# BIOLOGICAL CONTROL OF *Rhizoctonia solani* Kuhn ON RICE USING MYCOPARASITES

BY

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1989

# DECLARATION

I hereby declare that this thesis entitled "Biological controlof *Rhizoctonia solani* Kuhn on rice using mycoparasites" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellayani,
15-12-1989.

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# CERTIFICATE

Certified that this thesis entitled "Biological control of *Rhizoctonia solani* Kuhn on rice using mycoparasites" is a record of the research work done independently by Shri Viswakumar, P under my guidance and supervision and that it has not previously formed the basis for the award of any Degree, Diploma, Fellowship or Associateship to him.

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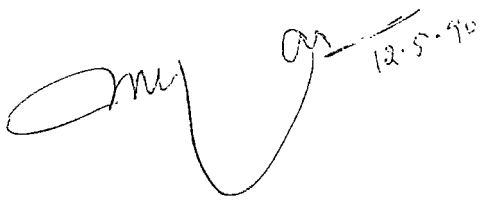

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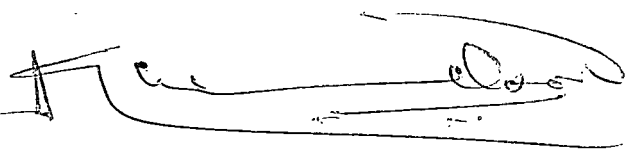

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*Dedicated to my beloved parents*
*and*
*grand parents*

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# INTRODUCTION

# INTRODUCTION

Sheath blight caused by Rhizoctonia solani Kuhn is one of the most prevalent and destructive diseases of rice in Kerala.

The widely adopted method of control of this disease is the use of fungicides and eventhough various fungicides are known to be effective against the disease, it is often a common observation that fungicidal umbrella is ineffective in managing the disease effectively.

As a result of the excessive use of chemicals for plant disease control, the pathogens have become more and more resistant to fungicides. In addition to this problem there is also the danger of destruction of beneficial microbes including antagonists in the environment along with pathogen. Moreover, in recent years environmental hazards due to imprudent use of chemicals have also been well demonstrated.

Rhizoctonia solani, mainly being a soil borne pathogen is not amenable to the usual control measures. Hence, more satisfactory method of control can be expected only from an integrated approach where chemical, biological and cultural methods are combined. Biological control of R. solani involves the use of other micro-organisms which are effective antagonists of the pathogen.

Biological control of R. solani using mycoparasites has been reported by many workers from all over the world on a number of crops (Velvis and Jager, 1983; Chand and Logan, 1984; Venkatasubbiah et al., 1984). Hence the present study has been undertaken with the following objectives.

Isolation of antagonistic fungi effective against the rice isolate of R. solani from the rhizosphere and phylloplane of rice plants. Identification of suitable carrier materialsfor mass multiplication of antagonistic organism and assessment of comparative efficacy of various antagonistic fungi alone and in combination with fungicidal treatments against sheath blight diseases, both in vitro and in vivo.

# REVIEW OF LITERATURE

# REVIEW OF LITERATURE

The first report of a disease under the name "Oriental Sheath blight and leafspot" caused by "Sclerotium irregulare" from Japan was given by Miyake (1910). Later, the disease has been reported from other countries like Philippines (Reinking, 1918; Palo, 1926), Sri Lanka (Park and Bertus, 1932) and China (Wei, 1934). Ryker and Gooch (1938) have reported the occurrence of sheath blight from the United States and now it is known to occur throughout the temperate and tropical rice growing areas of the world (Ou, 1984).

In India sheath blight was first observed by Butler (1918), but it was Paracer and Chahal (1963), who first gave a detailed description of sheath blight caused by Rhizoctonia solani Kuhn from Punjab. Later on Kohli (1967) also reported the occurrence of the disease from Punjab.

Mahendra Prabath (1971) was the first worker to report the occurrence of this disease in Kerala and to make a detailed study. Sheath blight in epidemic proportions, has been reported in Kerala, especially after the introduction of high yielding varieties.

## Symptomatology

Symptomatology was first detailed by Miyake (1910) as discoloured ellipsoidal spots on the sheath at or above the water level and also on the leaves. These spots later developed into oblong or irregularly elongated lesions, present on any part of the leaf sheath which often extends upto the leaf blade. Gradually the whole leaf blade rots and it can be pulled out easily. Singh and Pavgi (1969) reported the initial symptoms as oval or irregular straw coloured lesions on the leaf sheath near the leaf base, surrounded by a narrow reddish brown band. Lesions increased in size coalesced and covered the whole leaf lamina, which appeared as banded patches. Sclerotia were also reported to develop on the infected region. Kozaka (1970) observed that the lesions developed on the leaf sheath, were first greenish grey and ellipsoidal, 2 to 3 cm long or more, which gradually turned greyish white with a blackish narrow brown margin. Ou (1984) reported in the tropics the leaves of the plants are often severely infected and killed by the fungus.

## Causal Organism

Miyake (1910) described the organisms as Sclerotium irregulare. Later, a detailed study of the organism was

done by Sawada (1912) and he reported that the causal organism was identical with Hypochnus sasakii Shirai. Matsumoto et al. (1932) and Matsumoto (1934) studied the fungus employing a number of isolates. The organism was named as Corticium sasakii (Shirai) Matsumoto by Matsumoto (1934), and he considered this the most acceptable name. The morphological characters of the culture of the fungus obtained from China and Philippines were studied by Ryker and Gooch (1938) and they considered the rice pathogen to be the large sclerotial strain of Rhizoctonia solani. Talbot (1970) after detailed observations came to the conclusion that Thanatephorus cucumeris (Frank) Donk, is the perfect state (teleomorph) of R. solani and Hypochnus solani and H. filamentosus were considered synonyms of T. cucumeris. Saksena (1979) identified two distinct strains of R. solani in Indian soils, the root infecting and shoot infecting strains. The importance of aerial strains of R. solani in India was recognised only after the appearance and spread of sheath blight of rice causing severe blighting of the aerial parts of the plant.

The grouping of R. solani on the basis of hyphal anastomosis has gained much importance in the study of this versatile pathogen. According to Gregory (1984) hyphal fusion was first recorded by Tulasne and Tulasne

during 1861 to '65 (c.f. Buller, 1931). Major contribution to the understanding of hyphal fusion ~~were~~ was made in the works of Buller (1931; 1933), who distinguished 3 types of fusions, viz., vegetative fusion, sexual fusion and parasitic fusion. Capacity for hyphal fusion provides an indication of the relationship between isolates of R. solani. The first systematic grouping of R. solani based on anastomosis was done by Richter and Schneider (1953). As a result of the study, six morphological groups viz., A, B, C, D, E and F based on their capacity for hyphal fusion were identified. Parmeter et al.(1969) concreted the concept of anastomois of R. solani by elaborating the work of Richter and Schneider (1953). They grouped R. solani into AG1, AG2, AG3 and AG4 which are genitically isolated and incapable of nuclear exchange due to non-fusion between isolates of the different groups. Vijayan (1986) grouped 41 isolates of R. solani from Kerala into 4 morphological groups MG1, MG2, MG3 and MG4.

## Studies on mycoparasitism on Rhizoctonia solani

There are two types of mycoparasitism observed, necrotrophic or destructive mycoparasitism and biotrophic type, wherein a balanced relationship occur between the host and the parasite (Barnett, 1963; Barnett & Binder,1973).

R. solani which causes sheath blight of rice can be parasitised by necrotrophic as well as biotrophic mycoparasites. Trichoderma lignorum (Tode) Harz (Weindling, 1934; Chu & Wu, 1981; Chet & Baker, 1981). Penicillium vermiculatum Dang (Boosalis, 1956), Gliocladium roseum Bain (Pugh & Van Embden, 1969; Jager et al., 1979) Gliocladium virens Miller, Gidden & Foster (Tu & Vaartaja, 1981), Fusarium oxysporum Schlect, and Fusarium udum Butler (Arora & Dwivedi, 1980) are some of the examples.

It was reported that Penicillium vermiculatum could penetrate R. solani hyphae (Boosalis, 1956). Penetration and coiling of several isolates of Trichoderma has been reported by Dennis and Webster (1971 c). Granulation, vacuolation and finally disintegration of R. solani hyphae has been reported as a result of mycoparasitism by various isolates of Fusarium (Arora & Dwivedi, 1980).

Fusarium oxysporum was found to parasitise R. solani, causing, penetration, lysis and chlamydospore formation inside the host (Gupta et al., 1979).

The mycoparasitism of R. solani by Gliocladium virens leading to the formation of appressoria on contact with host cells, penetration, formation of intracellular hyphae and death of host cells has been reported by Tu (1980) and Tu and Vaartaja (1981). Lewis and Papavizas (1980) reported the hyphal invasion of R. solani by Trichoderma sp.

Chet et al. (1981) demonstrated the coiling and appressoria formation by hyphae of Trichoderma hamatum (Bonord) Bain, when they came into contact with R. solani hyphae.

Elad et al. (1983) conducted studies on the ultrastructural aspects of interaction between Trichoderma spp. and plant pathogenic fungi. The mycoparasites, T. harzianum and T. hamatum on interaction with R. solani caused enzymatic digestion of host cellwalls. As a result of invasion the host cell became empty of cytoplasm. The sclerotia of R. solani have also been reported to be hyperparasitised by Verticillium biguttatum Fr. (Velvis & Jager, 1983; Jager & Velvis, 1984). Chand and Logan (1984) reported the parasitism of R. solani by Penicillium cyclopium Westing and P. nigricans Bain.

Roy and Sayre (1984) conducted electron microscopic studies on the mycoparasitism of T. harzianum and T. viride on R. solani f. sp. sasakii. R. solani was parasitised by T. harzianum leading to the coiling of the mycoparasite on the mycohost. Hyper parasitism of antagonistic species of Aspergillus, Penicillium and Trichoderma on anastomosis groups AG1 and AG4 of R. solani was observed by Wu et al. (1986). It was also observed that there was no appreciable difference between the hyperparasitism on the two AG groups. They also observed that T. harzianum could erode

the hyphae of R. solani in natural field soils. Elad et al. (1987) observed that the hyphae of T. harzianum formed branches and grew directly towards the host.

Lewis and Papavizas (1987 a) reported that the water extracts of young actively growing hyphae of Trichoderma spp on bran medium affected the growth of R. solani in liquid cultures.

Gokulapalan (1989) tested different fungi for the mechanism of mycoparasitism. T. harzianum and T. viride proved to be the most efficient parasites of R. solani. These fungi were found to cause excessive granulation, vacuolation and finally disintegration of the host hyphae. Aspergillus aculeatus, A. versicolor and Chaetomium globosum were also reported to cause granulation, vacuolation and finally disintegration of the host hyphae.

## Use of Mycoparasites in the biological control of Rhizoctonia solani

The group of mycoparasites which are well documented as antagonists against R. solani are the species of Trichoderma, since the pioneering work of Weindling (1932). He was the first worker to demonstrate the antagonistic and parasitic activity of Trichoderma lignorum towards R. solani. The fungus was found to readily parasitise and kill the hyphae of R. solani.

Naim and El-Esawy (1965) suggested that biological control of R. solani might be best achieved by applying Aspergillus terreus at a soil temperature of 35°C and a pH of 4 or less. Neweigy et al. (1982) stated that two species of Aspergillus and three species of Trichoderma were most effective against some pathogens attacking Faba bean cultivars, namely Fusarium solani, Rhizoctonia solani and Sclerotium rolfsii. Venkatasubbiah and Safeeullah (1984) demonstrated that under glass house and field conditions seed treatment with Aspergillus niger reduced incidence of collar rot in coffee seedlings.

Chaetomium spp., have been found to show antagonism against R. solani, especially C. globosum and C. cochlioides were the two species which showed significant antagonistic activity (Tviet & Moore, 1954; Harman et al., 1980; Sezgin et al., 1982).

The role of species of Penicillium in inhibiting the growth of R. solani was demonstrated by various workers. Many species of Penicillium including P. clavariaforme Bain, P. patulum, P. cyclopium Weslling, P. nigricans (Bain) Thom. and P. oxalicum Currie & Thom., were also reported to have antagonistic activities against R. solani (Wood, 1951; Chu and Wu, 1981; Chand and Logan, 1984; Gupta et al., 1985; Lulu Das, 1986).

Endo et al.(1973) observed the reduced incidence of Corticum sasakii, the causal agent of sheath blight on rice brought about by Neurospora crassa Shear and Dodge.

Among the many potential anatagonistic soil micro-organisms, members of the genus Trichoderma have gained considerable importance. There are many reports of the effective application of Trichoderma spp. for the control of diseases caused by R. solani. Weindling (1932) demonstrated that the damping off of citrus seedlings caused by R. solani can be controlled by inoculating with Trichoderma spp. Hino and Endo (1940) observed that Trichoderma viride Pers ex can parasitise and destroy the mycelium of Corticium solani. Tisdale and Foster (1948) from a green house test observed that Trichoderma sp. can reduce the pathogenicity of R. solani. Evans and Gottlieb (1952) found that damping off of pea caused by R. solani was controlled in sterile and nonsterile soils containing T. viride. Roy (1977) showed that when T. viride was incorporated in sterilized soil together with Corticium sasakii sheath blight of rice was slightly reduced.

Hadar et al. (1979) observed that an isolate of Trichoderma harzianum could directly attack R. solani and that a wheat bran culture of the fungus could control damping off of bean, tomato and egg plant seedlings caused by R. solani.

Wu (1980) used Trichoderma pseudokoningii Rifai and T. harzianum for seed treatment of Soybean to control pre-emergence damping off caused by R. solani.

Harman et al. (1980) found that Trichoderma hamatum effectively reduced the seedling disease of radish and pea caused by R. solani under field conditions.

Tu and Chang (1981) studied the ecology of rice sheath blight pathogen, R. solani emphasising its biological control, using Trichoderma sp.

Elad et al.(1981 b) achieved biocontrol of R. solani in Carnations using the antagonist T. harzianum.

Elad et al. (1981 a) used T. harzianum for controlling R. solani causing black root rot of strawberries under field conditions.

Chu and Wu (1981) found that species of Trichoderma, Penicillium and Aspergillus were efficient for the biological control of black scurf of potato caused by R. solani.

Chet and Elad (1982 a) discussed the possibility of using antagonistic microorganisms as a substitute or as an additive to fungicides for the control of plant pathogenic fungi. They successfully used wheat bran culture of T. harzianum for the biocontrol of damping off of beans, peanuts and egg plants caused by R. solani or Sclerotium rolfsii.

Chet et al. (1982 b) controlled R. solani in Iris, using T. harzianum along with soil solarisation.

Coating of cotton seeds with Trichoderma sp. was done for the biological control of infection by R. solani (Elad et al. 1982) Marshall (1982) effectively reduced damping off of snap beans caused by R. solani using T. harzianum.

Sportelli et al. (1983) reported the use of Trichoderma viride for biological control of fungal diseases of tomato caused by Fusarium oxysporum, Verticillium dahliae Kleb and R. solani. They found that the quantity of Trichoderma could be reduced by the direct application of the biocontrol agent to the root zone of tomatoes. They also observed that broadcast application was better than row application, so also seed coating enabled the application of the biocontrol agent at the most susceptible sites of the plant which are especially effective for controlling pre and post-emergence diseases.

Velvis and Jager (1983) conducted extensive studies on the biological control of R. solani on potatoes by using antagonists like Verticillium biguttatum, Trichoderma hamatum and Gliocladium roseum. From these studies, V. biguttatum emerged as an efficient mycorparasite of R. solani. This proved to be a valuable tool for biological control of pathogen. Van den Boogert and Jager (1983)

reported that the addition of live mycelium of R. solani to the soil markedly stimulated the growth of the hyper-parasitic fungi Gliocladium roseum and V. biguttatum.

Mew and Rosales (1984) reported the higher 'Cellulolytic adequacy index' of T. harzianum over R. solani, there by increasing its ability to decompose rice straw, thus affecting the survival of R. solani under natural conditions.

Venkatasubbiah et al. (1984) found that T. harzianum was an effective biocontrol agent for R. solani, the incitant of collar rot of coffee seedlings. Seed treatment with T. harzianum even gave better results.

Gokulapalan and Nair (1984) tried various isolates of antagonistic fungi obtained from soil, and irrigation water from paddy fields against the sheath blight organism. Of the various isolates tested Aspergillus niger and Trichoderma viride were found to be effective against R. solani.

