Imidachloprid with 37.42 percent inhibition at 50 ppm and gradually increased as the concentration increased and completely inhibiting the growth at 750 ppm. At 250 ppm, Endosulfan, Carbofuran and Phorate were not inhibitive to *R.bataticola*. However, Carbofuran and Phorate recorded 71.55 and 66.54 per cent inhibition at 750 ppm respectively. In case of Endosulfan, it was lowest at 750 ppm i.e., 40.82 per cent inhibition. The mean values indicated that, Chlopyrifos (66.83%) followed by Imidacloprid (66.83%) were suppressive for the growth of *R.bataticola*.

#### **4.13.5. Effect of insecticides against *Sclerotium rolfsii***

Among five insecticides tested against *Sclerotium rolfsii* at six levels of concentrations i.e., 50, 100, 200, 250, 500 and 750, Chlorpyrifos recorded highest percent inhibition (77.48%), followed by Imidacloprid (56.63%), mean of all concentrations as presented in Table 26 and Figure 15. Chlorpyrifos had cent percent inhibition at 500 and 750 ppm, but Imidachloprid recorded same result at 750 ppm. Among the rest of three insecticides Carbofuran, Phorate and Endosulfan were in the order of merit with mean percent inhibitions of 37.06, 28.69 and

**Table 25: Effect of insecticides against *Rhizoctonia bataticola***

Sl. No.	Concentration (ppm)	Phorate	Carbofuran	Endosulfan	Imidacloprid	Chlorpyrifos	Control	Mean
1.	50	2.99 (2.69)	2.71 (2.44)	1.55 (1.40)	37.42 (33.68)	100.00 (90.00)	0.00 (0.00)	24.11 (21.70)
2.	100	3.00 (2.70)	2.93 (2.63)	1.59 (1.43)	39.09 (35.18)	100.00 (90.00)	0.00 (0.00)	24.43 (21.99)
3.	200	8.31 (7.47)	6.88 (6.19)	2.70 (2.43)	59.53 (53.58)	100.00 (90.00)	0.00 (0.00)	29.57 (26.61)
4.	250	21.88 (19.69)	24.69 (22.22)	10.76 (9.68)	79.05 (71.14)	100.00 (90.00)	0.00 (0.00)	39.39 (35.45)
5.	500	40.48 (36.43)	48.66 (43.79)	26.44 (23.80)	85.92 (77.33)	100.00 (90.00)	0.00 (0.00)	50.25 (45.22)
6.	750	66.54 (59.88)	71.55 (64.39)	40.82 (36.74)	100.00 (90.00)	100.00 (90.00)	0.00 (0.00)	63.15 (56.83)
	<b>Mean</b>	<b>23.86</b> <b>(21.48)</b>	<b>26.23</b> <b>(23.61)</b>	<b>13.98</b> <b>(12.58)</b>	<b>66.83</b> <b>(60.15)</b>	<b>100.00</b> <b>(90.00)</b>	<b>0.00</b> <b>(0.00)</b>	<b>38.48</b> <b>(34.63)</b>

Note: Figures in the parenthesis are the arc-sin values

SEm ± : 0.353  
 CD at 1% Insecticide : 0.79  
 CD at 1% Concentration : 0.79  
 CD at 1% Insecticide V/s Concentration : 0.98  
 CV % : 1.77

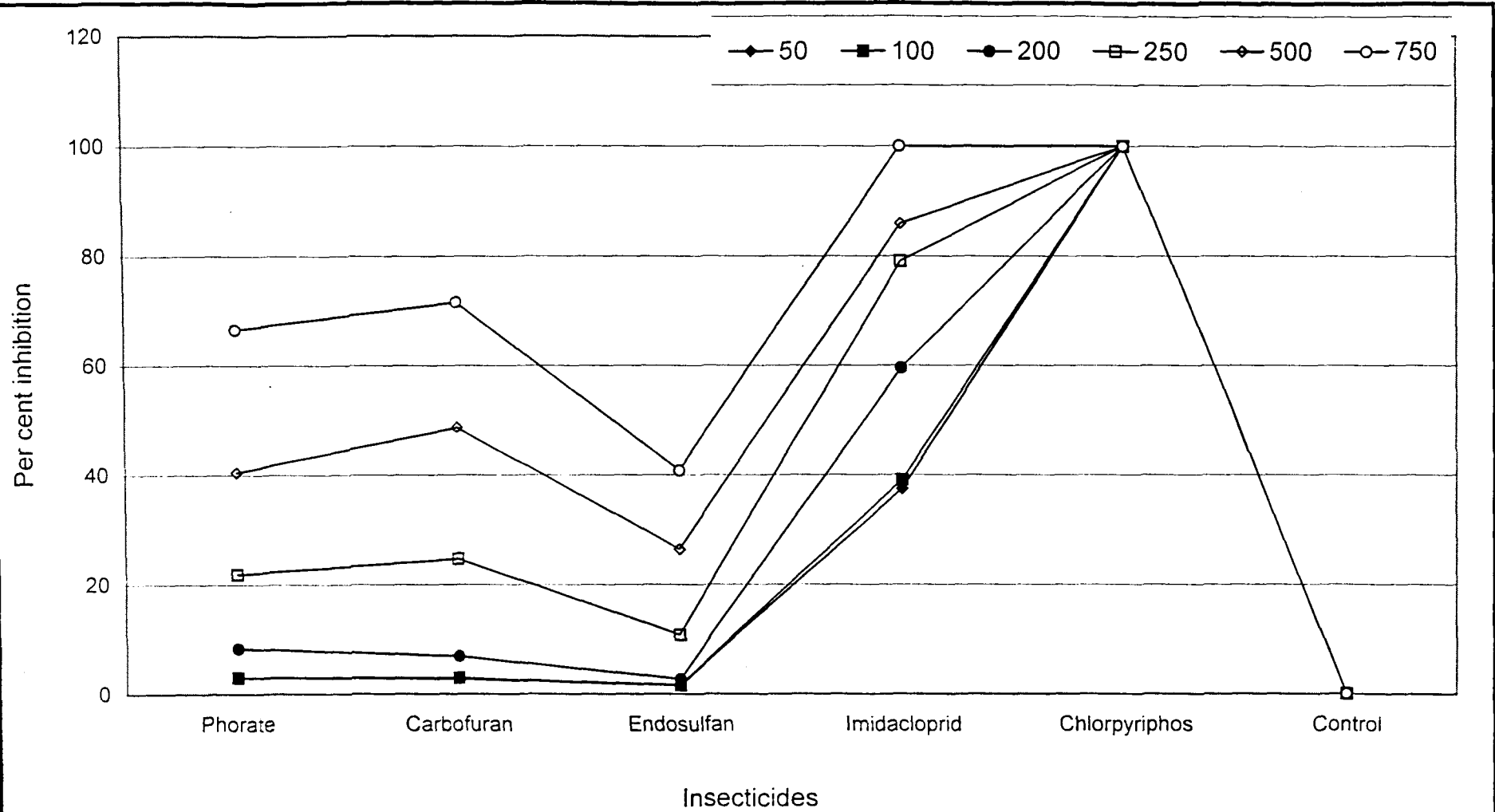


Fig. 14. Effect of insecticides against *Rhizoctonia bataticola*

**Table 26: Effect of insecticides against *Sclerotium rolfsii***

Sl. No.	Concentration (ppm)	Phorate	Carbofuran	Endosulfan	Imidacloprid	Chlorpyriphos	Control	Mean
1.	50	4.42 (3.97)	6.95 (6.26)	2.17 (1.95)	26.77 (24.09)	46.40 (41.76)	0.00 (0.00)	14.45 (13.00)
2.	100	4.66 (4.19)	7.07 (6.36)	2.42 (2.18)	29.77 (26.79)	51.05 (45.94)	0.00 (0.00)	15.83 (14.24)
3.	200	15.73 (14.16)	18.73 (16.87)	5.58 (5.02)	42.42 (38.17)	79.77 (71.79)	0.00 (0.00)	27.04 (24.33)
4.	250	25.94 (23.34)	35.78 (32.20)	7.01 (6.30)	58.36 (52.52)	87.71 (78.94)	0.00 (0.00)	35.80 (32.22)
5.	500	46.90 (42.21)	68.38 (61.54)	21.63 (19.47)	82.47 (74.22)	100.00 (90.00)	0.00 (0.00)	53.23 (47.90)
6.	750	74.51 (67.05)	85.48 (76.93)	36.40 (23.76)	100.00 (90.00)	100.00 (90.00)	0.00 (0.00)	64.40 (57.96)
	<b>Mean</b>	<b>28.69</b> <b>(6.26)</b>	<b>37.06</b> <b>(33.36)</b>	<b>10.87</b> <b>(9.78)</b>	<b>56.63</b> <b>(50.97)</b>	<b>77.48</b> <b>(69.74)</b>	<b>0.00</b> <b>(0.00)</b>	<b>35.12</b> <b>(31.61)</b>

Note: Figures in the parenthesis are the arc-sin values

SEm ± : 0.658

CD at 1% Insecticide : 1.81

CD at 1% Concentration : 1.81

CD at 1% Insecticide V/s Concentration : 1.82

CV % : 3.61

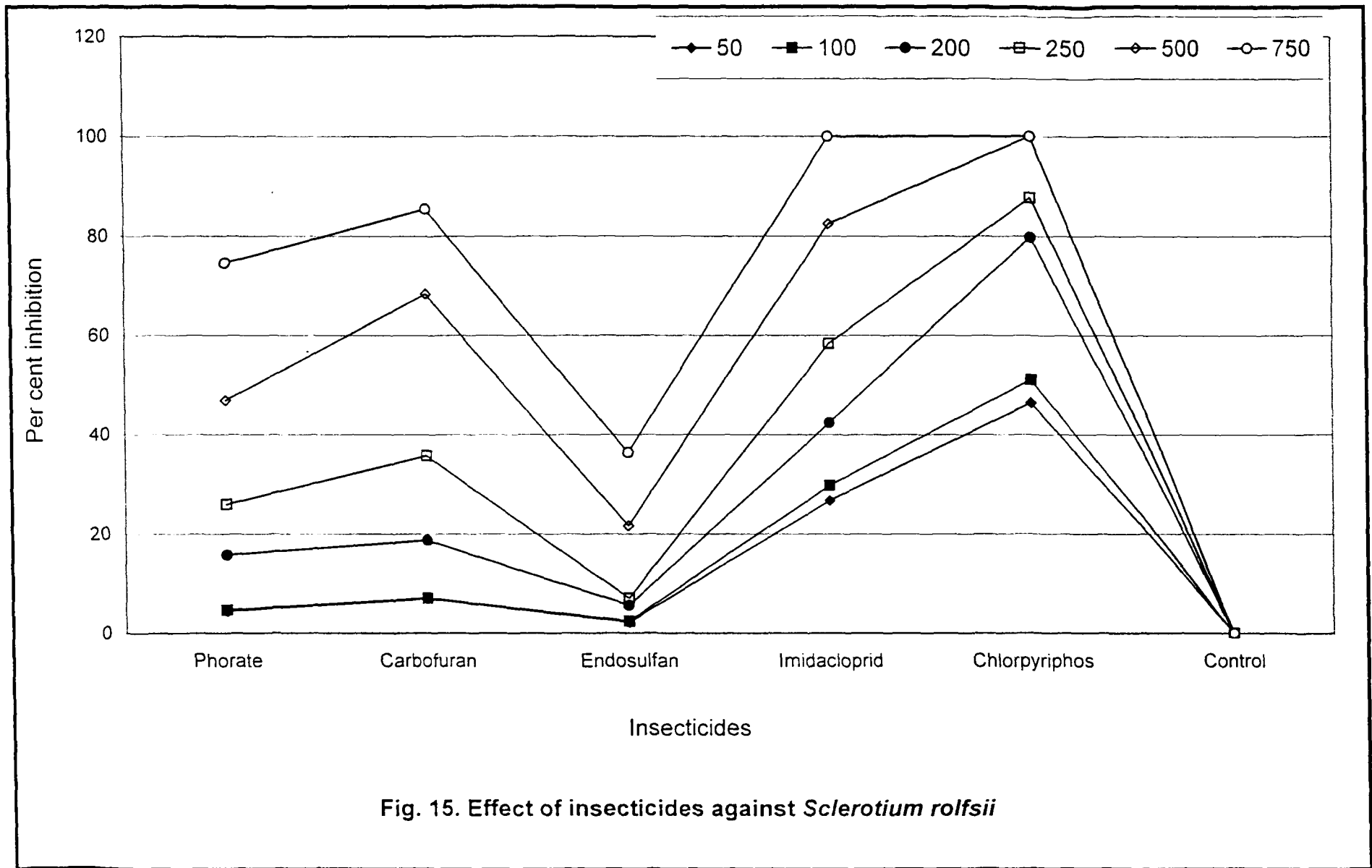


Fig. 15. Effect of insecticides against *Sclerotium rolfsii*

10.87 respectively. Later three insecticides were not effective at low concentrations tested. Overall, Chlorpyrifos and Imidachloprid were highly effective against *S.rolfsii*.

#### **4.14. Effect of herbicides against biofungicide and pathogens**

##### **4.14.1. Effect of herbicides against *Trichoderma harzianum***

Five herbicides at six concentrations i.e., 50, 100, 200, 500, 750 and 1000 ppm were tested for their effect against *Trichoderma harzianum* under 'in vitro' conditions as presented in material and methods. The per cent inhibitions were recorded (Table 27 and Figure 16). The results were statistically significant. Alachlor was found to be highly inhibitory to growth of *T.harzianum* even at the lowest concentration of 50 ppm. Next effective herbicides were Paraquat (87.51% at 200 ppm and 100% at 500 ppm) and Glyphosate (67.80% at 200 ppm and 100% at 500 ppm). Diuron recorded least among test herbicides from 50 ppm (29.54%) to 750 ppm (84.39%). Pendimethalin was also effective with 65.59 per cent inhibition at 500 ppm and cent percent inhibition from 750 ppm onwards. Over all Alachlor (100.00%) followed by Paraquat (84.58%), Glyphosate (73.81%) and Pendimethalin (63.73%) were found suppressive for the growth of *T.harzianum*.