Strashnov et al. (1985) achieved integrated control of R. solani on lupine with methyl bromide and T. harzianum under field conditions.

Lewis and Papavizas (1985) reported the inhibitory effect of mycelial preparations of Trichoderma and Gliocladium on populations of R. solani and incidence of

damping off in sugarbeet. The survival of R. solani was reduced at least 50 per cent. Mihuta and Rowe (1985) reported fluid drilling Trichoderma hamatum along with seeds and a gel matric gave the best control of R. solani on radish.

Lifhitz et al.(1985) reported the decrease in the incidence of pre-emergence damping off of radish caused by R. solani by the use of T. harzianum.

Lewis and Papavizas (1987 b) reported the reduction of R. solani inoculum in soil by the application of wheat bran culture of Trichoderma hamatum containing actively growing mycelium.

Studies on substrates for mass multiplication of antagonistic fungi

Many workers have tried out different substrates for the mass multiplication of antagonists.

T. viride and T. harzianum were mass cultured successfully in a substrate made up of diatomaceous earth granules and black strap molasses (Backman and Rodrignez-Kabana,1975). A wheat bran, sawdust and water mixture in the ratio 3:1:4 has been used successfully for the mass multiplication of T. harzianum, used in the biological control of R. solani on bean and tomatoes by Elad et al. (1980). A wheat bran

and peat soil mixture in the ratio 1:1 (v/v) with 50 per cent moisture has been used as a substrate for mass multiplication of T. viride and T. harzianum by Sivan et al. (1984). Jones et al.(1984) used a lignite and stillage carrier as a substrate for T. harzianum and Gliocladium virens. Here liquid stillage and granular lignite were used in the ratio 1:2. Upadhyay and Mukhopadhyay (1986) used a sorghum grain substrate for mass culturing T. harzianum, against Sclerotium rolfsii causing root rot of sugarbeet. Padmanabhan and Alexander (1987) tried a sand-sorghum medium for application of T. viride in the field for control of root rot of sugarcane seedlings. A tapioca rind or thippi substrate was tried out for mass multiplication of T. viride and T. harzianum in the control of root rot of blackgram (Kousalya Gangadharan and Jeyrajan, 1988). Gokulapalan(1989) reported that wheat bran and rice bran cultures of Trichoderma sp. were efficient in controlling sheath blight of rice caused by Rhizoctonia solani.

# MATERIALS AND METHODS

# MATERIALS AND METHODS

## 1. Isolation of *Rhizoctonia solani*

An isolate of *R. solani* causing sheath blight of rice was obtained from naturally infected rice plants of the variety Jyothi, collected from the rice fields of the College of Agriculture, Vellayani. Specimens of the infected sheath showing characteristic symptoms of sheath blight were collected, cut into small bits, surface sterilized with 0.1 per cent Mercuric chloride solution for two minutes and washed with three changes of sterile distilled water. The bits were then planted on potato dextrose agar (PDA), poured in sterile petri dishes and incubated under laboratory conditions ($28 \pm 2^{o}C$). After about 48-72 h, the fungal growth of the infected tissue was transferred to sterile PDA slants. The isolate of *R. solani* thus obtained was purified by hyphal tip method and the organism was maintained on PDA by subculturing periodically.

The isolate was cultured on PDA in 9 cm sterile petri dishes under laboratory conditions. The morphological characters of the pathogen, such as hyphal thickness, number of sclerotia etc. were recorded.

The pathogenicity of the isolate was also proved by following Koch's postulates. Rice plants of the variety 'Jyothi' was raised in earthen pots of size 32 x 38 cm. The plants were inoculated by placing two sclerotia of the fungus from a two week old culture, in between sheaths. A bit of moist cotton wool was placed on top of the sclerotia to provide high percentage of relative humidity. The inoculated plants were maintained under high humidity conditions for 48-72 h by giving periodical water sprays and covering with polythene bags. The fungus was then reisolated from portions showing typical symptoms and maintained on PDA slants after purification by hyphal tip method. The pure culture of this fungus was used throughout the course of study.

II. Isolation of mycoflora from rhizosphere and phylloplane of rice plants

A. Rhizosphere.

Isolation of mycoflora was done from rhizosphere soil samples collected from four different localities in Kerala, viz., Vellayani, Kayamkulam, Moncompu and Wynad.

Isolation was done using the serial dilution plate technique (Johnson & Curl, 1972). Representative samples were collected from the rice growing localities of each area. A composits sample was then prepared for each locality and this was used for isolation purposes.

One gram of the soil was taken into a 250 ml Erlenmeyer Flask containing 100 ml of sterile water. The contents of the flask were shaken well in a shaker for 20 minutes. One ml of this was taken using a sterile pipette and transferred into test tubes containing 9 ml of sterile water in each, so that the final dilution became $10^{-3}$ and $10^{-4}$ respectively. One ml from each of these dilutions ($10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$) was transferred into sterile petri plates and rose bengal streptomycin agar was added. The representative colonies of soil fungi thus obtained from each dilution plate were maintained on PDA slants after purification by the hyphal tip method. Identification of the fungi thus obtained were tentatively done referring to relevant literature and confirmed by referring to the Common Wealth Agricultural Bureaux, International Mycological Institute, Kew, Surrey, England.

B. Phylloplane

The phylloplane mycoflora from rice plants of the above mentioned four localities were also isolated. Leaf samples were drawn randomly from ten different places from standing crop in each of the rice growing locality. A composite sample was then made for each locality.

A leaf washing and dilution technique (Waksman, 1922) was used to study the qualitative aspects of the mycoflora of the leaf surface. The leaf samples were collected using sterile scissors and brought to the laboratory in fresh polythene bags. Efforts were made to avoid contamination in the field as well as in the laboratory. A sample of 10 leaves ~~were~~ was transferred aseptically into 250 ml flasks containing 100 ml of sterile water and shaken for 20 minutes in a mechanical shaker to detach the propagules from the leaf surface. Samples of the mycoflora were obtained by plating 0.5 ml aliquots of leaf washings in 20 ml of rose bengal streptomycin agar medium in sterile petri dishes. The representative phylloplane mycoflora obtained were maintained on PDA, after hyphal tip purification for further studies. Identification of these fungi was done as mentioned earlier and got confirmed by the Common Wealth Agricultural Bureaux, International

Mycological Institute, Kew, Surrey, England.

III. Antagonism of rhizosphere and phylloplane mycoflora towards sheath blight pathogen.

The fungal cultures thus obtained from the rhizosphere and phylloplane were tested for their antagonism towards Rhizoctonia solani. Methods outlined by Purkayastha and Bhattacharya (1982) were followed for studying the antagonistic action against R. solani. Agar blocks of 3 mm diameter, containing 7 day old growth of mycelia of both R. solani and the test fungi were placed 3.5 cm apart on PDA in a 9 cm petri dish and incubated at 28 $\pm$ 2°C for 20 days. The paired cultures were examined at regular intervals throughout the incubation period and the nature of the reaction noted. Colony development was observed and assessment made of interactions between the organisms, when the growth pattern became stable. Interaction types were assigned into four categories following Purkayastha and Bhattacharya (1982).

A. Homogenous : Free intermingling of hyphae.

B. Overgrowth : R. solani overgrown by the test organism.

C. Cessation of growth at line of contact.

D. Aversion : development of a clear zone of inhibition.

IV. Mycoparasitism of the antagonists

In order to study the mechanism of mycoporasitism of rhizosphere and phylloplane mycoflora on R. solani the dual culture technique of Dennis and Webster (1971 c) was followed. Sterile PDA was poured out in 90 mm sterile petri dishes and it was allowed to solidify. Sterilized cellophane discs of 90 mm diameter were placed over this so as to lie flat on the medium using a pair of sterile forceps. An agar disc of 5 mm diameter of R. solani taken from an actively growing culture of the fungus was placed at one end of the petri dish and a 5 mm agar disc of the test fungus was placed 2 cm away from it. The plates were incubated at 28 $\pm$ 2°C for three to seven days. Direct observation was carried out after the incubation period under a light microscope at the zone of hyphal contact. Microscopic observation for hyphal interaction was also made by cutting one $cm^2$ portions of cellophane containing intermingling hyphal growth after mounting in glycerine.

V. Production of diffusible inhibitory substances by mycoparasites.

The effect of diffusible substances produced by the mycoparasites on the growth of R. solani was studied

using the method of Gibbs (1967) and Dennis and Webster (1971 a). Sterile petri dishes of 90 mm diameter were used for pouring PDA which after solidification was covered with 90 mm sterile cellophane discs. This was left overnight in order to allow the surplus moisture to evaporate. The following morning each plate was inoculated centrally with a 5 mm disc cut from a one week old culture of the selected mycoparasite. The plates were incubated at 28 $\pm$ 2°C for three to seven days so that full growth was obtained. Then the cellophane sheet and adhering fungus were removed. Immediately a 5 mm disc of R. solani inoculum cut from the margin of an actively growing colony was placed on the medium at the position previously occupied by the antagonist. After a further incubation period of 3 days the diameter of the colony of R. solani was measured. Controls were provided by plates on which R. solani was inoculated without prior growth of the antagonist. The inhibition percentage was calculated using the formula

$$\frac{C - T}{C} \times 100 = \text{Percentage inhibition over control}$$

C - Colony diameter of R. solani in control

T - Colony diameter of R. solani in treatment

Six replicates were maintained for each mycoparasite.

VI. Production of volatile inhibitory substances by mycoparasites.

The effect of volatile substances produced by mycoparasites on the growth of R. solani was studied using the technique of Dennis and Webster (1971 b).

Sterile petri plates of 90 mm diameter were used, in which PDA was poured. After solidification of the media these plates were centrally inoculated with agar discs cut from cultures of the concerned mycoparasite. These plates were then incubated at 28 $\pm$ 2°C for four to five days, during the time they attained full growth. After four to five days the lids of each plate were replaced by a bottom containing PDA inoculated with R. solani. The lids of the control plates which had not been inoculated with the selected mycoparasite were also replaced in a similar way. The colony diameter of R. solani in the treatments as well as in control was measured. Six replicates of each mycoparasite were maintained for taking observations. In this study also the growth inhibition of R. solani was expressed as a percentage following the formula mentioned above.

## VII. Efficacy of R. solani as a mycohost for the antagonistic fungi

The efficacy of utilisation of R. solani as a mycohost by various mycoparasites obtained from the rhizosphere and phylloplane was studied. Mycelial mat of R. solani was produced by inoculating PDA broth with agar discs of the fungus. The PDA broth was inoculated aseptically with agar discs containing inoculum of R. solani and incubated at 28 $\pm$ 2°C for seven to twelve days. The mycelial mat thus produced was then transferred into damp chambers. The damp chambers were made out of sterile petri dishes in which a layer of moist filter paper is placed so as to prevent the mat from drying up. The mycelial mats were then inoculated with 5 mm agar discs of the mycoparasite. They were then incubated at 28 $\pm$ 2°C for five to seven days. After incubation, when the growth of the mycoparasite was completed with profuse sporulation, 5 mm discs were cut out of the mycelial mats and spore suspensions were made out of sterile water blanks. The number of spores per microscopic field under high power of a light microscope were then counted and recorded. Twelve representative fields were counted and their average was found for each replicate. Six replicates were maintained for each mycoparasite.

## VIII. Comparative efficacy of various substrates for preparation of mycoparasite inocula.

A pot culture experiment was conducted at the College of Agriculture, Vellayani to study the efficacy of different mycoparasite inocula in reducing sheath blight. Here the mycoparasite *Trichoderma viride* was used. This was mass multiplied on different materials.

Layout

Design : Completely randomised design

Replication : Three

Variety : Jyothi

Treatments

$T_0$ - Control

$T_1$ - Rice bran

$T_2$ - Wheat bran

$T_3$ - Saw dust

$T_4$ - Coir waste

$T_5$ - Saw dust + Rice bran (3:1)

$T_6$ - Saw dust + Coir waste (3:1)

$T_7$ - Coir waste + Rice bran (3:1)

The experimental pots were uniformly filled with wet land soil collected from a field infested with *R. solani*. The organism *Trichoderma viride* was mass cultured in all the above mentioned materials and their combinations.

In each case a 1:2 mixture of the material with tap water was prepared, which was then autoclaved for 1 hour at a pressure of 1.2 kg/cm$^2$ for two successive days (Hadar *et al*. 1979). One litre Erlenmeyer flasks containing the media inoculated with *T. viride* were incubated at room temperature for 8-10 days.

The inoculum thus prepared was incorporated into soil at the rate of 100 g/pot. Fertilizers were also added before hand according to Package of Practices Recommendations (Kerala Agricultural University, 1982).

After a week, when the organism was well established eighteen day old seedlings were transplanted into the pots. A week later the plants were inoculated with *R. solani* both on the leaf sheath and base. The reaction of the rice plant to symptom development was noted by recording the intensity of sheath blight.

The intensity of the disease was assessed by scoring all the infected hills based on the Standard Evaluation System for Rice Disease (IRRI, 1976).

Description

Grade:

1. lesions limited to lower 25 per cent of leaf sheath
3. lesions present on lower 50 per cent of leaf sheath
5. lesions present on more than 50 per cent of leaf sheath
7. lesions present on more than 75 per cent of leaf sheath
9. lesions reaching top of tillers, severe infection on all leaves

Spore suspensions from each of the treatments were also prepared and the spore count was also recorded using high power of a light microscope. A total of 12 fields were counted for each treatment and their averages recorded.

IX. Efficacy of various mycoparasite-fungicide combinations in checking sheath blight disease of rice.

A. pot culture experiment

A pot culture experiment was laid out in the College of Agriculture, Vellayani in order to assess the effect of different mycoparasite-fungicidal combinations on the intensity of sheath blight disease of rice.

The details of the experiment are as follows:

Design : 4 x 3 factorial experiment in completely Randomised Design.

Variety : Jyothi

Replication : 3

Factor A : Antagonistic microorganisms

Factor B : Commonly recommended fungicides

Levels of Factor A

$A_0$ - Control

$A_1$ - *Trichoderma harzianum*. Rifai,

$A_2$ - *Trichoderma viride*. Pers. ex. Fr.

$A_3$ - *Aspergillus niger*. Van Tieghem.

Levels of Factor B

$B_0$ - Control

$B_1$ - Carbendazim (500 g /ha)

$B_2$ - Edifenphos (500 ml/ha)

Treatment combinations

$A_0B_0$

$A_0B_1$

$A_0B_2$

$A_1B_0$

$A_1B_1$

$A_1B_2$

$A_2B_0$

$A_2B_1$

$A_2B_2$

$A_3B_0$

$A_3B_1$

$A_3B_2$

Pots of standard size were filled with R. solani infested wet land soil. Recommended dose of fertilizers were added according to Package of Practices Recommendations (Kerala Agricultural University, 1982)

The population of the rhizosphere mycoflora already present in soil ~~were~~ was assessed. Soil samples were collected from each pot, serial dilutions were prepared and $10^{-4}$ dilutions were plated for assessment of the rhizosphere mycoflora. The medium used was rose bengal streptomycin agar. Thus the total count of the fungal colonies from each pot was assessed (Johnson & Curl, 1972).

The mycoparasites were mass cultured in Rice bran following the techniques of Hadar et al. (1979) as stated earlier. The rice bran was incorporated as per schedule of treatments at the rate of 100 g/pot. One week later, when the mycoparasites were well established, the soil was thoroughly puddled and 18 days old seedlings of the rice variety Jyothi was transplanted at the rate of three seedlings/clump. The rice plants were then inoculated with the sheath blight pathogen after a week. The phylloplane mycoflora before the spraying of chemicals were assessed 12 days after transplanting. This was done

following the method of Waksman (1922) as described earlier. The fungal counts were expressed as colony forming units/ $cm^2$ of leaf area by working out the average leaf area using an area meter. (Li-3000, LI-COR Ltd., Lincoln, USA). Spraying of chemicals were done as per schedule of treatments, 28 days after transplanting. The phylloplane population of mycoflora after spraying was assessed at 32 days after transplanting.

The assessment of disease intensity was made following the procedure given in the Standard Evaluation System for Rice (IRRI, 1976), following the 0-9 scale as stated elsewhere. Assessment of sheath blight was made at 35 days after transplanting and also at 49 days after transplanting. The percentage disease incidence was also recorded at this time.