##### **4.14.2. Effect of herbicides against *Fusarium solani***

Five herbicides were tested against *Fusarium solani* at six concentrations under 'in vitro' conditions. Observations on per cent inhibition of *F.solani* growth are presented in Table 28 and Figure 17. The results were statistically significant. At 500 ppm Alachlor, Diuron and Paraquat found to be cent per cent inhibitory to *F.solani*. The mean

**Table 27: Effect of herbicides against *Trichoderma harzianum***

Sl. No.	Concentration (ppm)	Alachlor	Glyphosate	Diuron	Pendimethalin	Paraquat	Control	Mean
1.	50	100.00 (90.00)	34.43 (30.99)	29.54 (26.59)	55.25 (31.73)	59.03 (53.13)	0.00 (0.00)	43.04 (38.74)
2.	100	100.00 (90.00)	40.68 (36.61)	28.95 (26.05)	38.29 (34.46)	60.95 (54.85)	0.00 (0.00)	44.81 (40.33)
3.	200	100.00 (90.00)	67.80 (61.02)	34.52 (31.07)	43.25 (38.92)	87.51 (78.76)	0.00 (0.00)	55.51 (49.96)
4.	500	100.00 (90.00)	100.00 (90.00)	62.46 (56.21)	65.59 (59.03)	100.00 (90.00)	0.00 (0.00)	71.34 (64.20)
5.	750	100.00 (90.00)	100.00 (90.00)	84.39 (75.95)	100.00 (90.00)	100.00 (90.00)	0.00 (0.00)	80.73 (72.65)
6.	1000	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	0.00 (0.00)	83.33 (75.00)
	<b>Mean</b>	<b>100.00</b> <b>(90.00)</b>	<b>73.81</b> <b>(66.43)</b>	<b>56.64</b> <b>(50.98)</b>	<b>63.73</b> <b>(57.36)</b>	<b>84.58</b> <b>(76.12)</b>	<b>0.00</b> <b>(0.00)</b>	<b>63.13</b> <b>(56.81)</b>

Note : Figures in the parenthesis are the arc-sin values

SEm ± : 0.527  
 CD at 1% Herbicide : 1.18  
 CD at 1% Concentration : 1.49  
 CD at 1% Herbicide V/s Concentration : 1.20  
 CV % : 1.32

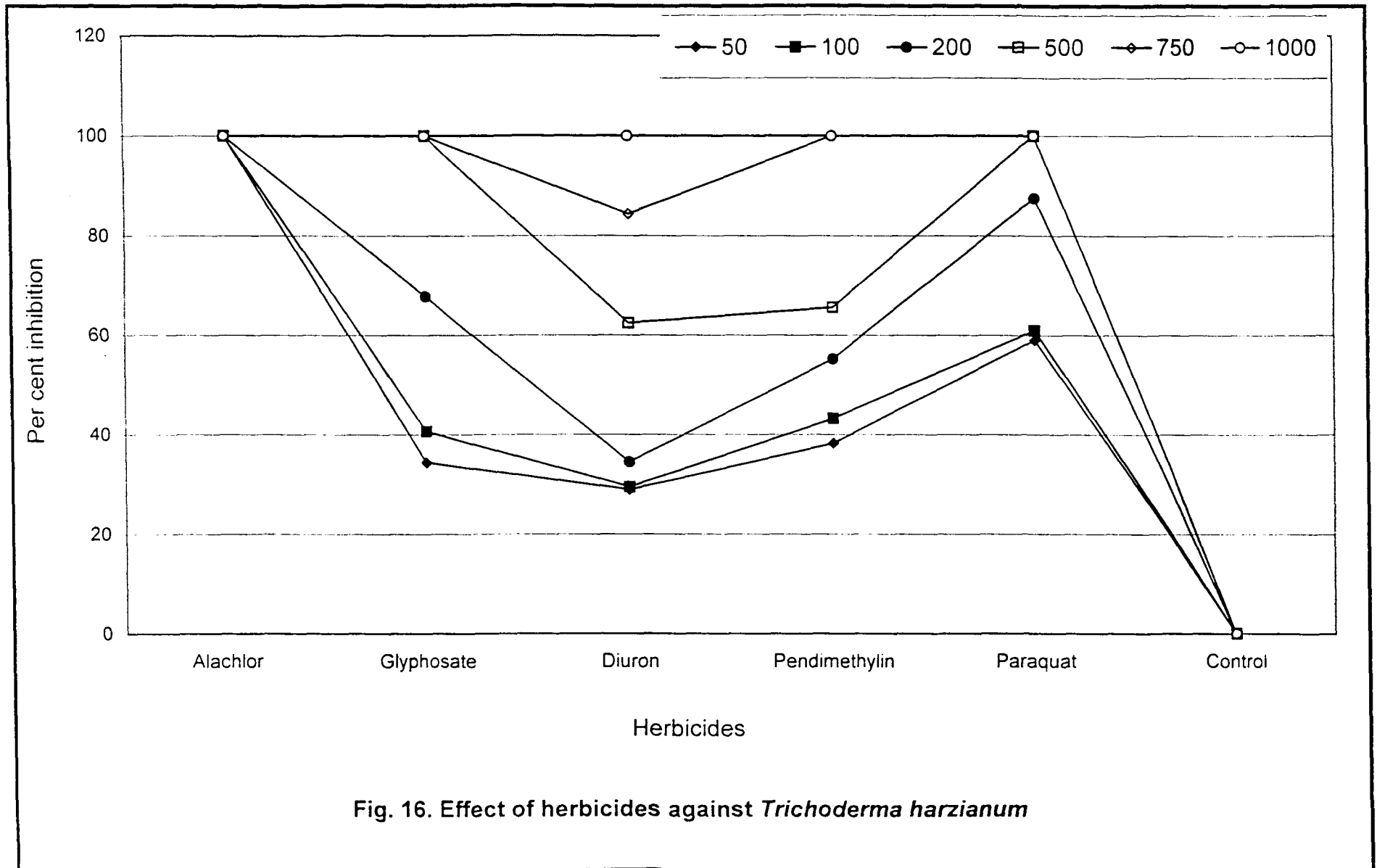


Fig. 16. Effect of herbicides against *Trichoderma harzianum*

**Table 28: Effect of herbicides against *Fusarium solani***

Sl. No.	Concentration (ppm)	Alachlor	Glyphosate	Diuron	Pendimethalin	Paraquat	Control	Mean
1.	50	48.87 (43.98)	45.28 (40.75)	52.12 (46.90)	35.66 (32.10)	51.41 (46.27)	0.00 (0.00)	38.89 (35.00)
2.	100	51.51 (46.36)	50.30 (45.27)	61.25 (55.13)	39.48 (35.53)	70.59 (63.53)	0.00 (0.00)	45.52 (40.97)
3.	200	70.70 (63.63)	68.42 (61.57)	77.24 (69.51)	49.24 (44.31)	83.08 (74.77)	0.00 (0.00)	58.11 (52.30)
4.	500	100.00 (90.00)	75.19 (67.67)	100.00 (90.00)	68.02 (61.21)	100.00 (90.00)	0.00 (0.00)	73.86 (66.48)
5.	750	100.00 (90.00)	85.78 (77.20)	100.00 (90.00)	75.03 (67.77)	100.00 (90.00)	0.00 (0.00)	76.84 (69.16)
6.	1000	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	0.00 (0.00)	83.33 (75.00)
	<b>Mean</b>	<b>78.51</b> <b>(70.66)</b>	<b>70.83</b> <b>(63.74)</b>	<b>81.76</b> <b>(73.59)</b>	<b>61.28</b> <b>(55.15)</b>	<b>84.18</b> <b>(75.76)</b>	<b>0.00</b> <b>(0.00)</b>	<b>62.76</b> <b>(56.48)</b>

Note: Figures in the parenthesis are the arc-sin values

SEm ± : 0.556  
 CD at 1% Herbicide : 0.690  
 CD at 1% Concentration : 0.690  
 CD at 1% Herbicide V/s Concentration : 1.540  
 CV % : 1.70

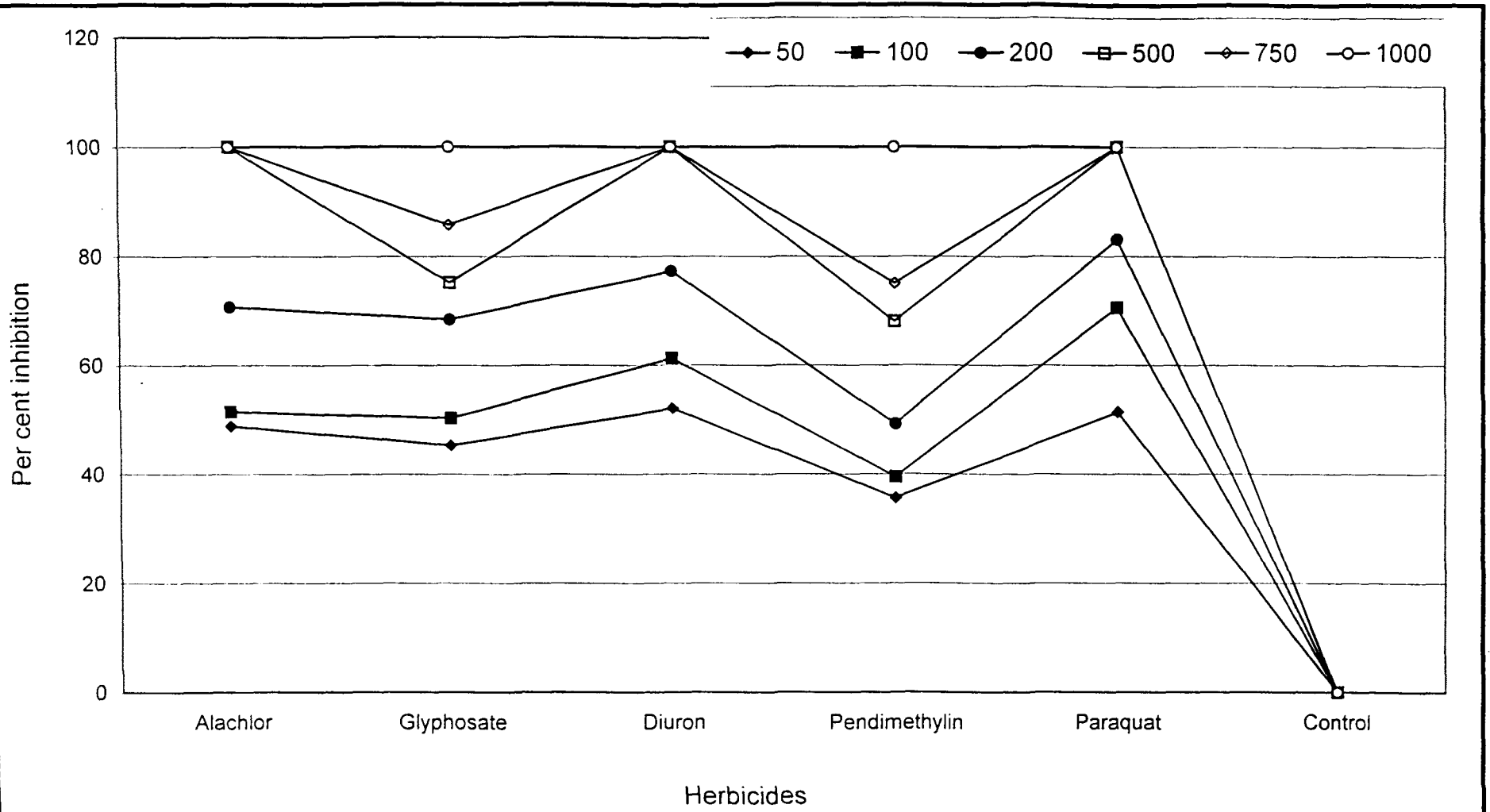


Fig. 17. Effect of herbicides against *Fusarium solani*

values of these herbicides exhibited that Paraquat was the highest (84.18%), followed by Diuron (81.76%) and Alachlor (78.51%). Glyphosate (70.83%) and Pendemethylin (61.28%) were in the next order of merit to inhibit the growth of *F.solani*. Overall, Paraquat (84.18%) followed by Diuron (81.76%), Alachlor (78.51%) and Glyophosate (70.83%) were found inhibitory to the growth of *F.solani*.

#### **4.14.3. Effect of herbicides against *Rhizoctonia solani***

The five herbicides, Alachlor, Glyphosate, Diuron, Pendimethalin and Paraquat at six levels of concentrations 50, 100, 200, 500, 750 and 1000 ppm were tested against *Rhizoctonia solani* for per cent inhibition of growth as given in material and methods. Observations were recorded and the results are presented in Table 29 and Figure 18. Results were statistically significant. Alachlor and Diuron herbicides ranked as number one and two herbicides to inhibit the growth of *R.solani* under the study with mean values of 69.76 and 67.20 respectively. Next was Paraquat with a mean value of 63.29 per cent inhibition. Pendimethalin (52.14%) was least amongst test herbicides followed by Glyphosate (58.13%). Overall, Alachlor, Diuron and Paraquat were found inhibitory to the growth of *R.solani*.

#### **4.14.4. Effect of herbicides against *Rhizoctonia bataticola***

The same herbicides and at same levels of concentrations were tested against *Rhizoctonia bataticola* also as given in material and methods. Recorded observations on percent inhibition of *R.bataticola* were analysed statistically and found significant (Table 30 and Figure 19). Among the herbicides Alachlor and Diuron were highly inhibitory

**Table 29: Effect of herbicides against *Rhizoctonia solani***

Sl. No.	Concentration (ppm)	Alachlor	Glyphosate	Diuron	Pendimethalin	Paraquat	Control	Mean
1.	50	38.32 (34.48)	27.55 (24.79)	31.85 (28.66)	20.55 (18.50)	35.10 (31.59)	0.00 (0.00)	25.56 (23.00)
2.	100	46.86 (42.17)	31.24 (28.11)	47.55 (42.79)	23.32 (20.98)	42.73 (38.46)	0.00 (0.00)	31.95 (28.75)
3.	200	61.94 (55.74)	39.57 (35.61)	55.02 (49.51)	30.83 (27.75)	51.12 (46.01)	0.00 (0.00)	39.74 (35.77)
4.	500	71.49 (64.34)	67.15 (60.43)	68.83 (61.94)	62.10 (55.89)	62.13 (55.92)	0.00 (0.00)	55.28 (49.75)
5.	750	100.00 (90.00)	83.03 (74.97)	100.00 (90.00)	76.06 (68.45)	88.65 (79.78)	0.00 (0.00)	74.66 (67.20)
6.	1000	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	0.00 (0.00)	83.33 (75.00)
	<b>Mean</b>	<b>69.76</b> <b>(62.79)</b>	<b>58.13</b> <b>(52.32)</b>	<b>67.20</b> <b>(60.48)</b>	<b>52.14</b> <b>(46.93)</b>	<b>63.29</b> <b>(56.96)</b>	<b>0.00</b> <b>(0.00)</b>	<b>51.75</b> <b>(46.58)</b>

Note: Figures in the parenthesis are the arc-sin values

SEm ± : 0.585

CD at 1% Herbicide : 0.726

CD at 1% Concentration : 0.726

CD at 1% Herbicide V/s Concentration: 1.620

CV % : 2.17

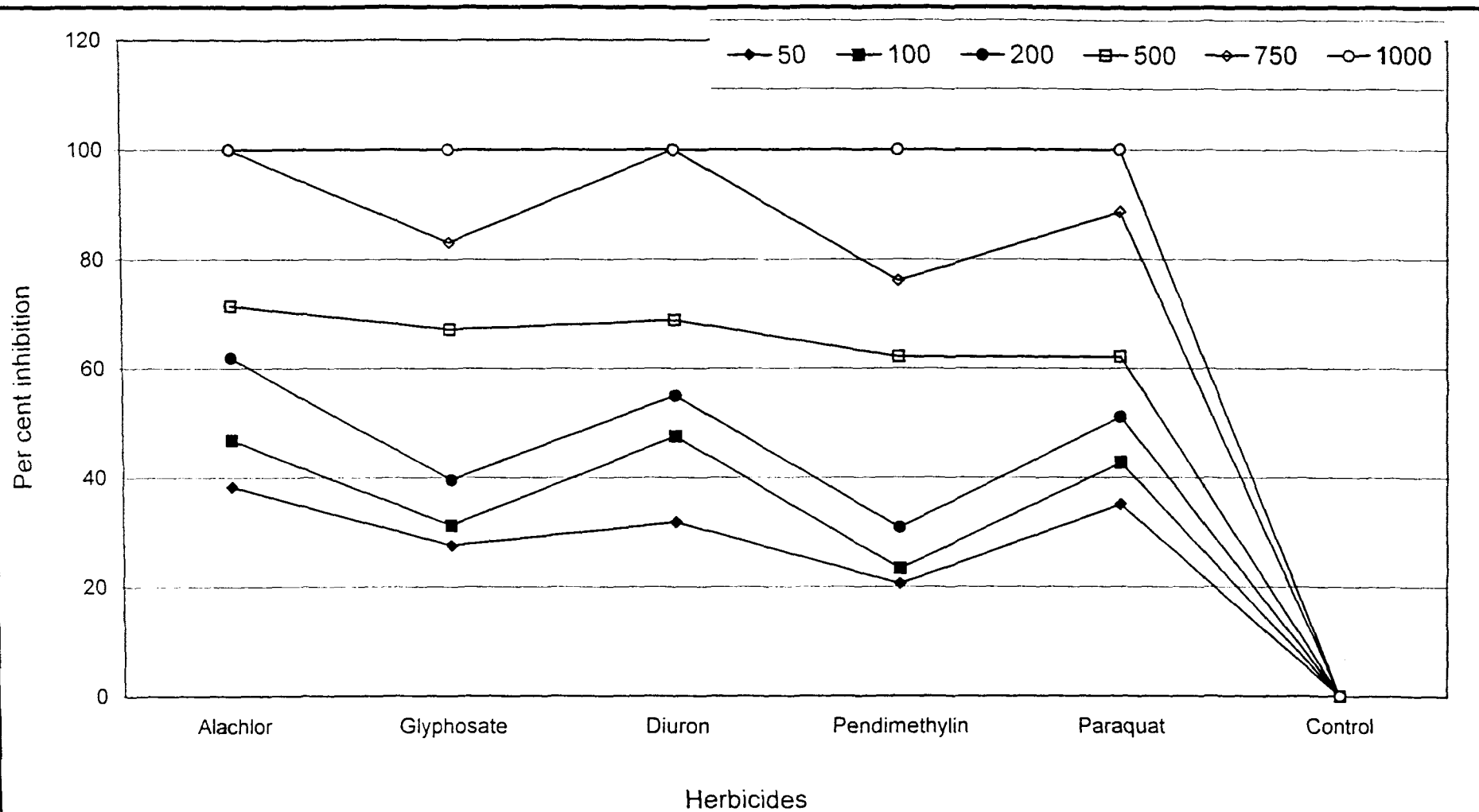


Fig. 18. Effect of herbicides against *Rhizoctonia solani*

**Table 30: Effect of herbicides against *Rhizoctonia bataticola***

Sl. No.	Concentration (ppm)	Alachlor	Glyphosate	Diuron	Pendimethalin	Paraquat	Control	Mean
1.	50	49.28 (44.35)	25.92 (23.32)	46.44 (41.80)	19.34 (17.40)	46.02 (41.42)	0.00 (0.00)	31.17 (28.05)
2.	100	62.25 (56.03)	27.98 (25.18)	58.75 (52.87)	23.86 (21.48)	59.59 (53.63)	0.00 (0.00)	38.74 (34.86)
3.	200	71.57 (64.41)	41.25 (37.12)	67.81 (61.03)	28.92 (26.03)	70.51 (63.45)	0.00 (0.00)	46.67 (42.01)
4.	500	100.00 (90.00)	61.55 (55.39)	100.00 (90.00)	58.44 (52.59)	89.17 (80.25)	0.00 (0.00)	68.19 (61.37)
5.	750	100.00 (90.00)	82.19 (73.97)	100.00 (90.00)	74.81 (67.32)	100.00 (90.00)	0.00 (0.00)	76.16 (63.55)
6.	1000	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	0.00 (0.00)	83.33 (75.00)
	<b>Mean</b>	<b>80.51</b> <b>(72.46)</b>	<b>56.48</b> <b>(50.83)</b>	<b>78.83</b> <b>(70.95)</b>	<b>50.89</b> <b>(45.80)</b>	<b>77.55</b> <b>(69.79)</b>	<b>0.00</b> <b>(0.00)</b>	<b>57.38</b> <b>(51.64)</b>

Note : Figures in the parenthesis are the arc-sin values

SEm ± : 0.400  
 CD at 1% Herbicide : 0.89  
 CD at 1% Concentration : 0.89  
 CD at 1% Herbicide V/s Concentration : 1.11  
 CV % : 1.35

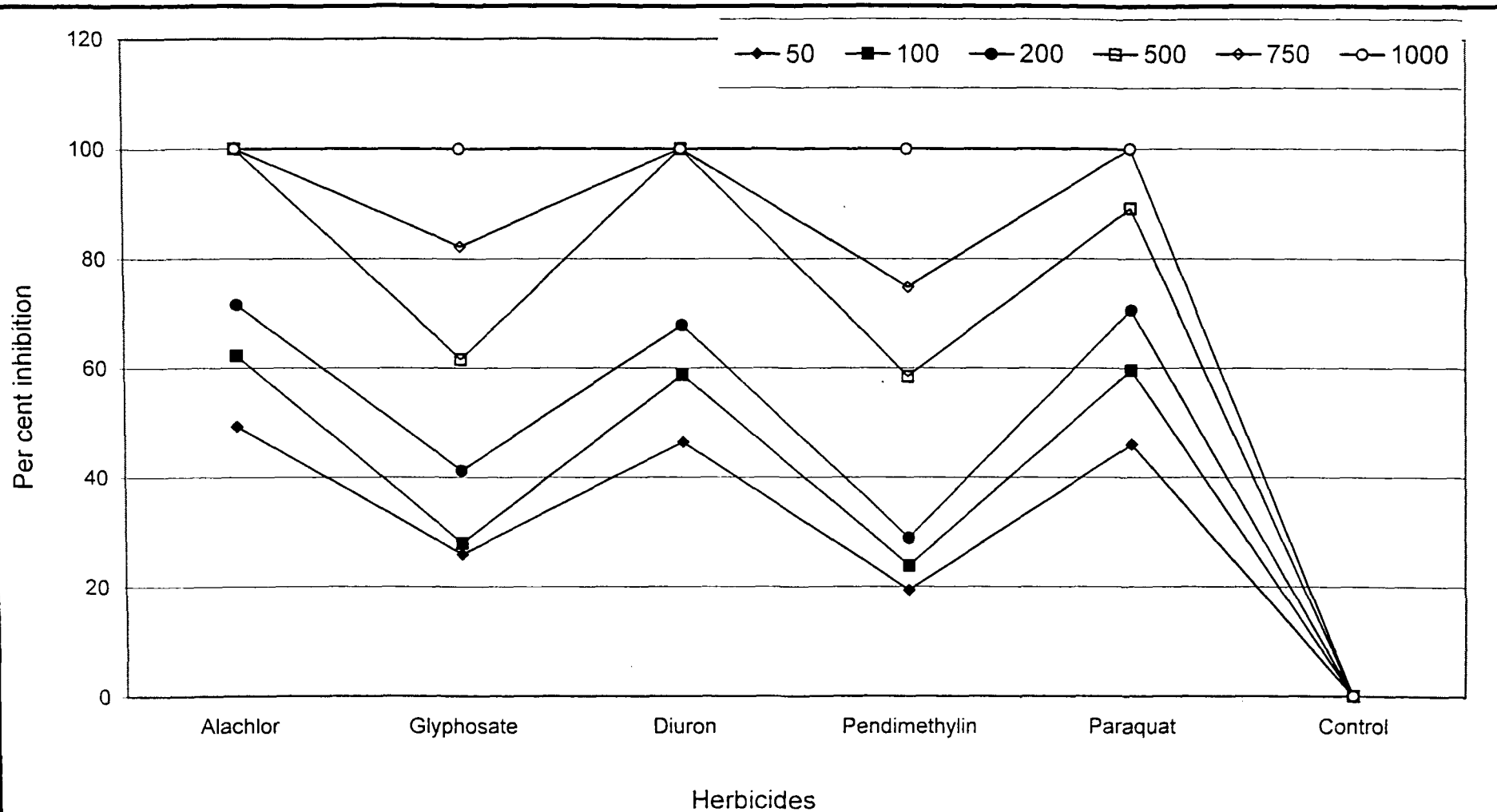


Fig. 19. Effect of herbicides against *Rhizoctonia bataticola*

with mean values of 80.51 percent and 78.83 percent. However, Paraquat was also on par with mean value of 77.55 percent. Both Glyphosate and Pendimethalin recorded more than 50 percent inhibition with values of 56.48 and 50.89 respectively and finally, Alachlor, Diuron and Paraquat were found to be inhibiting the growth of *R.bataticola*.

#### **4.14.5. Effect of herbicides against *Sclerotium rolfsii***

Five herbicides at six levels of concentrations were tested against *Sclerotium rolfsii*. Percent inhibitions of growth of *S.rolfsii* were recorded. The results are presented in Table 31 and Figure 20. The results were statistically significant. In case of *S.rolfsii*, Paraquat and Diuron were highly inhibitory to the mycelial growth of *S.rolfsii* with mean values of 82.97 percent and 80.65 respectively and these two herbicides were on par. Alachlor inhibited cent percent growth at 750 and 1000 ppm and with a mean value of 69.64. Pendimethalin and Glyphosate recorded less than 50 per cent inhibition with the mean values of 46.80 percent and 46.52 percent respectively and finally, Paraquat and Diuron were the two herbicides were found highly inhibitory to the growth of *S.rolfsii*.