B. Field experiment

A field experiment was laid out at the College of Agriculture, Vellayani, during the first crop season of 1989 to study the effect of different Mycoparasite-fungicidal combinations on the intensity and incidence of sheath blight disease.

The details of the field experiment was as follows.

Design : 4 x3 factorial experiment laid out in Randomised Block Design.

Variety : Jyothi

Season : Punja 1989

Replications : Three

Plot size : 1 x 1.5 m

Spacing : 15 x 10 cm

Location : College of Agriculture, Vellayani

Factor A : Antagonistic microorganisms.

Factor B : Commonly recommended fungicides

Levels of Factor A

$A_0$ - Control

$A_1$ - Trichoderma harzianum. Rifai.

$A_2$ - Trichoderma viride Pers. ex Fr.

$A_3$ - Aspergillus niger - Van Tieghem.

Levels of Factor B

$B_0$ - Control

$B_1$ - Carbendazim ( 500 g/ha)

$B_2$ - Edifenphos ( 500 ml/ha)

Treatment combinations

$A_0B_0$
$A_0B_1$
$A_0B_2$
$A_1B_0$
$A_1B_1$
$A_1B_2$
$A_2B_0$
$A_2B_1$
$A_2B_2$
$A_3B_0$
$A_3B_1$
$A_3B_2$

Nursery

Seedlings of the rice variety Jyothi were raised in a wet land nursery in an area of 20 $m^2$. Eighteen day old seedlings were used for the experiment. A bulk crop was also raised around the main field to facilitate the uniform spread of sheath blight disease.

Main field

The crop was raised following the Package of Practices Recommendation (Kerala Agricultural University, 1982). After the thorough preparation and lay out of the main field the antagonistic organisms, mass multiplied in rice bran as described earlier, were incorporated at the rate of 250 g into the experimental plots(1.5 sq.m.) This was done as per schedule of treatments. A week

later eighteen day old seedlings were transplanted into the main field. The commonly recommended fungicides were then sprayed as per schedule of treatments at 27 days after transplanting.

Observations recorded

Observations were made regarding the population of rhizosphere mycoflora before and after the treatments. The observation after treatments were done at 35 days after transplanting. The Rhizosphere mycofloral count was estimated as stated earlier. Similarly the phylloplane mycofloral population was also estimated both before and after the treatments as stated elsewhere. Estimation before treatments were done 12 days after transplanting and after treatments at 32 days after transplanting respectively.

Observations on sheath blight intensity and incidence were recorded at 42 days after transplanting. Scoring for disease intensity was done for all the hills in a plot. After harvest the grain and straw yield of the crop were also recorded plot wise.

# RESULTS

# RESULTS

## I (i) Isolation of the pathogen

The fungus Rhizoctonia solani Kuhn for the study was isolated from the sheaths of naturally infected rice plants collected from the rice fields attached to the Instructional Farm, College of Agriculture, Vellayani and it was made into pure culture. The pathogenicity of the isolate was tested and confirmed following Koch's postulates.

## (ii) Morphology of the pathogen

Morphological characters of Rhizoctonia solani isolated was studied. The characters are enumerated in Table 1.

Table 1. Morphological characters of Rhizoctonia solani isolated from rice used for further studies.

| Sl. No. | Characters | Range | Average |
|---|---|---|---|
| 1. | Number of Sclerotia formed per plate of 9 cm diameter. | 100 to 150 | 115 |
| 2. | Hyphal thickness | 8.5 to 10.5 /um | 9.5 /um |
| 3. | Size of macrosclerotia | | |
| | length | 920 to 2685 /um | 1802.5 /um |
| | Breadth | 703 to 2528 /um | 1615.5 /um |

## II. Isolation of mycoflora from the rhizosphere and phylloplane of rice plants

The fungi isolated from the rhizosphere and phylloplane of rice plants were identified and they are listed out in Table 2.

## III. Antagonism of rhizosphere and phylloplane mycoflora towards the sheath blight pathogen

When the different rhizosphere and phylloplane fungi were paired with *Rhizoctonia solani* varied reactions were observed (Table 3).

A clear zone of inhibition between the paired cultures was observed in the case of *Chaetomium globosum*, *Aspergillus fumigatus*, *A. melleus*, and *A. ochraceaus* (Plate 1). Overgrowth by antagonists were observed in the case of fungi like *A. japonicus*, *A. niger* (Plate II A), *Trichoderma viride*, *T. harzianum* and *Talaromyces stipitatus* (Plate II B).

Some fungi were found to cause cessation of growth at the point of contact, e.g., *Aspergillus terreus*, *A. tamarii*, *A. niveus*, *Penicillium citrinum*, *A. flavus* (Plate III A & B). Few other fungi were

Table 2. Fungi isolated from the rhizosphere and phylloplane of rice plants.

| Sl. No. | Herb.IMI* No. | Fungi |
|---|---|---|
| 1 | 333279 | Aspergillus carneus (v.Tiegh)Blotchw |
| 2 | 333276 | A. flavus Link ex Fr |
| 3 | 333270 | A. fumigatus Fres |
| 4 | 333278 | A. japonicus Saito |
| 5 | 333292 | A. melleus Yukawa |
| 6 | - | A. niger V. Tiegh |
| 7 | 333290 | A. nidulans (Eidam) Wingte |
| 8 | - | A. niveus Blotchw |
| 9 | 333293 | A. ochraceus Withelm |
| 10 | 333281 | A. sydowii (Bainier & Sart) Thom & Church |
| 11 | 333283 | A. tamarii Kita |
| 12 | - | A. terreus Thom |
| 13 | - | Chaetomium globosum Kunze & Schm |
| 14 | - | Cochliobolus miyabeanus (Ito & Kuribayashi) Drechsler ex Dastur |
| 15 | 333284 | Curvularia lunata (Wakker)Boedijn |
| 16 | - | Mucor hiemalis Wehmeyer |
| 17 | 333272 | Penicillium citrinum Thom |
| 18 | 333274 | P. corylophilum Dierckx |
| 19 | 333277 | P. minioluteum Dierckx |
| 20 | - | P. oxalicum Currie & Thom |
| 21 | - | P. pinophilum Hedgcock |
| 22 | 333275 | P. purpurogenum Stoll |
| 23 | 333282 | P. spinulosum Thom |
| 24 | 333288 | Talaromyces stipitatus (Thom) Benjamin |
| 25 | 333280 | T. flavus (Klocker)Stolk & Samson |
| 26 | 333271 | Trichoderma harzianum Rifai |
| 27 | | T. viride Pers ex Fr |

* Accession Numbers of cultures deposited at the C.A.B. International Mycological Institute, U.K.

PLATE I

Key

0 - Rhizoctonia solani

1 - Aspergillus ochraceus

2 - Interaction

0 - Rhizoctonia solani

1 - Aspergillus mellens

2 - Interaction

PLATE I

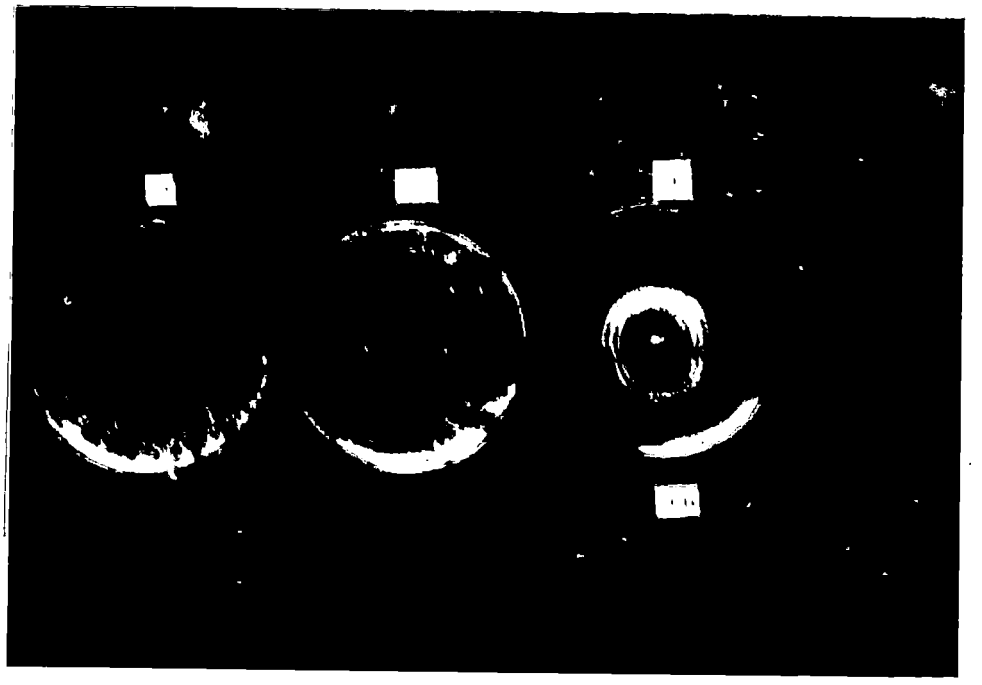


PLATE II A

Key

0 - *Rhizoctonia solani*

1 - *Aspergillus niger*

2 - Interaction

0 - *Rhizoctonia solani*

1 - *Aspergillus japonicus*

2 - Interaction

PLATE II A

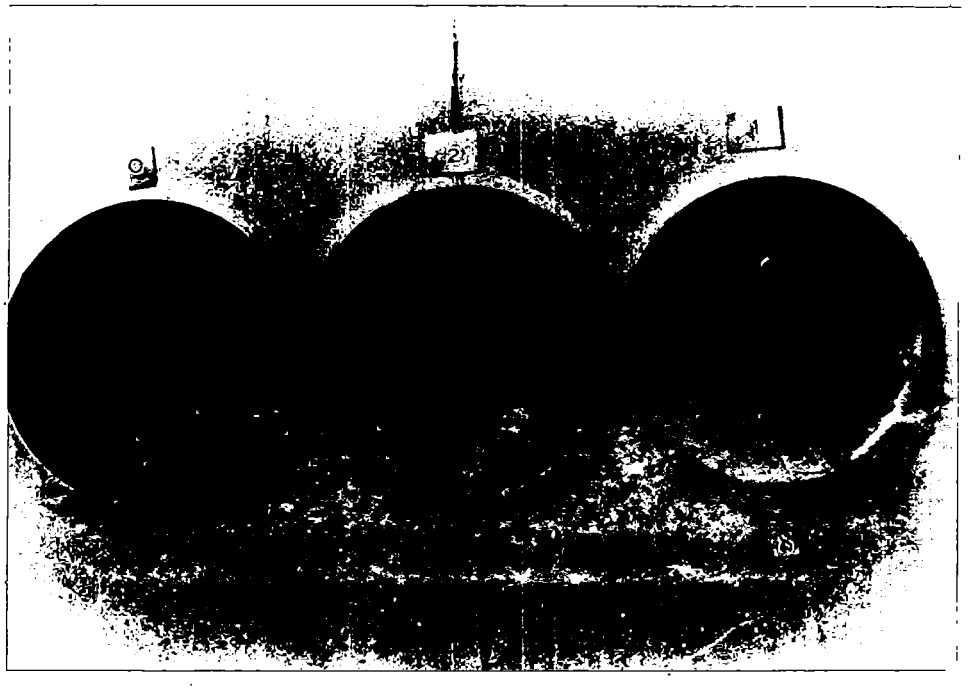


PLATE II B

Key

0 - *Rhizoctonia solani*

1 - *Trichoderma viride*

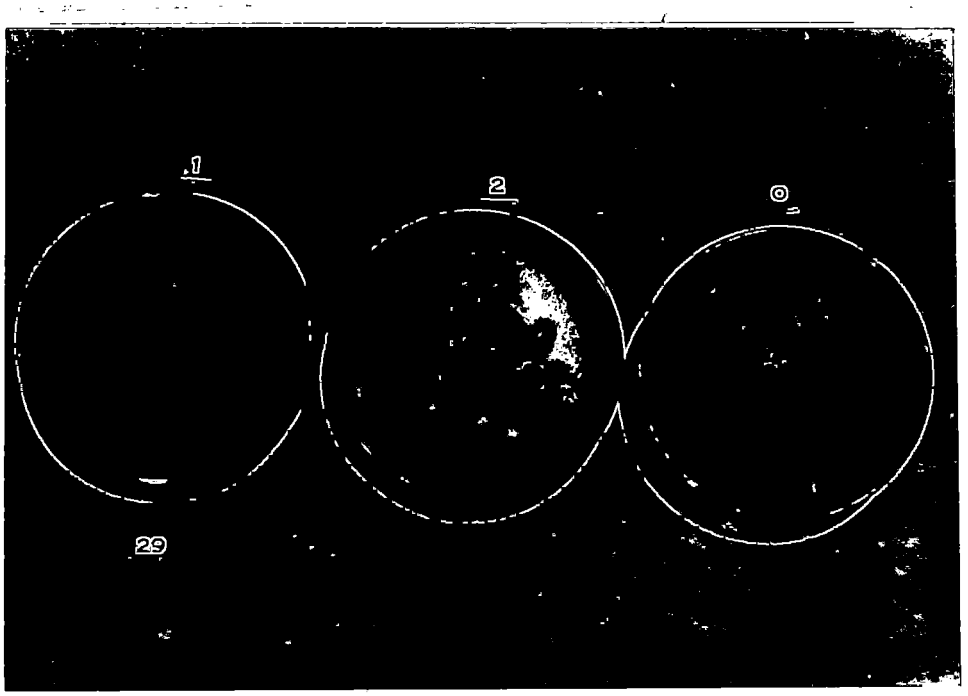
2 - Interaction

0 - *Rhizoctonia solani*

1 - *Trichoderma harzianum*

2 - Interaction

PLATE II B



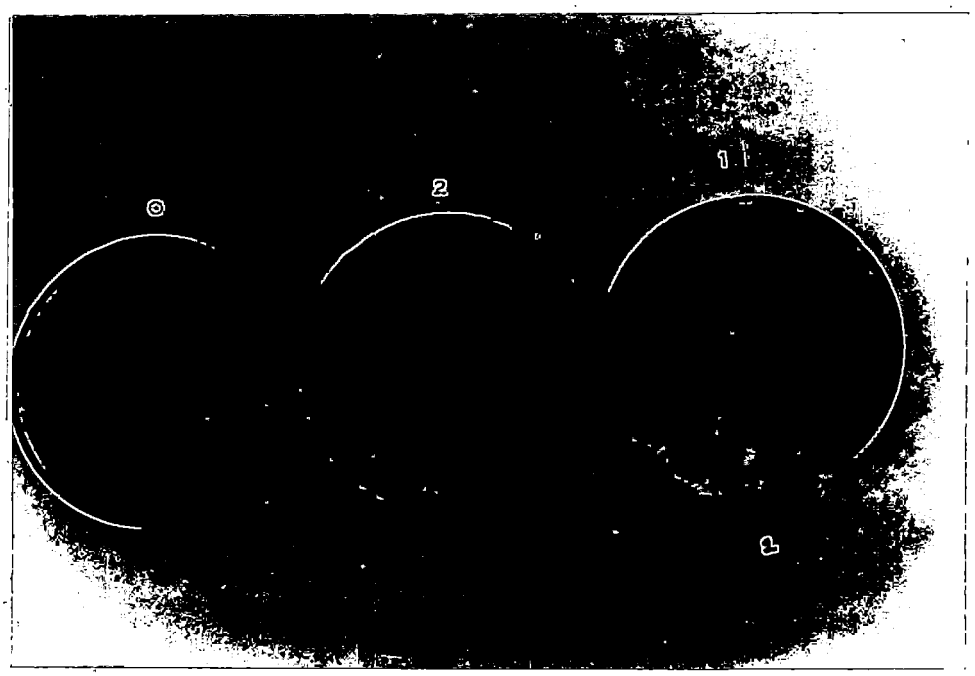



PLATE II B

0 - *Rhizoctonia solani*

1 - *Talaromyces stipitatus*

2 - Interaction

PLATE II B

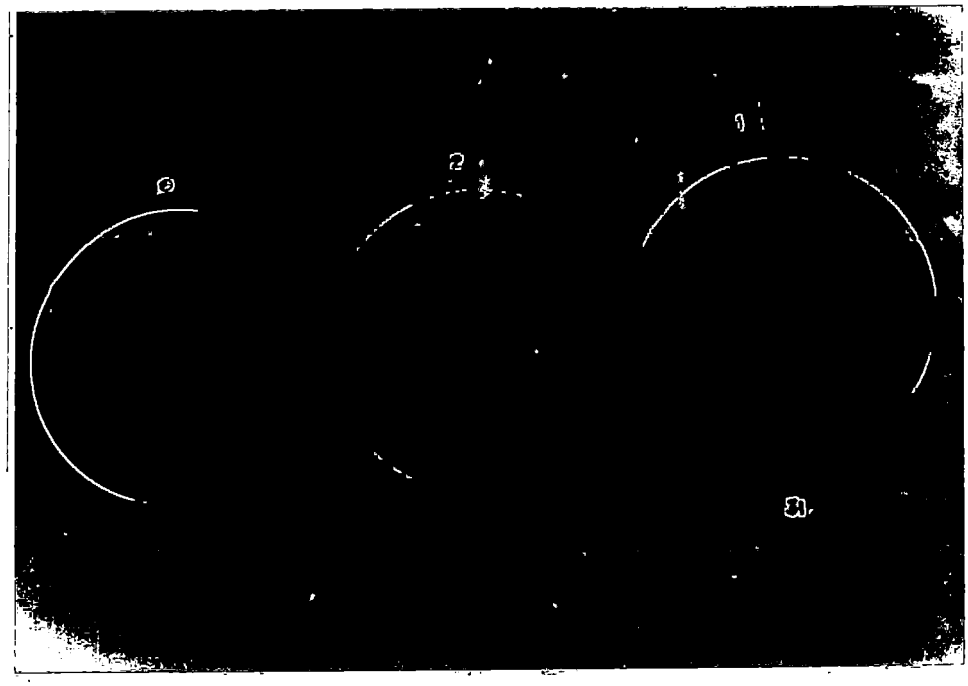


PLATE III A

Key:

0 - *Rhizoctonia solani*

1 - *Aspergillus tamarii*

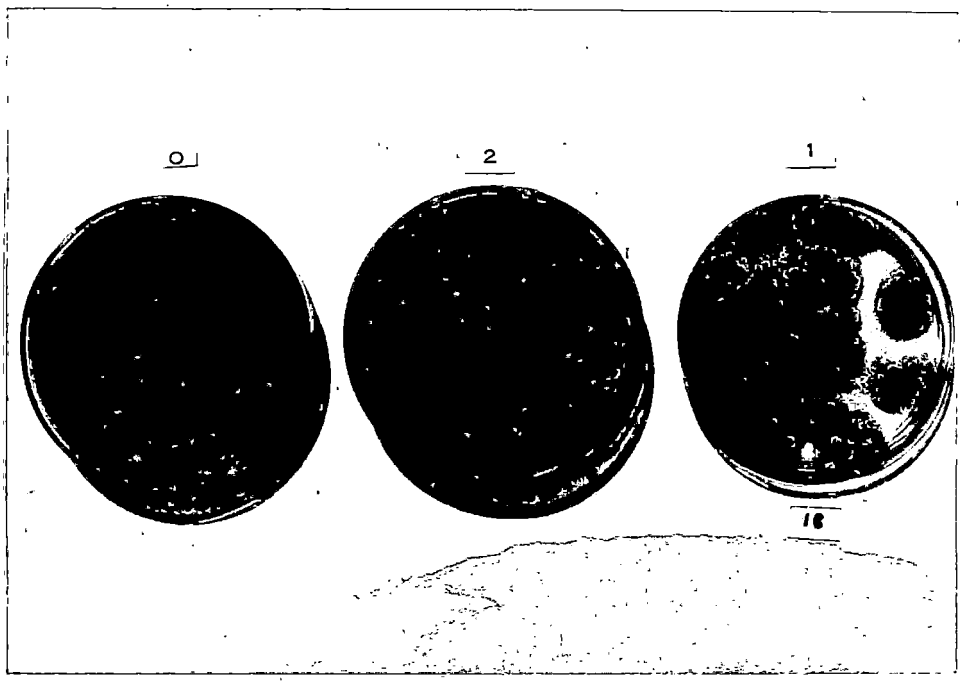
2 - Interaction

0 - *Rhizoctonia solani*

1 - *Aspergillus terreus*

2 - Interaction

PLATE III A



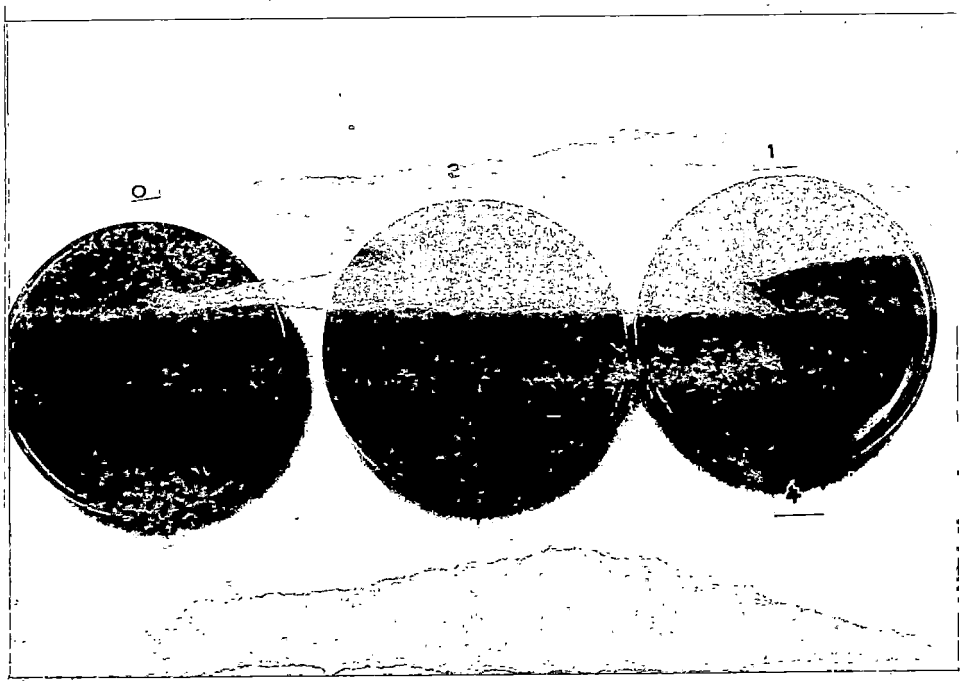



PLATE III B

Key

0 - Rhizotonia solani

1 - Pencillium citrinum

2 - Interaction

0 - Rhizoctonia solani

1 - Aspergillus nivens

2 - Interaction

PLATE III B


PLATE III B

Key:

0 - *Rhizoctonia solani*

1 - *Aspergillus flavus*

2 - Interaction

PLATE III B

Table 3. Antagonistic reaction of rhizosphere and phylloplane fungi isolated from rice, against the sheath blight pathogen R. solani.

| Sl. No. | Test fungus | Type of reaction |
|---|---|---|
| 1. | Aspergillus carneus | A |
| 2. | A. flavus | C |
| 3. | A. fumigatus | D |
| 4. | A. japonicus | B |
| 5. | A. melleus | D |
| 6. | A. niger | B |
| 7. | A. nidulans | A |
| 8. | A. niveus | C |
| 9. | A. ochraceus | D |
| 10. | A. sydowii | A |
| 11. | A. tamarii | C |
| 12. | A. terreus | C |
| 13. | Chaetomium globosum | D |
| 14. | Cochliobolus miyabeanus | C |
| 15. | Curvularia lunata | A |
| 16. | Mucor hiemalis | C |
| 17. | Penicillium citrinum | C |
| 18. | P. corylophilum | A |
| 19. | P. minioluteum | A |
| 20. | P. oxalicum | C |
| 21. | P. pinophilum | C |
| 22. | P. purpurogenum | B |
| 23. | P. spinulosum | A |
| 24. | Talaromyces stipitatus | B |
| 25. | T. flavus | C |
| 26. | Trichoderma harzianum | B |
| 27. | T. viride | B |

A - Homogenous: Free intermingling between pairing organism.
B - Overgrowth: R. solani overgrown by the test organism.
C - Cessation of growth: at the line of contact of cultures.
D - Aversion: A clear zone of inhibition between the two organisms.

found to intermingle freely with R. solani. They are Penicillium corylophilum, P. minioluteum, Curvularia lunata etc. (Table 3).

IV. Mycoparasitism of the rhizosphere and phylloplane fungi on R. solani.

The mycoparasites were tested for studying their effects on the hyphae of R. solani. Those fungi showing antagonistic action have also in most caused granulation, vacuolation, and finally disintegration of host hyphae. The results are presented in Table 4.

V. Effect of diffusible metabolites produced by rhizosphere and phylloplane fungi antagonistic on the growth of R. solani.

The effect of diffusible metabolites produced by a few of the antagonistic fungi, on the radial growth of R. solani was studied. It can be seen from the data presented in Table 5 that considerable inhibition of growth was obtained in the case of Trichoderma viride followed by Aspergillus melleus, Penicillium oxalicum and T. harzianum.

Table 4. Mycoparasitic reactions of rhizosphere and phylloplane fungi on R. solani.

| Sl. No. | Test fungus | Type of reaction |
|---|---|---|
| 1 | Aspergillus carneus | - |
| 2 | A. flavus | + |
| 3 | A. fumigatus | - |
| 4 | A. japonicus | - |
| 5 | A. melleus | - |
| 6 | A. niger | + |
| 7 | A. nidulans | - |
| 8 | A. niveus | - |
| 9 | A. ochraceus | - |
| 10 | A. sydowii | - |
| 11 | A. tamarii | + |
| 12 | A. terreus | + |
| 13 | Chaetomium globosum | + |
| 14 | Cochliobolus miyabeanus | - |
| 15 | Curvularia lunata | - |
| 16 | Mucor hiemalis | + |
| 17 | Penicillium citrinum | + |
| 18 | P. corylophilum | - |
| 19 | P. minioluteum | - |
| 20 | P. oxalicum | + |
| 21 | P. pinophilum | + |
| 22 | P. purpurogenum | - |
| 23 | P. spinulosum | - |
| 24 | Talaromyces stipitatus | - |
| 25 | T. flavus | + |
| 26 | Trichoderma harzianum | + |
| 27 | T. viride | + |

Note:
Positive (+): Granulation, vacuolation and disintegration of hyphae
Negative (-) : No reaction.

Table 5. Inhibition of growth of R. solani by diffusible metabolites produced by antagonistic fungi.

| Sl. No. | Test fungus | Percentage inhibition over control |
|---|---|---|
| 1 | Aspergillus carneus | 0 |
| 2 | A. flavus | 12.10 |
| 3 | A. fumigatus | 22 |
| 4 | A. japonicus | 43.50 |
| 5 | A. melleus | 94.40 |
| 6 | A. niger | 26.80 |
| 7 | A. nidulans | 0 |
| 8 | A. niveus | 16.60 |
| 9 | A. ochraceus | 65.70 |
| 10 | A. sydowii | 0 |
| 11 | A. tamarii | 80.30 |
| 12 | A. terreus | 26 |
| 13 | Chaetomium globosum | 12.04 |
| 14 | Cochliobolus miyabeanus | 34.33 |
| 15 | Curvularia lunata | 0 |
| 16 | Mucor hiemalis | 43.55 |
| 17 | Penicillium citrinum | 12.08 |
| 18 | P. corylophilum | 0 |
| 19 | P. minioluteum | 0 |
| 20 | P. oxalicum | 91.60 |
| 21 | P. pinophilum | 77.70 |
| 22 | P. purpurogenum | 15.50 |
| 23 | P. spinulosum | 20.40 |
| 24 | Talaromyces stipitatus | 73.30 |
| 25 | T. flavus | 40.70 |
| 26 | Trichoderma harzianum | 81.55 |
| 27 | T. viride | 94.44 |

VI. Effect of volatile metabolites produced by mycoparasites on the growth of R. solani.

The effect of volatile metabolites produced by the mycoparasites on the radial growth of R. solani has been studied and the results presented in Table 6. It is seen that the inhibition of R. solani is highest in the case of metabolites secreted by Trichoderma viride.

VII. Efficacy of R. solani as mycohost of antagonistic fungi

The efficiency of utilisation of mycelial mass of R. solani as a growth substrate, was studied. The ability of the mycoparasite to grow and sporulate on the mycelial substrate of R. solani was studied and rate of growth was measured by assessing the spore load from spore suspensions of the growth substrate. The results of the study are presented in Table 7. It can be seen that Aspergillus tamarii show the highest spore load, whereas Cochliobolus miyabeanus the lowest.

VIII. a. Comparative efficacy of various substrates for preparation of mycoparasite inocula

A pot culture experiment was conducted using the rice variety Jyothi in order to screen different materials

Table 6. Effect of volatile metabolites produced by mycoparasites on the inhibition of *R. solani*.

| Sl. No. | Test fungus | Percentage inhibition over control |
|---|---|---|
| 1 | *Aspergillus carneus* | 10.80 |
| 2 | *A. flavus* | 17.30 |
| 3 | *A. fumigatus* | 8.50 |
| 4 | *A. japonicus* | 10.80 |
| 5 | *A. melleus* | 15.30 |
| 6 | *A. niger* | 28.40 |
| 7 | *A. nidulans* | 0 |
| 8 | *A. niveus* | 18.80 |
| 9 | *A. ochraceus* | 9.50 |
| 10 | *A. sydowii* | 0 |
| 11 | *A. tamarii* | 13.50 |
| 12 | *A. terreus* | 4.30 |
| 13 | *Chaetomium globosum* | 14.90 |
| 14 | *Cochliobolus miyabeanus* | 13 |
| 15 | *Curvularia lunata* | 0 |
| 16 | *Mucor hiemalis* | 10.50 |
| 17 | *Penicillium citrinum* | 11.30 |
| 18 | *P. corylophilum* | 16.10 |
| 19 | *P. minioluteum* | 0 |
| 20 | *P. oxalicum* | 14.30 |
| 21 | *P. pinophilum* | 10.60 |
| 22 | *P. purpurogenum* | 11 |
| 23 | *P. spinulosum* | 12.50 |
| 24 | *Talaromyces stipitatus* | 0 |
| 25 | *T. flavus* | 12.20 |
| 26 | *Trichoderma harzianum* | 23.40 |
| 27 | *T. viride* | 63.70 |

Table 7. Sporulation of rhizosphere and phylloplane fungi on mycelia of *R. solani*.

| Sl. No. | Test fungus | Average number of spores/field (High power) |
|---|---|---|
| 1 | *Aspergillus carneus* | 35.33 |
| 2 | *A. flavus* | 54.83 |
| 3 | *A. fumigatus* | 107.33 |
| 4 | *A. japonicus* | 98.76 |
| 5 | *A. melleus* | 114.76 |
| 6 | *A. niger* | 124 |
| 7 | *A. nidulans* | 44.70 |
| 8 | *A. niveus* | 66.66 |
| 9 | *A. ochraceus* | 85.35 |
| 10 | *A. sydowii* | 43.90 |
| 11 | *A. tamarii* | 139.83 |
| 12 | *A. terreus* | 60.00 |
| 13 | *Chaetomium globosum* | 53 |
| 14 | *Cochliobolus miyabeanus* | 35.66 |
| 15 | *Curvularia lunata* | 0 |
| 16 | *Mucor hiemalis* | 0 |
| 17 | *Penicillium citrinum* | 75.83 |
| 18 | *P. corylophilum* | 20.33 |
| 19 | *P. minioluteum* | 21.60 |
| 20 | *P. oxalicum* | 77.33 |
| 21 | *P. pinophilum* | 102.33 |
| 22 | *P. purpurogenum* | 73.50 |
| 23 | *P. spinulosum* | 33.80 |
| 24 | *Talaromyces stipitatus* | 44.66 |
| 25 | *T. flavus* | 85.66 |
| 26 | *Trichoderma harzianum* | 135.35 |
| 27 | *T. viride* | 117.16 |

suitable as substrates for mass multiplication of mycoparasites and also to assess their comparative efficacy to reduce sheath blight incidence.

The mean data of the comparative efficacy of various substrates in reducing sheath blight are given in Table 8.