#### **4.15. Organic amendments, seed treatment with fungicide, biofungicide on *Fusarium solani*, *Rhizoctonia solani* and *Rhizoctonia bataticola***

Separate pot culture experiments were conducted with five organic amendments viz., FYM, Groundnut cake, Neem cake, Poultry manure and Vermicompost, Captan and *Trichoderma harzianum* seed treatments against for each of the pathogens under study i.e., *Fusarium solani*,

**Table 31: Effect of herbicides against *Sclerotium rolfsii***

Sl. No.	Concentration (ppm)	Alachlor	Glyphosate	Diuron	Pendimethalin	Paraquat	Control	Mean
1.	50	48.89 (44.00)	15.34 (13.81)	46.98 (42.28)	17.91 (16.11)	51.00 (45.90)	0.00 (0.00)	30.02 (27.02)
2.	100	46.08 (41.47)	18.08 (16.27)	56.80 (51.12)	18.57 (16.71)	62.19 (55.97)	0.00 (0.00)	33.62 (30.26)
3.	200	46.77 (42.09)	31.80 (28.62)	80.16 (72.14)	26.21 (23.58)	84.63 (76.16)	0.00 (0.00)	44.93 (40.43)
4.	500	76.11 (68.49)	45.32 (40.78)	100.00 (90.00)	46.72 (42.04)	100.00 (90.00)	0.00 (0.00)	61.35 (55.22)
5.	750	100.00 (90.00)	68.62 (61.75)	100.00 (90.00)	71.41 (64.26)	100.00 (90.00)	0.00 (0.00)	73.33 (66.00)
6.	1000	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	0.00 (0.00)	83.33 (75.00)
	<b>Mean</b>	<b>69.64</b> <b>(62.68)</b>	<b>46.52</b> <b>(41.87)</b>	<b>80.65</b> <b>(72.59)</b>	<b>46.80</b> <b>(42.12)</b>	<b>82.97</b> <b>(74.67)</b>	<b>0.00</b> <b>(0.00)</b>	<b>54.43</b> <b>(48.99)</b>

Note: Figures in the parenthesis are the arc-sin values

SEm ± : 0.596

CD at 1% Herbicide : 1.33

CD at 1% Concentration : 1.33

CD at 1% Herbicide V/s Concentration : 1.65

CV % : 2.11

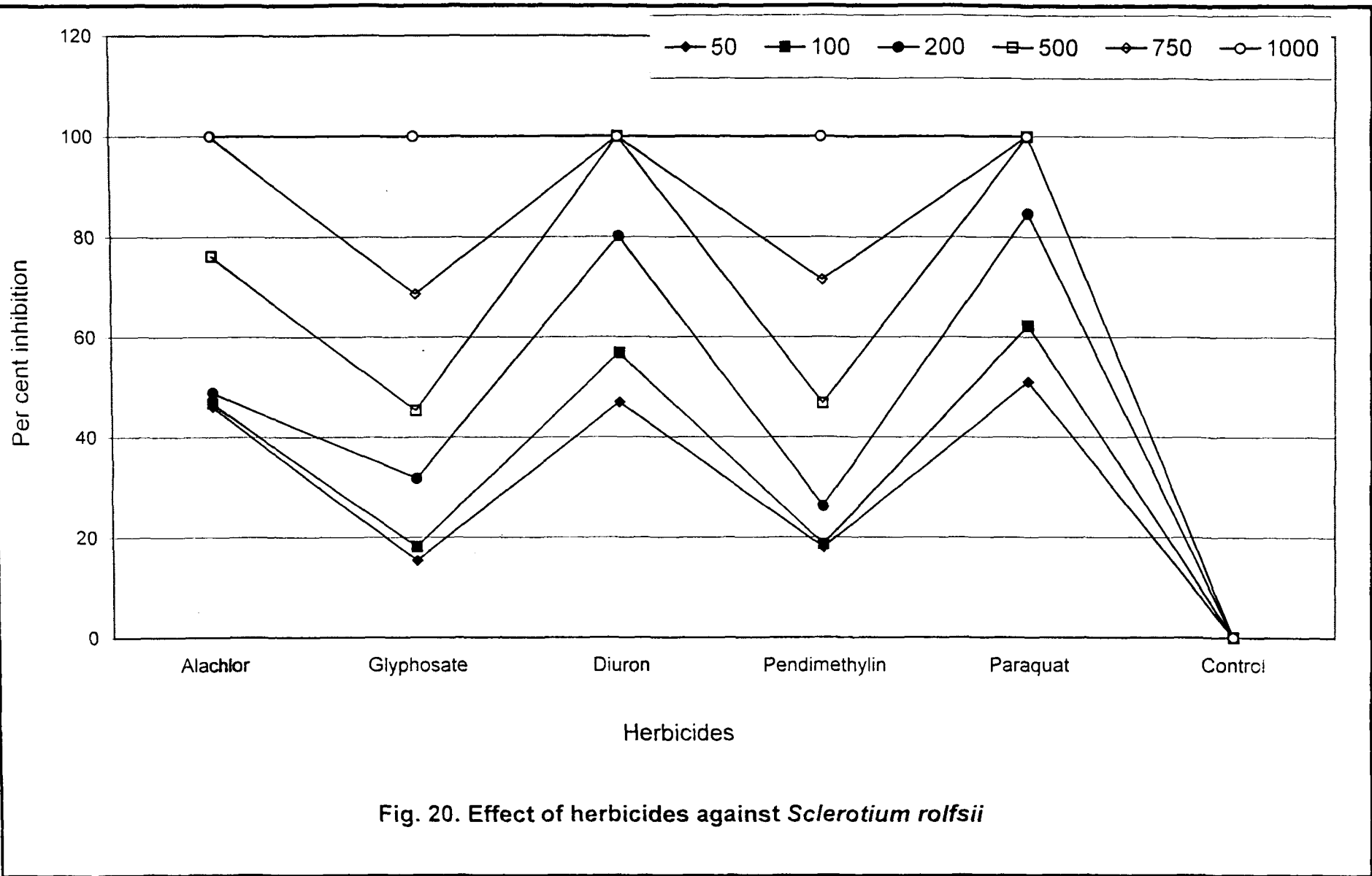


Fig. 20. Effect of herbicides against *Sclerotium rolfsii*

*Rhizoctonia solani* and *Rhizoctonia bataticola*. Observations on percent germination of cotton were recorded and the results are presented Table 32 and Figure 21.

In case of *Fusarium solani*, among eight treatments imposed, Captan seed treatment recorded highest germination (84.11%), followed by *Trichoderma harzianum* (71.86) . Among organic amendments Neem cake recorded the highest (45.79%), followed by Vermicompost (42.44%). Poultry manure recorded least germination percentage (25.29%), followed by Groundnut cake (37.45%) and FYM (34.40%).

Similar results were observed in *Rhizoctonia solani* and *Rhizoctonia bataticola* i.e., Captan seed treatment was the highest (80.03% and 80.02% respectively), followed by *T.harzianum*, 69.85 percent and 71.86 percent respectively. Poultry manure recorded the least i.e., 26.26 percent and 28.29 percent respectively. Over all Captan seed treatment (81.38% mean value) was the highest followed by *T.harzianum* (71.19% mean value).

#### **4.16. Organic amendments, seed treatment with fungicide, biofungicide on *Sclerotium rolfsii***

The pot culture studies were conducted for above pathogens, (particularly *Fusarium solani* ) to avoid these pathogens in ARS, Dharwad farm, but the *Sclerotium rolfsii* experiments were conducted in field during 1998-99 and 1999-2000 as it appears in cotton fields when taken after Groundnut. The treatments were imposed here also, i.e., FYM, Groundnut cake, Neem cake, Poultry manure, Vermicompost and seed treatment with Captan and seed treatment with *Trichoderma*

**Table 32: Management of wilt and root rots of cotton with organic amendments (Per cent germination of seedlings).**

Sl. No.	Treatments	<i>F. solani</i>	<i>R. solani</i>	<i>R. bataticola</i>	Mean
1.	FYM	34.40 (35.67)	28.28 (32.14)	29.28 (32.77)	30.53 (33.52)
2.	Groundnut cake	37.45 (37.76)	35.77 (36.75)	35.74 (36.69)	36.32 (37.05)
3.	Neem cake	45.79 (42.59)	44.18 (41.67)	45.79 (42.59)	45.25 (42.30)
4.	Poultry Manure	25.29 (30.20)	26.26 (30.85)	28.29 (32.14)	26.61 (31.05)
5.	Captan seed Tr.	84.11 (66.50)	80.03 (63.43)	80.02 (63.43)	81.38 (64.45)
6.	Vermicompost	42.44 (40.63)	37.48 (37.76)	35.64 (36.63)	38.52 (38.35)
7.	<i>T.harzianum</i>	71.86 (57.99)	69.85 (56.37)	71.86 (57.99)	71.19 (57.54)
8.	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	<b>SEm±</b>	<b>2.772</b>	<b>3.21</b>	<b>4.011</b>	
	<b>CD at 5%</b>	<b>8.035</b>	<b>9.298</b>	<b>11.626</b>	
	<b>CV %</b>	<b>14.42</b>	<b>17.83</b>	<b>21.97</b>	

Note : Figures in the paranthesis are the arc-sin values

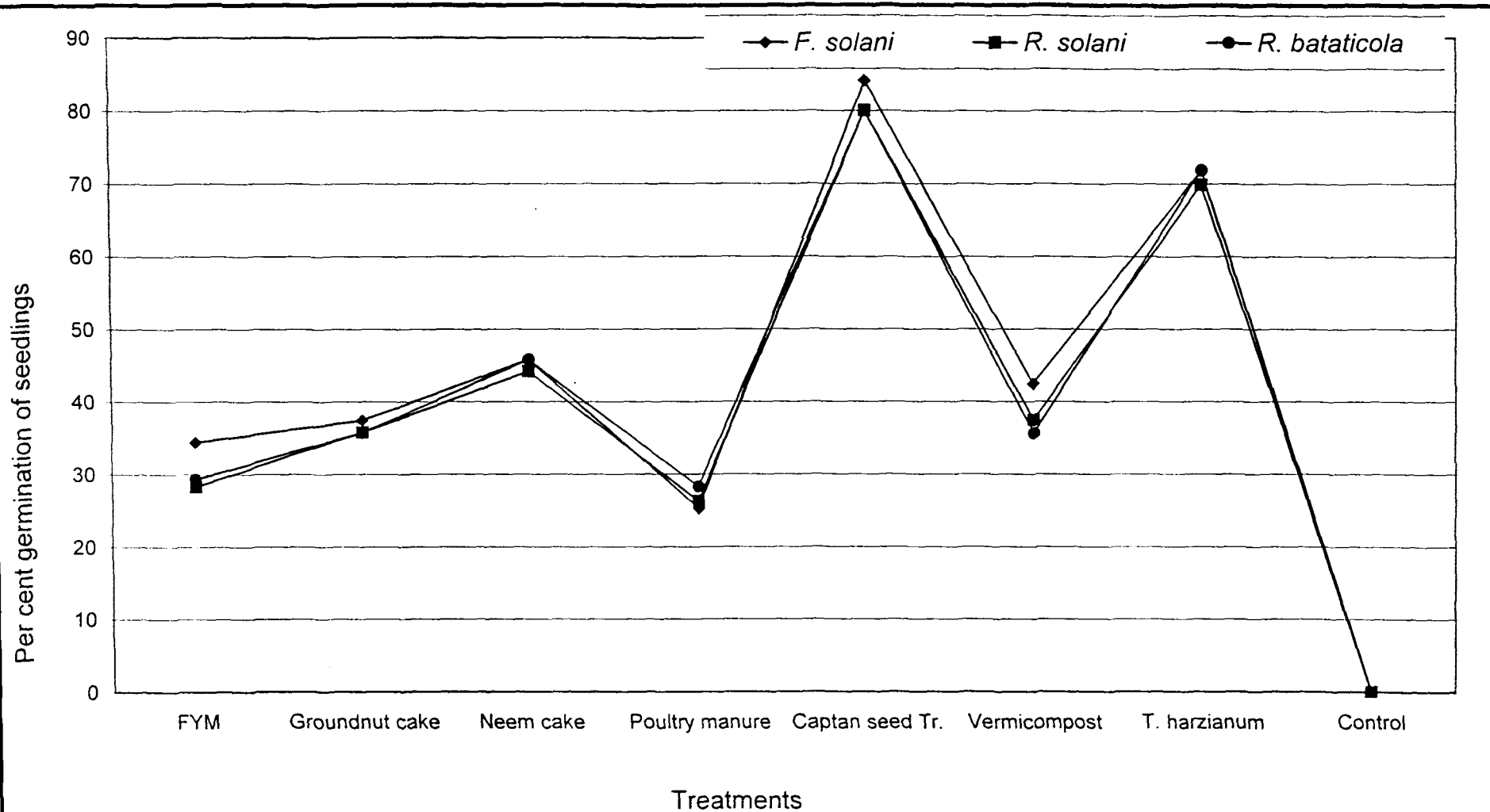


Fig. 21. Management of wilt and root rots of cotton with organic amendments (per cent germination of seedlings)

*harzianum*. The observations were recorded on per cent mortality, plant height, number of monopodia, number of sympodia, number of squares, number of fruiting points and yield q/ ha ( Table 33 and Table 34). The three best treatments were Captan seed treatment, *Trichoderma harzianum* seed treatment and Neem cake with respect to the per cent mortality of seedlings and with respect to the yield also. The results were statistically significant to the rest of treatments. Further, the yield contributing factors i.e., the plant height, number of monopodia, number of sympodia, number of squares and number of fruiting points were statistically significant.

The per cent mortality was more during 1999-2000 compared to 1998-1999 i.e., 73.88 per cent and 19.26 per cent respectively. These results have reflected on yield. The three treatments i.e., the *Trichoderma harzianum* and Captan seed treatments and the Neem cake have shown same trend with respect yield, but the former two treatments were statistically on par. Poultry manure, Vermicompost, FYM and the Groundnut cake when applied alone recorded the low yields.