The data revealed the superiority of wheat bran culture over all other treatments in reducing the intensity of sheath blight at 31 days after planting. But is effect was found to be on par with rice bran treatments at 31 days after planting. Treatments with rice bran cultures recorded the lowest disease intensity at 52 days after planting and this treatment was found to be significantly superior to all other treatments tried. At 52 days after planting wheat bran treatments proved only second to rice bran. At both 31 days after planting and 52 days after planting the control plots recorded the highest disease intensity.

Among the treatment combinations, sawdust + rice bran in 3:1 ratio, recorded the lowest disease intensity and this was found to be statistically on par with rice bran and wheat bran treatments at 31 days after planting. At 52 days after planting also the lowest

Table 8. Efficacy of different substrates for mass multiplication of *T. viride* (assessed by the intensity of sheath blight incidence)

| Sl. No. | Treatments | Disease Intensity | | | |
|---|---|---|---|---|---|
| | | 31 DAP* | Rank | 52 DAP* | Rank |
| 1. | Rice bran | 1.23(1.11)** | II | 1.38(1.17) | I |
| 2. | Wheat bran | 1.22(1.10) | I | 2.30(1.52) | II |
| 3. | Saw dust | 3.10(1.76) | VI | 3.64(1.90) | VI |
| 4. | Coir waste | 3.66(1.91) | VII | 4.39(2.09) | VII |
| 5. | Saw dust + Rice bran | 1.53(1.23) | III | 3.08(1.75) | III |
| 6. | Saw dust +Coir waste | 1.96(1.40) | V | 3.33(1.82) | IV |
| 7. | Coir waste +Rice bran | 1.56(1.25) | IV | 3.46(1.86) | V |
| 8. | Control | 4.72(2.17) | VIII | 5.62(2.37) | VIII |
| | CD(5%) | 0.399 | | 0.317 | |

* DAP - Days after planting

** Values in parentheses after $\sqrt{x}$ - transformation.

Table 9. Growth and sporulation of *I. viride* on various substrates.

| Sl. No. | Treatments | Mean spore count per field (high power) | Mycelial growth* |
|---|---|---|---|
| 1. | Rice bran | 52.58 | ++ |
| 2. | Wheat bran | 19.5 | +++ |
| 3. | Saw dust | 14.5 | + |
| 4 | Coir waste | 13.5 | + |
| 5 | Saw dust + Rice bran | 22.58 | ++ |
| 6 | Saw dust +Coir waste | 12.6 | + |
| 7 | Coir waste + Rice bran | 25.08 | ++ |

* + poor growth
++ medium growth
+++ high growth

disease intensity was exhibited, among treatment combinations in case of sawdust + rice bran.

## IX. Efficacy of various mycoparasite fungicide combinations in checking sheath blight disease of rice

### A. Pot culture experiment

#### A.1 Disease intensity

The mean data of the effect of different mycoparasite fungicidal combinations on the intensity of sheath blight disease is presented in Table 10.

The results revealed that both at 53 days after planting and 67 days after planting application of antagonists *Trichoderma viride* and *T. harzianum* recorded least disease intensity (Plate IV A, B, C).

Among the commonly recommended fungicides edifenphos proved to be the most effective, both at 53 and 67 days after planting, by recording the lowest disease intensity during these periods. Application of carbendazim proved to be the second best treatment, but it was found to be on par with control, both at 53 and 67 days after planting (Plate IV D, E).

Table 10. Comparative efficacy of various mycoparasite-fungicide combinations on the intensity of sheath blight (pot culture experiment)

| Treatments | Disease Intensity* | |
|---|---|---|
| | 53 DAP** | 67 DAP |
| Antagonists | | |
| $A_0$ - control | 1.80 | 1.97 |
| $A_1$ - *T. harzianum* | 1.77 | 2.07 |
| $A_2$ - *T. viride* | 1.33 | 1.58 |
| $A_3$ - *A. niger* | 1.87 | 2.14 |
| CD(5%) | 0.321 | 0.404 |
| Fungicides | | |
| $B_0$ - control | 1.88 | 2.17 |
| $B_1$ - carbendazim | 1.76 | 2.01 |
| $B_2$ - edifenphos | 1.44 | 1.65 |
| CD(5%) | 0.278 | 0.349 |
| Combinations | | |
| $A_0B_0$ | 2.35 | 2.69 |
| $A_0B_1$ | 1.65 | 1.76 |
| $A_0B_2$ | 1.40 | 1.45 |
| $A_1B_0$ | 1.92 | 2.21 |
| $A_1B_1$ | 1.76 | 2.00 |
| $A_1B_2$ | 1.63 | 2.02 |
| $A_2B_0$ | 1.28 | 1.43 |
| $A_2B_1$ | 1.53 | 1.92 |
| $A_2B_2$ | 1.18 | 1.38 |
| $A_3B_0$ | 1.96 | 2.34 |
| $A_3B_1$ | 2.10 | 2.35 |
| $A_3B_2$ | 1.56 | 1.73 |
| CD(5%) | - | - |

* Values after $\sqrt{X + 1}$ transformation.

** DAP - Days after planting

PLATE IV A

MoFO - Control

PLATE IV C

$M_1F_o$ - *Trichoderma harzianum* + control

PLATE IV A

MoFO - Control

PLATE IV A

PLATE IV B

$M_2Fo$ - *Trichoderma viride* + control

PLATE IV B

PLATE IV C

PLATE IV D

$M_oF_2$ - Control + Edifenphos

PLATE IV D

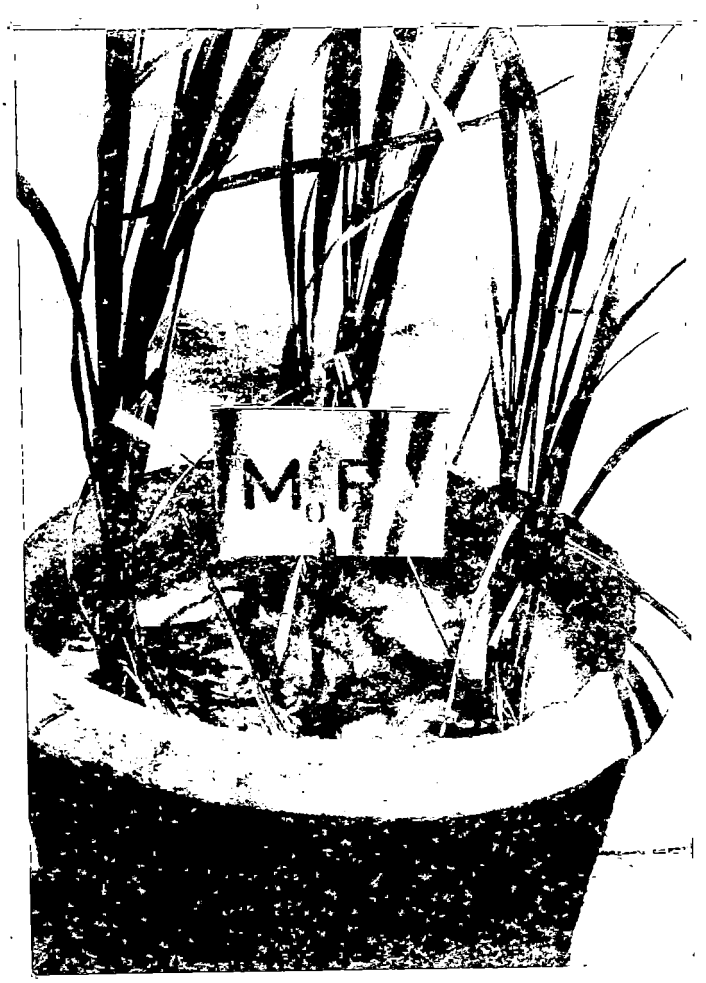
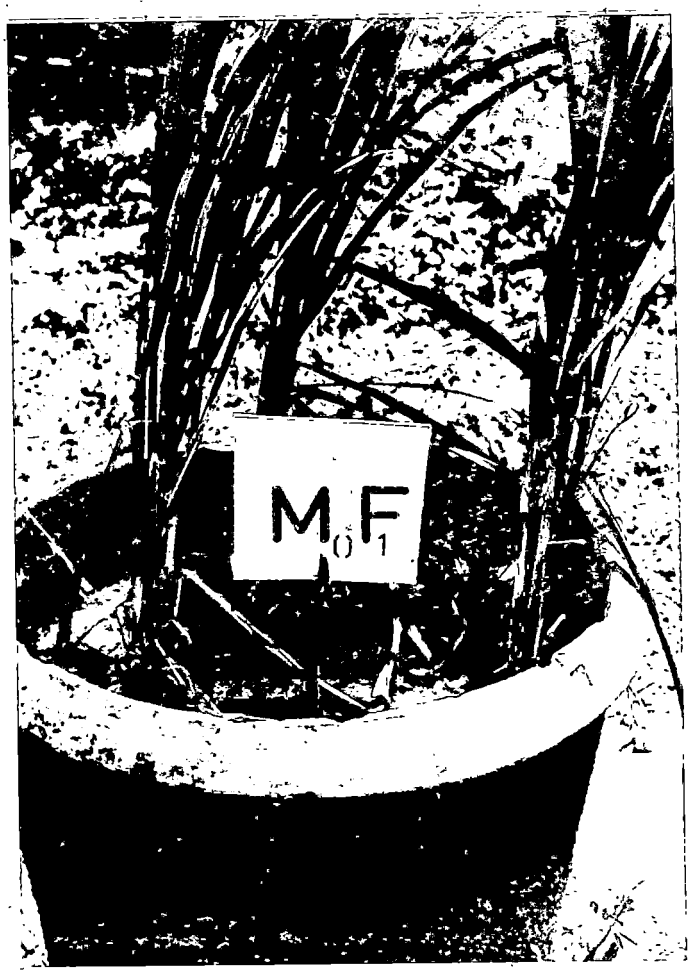


PLATE IV E

$M_oF_1$ - Control + carbendazim

PLATE IV E



Among the mycoparasite-fungicide combinations the lowest disease intensity was recorded by Trichoderma viride + edifenphos and the highest by control even though the results were not statistically significant.

A.2 Disease incidence

The mean data of the effect of application of different mycoparasite-fungicide combinations on percentage disease incidence ~~is~~ are presented in Table 11.

Among the mycoparasites tried Trichoderma viride proved to be the best by recording the lowest incidence of sheath blight. This was followed by the mycoparasite T. harzianum. Even though application of Aspergillus niger resulted in a lower disease incidence than control, it was found to be statistically on par with the same.

Among the fungicides tried application of edifenphos proved to be superior, but it was on par with application of carbendazim. Both these treatments recorded significantly lower disease incidence values than control.

Table 11. Effect of different mycoparasite fungicidal combinations in the percentage disease incidence of sheath blight (pot culture experiment)

| Treatments | Percentage disease incidence* |
|---|---|
| Antagonists | |
| $A_0$ - control | 47.13 |
| $A_1$ - T. harzianum | 39.59 |
| $A_2$ - T. viride | 28.88 |
| $A_3$ - A. niger | 40.21 |
| CD(5%) | 8.580 |
| Fungicides | |
| $B_0$ - control | 54.34 |
| $B_1$ - carbendazim | 33.78 |
| $B_2$ - edifenphos | 28.74 |
| CD(5%) | 7.430 |
| Combinations | |
| $A_0B_0$ | 90.00 |
| $A_0B_1$ | 23.30 |
| $A_0B_2$ | 28.11 |
| $A_1B_0$ | 43.91 |
| $A_1B_1$ | 39.61 |
| $A_1B_2$ | 34.24 |
| $A_2B_0$ | 37.42 |
| $A_2B_1$ | 30.49 |
| $A_2B_2$ | 18.74 |
| $A_3B_0$ | 40.64 |
| $A_3B_1$ | 41.73 |
| $A_3B_2$ | 32.86 |
| CD(5%) | 14 |

* Values after angular transformation.

Among the mycoparasite-fungicide combinations, T. viride + edifenphos proved to be the best by recording the lowest percentage of disease incidence. But it was found to be on par with control + carbendazim, control + edifenphos, T. viride + carbendazim, and Aspergillus niger + edifenphos. All these treatment combinations recorded significantly lower values than control.

A.3 Effect on rhizosphere mycoflora of rice

The mean data of the effect of various mycoparasite fungicide combinations on the rhizosphere mycoflora in pot culture experiments is given in Table 12.

Application of mycoparasites enhanced the mycofloral population in rhizosphere of rice plants, compared with control. The highest increase was registered in the case of Trichoderma viride, which recorded the highest mean count of 6.459. This was followed by T. harzianum which was on par with Aspergillus niger.

Table 12. Effect of different mycoparasite-fungicide combinations on the rhizosphere mycoflora of rice (pot culture experiment)

| Treatments | Mean colony count of Rhizosphere mycoflora* |
|---|---|
| Antagonists | |
| $A_0$ - control | 4.57 |
| $A_1$ - *T. harzianum* | 5.74 |
| $A_2$ - *T. viride* | 6.45 |
| $A_3$ - *A. niger* | 5.62 |
| CD(5%) | 0.215 |
| Fungicides | |
| $B_0$ - control | 5.48 |
| $B_1$ - carbendazim | 5.45 |
| $B_2$ - edifenphos | 5.85 |
| CD(5%) | 0.196 |
| Combinations | |
| $A_0B_0$ | 4.73 |
| $A_0B_1$ | 4.69 |
| $A_0B_2$ | 4.77 |
| $A_1B_0$ | 5.66 |
| $A_1B_1$ | 5.75 |
| $A_1B_2$ | 6.29 |
| $A_2B_0$ | 6.62 |
| $A_2B_1$ | 6.33 |
| $A_2B_2$ | 6.92 |
| $A_3B_0$ | 5.58 |
| $A_3B_1$ | 5.66 |
| $A_3B_2$ | 6.07 |
| CD(5%) | - |

* Values after $\sqrt{X}$ transformation.

Among the fungicides tried edifenphos proved to be the treatment showing the highest mean colony count. Application of carbendazim proved to be second in rank, but it was on par with no fungicidal spray.

A.4 Effect on the phylloplane mycoflora of rice

The mean data of the effect of different mycoparasite fungicide combinations on the population of phylloplane mycoflora of rice is given in Table 13.

Application of mycoparasites suppressed the phylloplane mycofloral population. Control plants had the maximum phylloplane mycofloral population. The lowest phylloplane mycofloral population was registered in the case of application with Aspergillus niger.

Application of edifenphos increased the phylloplane mycofloral population, compared with control. But the effect was not statistically significant. Application of carbendazim on the other hand suppressed the phylloplane mycoflora.

Table 13. Effect of application of various mycoparasite-fungicide combinations on the phylloplane mycoflora of rice (pot culture experiment)

| Treatment | Phylloplane mycofloral count (cfu/$cm^2$)* |
|---|---|
| Antagonists | |
| $A_0$ - control | 3.60 |
| $A_1$ - *T. harzianum* | 3.30 |
| $A_2$ - *T. viride* | 3.15 |
| $A_3$ - *A. niger* | 2.60 |
| CD(5%) | 0.411 |
| Fungicides | |
| $B_0$ - control | 3.42 |
| $B_1$ - carbendazim | 2.43 |
| $B_2$ - edifenphos | 3.63 |
| CD(5%) | 0.356 |
| Combinations | |
| $A_0B_0$ | 5.87 |
| $A_0B_1$ | 4.87 |
| $A_0B_2$ | 6.23 |
| $A_1B_0$ | 5.40 |
| $A_1B_1$ | 5.03 |
| $A_1B_2$ | 5.39 |
| $A_2B_0$ | 5.24 |
| $A_2B_1$ | 5.52 |
| $A_2B_2$ | 5.71 |
| $A_3B_0$ | 5.13 |
| $A_3B_1$ | 3.65 |
| $A_3B_2$ | 5.26 |
| CD(5%) | N.S |

* Values after $\sqrt{X}$ transformation.

Among the mycoparasite-fungicide combinations, the highest phylloplane mycofloral population was recorded in the case of control + edifenphos and the lowest in the case of Aspergillus niger + carbendazim, even though the results were statistically insignificant.

B. Field experiment

B.1 Efficacy of different mycoparasite-fungicide combination in checking sheath blight disease in rice at 60 DAP.

The mean data on the efficacy of different mycoparasite-fungicide combinations in checking sheath blight disease are presented in Table 14.

Trichoderma viride was found to be the most effective mycoparasite in checking the disease intensity and incidence by recording the lowest values of 1.17 and 37.83 respectively. This is followed by T. harzianum for both the parameters, viz., disease intensity and incidence, but it was found to be on par with application of the mycoparasite Aspergillus niger and control.

Among the fungicides tried carbendazim was found to be superior in reducing disease intensity, whereas edifenphos was found to be most superior in checking the disease incidence.

Table 14. Efficacy of different mycoparasite-Fungicide combinations in checking sheath blight disease ( field trial)

| Treatment | Disease intensity* | Disease incidence** |
|---|---|---|
| Antagonists | | |
| $A_0$ - control | 1.84 | 48.93 |
| $A_1$ - *T. harzianum* | 1.74 | 46.18 |
| $A_2$ - *T. viride* | 1.17 | 37.83 |
| $A_3$ - *A. niger* | 1.90 | 49.00 |
| CD(5%) | 0.408 | 7.923 |
| Fungicides | | |
| $B_0$ - control | 1.97 | 58.18 |
| $B_1$ - carbendazim | 1.49 | 49.40 |
| $B_2$ - edifenphos | 1.52 | 39.89 |
| CD(5%) | 0.354 | 6.862 |
| Combinations | | |
| $A_0B_0$ | 2.46 | 66.60 |
| $A_0B_1$ | 1.77 | 45.37 |
| $A_0B_2$ | 1.30 | 34.82 |
| $A_1B_0$ | 1.39 | 41.43 |
| $A_1B_1$ | 1.97 | 51.12 |
| $A_1B_2$ | 1.87 | 46.00 |
| $A_2B_0$ | 1.79 | 46.89 |
| $A_2B_1$ | 0.84 | 37.98 |
| $A_2B_2$ | 0.87 | 28.63 |
| $A_3B_0$ | 2.25 | 57.79 |
| $A_3B_1$ | 1.39 | 39.13 |
| $A_3B_2$ | 2.05 | 50.09 |
| CD(5%) | 0.708 | 13.724 |

* Values after $\sqrt{X}$ transformation.

** Values after angular transformation.

In the case of treatment combinations application of Trichoderma viride + carbendazim was found to be the most effective in reducing disease intensity, but it was found to be on par with application of edifenphos, Trichoderma harzianum + control, Aspergillus niger + carbendazim, T. viride + edifenphos. In the case of disease incidence T. viride + edifenphos was found to be superior to all other treatment combinations tried, but was found to be statistically on par with Aspergillus niger + carbendazim, control + edifenphos, and T. harzianum + control.

B.2 Effect of different mycoparasite-fungicide combinations on the per plot grain yield

The mean data on the grain yield per plot are presented in Table 15.

Among the mycoparasites application of T. viride recorded the highest per plot grain yield of 507.77, followed by T. harzianum. Lowest per plot yield was recorded in case of application of Aspergillus niger which was on par with control.

Table 15. Effect of different mycoparasite- fungicide combinations on the plot yield of grains

| Treatment | | Grain yield per plot (gram) |
|---|---|---|
| Antagonists | | |
| $A_0$ | - control | 360.22 |
| $A_1$ | - *T. harzianum* | 466.66 |
| $A_2$ | - *T. viride* | 507.77 |
| $A_3$ | - *A. niger* | 374.44 |
| CD(5%) | | 81.403 |
| Fungicides | | |
| $B_0$ | - control | 363.25 |
| $B_1$ | - carbendazim | 447.16 |
| $B_2$ | - edifenphos | 471.41 |
| CD(5%) | | 70.497 |
| Combinations | | |
| $A_0B_0$ | | 295.00 |
| $A_0B_1$ | | 375.33 |
| $A_0B_2$ | | 408.33 |
| $A_1B_0$ | | 426.66 |
| $A_1B_1$ | | 529.00 |
| $A_1B_2$ | | 444.33 |
| $A_2B_0$ | | 393.00 |
| $A_2B_1$ | | 527.33 |
| $A_2B_2$ | | 603.00 |
| $A_3B_0$ | | 338.33 |
| $A_3B_1$ | | 355.00 |
| $A_3B_2$ | | 430.00 |
| CD(5%) | | - |

Among the fungicides, edifenphos application recorded the highest per plot yield of grains, followed by application of carbendazim. Control plot had the lowest grain yield.

Among the mycoparasite-fungicide combinations highest yield was recorded in case of application of T. viride + edifenphos and lowest in control, even though the treatments did not show any significant difference statistically.

B.3 Effect of different mycoparasite-fungicide combinations on the per plot straw yield

The mean data of the straw yield per plot is given in Table 16.

The effect of the mycoparasite Trichoderma viride in influencing the per plot yield of straw was highly significant, and the highest straw yield was recorded in this case. The second best yield was obtained in the case of T. harzianum, but was found to be statistically on par with Aspergillus niger and control. A. niger recorded a lower per plot yield when compared with control.

Table 16. Effect of different mycoparasite- fungicide combinations on the straw yield.

| Treatment | | Per plot straw yield (gram) |
|---|---|---|
| Antagonists | | |
| $A_0$ | - control | 1108.88 |
| $A_1$ | - *T. harzianum* | 1174.44 |
| $A_2$ | - *T. viride* | 1733.33 |
| $A_3$ | - *A. niger* | 1096.66 |
| CD(5%) | | 230.023 |
| Fungicides | | |
| $B_0$ | - control | 1068.33 |
| $B_1$ | - carbendazim | 1342.91 |
| $B_2$ | - edifenphos | 1423.75 |
| CD(5%) | | 199.206 |
| Combinations | | |
| $A_0B_0$ | | 970.00 |
| $A_0B_1$ | | 1161.66 |
| $A_0B_2$ | | 1195.00 |
| $A_1B_0$ | | 1163.33 |
| $A_1B_1$ | | 1210.00 |
| $A_1B_2$ | | 1150.00 |
| $A_2B_0$ | | 1166.66 |
| $A_2B_1$ | | 1966.66 |
| $A_2B_2$ | | 2066.66 |
| $A_3B_0$ | | 973.33 |
| $A_3B_1$ | | 1033.33 |
| $A_3B_2$ | | 1283.33 |
| CD(5%) | | 398.412 |

Among the fungicides, edifenphos gave the highest value for this parameter, followed by carbendazim. Both these treatments were found to be significantly superior to control.

In the case of mycoparasite-fungicide combinations T. viride + edifenphos recorded the highest per plot straw yield, but it was found to be on par with T. viride + carbendazim. Lowest per plot yield was registered in control plots.

B.4 Effect of different mycoparasite-fungicide combinations on the rhizosphere mycoflora of rice

The mean data of the effect of different mycoparasite-fungicide combinations on the rhizosphere mycoflora of rice are presented in Table 17.

The data reveal that application of Trichoderma viride has significant influence in enhancing the rhizosphere mycoflora of rice, by registering the highest value. Application of Aspergillus niger suppressed the rhizosphere mycofloral population by registering a lower value than control.

Table 17. Effect of different mycoparasite-Fungicide combinations on Rhizosphere mycoflora of rice

| Treatment | Mean colony count of rhizosphere mycoflora * | Percentage increase or decrease over control |
|---|---|---|
| Antagonists | | |
| $A_0$ - control | 5.00 | |
| $A_1$ - *T. harzianum* | 5.78 | +15.60 |
| $A_2$ - *T. viride* | 6.10 | +22.00 |
| $A_3$ - *A. niger* | 4.93 | - 1.40 |
| CD(5%) | 0.504 | |
| Fungicides | | |
| $B_0$ - control | 5.44 | |
| $B_1$ - carbendazim | 5.48 | + 0.73 |
| $B_2$ - edifenphos | 5.45 | + 0.18 |
| CD(5%) | N.S | |
| Combinations | | |
| $A_0B_0$ | 5.01 | |
| $A_0B_1$ | 5.30 | + 5.78 |
| $A_0B_2$ | 5.78 | +15.36 |
| $A_1B_0$ | 6.07 | +21.15 |
| $A_1B_1$ | 6.21 | +23.95 |
| $A_1B_2$ | 6.17 | +23.15 |
| $A_2B_0$ | 6.79 | +35.52 |
| $A_2B_1$ | 6.87 | +37.12 |
| $A_2B_2$ | 6.75 | +34.73 |
| $A_3B_0$ | 5.35 | + 6.78 |
| $A_3B_1$ | 4.99 | - 2.19 |
| $A_3B_2$ | 5.57 | +11.17 |
| CD(5%) | N.S | N.S |

* Values after $\sqrt{X}$ transformation.

In the case of fungicides, it was found that the mycofloral population in the rhizosphere did not have any significant change due to their application.

The highest population of rhizosphere mycoflora was recorded in the case of Trichoderma viride + carbendazim and the lowest in the case of Aspergillus niger + carbendazim among treatment combinations. But the results were found to be statistically insignificant.

B.5 Effect of different mycoparasite-fungicide combinations on the phylloplane mycoflora of rice

The mean data of phylloplane mycoflora count are presented in Table 18.

It is seen from the data that all the treatments have a suppressive effect on the phylloplane mycofloral count. Control exhibited the highest value, mycoparasitic treatments exhibited lower values, the lowest being in the case of Aspergillus niger.

Among the fungicides tried out edifenphos did not have any appreciable effect on the phylloplane mycoflora count, on the other hand carbendazim was found to suppress mycolfloral population on the leaf surface to a greater extent.

Table 18. Effect of mycoparasite-fungicide combinations on phylloplane of rice

| Treatment | Phylloplane mycoflora count* cfu/ $cm^2$ | Percentage increase or decrease over control |
|---|---|---|
| Antagonists | | |
| $A_0$ - control | 6.11 | |
| $A_1$ - *T. harzianum* | 5.75 | - 5.89 |
| $A_2$ - *T. viride* | 5.71 | - 6.54 |
| $A_3$ - *A. niger* | 5.58 | - 8.67 |
| CD(5%) | 0.224 | - |
| Fungicides | | |
| $B_0$ - control | 6.19 | |
| $B_1$ - carbendazim | 5.15 | -16.80 |
| $B_2$ - edifenphos | 6.02 | - 2.74 |
| CD(5%) | 0.194 | - |
| Combinations | | |
| $A_0B_0$ | 6.42 | |
| $A_0B_1$ | 5.26 | -18.06 |
| $A_0B_2$ | 6.39 | - 0.46 |
| $A_1B_0$ | 6.27 | - 2.33 |
| $A_1B_1$ | 4.75 | -26.01 |
| $A_1B_2$ | 5.94 | - 7.47 |
| $A_2B_0$ | 5.90 | - 8.09 |
| $A_2B_1$ | 5.24 | -18.38 |
| $A_2B_2$ | 5.73 | -10.74 |
| $A_3B_0$ | 5.82 | - 9.3 |
| $A_3B_1$ | 4.97 | -22.58 |
| $A_3B_2$ | 5.66 | -11.83 |
| CD(5%) | 0.388 | - |

* Values after $\sqrt{X}$ transformation.

*Trichoderma harzianum* + control and edifenphos were found to exert no appreciable change in the mycofloral count. *T. harzianum* + edifenphos and *A. niger* + carbendazim was found to have more pronounced suppressive effect on the mycoflora on the rice leaf surface, among the various treatment combinations.

# DISCUSSION

# DISCUSSION

A large number of diseases affecting rice plants are known to be caused by fungi. One of the most important fungal diseases noticed in Kerala in the recent times is sheath blight caused by the anaphase Rhizoctonia solani Kuhn of the teleophase Thanatephorus cucumeris (Frank) Donk. The disease is now mainly managed by the application of chemicals. For this purpose a number of chemicals have been recommended to the growers in Kerala in the "Package of Practices Recommendations" published by the Kerala Agricultural University (Kerala Agricultural University, 1982). Not much effort has been made to study the effect of plant protection chemicals on the rhizosphere and phylloplane mycoflora of rice. It is a known fact that there are many rhizosphere and phylloplane mycoflora having antagonistic action against the pathogen R. solani. Baker and Cook (1974) have stated that "as a result of introduction of plant protection chemicals into the environment of the plant to alleviate diseases, the disease may be cured or prevented, but it may eliminate some harmless saprophytes, which may ultimately lead to an imbalance in the natural ecosystem."

In the present study, rhizosphere and phylloplane mycoflora of rice were isolated and identified. Efficient mycoparasites were identified from among the fungi thus obtained, following standard techniques. The most efficient of these mycoparasites were tested for their efficacy in reducing sheath blight disease of rice under *in vivo* conditions, so as to formulate integrated control measures against the disease. Studies were also undertaken to assess the effect of commonly recommended fungicides on the phylloplane and rhizosphere mycoflora. A highly virulent isolate obtained from sheath blight infected rice was used for the study. The morphological characters of the same were studied in detail. *R. solani* is a versatile fungus and is known to vary much morphologically. With four isolates of *R. solani* in Kerala, Lakshmanan(1979) showed that they differed in the morphological characters and also in pathogenicity. The characters of the present isolate was almost identical with those of the rice isolate reported by Lakshmanan (1979) and also those of the MG4 group reported by Vijayan (1986).

The naturally occurring mycoflora which are found to be enormous on the rhizosphere and phylloplane of rice plants were isolated following standard procedures. Among the fungi isolated from the rhizosphere and phylloplane, most of them are found to be efficient mycoparasites on R. solani.

The different rhizosphere and phylloplane mycoflora were tested for their antagonism towards R. solani. These fungi were found to exhibit varying degrees of antagonism towards the sheath blight pathogen. Some of the fungi were noticed to have a smothering effect on R. solani. They were Aspergillus niger, A. japonicus, Trichoderma viride, T. harzianum and Penicillium purpurogenum. Similar results have been reported by Gokulapalan (1989) from this laboratory. There has been very early reports regarding the antagonism of Zygomycetous fungi on R. solani.(Endo, 1931; 1932). Mucor hiemalis was found to cause cessation of growth of R. solani at the point of contact of the colonies. Other fungi exhibiting this type of reaction were Penicillium citrinum and P. oxalicum. A clear zone of inhibition between the paired cultures were noticed in the case of Chaetomium globosum, Aspergillus fumigatus,

A. ochraceus. Gokulapalan (1989) reported similar reactions in the case of C. globosum.

The mycoparasites obtained from the rhizosphere and phylloplane were tested for studying their effects on the hyphae of R. solani, in vito. It was noticed that in most cases, the resultant granulation, vacuolation and disintegration of R. solani hyphae caused by various mycoparasites may be a necrotrophic type of interaction where the antagonizing fungus is obtaining nutrients from the dead host cells (Barnett & Binder, 1973).

The mycoparasites were also tested to find out the effects of diffusible metabolites produced by them on the growth of R. solani. It was observed that maximum inhibition of growth of R. solani was due to production of metabolites by Trichoderma viride, and an inhibition of 94.4 per cent when compared with control was observed. Similarly the, diffusible metabolites produced by Aspergillus melleus and Penicillium oxalicum also exhibited significant inhibition of growth of R. solani. The antagonistic action of Trichoderma spp., due to the production of such metabolites have been well documented (Weindling, 1932; 1934). The production of two very

active metabolities such as gliotoxin, (Weindling & Emerson, 1936; Brian & Hemming, 1945), and Viridin (Brian et al., 1946), have been reported in the case of Trichoderma spp. Webster and Lomas (1964) suggested that antagonism exhibited by Trichoderma is due to the production of antibiotic, which may neither be gliotoxin nor viridin. The production of such metabolites by Trichoderma ~~have~~ has been reported by Dennis and Webster (1971 a).

A study was conducted in order to assess the inhibitory effect of volatile metabolites produced by mycoparasites on R. solani, Trichoderma viride recorded 63.7 per cent inhibition of growth of R. solani followed by T. harzianum, showing 23.4 per cent inhibition. In contrast to the wide interest in the production of diffusible metabolites, very little work has been carried out on the production of volatile substances by fungi. It has been shown that isolates of Trichoderma especially isolates of T. viride are capable of producing volatile metabolites which inhibit mycelial growth of R. solani (Dennis & Webster, 1971 b).

The chemical nature of the volatile metaboites ~~were~~ was investigated by Robinson and Park (1966) and they concluded that in T. viride acetaldehyde was the main compound present. Similarly the inhibitory nature of volatile metabolites produced by T. harzianum was also reported. This fungus was reported to produce the volatile metabolites 6 n Pentyl 2 H Pyran 2-one and 6 n pentenyl 2 H pyran 2-one (Claydon et al. 1987). This might have been the reason for the high percentage of inhibition of growth of R. solani by T. viride and T. harzianum. The inhibition exhibited by other fungi may also be due to the production of substances similar to this.