#### **4.17. Storage studies on *Trichoderma harzianum***

Two formulations i.e., Wheat bran:sand:water (F<sub>1</sub>) and Talc formulations of *Trichoderma harzianum* were used for the viability at five different storage conditions i.e., 0° C, 5° C, 15° C, room temperature (26± 1° C) and in earthen pot was studied for four periods i.e., 60, 90, 120 and 150 days after preparation as presented in material and methods. The number of cfu (10<sup>-5</sup>) g<sup>-1</sup> was recorded (Table 35). The results were statistically significant. The wheat bran : sand : water (F<sub>1</sub>) (140.70 mean cfu) was superior to talc formulation 86.58 mean cfu). It was observed

**Table 33: Field experiment on influence of organic amendments on *Sclerotium rolfsii* (1998-99)**

Sl. No.	Treatment	Percent Mortality	Plant Height	No. of Monopodia	No. of Sympodia	No. of Squares	No. of fruiting points	Yield (q/ha)
1.	FYM	16.11 (23.31)	97.16 (81.57)	0.70 (4.8)	12.73 (20.9)	1.63 (7.30)	39.74 (39.0)	8.27
2.	Groundnut cake	16.14 (23.64)	95.46 (79.05)	1.30 (6.52)	13.70 (21.7)	2.80 (9.62)	52.1 (46.19)	5.42
3.	Neem cake	5.37 (13.34)	101.6 (86.84)	1.73 (7.56)	16.70 (24.11)	2.33 (8.74)	58.72 (50.04)	8.15
4.	Poultry Manure	11.11 (19.38)	100.50 (86.30)	1.20 (6.2)	13.40 (21.44)	2.00 (8.0)	61.45 (51.62)	5.64
5.	Captan seed Tr.	6.11 (14.31)	99.46 (85.56)	1.43 (6.9)	15.60 (23.3)	2.73 (9.5)	58.01 (49.60)	9.56
6.	Vermicompost	10.00 (18.4)	99.96 (86.26)	0.83 (5.22)	13.60 (21.61)	2.33 (8.74)	54.4 (47.53)	6.95
7.	<i>T.harzianum</i> seed Tr.	5.37 (13.34)	99.76 (84.76)	1.33 (6.62)	14.43 (22.30)	3.13 (10.2)	58.04 (49.65)	9.95
8.	Control	19.26 (26.01)	98.53 (84.62)	0.80 (5.22)	14.43 (23.32)	2.46 (9.0)	53.75 (46.81)	5.64
	<b>SEm+</b>	<b>1.061</b>	<b>2.213</b>	<b>0.097</b>	<b>0.816</b>	<b>0.376</b>	<b>8.633</b>	<b>12.13</b>
	<b>CD at 5%</b>	<b>3.218</b>	<b>NS</b>	<b>0.294</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>12.44</b>
	<b>CV %</b>	<b>18.51</b>	<b>3.87</b>	<b>14.38</b>	<b>9.88</b>	<b>27.46</b>	<b>22.62</b>	<b>1.62</b>

Note: Figures in the parenthesis are the arc-sin values

**Table 34: Field experiment on influence of organic amendments on *Sclerotium rolfsii* (1999-2000)**

Sl. No.	Treatment	Percent Mortality	Plant Height	No. of Monopodia	No. of Sympodia	No. of Squares	No. of fruiting points	Yield (q/ha)
1.	FYM	57.21 (49.21)	105.53 (90.00)	1.53 (7.05)	14.53 (22.40)	2.33 (8.51)	53.50 (47.1)	4.91
2.	Groundnut cake	53.33 (46.87)	97.26 (82.61)	1.70 (7.4)	15.30 (23.0)	3.46 (10.63)	62.53 (52.71)	5.09
3.	Neem cake	46.66 (43.11)	104.8 (87.73)	1.80 (7.71)	16.00 (23.48)	2.66 (9.38)	17.53 (57.15)	7.89
4.	Poultry Manure	57.77 (49.49)	102.66 (86.45)	1.50 (6.82)	16.80 (24.2)	2.53 (9.06)	77.70 (62.7)	6.20
5.	Captan seed Tr.	20.55 (26.99)	103.66 (85.04)	1.33 (6.6)	15.53 (23.21)	3.20 (10.03)	70.73 (58.2)	8.91
6.	Vermicompost	55.55 (48.18)	101.26 (90.00)	0.90 (5.33)	16.40 (23.9)	3.36 (10.26)	72.30 (58.31)	5.87
7.	<i>T.harzianum</i> seed Tr.	26.66 (31.66)	100.66 (82.98)	2.00 (8.00)	15.20 (23.0)	2.80 (9.14)	56.30 (48.8)	9.25
8.	Control	73.88 (59.21)	93.33 (81.00)	1.40 (6.75)	16.00 (23.24)	3.06 (9.76)	65.30 (54.14)	4.90
	<b>SEm+</b>	<b>1.236</b>	<b>3.496</b>	<b>0.288</b>	<b>0.879</b>	<b>0.885</b>	<b>8.633</b>	<b>9.66</b>
	<b>CD at 5%</b>	<b>4.021</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>15.15</b>
	<b>CV %</b>	<b>5.19</b>	<b>5.94</b>	<b>33.24</b>	<b>9.70</b>	<b>52.30</b>	<b>22.62</b>	<b>1.76</b>

Note: Figures in the parenthesis are the arc-sin values

**Table 35: Storage studies on *Trichoderma harzianum* with two formulations (cfu (10<sup>-5</sup>) g<sup>-1</sup>)**

Sl. No.	Treatment	F <sub>1</sub> (Wheat), DAS					F <sub>2</sub> (Talc), DAS				
		60	90	120	150	Mean	60	90	120	150	Mean
1.	0°	170.67	163.33	150.67	120.67	151.33	123.67	90.67	82.00	78.67	93.75
2.	5°	163.33	144.67	139.00	114.67	140.42	113.67	88.67	80.33	72.00	88.67
3.	15°	149.67	139.00	128.33	108.33	137.33	97.33	83.67	75.33	74.67	82.75
4.	Room temp.	152.33	138.67	127.00	112.67	132.67	87.67	87.67	78.33	73.33	80.25
5.	Earthen pot	168.33	161.67	144.33	116.67	147.75	109.67	87.67	79.33	73.33	87.50
	<b>Mean</b>					<b>140.70</b>					<b>86.58</b>

Note: Figures in the parenthesis are the arc-sin values

SEm ± : 0.438  
 CD at 1% Formulation : 1.52  
 CD at 1% Temperature : 3.03  
 CD at 1% Days : 1.52  
 CD at 1% Formulation V/s Temperature : 1.52  
 CD at 1% Formulation V/s Days : 3.03  
 CD at 1% Temperature V/s Days : 3.03  
 CD at 1% Formulation V/s Temperature V/s Days : 1.12  
 CV % : 9.271

that, as the temperature increased there was reduction in number of cfu and 0° C recorded the highest cfu of 151.33 and 93.75 in F<sub>1</sub> and F<sub>2</sub> respectively. Further, it was observed that, as the storage period increased from 60 DAP to 150 DAP, the number of cfu reduced. The earthen pot storage recorded a mean of 147.75 cfu in F<sub>1</sub> and 84.17 in F<sub>2</sub>. Overall, the wheat bran sand water formulation was better than the talc formulation. Further, storing in low temperature conditions increased the viability of *T.harzianum* for a long time. Further, storing upto 120 days is optimum for better viability of biofungicide.

#### **4.18. Shelf life studies on *Trichoderma harzianum***

The two formulations Wheat bran : sand: water (F<sub>1</sub>) and Talc formulation (F<sub>2</sub>) were further used for their shelf life studies of *Trichoderma harzianum*. The number of cfu (10<sup>-5</sup>) g<sup>-1</sup> were recorded at regular interval of one month i.e., two, three, four, five, six, seven, eight, nine and ten months as given in material and methods. The results are presented Table 36. The statistical analysis of the results was significant for both the formulations of F<sub>1</sub> and F<sub>2</sub> and also for periods. In both the formulations i.e., F<sub>1</sub> and F<sub>2</sub> high cfu was recorded at two months with values of 155.22 and 99.22 and at tenth month there was drastic reduction of cfu with values of 26.82 and 18.33 respectively. But the reduction was gradual from two months to ten months. The study indicated that, *T.harzianum* can be stored upto four months after there is drastic reduction in the cfu of *T.harzianum*.

**Table 36: Storage studies on *Trichoderma harzianum* in two formulations (mean cfu 10<sup>-5</sup> g<sup>-1</sup>)**

Sl. No.	Storage months	Formulation		Mean
		F <sub>1</sub>	F <sub>2</sub>	
1.	Two	155.22	99.22	127.22
2.	Three	140.51	98.77	119.64
3.	Four	127.31	85.42	106.36
4.	Five	116.19	77.07	50.03
5.	Six	79.55	50.03	64.79
6.	Seven	70.03	45.51	57.71
7.	Eight	49.35	31.15	40.25
8.	Nine	45.31	27.34	36.33
9.	Ten	26.82	18.83	22.83
	<b>Mean</b>	<b>90.03</b>	<b>59.26</b>	<b>74.65</b>

SEm ± : 0.601  
 CD at 1% Formation : 2.94  
 CD at 1% Months : 1.04  
 CD at 1% Formation V/s Pathogen : 3.05  
 CV % : 1.39

#### 4.19. Studies on culture filtrate of isolates of *Trichoderma* spp., and isolates of *Gliocladium virens* on seed germination

Two cotton genotypes viz., Jayadhar and Abhadita seeds with two sub-treatments i.e., delinted and fuzzed were subjected to culture filtrates of *Trichoderma* isolates and *Gliocladium virens* isolates, for seed germination. Germination percentage was recorded and analysed statistically. The results are presented in Table 37. Results were highly significant. The average germination percentage was higher in delinted (65.30) was more than that of Jayadhar (54.50). Among these *Trichoderma* culture filtrates T<sub>8</sub> of Dharwad isolate (*Trichoderma harzianum*) recorded highest mean germination percentage of 67.33, but it was highest in T<sub>12</sub> (*Trichoderma reesai*) for Abhadita (73.71%) and T<sub>1</sub> (*Trichoderma harzianum* of TNAU, Coimbatore) for Jayadhar (66.93%). The lowest germination percentage was in T<sub>11</sub> (*Trichoderma viride* of Agharkar Research Institute of Pune (49.29 mean per cent germination value) followed by T<sub>9</sub> (*Trichoderma harzianum* TNAU, Coimbatore) of (49.69%). Among *Gliocladium virens* isolates G<sub>1</sub> (TNAU, Coimbatore) recorded the highest (68.72%) as compared to G<sub>2</sub> (63.48%) CICR, Sirsa.

**Table 37: Effect of culture filtrates of isolates of *Trichoderma* spp, and isolates of *Gliocladium virens* on cotton seed germination**

Sl. No.	Isolate	Jayadhar			Abadhita		
		Delinted	Fuzzed	Mean	Delinted	Fuzzed	Mean
1.	T <sub>1</sub>	96.38 (78.95)	66.98 (54.90)	84.65 (66.93)	97.48 (80.87)	55.59 (48.21)	81.52 (64.54)
2.	T <sub>2</sub>	92.78 (74.38)	74.09 (59.42)	84.62 (66.90)	99.38 (85.43)	66.15 (53.84)	87.90 (69.64)
3.	T <sub>3</sub>	59.29 (50.35)	42.50 (40.69)	50.90 (45.52)	91.82 (74.38)	70.59 (57.16)	83.15 (65.77)
4.	T <sub>4</sub>	87.15 (68.98)	72.35 (58.27)	80.28 (63.63)	96.35 (78.95)	68.64 (55.94)	85.29 (67.45)
5.	T <sub>5</sub>	89.30 (70.91)	76.26 (60.84)	83.42 (65.97)	98.75 (83.51)	49.95 (44.99)	81.12 (64.25)
6.	T <sub>6</sub>	40.65 (39.61)	57.45 (49.28)	49.08 (44.45)	93.71 (75.47)	64.85 (53.66)	81.55 (64.57)
7.	T <sub>7</sub>	83.59 (66.10)	42.50 (40.69)	64.45 (53.40)	89.30 (70.91)	78.51 (62.39)	84.29 (66.65)
8.	T <sub>8</sub>	92.78 (74.38)	57.45 (49.28)	77.75 (61.84)	96.35 (78.95)	84.35 (66.69)	91.25 (72.82)
9.	T <sub>9</sub>	42.50 (40.69)	36.84 (37.37)	39.66 (39.04)	90.95 (72.46)	55.59 (48.21)	75.51 (60.34)
10.	T <sub>10</sub>	61.22 (51.46)	44.39 (41.77)	52.82 (46.62)	95.25 (77.39)	57.45 (49.28)	79.88 (63.34)
11.	T <sub>11</sub>	40.65 (39.61)	35.09 (36.29)	37.82 (37.95)	89.30 (70.91)	59.29 (50.35)	75.92 (60.62)
12.	T <sub>12</sub>	85.25 (67.43)	72.55 (58.39)	79.28 (62.91)	99.35 (85.43)	77.98 (61.98)	92.12 (73.71)
13.	G <sub>1</sub>	89.30 (70.91)	74.59 (59.73)	82.58 (65.32)	98.73 (83.51)	76.24 (60.75)	90.58 (72.13)
14.	G <sub>2</sub>	85.26 (67.43)	66.98 (54.90)	76.75 (61.17)	96.35 (78.95)	63.20 (52.65)	83.18 (65.79)
15.	Control	53.75 (47.14)	17.38 (24.62)	34.35 (35.87)	92.75 (74.38)	13.11 (21.23)	54.90 (47.81)

Note: Figures in the parenthesis are the arc-sin values

SEm ±	: 2.966	CD at 1% Variety	: 11.10
CD at 1% Lint	: 11.10	CD at 1% Treatment	: 2.97
CD at 1% Variety V/s Lint	: 2.97	CD at 1% Variety V/s Treatment	: 11.10
CD at 1% Lint V/s Treatment	: 2.97	CD at 1% Variety V/s Lint V/s Treatment	: 5.07
CV %	: 8.58		

## *Discussion*

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## V. DISCUSSION

Cotton suffers not only from foliar diseases but also from soil borne pathogens viz., *Fusarium solani*, *Fusarium oxysporum* f.sp. *vasinfectum*, *Verticillium dahliae*, *Rhizoctonia solani*, *Rhizoctonia bataticola* and *Sclerotium rolfsii*. The losses due to diseases have been well documented in USA. Under Indian multifaceted Agriculture it is little difficult to have a concrete account of losses. However some diseases have been managed through different means. Among these *Fusarium oxysporum* f.sp.*vasinfectum* and *V.dahliae* have been managed through resistant sources. *F.solani* is posing serious threat to cotton cultivation in command or the irrigated areas. This pathogen must be existing in soil and as a simple interest disease as the inoculum is being accumulated year after year *R.solani*, *R.bataticola* and *S.rolfsii* do pose serious threats but compared to *F.solani* they can be the threat only under congenial conditions. *S.rolfsii* is being polyphagous, and if cotton is taken after groundnut, the chances of *S.rolfsii* root rot are more that too under rainfed conditions. Since *Fusarium solani*, *Rhizoctonia solani*, *Rhizoctonia bataticola* and *Sclerotium rolfsii* are four important soil borne pathogens on cotton, research investigations were under taken on management of these important soil borne pathogens.

Surveys during 1997-98 and 1998-99 were conducted in cotton growing areas of Karnataka and the samples collected during 1997-98 were used for future studies. Incidence of *F.solani* wilt was 20 percent and the disease was more prevalent in command areas or under irrigated conditions. The incidence of *R.solani*, *R.bataticola* and *S.rolfsii* varied from 0.00 to 6.00 percent in the former two and *S.rolfsii* was in traces to one percent. This study helped that, *F.solani* is the major pathogen to be

managed than the other soil borne pathogens under study. The research studies were conducted to manage the wilt and root rots of cotton with special reference to biofungicide as the biological control is cheaper, self sustaining, ecofriendly i.e., non hazardous as compared to chemicals and fits well as one of the components in integrated disease management and the use of biofungicide is the need of the hour.