The efficacy of R. solani as a mycohost for the antagonistic fungus was indirectly assessed by determining the sporulation of these fungi on the mycelia of R. solani. Profuse sporulation was observed in the case of Aspergillus tamarii, T. viride, T. harzianum and also in the case of A. niger. The ability of mycoparasites such as T. viride, T. harzianum and Pencillium oxalicum to sporulate profusely on R. solani has been recorded by Gokulapalan (1989).

In order to select suitable carrier materials for mass culturing the effective antagonistic organism, T. viride for application in rice fields, a study was carried out to assess the comparative merits of commonly used carrier materials like ricebran, wheatbran, sawdust, coirwaste and some of their combinations. The maximum sporulation was observed in culture on ricebran followed by coirwaste and ricebran combination. The least spore count was observed on a combined medium of sawdust and coirwaste. The mass cultures of the antagonistic organism on different substrates, were added to rice in a pot culture experiment and their comparative efficacy assessed by scoring sheath blight incidence at two intervals and it was found that ricebran as well as wheatbran were the most efficient carrier material. However, ricebran was found to be more effective in reducing the disease intensity at 52 DAP also.

The superiority of wheatbran as a carrier material for Trichoderma spp. has been well documented (Hadar et al., 1979; Chet and Elad, 1982 a, Elad et al. 1983; Mukhopadhyay, 1987). It can be seen that among the various carrier materials of antagonistic organisms, ricebran is as efficient as wheatbran in controlling

sheath blight. The efficacy of ricebran as a substrate has been established by Gokulapalan (1989).

The sporulation of T. viride on ricebran was higher than in wheat bran. The capacity of wheat bran to reduce sheath blight was same as that of rice bran. The mycelial growth of T. viride on wheat bran was very profuse even though the sporulation was poor. The ability of young actively growing hyphae of Trichoderma spp. on bran to control R. solani has been reported by Lewis and Papavizas (1987 a).

The study on the comparative efficacy of various mycoparasite fungicide combinations on the intensity of sheath blight in pot culture showed that 53 days after planting T. viride proved to be the best mycoparasite followed by T. harzianum. The superiority of Trichoderma spp. in controlling diseases caused by R. solani has been reported by many workers (Hadar et al. 1979; Wu, 1980; Tu and Chang 1981; Elad et al., 1981 a; Berberich, 1987).

Among the fungicides used edifenphos was found to be very efficient in controlling sheath blight intensity at both 53 and 67 days after planting. Similar results have been reported by many workers and it is a routinely recommended fungicide for sheath blight control (Jaganmohan, 1977; Mathai, 1975; Gokulapalan, 1989).

The increased efficacy of treatment combination, T. viride + edifenphos in controlling sheath blight intensity as well as incidence can be due to a supplementary effect of both of the individual treatments.

The effect of different mycoparasite fungicide combinations on the rhizosphere mycoflora has been assessed. Among mycoparasites when compared with control, the highest increase was seen in the case of T. viride, followed by T. harzianum and Aspergillus niger The increase in mycofloral population can be attributed to the successful establishment of antagonists applied to soil.

Among the fungicides tested, edifenphos showed an enhancement in soil mycoflora population. Similar results have been reported by Lulu Das (1986). The enhancement of mycofloral population by application of

edifenphos can effect an inhibition of growth of R. solani in soil which may be due to competition for nutrients and or due to increase in antagonistic organisms in soil. This may be the reason why a combined treatment of T. viride and edifenphos resulted in lowest disease incidence.

The effect of different mycoparasite fungicidal combinations on the phylloplane mycoflora of rice was assessed and it was found that application of mycoparasites suppressed the phylloplane mycofloral population.

In the case of fungicides, application of edifenphos enhanced the phylloplane mycofloral population, compared with control. However, a reverse trend has been reported by Gokulapalan (1989).

The increased efficiency of the fungicide edifenphos to control sheath blight may be mainly due to its fungicidal ability, however, its indirect influence by enhancing the level of antagonistic mycoflora on the phylloplane, cannot also be ignored. Saprophytes of the phylloplane have also been suggested as being involved in the production of phytoalexins and possibly changing the reaction of the host plant to the pathogen (Blakeman, 1973; Mehan, 1978; Sinha, 1965). Thus the increased effectiveness

of edifenphos may alsobe attributed to inducing production of phytoalexins by antagonistic organisms.

The effectiveness of T. viride as a mycoparasite against sheath blight of rice, observed in pot culture experiment has been further confirmed by the results obtained in the field experiment.

In the case of fungicides, also edifenphos was found to be superior in reducing the percentage of disease incidence while carbendazim was found to be superior in reducing the disease intensity.

Among treatment combinations, the most effective one was found to be T. viride + carbendazim. But T. viride + edifenphos, T. harzianum + control and Aspergillus niger + carbendazim were found to be equally effective in controlling disease intensity. In the case of disease incidence T. viride + edifenphos was found to be the best treatment combination. Aspergillus niger + carbendazim, control + edifenphos and T. harzianum + control were found to be equally effective in checking disease incidence (Fig.1).

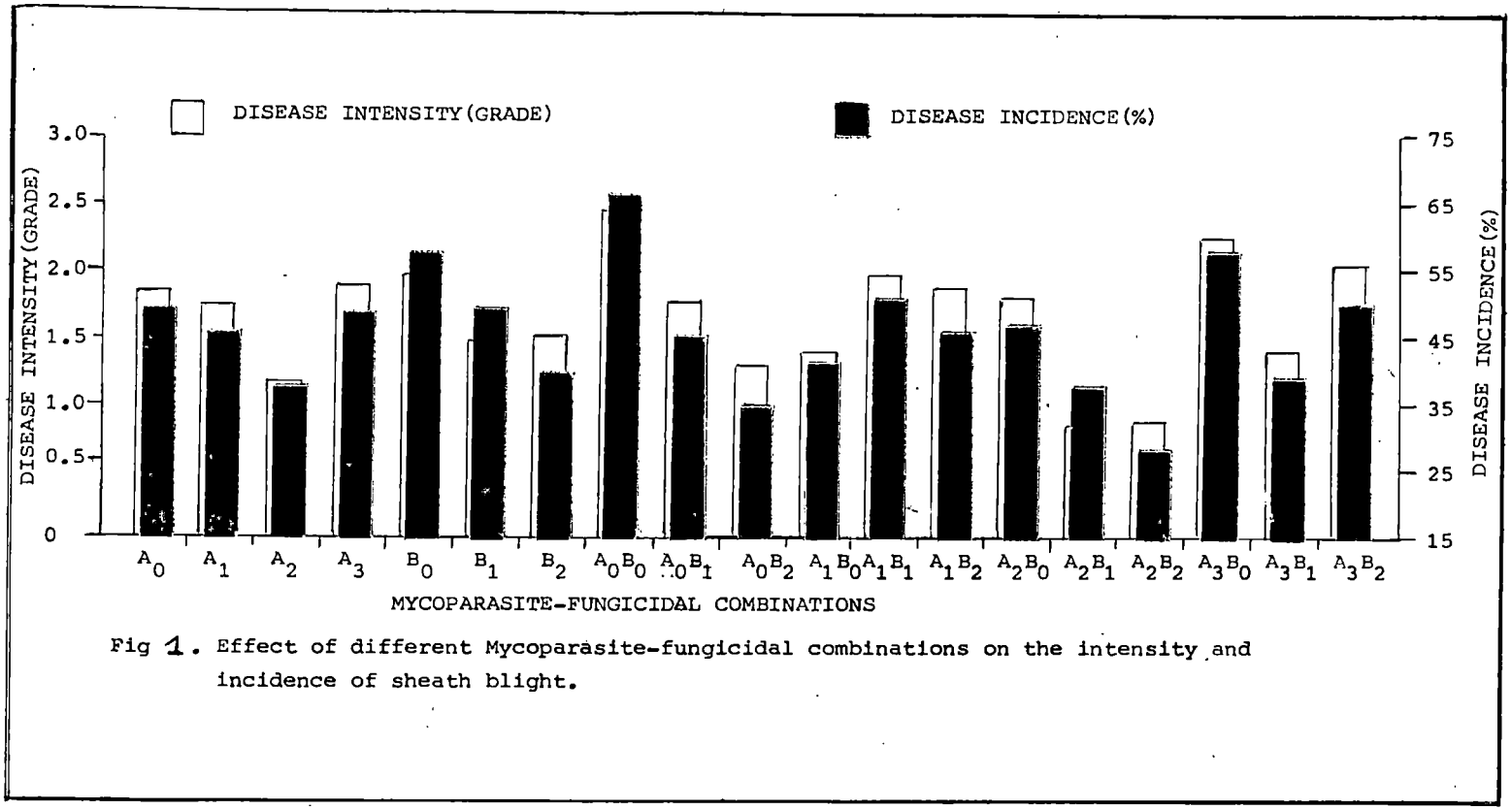



Fig 1. Effect of different Mycoparasite-fungicidal combinations on the intensity and incidence of sheath blight.

In the case of grain yield in the field experiment among mycoparasites. T.viride recorded the highest yield. This can be attributed to lesser disease intensity and incidence. Among fungicides edifenphos application recorded the highest yield, followed by carbendazim.

The highest straw yield also was recorded in the case of treatment with T. viride, followed by T. harzianum. Among the fungicides edifenphos recorded the highest straw yield followed by carbendazim (Fig.3).

The high grain and straw yield in the case of edifenphos can be attributed to a lower intensity and incidence of sheath blight disease. Regarding straw yield among treatment combinations the highest was recorded in case of T. viride + edifenphos. This can be attributed to the supplementary effect of individual treatments.

In the field trial it was found that the highest increase in rhizosphere mycoflora was in the case of application of T. viride. So the increased mycofloral population in the rhizosphere can be one of the reasons for the control of soil borne R. solani. The reduction

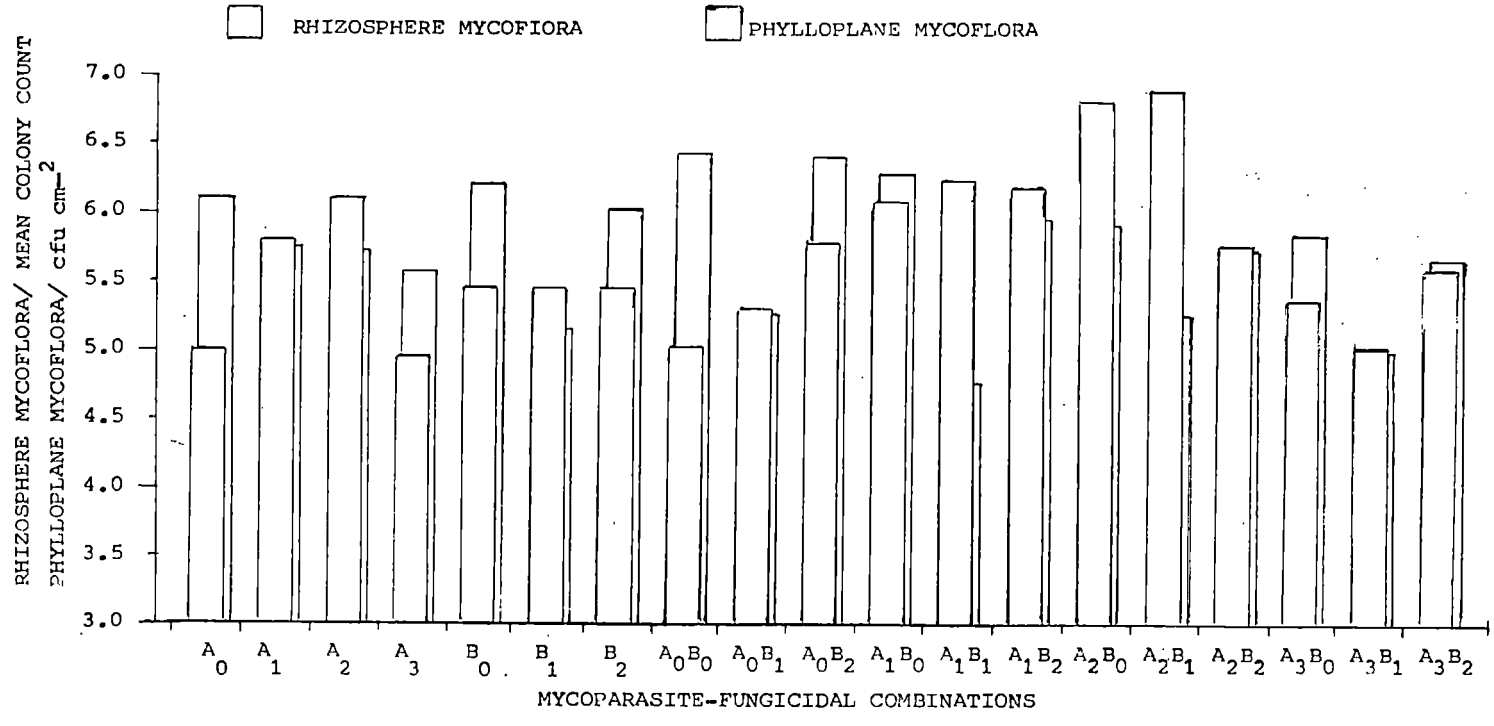



Fig 2 . Effect of different Mycoparasite -fungicidal combinations on the rhizosphere and phylloplane mycoflora.

of disease intensity and incidence of sheath blight in the case of *T. viride* treatments may be attributed to the above fact also. The addition of fungicides and mycoparasite fungicide combinations did not have any significant effect on the rhizosphere mycofloral population.

In the case of phylloplane mycofloral population, application of mycoparasites suppressed the phylloplane mycoflora as in the case of pot culture experiment. Though the fungicide edifenphos did not change the phylloplane mycofloral population, but carbendazim was found to suppress the phylloplne mycofloral population. Similar observations have been reported by Gokulapalan (1989), where such fungicides were found to have a depressing effect on non target phylloplane mycoflora (Fig.2).

Among treatment combinations *T. harzianum* + edifenphos and *A. niger* + carbendazim were found to have a suppressive effect on the mycofloral count.

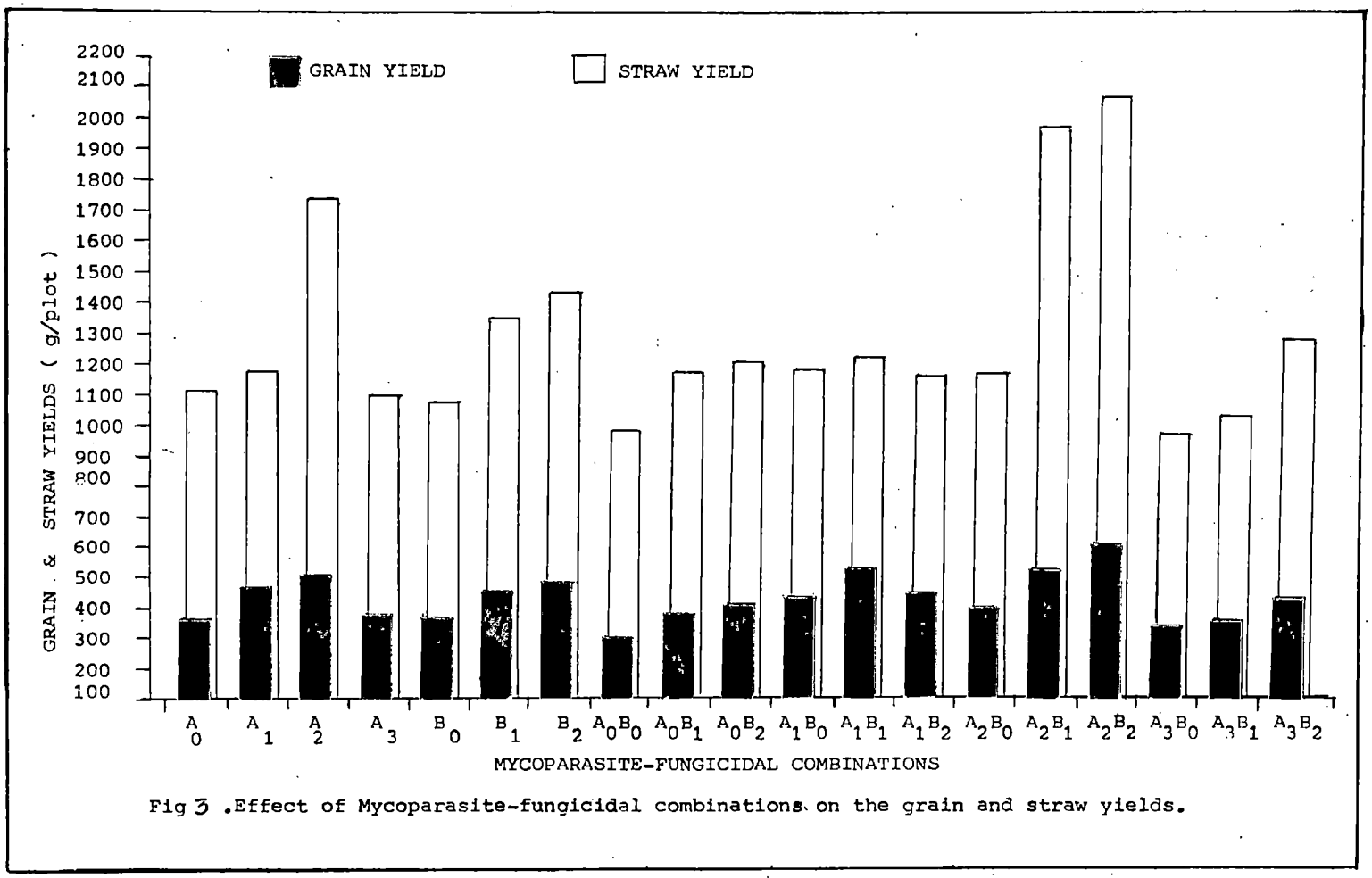



Fig 3 .Effect of Mycoparasite-fungicidal combinations on the grain and straw yields.