In view of the threat due to *F.solani*, as per the survey conducted isolates were collected from cotton growing areas of Karnataka. *R.solani*, *R.bataticola* and *S.rolfsii* were obtained from ARS, Dharwad Farm and adjacent cotton fields.

Commonly field symptoms of *F.solani* may appear at any stage of the crop growth. In the early seedling stage, death may be sudden and may be mistaken to be due to damping-off. Even the true leaves of young plants may show yellow coloured leaves and drop off. At this stage, vascular browning may be observed but there is no discoloration or necrosis of the roots as in other root diseases like damping-off or root rot. In older plants, while the disease was prominent at square formation and flowering. The margins of the leaves wilt, and leaves were chlorotic and brown, ultimately shed. The entire plant or only one side was found affected. Often the bark of the stem turned dark brown either uniformly or on one side from the base upwards. Vascular browning was traced continuously from the main stem into the branches. While there was a marked discoloration of the bark and stem in young plants, the discoloration was seen only on peeling the bark in older plants. Microscopic examination of sections of the roots and stems showed fungal hyphae inside the xylem, particularly in the lower part of the plant.

The *R.solani* was found affecting the collar, roots and leaves. It is distinguished from damping-off by the affected young plants not falling over but standing erect. The diseased lesions are not water-soaked. The plants are killed when the collar is girdled and the roots are also damaged. The seedlings are easily pulled out without the taproot coming apart. The bark at the basal part of the stem and upper part of the root is shredded in severely attacked plants. The wood is discoloured brown or black. The upper portion of the taproot is also discoloured but without shrinking.

In case of *R.bataticola* there was stunting of the main stem though there was normal growth of some of the lower branches. The disease was found occurring in circular patches. In young seedlings when the stem is still soft, a yellow patch appears on the lower part of the stem and if the attack is severe the seedling was found collapsing. In older seedlings which have developed some wood, the patch becomes blackened at ground level and the seedlings dry up and wither. The roots were found decayed. The bark was shredded on the roots and basal part of the stem.

The *S.rolfsii* root rot affected plants are attacked at or near the soil surface causing a typical girdling of the stem at soil level, which is followed by decay of the roots and wilting of the plants. A white mass of mycelium with numerous spherical, black sclerotia of the size of mustard seeds is seen at the base of the stem and top of the roots. Young plants are killed quickly but on older plants the leaves turn yellow and then wilt. Affected plants rarely topple over and generally stand erect. The disease is more at seedling stage than the adult plant and further, the disease is more prevalent in dry conditions than the moist or the irrigated conditions.

After collecting the diseased cotton samples, the pathogens were isolated on Potato Dextrose Agar medium. For pathogenecity cotton seeds were crushed and mixed with little water, sterilized twice and inoculated with *F.solani*, *R.solani*, and *R.bataticola* separately and were allowed for mass multiplication. These cultures were mixed with double sterilized soil in pots. DCH-32 was used for pathogenicity test. Pots were watered regularly. *F.solani* produced yellowing of leaves and gradual wilting. Re-isolations were made and the *F.solani* was identified by Agharkar Research Institute, Pune. *R.bataticola* and *R.solani* produced brown discolouration of roots followed by shredding of cotton root bark. Minute sclerotial bodies were found in *R.bataticola*.

The giant culture medium (Maize and Sand 1:4) was prepared and mycelium of *S.rolfsii* was inoculated and allowed for sclerotia production in the medium. The culture was mixed with double sterilized soil. Seeds of DCH-32 were sown. Low soil moisture was maintained in the pots. Germinated seedlings were unhealthy and became yellow coloured within 2-3 days and they started drying. Uprooted seedlings showed white mycelial growth covered with whitish yellow, later on turned mustard seed like structures i.e., sclerotia.

Some fungi were found commonly occurring with cotton rhizosphere while isolating the pathogens from the cotton. These fungi were separated out and were identified by the Mycology Division, Agharkar Research Institute, Pune.

The nine isolates of *F. solani* were identified based on the principal morphological and cultural characters described by Booth (1971) further

the identity was confirmed by the Mycology Division, Agharkar Research Institute, Pune.

The identification of *R.solani* and *R.bataticola* was made on the basis of morphology and formation of sclerotia as described by Ashby (1927) and Goidanich (1947) and that of *S.rolfsii* was based on the characters given by Domsh *et al* (1980).

The potential pathogen often blessed with biodiversity within its population. Basically variation in pathogen is desirable trait for its existence in nature. In the present study, the amount of variability is available in *Fusarium solani* isolates was attempted through study of their morphological and cultural characters. There was definite variability in the isolates with their morphological and sporulation characters. Variability within the species may be attributed to long term influence of soil environment and weather conditions of the location and ability of the pathogen to adopt to the every changing conditions surrounding it. Among the all nine isolates of *F.solani* were tested for virulence and *F.solani* of Raichur was more virulent than others. Variability between isolates of *Fusarium* has been reported by many workers. Bhatnagar and Prasad (1968) grouped isolates of *F.solani* in to two distinct groups. Khare *et al* (1975) reported variation in the growth characters and pigmentation of six isolates of *Fusarium*.

The mechanism of biological control does not need more emphasis as it is well documented in literature and commercial formulations of biofungicides (bioagents) are available in market. Different species of *Trichoderma* and also their isolates were procured from different sources and two isolates of *Gliocladium virens* along with one i.e., *Trichoderma*

*harzianum* isolated from the escape plants from the cotton rhizosphere of ARS Dharwad farm.

It was necessary to evaluate the isolates of both *Trichoderma* and *Gliocladium virens* against all four pathogens i.e., nine isolates of *F.solani* and one each of *R.solani*, *R.bataticola* and *S.rolfsii* so as to come out with the best biofungicide which would suppress the activities of all pathogens.

Among twelve isolates of *Trichoderma* evaluated against nine isolates of *F.solani* and *R.solani*, *R.bataticola* and *S.rolfsii*. *Trichoderma harzianum* of ARS, Dharwad was efficient, which could inhibit on an average 51.77 per cent inhibition followed by T<sub>7</sub> (*Trichoderma longi* of TNAU isolate) with 49.31 per cent inhibition, T<sub>1</sub> (*Trichoderma harzianum* of TNAU isolate with 45.52 per cent inhibition than the rest of the other *Trichoderma* cultures. Among *Gliocladium virens* cultures G<sub>2</sub> of CICR, Sirsa was the best with per cent inhibition of 47.01 than the G<sub>1</sub> of TNAU, Coimbatore with per cent inhibition of 46.03. Considering this interaction study T<sub>8</sub> (*T.harzianum*, Dharwad isolate) proved was found to be efficient. This variation in antagonist i.e., ability of isolates of *Trichoderma* spp has also been reported by Chu and Wu (1981).

Antagonistic potential of different species of *Trichoderma* was reported (Majumdar *et al.*, 1996, Pinola *et al.*, 1988; Samiyappan, 1988, Muthusamy, 1989, Maharishi and Kumavat, 1992, Nakkeeran, 1992 and Singh and Majumdar, 1995). Similarly, effective antagonism against *Rhizoctonia bataticola* which is sclerotial stage of *M.phaseolina* by *T.harzianum* was reported by several workers (Parkhia and Vaishnav, 1986, Bhaskaran and Seetharam, 1986, Upadhyay and Mukopadhyay,

1986; Jacob, 1989 and Selvarajan, 1990). These reports consistently support biological potential of antagonistic microorganisms.

Further, the organic farming or non-chemical agriculture is growing demand all over the world in addition to fungicides, other agricultural chemicals viz., insecticides and herbicides are commonly used. But compatibility of these chemicals with biofungicides and some times the chemicals may exhibit antifungal properties also. Therefore, it is essential to study the effect of synthetic chemicals on biofungicide that is advocated to the farming community.

After standardizing the efficient biofungicide further investigations were carried out with respect to standardization of growth period for *T.harzianum*, effect of carbon source on *T.harzianum*, effect of agricultural chemicals on both pathogens and biofungicide, screening of substrates for biofungicides, effect of storage bag colour, standardization of seed and soil application dosages, storage and shelf life studies, screening of organic amendments for biofungicide and pathogens and effect of cultural filtrates on cotton seed germination were undertaken.

The peak period at which *T.harzianum* could put up maximum mycelial growth was on 12<sup>th</sup> day. Among seven carbon sources studied for growth of *T.harzianum* sources, Maltose was recorded the highest followed by Dextrose i.e., the control with mycelial weight.

The mechanism controlling fungistasis has been attributed to a deficiency of endogenous nutrients (Lockwood, 1964) and the presence of unknown inhibitors of microbial origin (Allen and Young, 1965 ; Dobbs et al , 1960). The presence or absence of exogenous nutrients

appears critical. *Trichoderma* was found to be highly sensitive to fungistasis by Steiner and Lockwood (1969) and relatively insensitive by Dix (1967). Since various types of carbon sources are present in organic matter and organic amendments or the exogenous substances, the carbon source, which is utilised by *T.harzianum*, plays an important role. In the present study both disaccharide and monosaccharides have favoured the growth of *T.harzianum*. Danielson and Davey (1973) found that disaccharides are better favoured by *T.harzianum*. Further, they opined that, germination of *Trichoderma* must be dependent upon more than just the presence of an exogenous carbon source since better germination was obtained with complex materials, such as malt extract, yeast extract and peptone than with dextrose.

In the present studies, among different substrates tested wheat bran sand and talc formulation recorded the highest cfu of *T.harzianum*. Groundnut cake and Neem cake have recorded with the very low cfu values. Paddy husk, Poultry manure and Vermicompost were intermediary. Anahosur (1998b) mentioned that, treating talc formulation @ 4 g/kg seeds was useful in controlling various soil borne pathogens. Elad *et al.*, (1986) and Hussain *et al.*, (1990) reported effective nature of seed pelleting of *T.viride* and *T.harzianum* in bean, sunflower and mungbean for checking infection by *M.phaseolina*. Jeyarajan *et al.* (1994) reported that, root rots in urdbean, chickpea, peanut and sesamum were reduced by 66, 55, 77 and 67 per cent, respectively by seed treatment of talc formulation. Wheat bran saw dust formulation was common for soil application. Soil application was better than seed pelleting (Biswas and Das, 1999, Krishnamoorhty and Bhaskaran, 1990).

The quantity of *T.harzianum* required for seed treatment as well as soil application and their combinations are necessary for optimum quantity of application need to be standardised. In the present investigations seed treatment, soil application and their combinations, were studied against all four pathogens, *F.solani*, *R.solani*, *R.bataticola* and *S.rolfsii*. Among seed treatment dosages, there was increase in number of cfu as dosage increased at both 15 DAS and 45 DAS. However there was significant increase in treatments of 8g/kg and 10g/kg and were statistically superior over 4g/kg and 6g/kg. Among 8g/kg and 10g/kg there was not significant increase in cfu in all the fungi. It was highest in *R.solani* followed by *F.solani* in the treatment of 8 g/kg of seed at 15 DAS. Similar trend was at 45 DAS. Therefore, seed treatment @ of 8g/kg of seed was optimum as compared to the rest of the seed treatment dosages.

Among soil application method four percent application recorded the highest mean cfu for all four pathogens under study at 15 DAS and 45 DAS. Although, the treatments of three per cent and four per cent were statistically significant but, there was not much higher variation in the cfu values, therefore three per cent was the best as per cfu values than the rest of the soil application methods.

Considering 15 DAS and 45 DAS together, single seed treatment (T<sub>3</sub>) i.e., 8 g/kg was superior among the seed treatments, three per cent was the best among the soil application methods and T<sub>3</sub> + T<sub>5</sub> i.e., seed treatment @ 8 g/kg and soil application @ 1 per cent found to be better than rest of the combinations studied.

There are several reports regarding the reduction of wilt incidence by application of antagonist to soil. The application of selected antagonist to soil reduced incidence of *Fusarium* crown rot of tomato under field condition (Sivan *et al.*, 1987). *T.harzianum* controlled *Fusarium* wilt of cotton and melon under natural soil conditions (Shivan and Chet, 1986).

Improved stands and yields were also obtained in *Rhizoctonia* infested field by planting soybean seed treated with *T.pseudokoningii* (Wu, 1982) and seed of corn and soybean treated with *T.harzianum* (Kommedahl *et al.*, 1981). Good biocontrol of damping-off caused by *Rhizoctonia solani* and *Pythium* spp. was achieved by treating seeds with *T.hamatum*, (Chet and Baker (1981), *T.harzianum* (Harman *et al.*, 1989) and control of *Fusarium* crown rot of tomato by *T.harzianum* (Sivan *et al.*, 1987).

Several reports have been made on control of diseases by seed treatment of antagonists (Bhaskaran and Seetharam, 1986; Jeyarajan *et al.*, 1991; Kehri and Chandra, 1991; Haque and Gaffar, 1991, Umamaheshwari, 1991; Krishnaveni, 1991; Maharishi and Kumavat, 1992; Nakkeeran, 1992; Sankar, 1994; Vidya, 1995 and Raguchander *et al.*, 1993). Similarly, bacterial antagonists as seed treatment are successful as reported by several workers (Jacob, 1989, Sridar *et al.*, 1991, Selvarajan, 1990, Krishnaveni, 1991, Savitry and Gnanamanickam, 1987, Samiyappan *et al.*, 1987, Saxena and Saxena 1995 and Kulkarni, 2000). Soil application method of both fungal and bacterial antagonists was successful @ 130-160 kg/ha as applied in furrow, row and filling holes (Anahosur, 1998b).

Combined application of seed treatment and soil application was most effective in controlling soil borne diseases as reported by Vidyasekaran *et al.* (1996). Jeyarajan *et al.* (1991), Vidyasekaran and Muthamilan (1995) combination of organisms was also useful practice (Sankar and Jeyarajan, 1996, Rajendran and Renganathan, 1996, Kichadi and Sreenivasa, 1998, Duffy *et al.*, 1996 and McLarren *et al.*, 1994). The present study indicated that, combined application of seed treatment and soil application method was superior which may be due to presence of adequate inoculum of antagonists in the infection court, which might have helped to reduce all the parameters of *F.solani*, *R.solani*, *R.bataticola* and *S.rolfsii*.

The colour of the biofungicide storage bags does play an important role in the storage. In the present study the transparent polythene bag recorded the highest cfu of 57.44 followed by cream (53.51 cfu), pink (51.05 cfu) and blue (50.47 cfu) colours. Black polythene was least among polythene bags, however it was superior to paper cloth bag. Nakkeeran *et al.* (1997) observed that storage of *T.viride* in talc formulation in milky white bags at room temperature (20-30° C) for up to 75 days was observed (cfu 206-276 x 10<sup>6</sup> g<sup>-1</sup>). Temperature for storage was on par and optimum at 10-15° C but zero temp (°C) was better than 10°C.

Many synthetic agro-chemicals are used in cotton cultivation may be it is a fungicide, insecticide or herbicide, but their role on the activities of biofungicide and the fungal pathogens is very much essential as because ultimately the biofungicide or the pathogens have live and grow in different soil conditions. In the present studies, the commonly used

fungicides, insecticides and herbicides were studied for both biofungicide and the soil borne pathogens.

The effect of fungicides on *Trichoderma harzianum* was studied and also on pathogens of *Fusarium solani*, *Rhizoctonia solani*, *Rhizoctonia bataticola* and *Sclerotium rolfsii* in laboratory as well as in pots as seed treatment and soil drenching application.

Both Carbendazim and Benomyl were inhibitory to *Trichoderma harzianum*, even at the lowest concentration (50 ppm), Captan was inhibitory at higher concentration, otherwise no effect was seen at other concentrations. Copper-Oxy-Chloride and Methoxy Ethyl Mercury Chloride were low in inhibiting *T.harzianum* at lower dosages but Copper-Oxy-Chloride was inhibitory at higher concentration. Carboxin, Thiram and Mancozeb recorded similar trend of inhibition but were more toxic than Copper-Oxy-Chloride and Methoxy Ethyl Mercury Chloride.

The Benzamidazole fungicides are deleterious to the antagonistic *Trichoderma* have been well documented in literature. Papavizas *et al.* (1982) reported inhibitory effect of benomyl against *T.harzianum*. Abd-Ei-Moity *et al.* (1982) found strong inhibition of *T.harzianum* even at as low concentration of benomyl as 0.5 mg/litre. They also found captan to have no inhibitory effect against *T.harzianum*. Papavizas (1980) reported, induced tolerance of *T.harzianum* to fungicides. Ramdoss and Shivaprakasam (1987) reported, inhibitory effect of carbendazim at 10 ppm and thiram at 500 ppm concentrations. Further, they mentioned that, insecticidal inhibition was fungistatic in nature. However, Algarsamy and Sivaprakasam (1988) reported, no adverse effect of carbendazim on *T.harzianum*. Rai and Vijay (1992) reported that, 5 to 10 ppm

concentration of Carbendazim was lethal to *T.viride* and also inhibited hyphal growth.

Three fungicides Carbendazim, Carboxin and Benomyl completely inhibited the growth of *F.solani* in laboratory studies. In seed treatment, Benomyl recorded the highest germination followed by Carbendazim and Carboxin and were on par. While for soil drenching, Carbendazim proved to be the best followed by Carboxin and Benomyl. The pre emergence and post emergence together, Carbendazim was the best followed by Carboxin and Benomyl. Mancozeb was least effective for *F.solani*.

Many previous workers have studied the efficacy of fungicides as dry mix, on various soil-borne pathogens. Siddappa and Anilkumar (1985) reported carbendazim to be very effective when mixed dry against *F.solani* f.sp. *piperis*. This was also confirmed in subsequent studies on the pathogen (Ramaswamy and Anilkumar, 1989). In many other studies, carbendazim alone was found to be highly effective against fusaria when applied as dry mix. Emisan was very effective against certain other fusaria (Siddappa and Anilkumar, 1985).

Gawande and Shukla (1976) reported captan to be most effective against wilt of betelvine, while Haider *et al.* (1978) recorded best control of *Fusarium* wilt of pigenopea by carbendazim application. Captan in addition to carbendazim was found to be highly effective in checking soil population of *F.oxysporum* f.sp. *lycopersici* (Dwivedi and Pathak, 1981). Sen and Kappor (1974) also reported carbendazim to be the most effective fungicide in controlling *Fusarium* wilt of tomato and wilt of pea when used as soil drench. That emisan was effective in suppressing not

only *Fusarium* population but also reduced wilt incidence as reported in these studies was earlier found in case of Fusarial root rot of betelvine also (Siddappa and Anilkumar, 1985).

Four fungicides viz., Carboxin, Thiram, Methoxy Ethyl Mercury Chloride and Benomyl found to be highly effective for *Rhizoctona solani* and completely inhibiting growth even at the lowest concentration. However, Carbendazim proved low in inhibiting. Mancozeb and Copper-Oxy Chloride were least effective. In soil drenching similar trend was observed. Methoxy Ethyl Mercury Chloride was the best followed by Carboxin and Thiram. Captan was also good. Mancozeb was least effective. Over all Benomyl was the best followed by Carboxin and Copper Oxy-Chloride to control *R.solani*.

In case of *Rhizoctonia bataticola* although the results were similar to *R.solani* i.e., complete inhibition of growth was seen in respect of Carboxin, Thiram, Methoxy-Ethyl Mercury Chloride, and Benomyl but, Mancozeb was inhibitory at higher concentration. For Carbendazim same trend was observed except higher concentration it was little more inhibitory in *R.bataticola* than *R.solani*. At lower concentration Carbendazim was better to *R.solani* than *R.bataticola*.

In seed treatment, for *R.bataticola*, Carboxin was the highest followed by Thiram and Benomyl. Mancozeb was least effective. In soil drenching of fungicides, Carboxin recorded the highest germination percentage followed by Methoxy Ethyl Mercury Chloride and Benomyl and Thiram. Least germination was observed in Mancozeb. Over all Carboxin was the most effective fungicide followed by Benomyl.

Three fungicides namely Carboxin, Methoxy Ethyl Mercury Chloride and Benomyl were highly effective for *S.rolfsii* at the lowest concentration studied, followed by Copper-Oxy-Chloride and Thiram. Mancozeb was least effective at low concentrations. Although Carbendazim was also least in inhibiting at low concentration but gradually suppressed the growth.

The seed treatment studies, for *S.rolfsii* Carboxin was the best followed by Methoxy Ethyl Mercury Chloride, Copper-Oxy-Chloride, Benomyl and Captan. In soil drenching Carboxin recorded most effective followed by Methoxy Ethyl Mercury Chloride, Copper-Oxy-Chloride, Benomyl and Captan.

Similar results were observed by Kulkarni *et al* (1986) that the Vitavax and Bayton are effective in controlling the *S.rolfsii* of wheat. The effectiveness of Thiram as soil drench has been reported by Kumar and Pandurang Gowda (1984) and Patil *et al* (1986), while working with many crop plants due to *S.rolfsii*.

As mentioned earlier different agro-chemicals such as insecticides and herbicides are used in cotton cultivation and the effect of these agro-chemicals on biofungicide, *Trichoderma harzianum* and the pathogens after studying the fungicides as these agro-chemicals i.e., the insecticides, herbicides may have either compatible and non-compatible reactions with the bio fungicide and further, some times the agro-chemicals may themselves be acting on the pathogens.

Among the five insecticides studied for *Trichoderma harzianum* Chlorpyrifos was found more inhibitive to *T.harzianum* followed by

Imidachloprid. Phorate and Carbofuran were also inhibitory to *T.harzianum* but Phorate was more inhibitory than Carbofuran. Endosulfan was least effective.

Martinez – Toledo *et al.* (1992) reported, strong inhibition of *T.harzianum* by Chlorpyrifos. Sridar *et al.* (1995) reported inhibitory growth of *T.viride* at 500 and 100 ppm of Carbofuran. Jayaraj and Ramabadrnan (1996a) reported that, Phorate and Carbofuran inhibited the hyphal growth of *T.harzianum*. Kolb *et al.* (1996) reported, favourable effect of Imidacloprid as seed treatment in wheat and oats.

For *Fusarium solani* Chlorpyrifos and Imidachloprid were highly suppressive and were on par. Endosulfan, Carbofuran and Phorate were least in inhibiting the *Fusarial* growth. Chlorpyrifos was the most effective against *Rhizoctonia solani*, completely inhibiting the growth even at lowest concentration. Endosulfan, Carbofuran and Phorate were not effective at low concentration. Chlorpyrifos had same result on *Rhizoctonia bataticola* too, i.e., complete inhibition of growth followed by Imidachloprid and gradually increased as the concentration increased. In case of Endosulfan it was lowest.

Chlorpyrifos recorded highest cent per cent inhibition of *Sclerotium rolfsii* even at the lowest concentration tested followed by Imidachloprid. Among the rest of three insecticides, Carbofuran, Phorate and Endosulfan were in the order of merit to inhibit the growth of *S.rolfsii*.

Sridar *et al.* (1995) reported inhibition of fungi by Carbofuran at higher dosage of 1000 ppm and mycotonic effect at lower dosage of 100 ppm.

After testing the insecticides, the common herbicides were tested for their effect on bio fungicide, *Trichoderma harzianum*, and all four pathogens under study i.e., *Fusarium solani*, *Rhizoctonia solani*, *Rhizoctonia bataticola* and *Sclerotium rolfsii*.

Among the five herbicides tested for all fungi, Alachlor was found to be highly inhibitory to the growth of *T.harzianum* even at the lowest concentration followed by Paraquat. Diuron recorded least among test herbicides at low concentration.

For the pathogens under study, Alachlor, Diuron and Paraquat found to be cent per cent inhibitory to *Fusarium solani*, Alachlor and Diuron herbicides ranked number one and two to inhibit the growth of *R.solani*. Pendemethalin was least among test herbicides. For *R.bataticola* Alachlor and Diuron were highly inhibitory. Both Glyphosate and Pendemethalin recorded more than 50% inhibition at higher concentrations.

Russin *et al.* (1995) reported that, growth of *M.phaseolina* was less affected by Atrazine.

In case of *S.rolfsii*, Paraquat and Diuron were highly inhibitory to the mycelial growth of *S.rolfsii*. Pendemethalin and Glyphosate recorded less than 50 per cent inhibition at higher concentrations.

Anahosur *et al.* (1984) reported that, herbicides used in sorghum may not control charcoal rot. Atrazine and 2, 4-D were ineffective inhibitors to *M.phaseolina*. Glyphosate at higher concentrations (0.01 to

0.25%) reduced charcoal rot of sorghum (Gouvela *et al.*, 1993), whereas, Atrazine did not inhibit sclerotial germination of *M.phaseolina*.

The pot culture studies were conducted for three pathogens, viz., *Fusarium solani*, *Rhizoctonia solani*, and *Rhizoctonia bataticola* (*Sclerotium rolfsii* under *in vivo* conditions) with different organic amendments viz., FYM, Groundnut cake, Neem cake, Poultry manure, Vermicompost and seed treatment with Captan and seed treatment with *Trichoderma harzianum*. In these studies, Captan was the best followed by *T.harzianum* for all the four pathogens under study. However, among the organic amendments separately, Neem cake was the best in suppressing the activities of these pathogens.

Storage and stability with prolonged shelf life are major criteria for the success of biocontrol agent in the management of plant disease. In the present studies, it was observed that, as the temperature increased there was reduction in number of cfu and 0° C recorded the highest cfu of 151.33 and 93.75 in F<sub>1</sub> i.e., the wheat bran sand medium and F<sub>2</sub> i.e., the talc formulation respectively. Further, it was observed that, as the storage period increased from 60 days after preparation to 150 days after preparation, the number of cfu was reduced. It is concluded that, storing at 0°C will have longer shelf life and wheat sand bran medium is better than the talc formulation. Another study, that is storing the *T.harzianum* in marketable packets was studied in both formulations and the studies indicated that, *T.harzianum* can be stored upto five months without much losing the viable spores of it.

Vidhyasekaran and Muthamilan (1995) found that, *P.fluorescens* in talc formulation and peat based formulation survived upto 240 days

although population decline occurred after 30 days. Similarly, Sanker and Jayaraj (1996) reported talc based powder formulation of *Trichoderma* and *Gliocladium* retained more than 50 per cent population upto 120 days. Initial population was  $224 - 297 \times 10^6 \text{ g}^{-1}$  in *Trichoderma* spp and *Gliocladium* sp. Population declined in *Trichoderma* (19-21%) and *G.virens* (29.9%) after 7 days. Sanker and Jayaraj (1996) observed that storage as treated seeds of sesamum was better than treating during sowing. Antagonists remained viable without affecting seed germination. Prasad *et al.*, 1997 found that Molasses yeast medium and potato dextrose broth were best for *in vitro* multiplication of antagonists. Talc formulation supported growth upto 60 days. Viability upto 90 days of *T.harzianum* was achieved in talc, gypsum, vermiculite and wheat bran, three pesta granule formulation with wheat flour, wheat flour kaolin, wheat flour bentonite strong at 4° C was the best. Talc formulation of *T.viride* can be safely stored in milky white bags at room temperature (20-30° C) for upto 75 days. Similaly Saha and Pan (1998) reported that, 40° C temperature was lethal to survival of *Glicoladium virens*.

Lignite carrier system retained 90 per cent viability of antagonists (*G.virens*, *T.harzianum*) upto 120 days at 20° C (Jones *et al.*, 1984). Hader *et al.* (1979) reported, wheat bran with *T.harzianum* retained 80 per cent viability at 4° C. Similarly, Convey (1983) stored the conidia of *T.harzianum* in fluid drilling gels at four different temperature levels viz., 5, 16, 20 and 25° C for 10 days without affecting viability. Manmohan Das (1992) reported that, the nucleus inoculum of *T.harzianum* developed on FYM or gobar gas effluent retained, its viability even after 300 days of storage. Lower temperature of 20° C is better than 30° C to retain viability for longer periods.