The concept that emerges out of the study is as follows:-

1. Indiscriminate application of fungicides for the control of sheath blight may upset the balance of natural mycoflora including the antagonistic organisms of *R. solani*.

2. Efficient antagonists such as *T. viride* can be isolated and mass multiplied in materials like rice bran, for field application.

3. Mass cultured antagonists judiciously applied in conjunction with chemicals such as edifenphos will achieve integrated control of sheath blight of rice.

# SUMMARY

## SUMMARY

Sheath blight caused by Rhizoctonia solani is one of the most important diseases affecting rice in Kerala. The present investigation was taken up in order to assess the efficacy of mycoparasites isolated from the rhizosphere and phylloplane of rice in controlling the disease. Emphasis was placed in developing integrated control measures, using fungicides, in conjunction with mycoparasites.

Fungi isolated from the rhizosphere and phylloplane were tested for their antagonism against R. solani. Among the mycoparasites isolated, T. viride was found to be the most efficient antagonist exhibiting complete smothering of the sheath blight pathogen. Mechanism of mycoparasitism of the antagonist against R. solani was studied. Granulation, vacuolation, and disintegration of the R. solani hyphae ~~was~~ were observed in the case of mycoparasites like T. viride, T. harzianum and Aspergillus niger. The effect of diffusible and volatile metabolites produced by the antagonistic fungi on the growth of R. solani was assessed. The maximum

inhibition was observed in the case of T. viride, both in the case of diffusible metabolites as well as volatile substances. The utilisation of R. solani mycelia as a growth substrate was also assessed. Aspergillus tamarii showed the highest and Cochliobolus miyabeanus the lowest efficiency in utilising the same as growth substrate.

A study conducted to gauge the comparative efficacy of various growth substrates for mass multiplication of antagonists, showed that the best control of the disease was obtained by application of wheat bran as well as rice bran cultures. The high rate of sporulation was noticed when rice bran was used as substrate while the best mycelial growth was noticed in wheat bran culture.

The efficacy of various mycoparasite-fungicide combinations in controlling sheath blight was studied in pot culture. T. viride emerged as the best mycoparasite and edifenphos was found to be the best among fungicides for controlling sheath blight. Regarding the population of rhizosphere and phylloplane mycoflora, the application of mycoparasites enhanced rhizosphere mycofloral population, whereas it depressed the

phylloplane mycofloral population. Application of fungicides, especially edifenphos enhanced both rhizosphere as well as phylloplane population.

In the field trial, where the efficacy of various mycoparasite-fungicide combinations were tried out, in controlling sheath blight, T. viride emerged as the best mycoparasite, and edifenphos the best fungicide. T. viride + edifenphos were found effective in checking sheath blight along with T. viride + carbendazim. The grain yield and also the straw yield was also significantly higher on treatment with the mycoparasite T. viride, and the fungicide edifenphos. Treatment combination of T. viride + edifenphos exhibited the highest yield of both grain and straw.

Studies on the assessment of rhizosphere mycoflora of rice plants treated with various mycoparasites and fungicides revealed that application of the mycoparasite T. viride enhanced the total population. On the other hand phylloplane mycoflora showed a depressive effect not only by application of mycoparasites, but also fungicides. The fungicide carbendazim showed the

maximum depressive effect on phylloplane mycoflora.

The study revealed the fact that there is vast scope for an integrated approach in the control of sheath blight of rice utilising proper mycoparasites, complimentary to the appropriate fungicidal umbrella.

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# REFERENCES


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* Original not seen

# APPENDICES

## APPENDIX- I

### Potato dextrose agar

Potato - 200g
Dextrose- 20g
Agar - 20g
Distilled water - 1 L

### Rose Bengal streptomycin agar

Dextrose - 10g
Peptone - 5g
Potassium dihydrogen phosphate - 1g
Magnesium sulphate - 0.5g
Rose bengal - 1 partin 30,000 parts of the medium
Agar - 20g
Distilled water - 1 L

### Potato dextrose broth

Potato - 200g
Dextrose - 20g
Distilled water - 1 L

# APPENDIX- II

## Abstract of ANOVA - Pot culture studies

31 DAP

ANOVA

| Source | D.F | S.S. | M.S.S. | F |
|---|---|---|---|---|
| Treatments | 7 | 3.43 | 0.49 | 91.8* |
| Error | 16 | 0.85 | 5.33 | |
| Total | 23 | 4.28 | | |

SE - Treatments 0.133
CD - Treatments 0.399

52 DAP

ANOVA

| Source | D.F | S.S | M.S.S | F |
|---|---|---|---|---|
| Treatments | 7 | 2.68 | 0.38 | 11.43* |
| Error | 16 | 0.53 | 3.36 | |
| Total | 23 | 3.22 | | |

SE - Treatments 0.105
CD - Treatments 0.317

53 DAP

ANOVA

| Source | D.F | S.S. | M.S.S. | F |
|---|---|---|---|---|
| Treatments | 11 | 3.86 | 0.35 | 3.21* |
| A | 3 | 1.62 | 0.54 | 4.95* |
| B | 2 | 1.20 | 0.60 | 5.50* |
| AB | 6 | 1.03 | 0.17 | 1.57 |
| Error | 24 | 2.62 | 0.10 | |
| Total | 35 | 6.49 | | |

| SE values | CD values |
|---|---|
| A - 0.110 | A - 0.321 |
| B - 9.548 | B - 0.298 |
| AB - 0.190 | AB - 0.557 |

67 DAP

ANOVA

| Source | DF | S.S | M.S.S | F |
|---|---|---|---|---|
| Treatments | 11 | 5.60 | 0.50 | 2.95* |
| A | 3 | 1.72 | 0.57 | 3.32* |
| B | 2 | 1.71 | 0.85 | 4.98* |
| AB | 6 | 2.16 | 0.36 | 2.09 |
| Error | 24 | 4.13 | 0.17 | |
| Total | 35 | 9.74 | | |

| SE values | CD values |
|---|---|
| A - 0.138 | A - 0.404 |
| B - 0.119 | B - 0.349 |
| AB - 0.239 | AB - 0.699 |

ANOVA

| Source | DF | S.S | M.S.S | F |
|---|---|---|---|---|
| Treatments | 11 | 10753.49 | 977.590 | 12.57* |
| A | 3 | 1532.81 | 510.93 | 6.56* |
| B | 2 | 4415.09 | 2207.54 | 28.38* |
| AB | 6 | 4805.58 | 800.93 | 10.29* |
| Error | 24 | 1866.50 | 77.77 | |
| Total | 35 | 12620 | | |

SE values

A - 2.939
B - 2.545
AB - 5.091

CD values

A - 8.580
B - 7.430
AB - 14.861

ANOVA

| Source | DF | S.S. | M.S.S | F |
|---|---|---|---|---|
| Treatment | 11 | 18.07 | 1.64 | 33.98* |
| A | 3 | 16.42 | 5.47 | 113.23* |
| B | 2 | 1.18 | 0.59 | 12.29* |
| AB | 6 | 0.46 | 0.76 | 1.58 |
| Error | 24 | 1.16 | 4.83 | |
| Total | 35 | | | |

| SE values | CD values |
|---|---|
| A - 7.330 | A - 0.215 |
| B - 6.348 | B - 0.186 |
| AB - 0.126 | AB - 0.372 |

ANOVA

| Source | DF | S.S | M.S.S | F |
|---|---|---|---|---|
| Treatments | 11 | 14.53 | 1.32 | 7.46* |
| A | 3 | 4.36 | 1.45 | 8.21* |
| B | 2 | 8.49 | 4.24 | 2.40* |
| AB | 6 | 1.67 | 0.27 | 1.58 |
| Error | 24 | 4.24 | 0.17 | |

| SE values | | CD values | |
|---|---|---|---|
| A | - 0.140 | A | - 0.411 |
| B | - 0.121 | B | - 0.356 |
| AB | - 0.242 | AB | - 0.712 |

# ABSTRACT OF ANOVA- FIELD STUDIES

## Disease intensity (60 DAP)

ANOVA

| Source | DF | S.S | M.S.S | F |
|---|---|---|---|---|
| Replication | 2 | 0.65 | 0.32 | 1.85 |
| Treatments | 11 | 8.60 | 0.78 | 4.47* |
| A | 3 | 3.04 | 1.01 | 5.79* |
| B | 2 | 1.71 | 0.85 | 4.91* |
| AB | 6 | 3.84 | 0.64 | 3.66* |
| Error | 22 | 3.84 | 0.17 | |
| Total | 35 | 13.10 | | |

| SE Values | CD values |
|---|---|
| A - 0.139 | A - 0.408 |
| B - 0.120 | B - 0.354 |
| AB - 0.241 | AB - 0.708 |

## Disease incidence (60 DAP)

ANOVA

| Source | DF | S.S | M.S.S. | F |
|---|---|---|---|---|
| Replication | 2 | 54.19 | 27.09 | 0.41 |
| Treatments | 11 | 3490.10 | 317.28 | 4.83* |
| A | 3 | 749.38 | 249.79 | 3.80* |
| B | 2 | 1138.63 | 569.31 | 8.66* |
| AB | 6 | 1602.08 | 267.01 | 4.06* |
| Error | 22 | 1445.03 | 65.68 | |
| Total | 35 | 4989.33 | | |

| SE values | CD values |
|---|---|
| A - 2.701 | A - 7.923 |
| B - 2.339 | B - 6.862 |
| AB - 4.679 | AB - 13.724 |

ANOVA

| Source | DF | S.S. | M.S.S. | F |
|---|---|---|---|---|
| Replication | 2 | 57804 | 28902 | 4.16 |
| Treatments | 11 | 258589.50 | 23508.14 | 3.39* |
| A | 3 | 137876 | 45958.67 | 6.62* |
| B | 2 | 77320 | 38660 | 5.57* |
| AB | 6 | 43393.5 | 7232.25 | 1.04 |
| Error | 22 | 152512 | 6932.36 | |
| Total | | 468905.5 | | |

| SE values | CD values |
|---|---|
| A - 27.753 | A - 81.403 |
| B - 24.035 | B - 70.497 |
| AB - 48.070 | AB -140.995 |

ANOVA

| Source | DF | S.S | M.S.S | F |
|---|---|---|---|---|
| Replication | 2 | 182376 | 91188 | 1.64 |
| Treatments | 11 | 4232416 | 384765.10 | 6.95* |
| A | 3 | 2515788 | 838596 | 15.15* |
| B | 2 | 833004 | 416502 | 7.52* |
| AB | 6 | 883624 | 147270.70 | 2.66* |
| Error | 22 | 1217760 | 55352.73 | |
| Total | | 5632552 | | |

| SE values | CD values |
|---|---|
| A - 78.423 | A - 230.023 |
| B - 67.917 | B - 199.206 |
| AB -135.834 | AB- 398.412 |

ANOVA

| Source | DF | S.S. | M.S.S. | F |
|---|---|---|---|---|
| Replication | 2 | 0.57 | 0.28 | 1.07 |
| Treatments | 11 | 12.89 | 1.17 | 4.39* |
| A | 3 | 9.10 | 3.03 | 11.37* |
| B | 2 | 9.15 | 4.57 | 0.17 |
| AB | 6 | 3.78 | 0.63 | 2.36 |
| Error | 22 | 5.86 | 0.26 | |

| SE values | CD values |
|---|---|
| A - 0.172 | A- 0.504 |
| B - 0.149 | B- 0.437 |
| AB - 0.298 | AB- 0.874 |

ANOVA

| Source | DF | S.S | M.S.S | F |
|---|---|---|---|---|
| Replication | 2 | 0.32 | 0.16 | 3.09 |
| Treatments | 11 | 9.79 | 0.89 | 16.88 |
| A | 3 | 1.42 | 0.47 | 9.01 |
| B | 2 | 7.54 | 3.77 | 71.55 |
| AB | 6 | 0.81 | 0.13 | 2.58 |
| Error | 22 | 1.16 | 5.27 | |
| Total | | | | |

| SE values | CD values |
|---|---|
| A - 7.655 | A - 0.224 |
| B - 6.629 | B - 0.194 |
| AB - 0.132 | AB - 0.388 |

# BIOLOGICAL CONTROL OF *Rhizoctonia solani* Kuhn ON RICE USING MYCOPARASITES

BY

**VISWAKUMAR, P.**

ABSTRACT OF A THESIS
SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENT FOR THE DEGREE OF
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# ABSTRACT

The present investigation was undertaken to assess the effects of mycoparasite-fungicide combinations on the intensity of sheath blight disease, and also the effect of the same on the rhizosphere and phylloplane mycoflora of rice. An attempt has also been made to identify efficient mycoparasites of Rhzoctonia solani naturally present on the rhizosphere and phylloplane of rice plants.

Several fungi isolated from the rhizosphere and phylloplane of rice plants were found to exhibit strong antagonistic action against R. solani, in vitro. Antagonistic fungi such as Trichoderma viride, T. harzianum, Aspergillus niger caused granulation, vacuolation and finally disintegration of R. solani hyphae. The ability of the antagonists to produce volatile as well as diffusible metabolites was also assessed. T.viride was found to be very efficient in inhibiting the growth of R. solani, as a result of the diffusible as well as volatile metaboltes produced by the fungus. T. harzianum was also very efficient in inhibiting growth of R. solani.

When the antagonist T. viride was mass cultured in various growth substrates and tried for their efficacy as potential biocontrol agents of sheath blight disease of rice, rice bran as well as wheat bran turned out to be the best growth substrates. Under pot culture conditions, where the efficacy of various mycoparasite-fungicide combinations in checking sheath blight was assessed, T. viride was the best mycoparasite and edifenphos the best fungicide. The rhizosphere mycoflora was enhanced by application of mycoparasite, especially T. viride and T. harzianum. Among fungicides application of edifenphos resulted in increased rhizosphere mycofloral population. In the case of phylloplane mycoflora application of mycoparasites have a suppressive effect. Treatments of edifenphos increased phylloplane mycofloral population.

Under field conditions also T. viride emerged as the best mycoparasite and edifenphos as a superior fungicide in checking sheath blight. T. viride + edifenphos was also found to be very efficient in controlling sheath blight disease. The highest grain and straw yields were obtained in treatment with the mycoparasite T. viride, and fungicide edifenphos. The treatment combination T. viride + edifenphos also gave the highest yield.

In the case of rhizosphere mycoflora application of mycoparasites enhanced the population. Mycoparasites were found to have a suppressive effect on the fungal population on the leaf surface.