Among the culture filtrates of isolates of *Trichoderma* and *Gliocladium* culture filtrates T<sub>8</sub> of Dharwad isolate (*Trichoderma harzianum*) recorded highest mean germination percentage of 67.33, but it was highest in T<sub>12</sub> (*Trichoderma reesai*) for Abhadita (73.71%) and T<sub>1</sub> (*Trichoderma harzianum* of TNAU, Coimbatore) for Jayadhar (66.93%). The lowest germination percentage was in T<sub>11</sub> (*Trichoderma viride* of Agharkar Research Institute of Pune (49.29 mean per cent germination value) followed by T<sub>9</sub> (*Trichoderma harzianum* TNAU, Coimbatore) of (49.69%). Among *Gliocladium virens* isolates G<sub>1</sub> (TNAU, Coimbatore) recorded the highest (68.72%) as compared to G<sub>2</sub> (63.48%) CICR, Sirsa. In general, the culture filtrates of isolates of *Trichoderma* and *Gliocladium* were found suppressing the seed borne fungal activities thereby enhancing the germination of cotton seeds. The results further indicated that, the delinted seeds of either Jayadhar or Abhadita had better germination than the fuzzed seeds, it may be because the cotton fuzz contains more of fungal flora than the delinted seeds.

There are some reports that, metabolites of fungal antagonists affecting seed germination. Either soluble fractions of culture filtrates of *Chaetomium capreum* inhibited growth of several fungal pathogens and also delayed germination of soybean seeds (Yeh and Sinclair, 1980). Metabolites of *Trichoderma viride* and *Aspergillus*, *Fusarium* and *Penicillium* spp. have been reported to decrease the capacity of wheat, pea, rape and lettuce seeds to germinate under test conditions (Czachor; 1985). Kamal and Verma (1979) reported 100 per cent inhibition of pigeonpea seed germination by culture filtrate of *T. viride*.

# *Summary*

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## VI. SUMMARY

The importance of cotton under the Indian economical and social systems does not need more emphasis. In addition to the meeting of total requirement of the country, it is also exported as raw cotton, yarn and fabrics etc., earning a sizeable amount of foreign exchange. The pests and diseases are the greatest enemies of cotton and account for huge losses varying from 30-70 per cent. In the present investigations of survey carried out on soil borne diseases wilt caused by *Fusarium solani* alone has about 20 per cent incidence. Although *Rhizoctonia solani* and *Rhizoctonia bataticola* are present up to six per cent and the losses due to *Sclerotium rolfsii* are negligible. However, if cotton is cultivated after *S.rolfsii* affected groundnut crop, definitely this disease can be a serious threat. But the visible insects have shadowed the presence of the diseases. With the increasing commercial value of the crop, the limitations imposed by land use patterns, and the rising cost of seed and other inputs including labour wages, it is imperative that the maximum possible yield is realised from the crop and preventable losses through diseases to the minimum.

Several studies have been made for the management of cotton diseases. Most of the earlier researches and developed strategies employed chemicals for the control of diseases. Several chemicals effective against an individual pathogen are available, which in general can effectively control the specific disease caused by that pathogen. Quite often, more than one disease could occur simultaneously as mixed infection following one after other during the season. In such cases, application of chemical to control individual disease is neither feasible nor economical.

Scientists in both public and commercial institutions are investigating suppression of cotton pathogens by biofungicides or antagonists. To date, the search for disease control organisms in cotton has been limited to those associated with soil and the rhizosphere. Two fungal antagonists viz., *Trichoderma* and *Gliocladium* spp. and two bacterial viz., *Bacillus* and *Pseudomonas* spp. have been extensively exploited for the biocontrol of root rot and seedling diseases of cotton.

In view of these, the present investigations were carried out on four important soil borne pathogens of cotton with a special reference to biofungicides i.e., investigations were carried out on the management of cotton wilt caused by *F.solani* and root rots caused by *R.solani*, *R.bataticola* and *S.rolfsii* by employing various methods viz., application of fungicides, biofungicides, organic amendments, developing formulations of biofungicides, screening the substrates, storage, shelf life and effect of cultural filtrates of biofungicides on cotton seed germination during 1998 to 2002 at the Agricultural Research Station, Dharwad farm of UAS, Dharwad.

Survey on cotton diseases revealed that *F.solani* was in more severe form than that of *R.solani*, *R.bataticola* and *S.rolfsii*. In view of this, different isolates *F.solani* were collected from various cotton growing areas of Karnataka. The virulence of these isolates was studied. The Raichur isolate was found to be more severe than the other isolates. Twelve *Trichoderma* cultures and two cultures of *Gliocladium virens* were screened against all the four pathogenic cultures to ensure the most efficient biofungicide for all pathogens under study. *Trichoderma harzianum* of Dharwad isolate was the best among all the bioagents tested and was used for further studies. Among the *Gliocladium virens*

(G2) of TNAU, Coimbatore was the best followed by the CICR, Sirsa isolate, however the *T.harzianum* of Dharwad was the best among all the biofungicides tested and was taken for further studies.

Some fungi were found commonly associated with cotton rhizosphere, these fungi are saprophytic may be either affecting the pathogens or biofungicides present in the soil.

It is essential to study the growth period as it is preliminary study before proceeding for any nutritional studies. In this connection, *T. harzianum* reached its peak growth of mycelium 12 days after seeding and among the carbon sources Maltose and Dextrose were best for the growth of *T. harzianum*.

Among the substrates screened wheat bran formulation and talc formulation were superior for mass culturing and as the best carrier substances for *T.harzianum*.

The quantity of application of *Trichoderma harzianum* either as seed treatment or as soil application are necessary for optimum dosage of recommendation. In the seed treatment @ 8 g /kg was best and statistically superior over others. Similarly, soil application @ three per cent was superior and among the combinations of seed treatment and soil application studied, seed treatment @ 8 g / kg and one per cent of soil application gave better cfu.

As different types of agro-chemicals are used in cotton cultivation and effect of these agro-chemicals on both *T.harzianum* and the pathogens was carried out. In the first, fungicides were screened for *T.*

*harzianum* and four pathogens under the study. Carbendazim and Benomyl were inhibitory to *T.harzianum*. Carbendazim, Carboxin and Benomyl were effective against *F.solani*, Similarly, for *R.solani* Carboxin, Thiram,, Methoxy Ethyl Mercury Chloride and Benomyl were effective; for *R.bataticola* Carboxin, Thiram, Methoxy Ethyl Mercury Chloride and Benomyl and for *S. rolfsii*, Carboxin, Methoxy Ethyl Mercury Chloride and Benomyl were highly effective.

In pot culture experiments, Benomyl and Carbendazim were excellent for *F.solani*. Similarly, Benomyl, Methoxy Ethyl Chloride and Carboxin and Thiram and Methoxy Ethyl Mercury Chloride were superior to control *R.solani* and *R.bataticola* respectively.

The seed treatment studies, for *S.rolfsii* Carboxin was the best followed by Methoxy Ethyl Mercury Chloride, Copper-Oxy-Chloride, Benomyl and Captan. In soil drenching Carboxin recorded most effective followed by Methoxy Ethyl Mercury Chloride, Copper-Oxy-Chloride, Benomyl and Captan.

After testing the fungicides the commonly used insecticides in cotton cultivation were tested for both biofungicide and the pathogens. Among the five insecticides studied for *Trichoderma harzianum* Chlorpyriphos was found more inhibitive to *T.harzianum* followed by Imidachloprid. Phorate and Carbofuran were also inhibitory to *T.harzianum* but Phorate was more inhibitory than Carbofuran. Endosulfan was least effective.

For *Fusarium solani*, Chlorpyriphos and Imidachloprid were highly suppressive and were on par. Endosulfan, Carbofuran and Phorate

were least in inhibiting the *Fusarial* growth. Chlorpyrifos was the most effective against *Rhizoctonia solani*, completely inhibiting the growth even at lowest concentration. Endosulfan, Carbofuran and Phorate were not effective at low concentration. Chlorpyrifos had same result on *Rhizoctonia bataticola* too, i.e., complete inhibition of growth followed by Imidacloprid and gradually increased as the concentration increased. In case of Endosulfan it was lowest.

Chlorpyrifos recorded highest cent per cent inhibition of *Sclerotium rolfsii* even at the lowest concentration tested followed by Imidachloprid. Among the rest of three insecticides, Carbofuran, Phorate and Endosulfan were in the order of merit to inhibit the growth of *S.rolfsii*.

Overall, among the insecticides screened for both biofungicide and pathogens, Chlorpyrifos was highly inhibitory to *T.harzianum* followed by Imidachloprid. Same insecticides were also inhibitory to the pathogens i.e., *F.solani* , *R.solani*, *R. bataticola* and *S.rolfsii*. Cabofuran , Phorate and Endosulfan were less effective to both biofungicide and the pathogens.

Since the herbicides are directly applied to soil and their role on biofungicide and also the pathogens was studied. Among the herbicides tested, Alachlor was more effective to suppress the growth of *T.harzianum*. For *F.solani*, Alachlor, Diuron and Paraquat were inhibitory. Both *R. solani* and *R.bataticola* were inhibited by Alachlor and Diuron. In case of *S.rolfsii*, Paraquat and Diuron were inhibitory.

The organic amendments do have a role on fungal flora of the soil whether it is biofungicide or the pathogenic fungi. In this connection studies were carried out on commonly used organic amendments and seed treatment with Captan and biofungicide (*T.harzianum*) separately and the pot culture studies for three pathogens, viz., *Fusarium solani*, *Rhizoctonia solani*, and *Rhizoctonia bataticola* and field experiments for *S.rolfsii* with different organic amendments viz., FYM, Groundnut cake, Neem cake, Poultry manure, Vermicompost and seed treatment with Captan and seed treatment with *Trichoderma harzianum*. The studies indicated that, Captan was the best followed by *T.harzianum* for all the four pathogens. However, among the organic amendments separately, Neem cake was the best in suppressing the activities of these pathogens.

Storage and stability with prolonged shelf life are major criteria for the success of bio control agent in the management of plant disease. In the present studies, it was observed that, as the temperature increased there was reduction in number of cfu and 0° C recorded the highest cfu in both formulations of F<sub>1</sub> i.e., the wheat bran sand medium and F<sub>2</sub> i.e., the talc formulation. Further, it was observed that, as the storage period increased from 60 days after preparation to 150 days after preparation, the number of cfu was reduced. It is concluded that, storing at 0°C will have longer shelf life and wheat sand bran medium is better than the talc formulation. Another study, that is storing the *T.harzianum* in marketable packets was studied in both formulations and the studies indicated that, *T.harzianum* can be stored up to five months without much losing the viable spores in it.

There are reports that, some of the species of *Trichoderma* suppress the germination of seeds. In this context all twelve culture filtrates of *Trichoderma* and the two culture filtrates of *Gliocladium virens* were tested for their effect on cotton seed germination, taking two genotypes viz., Jayadhar and Abhadita against as delinted and fuzzed seeds. In the present investigation the culture filtrates of isolates of *Trichoderma* and *Gliocladium virens* did not affect the cotton seed germination, but rather had better seed germination, may be the culture filtrates are affecting the fungal flora of both delinted and fuzzed. It was more evident that, the delinted seeds germinated more than the fuzzed seeds.

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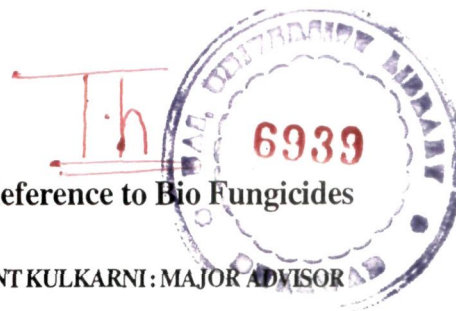
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## Management of Wilt and Root Rots of Cotton with Special Reference to Bio Fungicides

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2002

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Survey was revealed that wilt caused by *Fusarium solani* up to 20 per cent followed by *Rhizoctonia solani* and *Rhizoctoina bataticola* up to six per cent and *Sclerotium rolfsii* was negligible. Among nine isolates of *F. solani*, the Raichur isolate was more virulent. Among twelve cultures of *Trichoderma* and two cultures of *Gliocladium virens* against all four pathogens. *T. harzianum* of Dharwad isolate was the best and *G. virens* of TNAU was the best. In growth period studies, *T. harzianum* reached its peak on 12<sup>th</sup> day and maltose and dextrose were best to support the growth. Transparent polythene bag was superior. Among substrates wheat bran was superior. In seed treatments, @ 8g/kg of seed and among soil applications, three percent was superior. In the combination, seed treatment @ 8g/kg with 1% soil applicaiton was superior.

Among agro chemicals tested for both *T. harzianum* and pathogens, the fungicides, carbendazim and benomyl were inhibitory to *T. harzianum*. Benomyl and carbendaizim were excellent to control *F. solani*. Similarly, benomyl, methoxy ethyl mercury chloride (MEMC), carboxin, thiram and MEMC were superior to control *R. solani* and *R. bataticola* respectively. For *S. rolfsii*, carboxin was the best. Among insecticides, chlorpyriphos was inhibitory to *T. harzianum* followed by imidachloprid. Endosulfan was least inhibitory. Carbofuran, phorate and endosulfan were less effective to pathogens. Among herbicides, alachlor was more deleterious than paraquat. Diuron was effective against all four pathogens. Among organic amendments neem cake was superior in suppressing the growth of all four pathogens. In storage studies, increase in temperature lowered cfu and at 0°C it was maximum and wheat bran was the best and can be stored upto five months. Culture filtrates of all twelve *Trichoderma* and two *G. virens* on seed germination of Jayadhar and Abhadita with delinted and fuzzed seeds revealed that delinted seeds had better germination and culture filtrates enhanced seed germination.