EFFECT OF PLANT PROTECTION CHEMICALS ON FOLIAR PATHOGENS AND PHYLLOPLANE MICROFLORA OF RICE

By

C. GOKULAPALAN M.Sc. (Ag.)

THESIS

Submitted in partial fulfilment of the requirement
for the degree
DOCTOR OF PHILOSOPHY
Faculty of Agriculture
Kerala Agricultural University

Department of Plant Pathology
COLLEGE OF AGRICULTURE
Vellayani, Trivandrum

1989

DECLARATION

I hereby declare that this thesis entitled "Effect of plant protection chemicals on foliar pathogens and phylloplane microflora of rice" 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.

Gokulapalan C

(C. GOKULAPALAN)

Vellayani,

- -1989

CERTIFICATE

Certified that this thesis entitled "Effect of plant protection chemicals on foliar pathogens and phylloplane microflora of rice" is a record of research work done independently by Shri C. GOKULAPALAN 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.

(M. CHANDRASEKHARAN NAIR)
Chairman
Advisory Committee
Professor of Plant Pathology

APPROVED BY

Chairman

Dr. M. CHANDRASEKHARAN NAIR

Members

1. Dr. M.M. KOSHY

2. Dr. R.S. AIYER

3. Dr. S. BALAKRISHNAN

4. Dr. S K NAIR 29/4/1989

C O N T E N T S

Parakkode, Adoor, for help rendered during the field trials at the respective locations.

Dr. N.Sadanandan, Director, Post Graduate Studies, Kerala Agricultural University and Dr. K.I. Wilson, Professor and Head, Department of Plant Pathology are acknowledged for their active support and help rendered during the studies.

The author acknowledges the service rendered by Dr. S. Sulochana, Professor of Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Trichur in identifying the bacterial cultures isolated during the study.

He is thankful to Sri K.K.Ravindran Nair, Professor-in-Charge, Academic Cell for his valuable suggestions and help during the course.

He is indebted to Dr.P.Saraswathy, Associate Professor of Agricultural Statistics and Sri.C.E.Ajit Kumar, Junior Programmer, Department of Agricultural Statistics for their active involvement and help during the statistical analyses. He owes much to the Bharathy Computer Service, East of Court, Vanchiyoor, Trivandrum for the typing of the thesis.

Finally the facilities provided by the Kerala Agricultural University and ICAR for the successful completion of this work are acknowledged.

(C. GOKULAPALAN)

ACKNOWLEDGEMENTS

The author recalls with gratitude his indebtedness to the following persons/institutions in making this investigation fruitful.

He is extremely grateful to Dr. M. Chandrasekharan Nair, Professor of Plant Pathology, Chairman, Advisory Committee for suggesting the problem, constant encouragement, able guidance, and sustained interest during the course of investigations and for his creative criticism during the preparation of the thesis.

He is very much indebted to Dr.M.M.Koshy, Director, Centre for Excellence, Tropical Soils, Dr.R.S.Aiyer, Professor and Head, Department of Soil Science and Agricultural Chemistry, Dr.S.Balakrishnan, Professor of Plant Pathology and Dr. S.K.Nair, Associate Professor, Department of Plant Pathology as members of the Advisory Committee for the encouragement and support extended during the course of this investigation and preparation of the thesis.

His colleagues and others in the Department of Plant Pathology have been very helpful during the course of this study; their good will and support are gratefully acknowledged.

He is thankful to the staff of Cropping Systems Research Centre, Karamana and Sri R.S. Unnithan, Farmer,

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

INTRODUCTION

INTRODUCTION

In modern agriculture, biocides play an important role in the control of many of the rampantly occurring pests and diseases affecting rice. It is estimated that a total of 1059 tonnes of pesticides (technical grade) are being dumped into the rice fields of Kerala annually which may increase by about 30 per cent in the near future. However, very little attempt has been made so far to understand the influence of these chemicals on the rice ecosystem.

Sheath blight disease of rice caused by Rhizoctonia solani Kuhn (Thanatephorus cucumeris (Frank) Donk) and sheath rot disease of rice caused by Sarocladium oryzae Gams & Hawksworth have become major constraints facing rice production in Kerala, India. The only successful means to combat these diseases currently is by the routine application of fungicidal formulation like edifenphos, carbendazim and carboxin. The effects of these chemicals on the non-target microflora of the rice phylloplane has not yet been critically analysed. It is now a well known fact that substantial populations of microorganisms are present on leaf surfaces and many of these may possess antagonistic action against plant pathogens. Only very limited work has been done on the effect of fungicides on the phylloplane microflora of rice (Jagadeesh et al., 1978).

REVIEW OF LITERATURE

The present studies were taken up with a view to assess the following aspects:

1 The effects of plant protection chemicals on the important fungal pathogens affecting mainly the leaf sheath of the rice plant and its phylloplane microflora.

2 A qualitative study of the naturally existing microflora of rice phylloplane.

3 *In vitro* antagonism of the phylloplane microflora towards *R.solani*, the sheath blight pathogen.

4 *In vitro* effects of selected plant protection chemicals on antagonistic phylloplane mycoflora and *R.solani*.

5 Mycoparasitism of selected phylloplane antagonists towards *R.solani*.

6 Efficacy of a few mycoparasites of *R.solani* isolated from the rice phylloplane to control the sheath blight disease.

REVIEW OF LITERATURE

I FUNGAL PATHOGENS OF RICE FOLIAGE

The rice plant has been reported to harbour a large number of plant pathogenic microorganisms (Padwick, 1950). Most of the diseases affecting foliage and sheath are caused by deuteromycetous fungi, viz; Helminthosporium oryzae Breda de Haan; Cercospora oryzae Miyake; Pyricularia oryzae Cav., and Rhizoctonia solani Kuhn.

All these fungi are causing serious problems for rice growers and a comprehensive treatise on the important diseases of rice has been compiled by Ou (1972; 1985). In this work, the author has cited and discussed in detail about 20 fungal diseases affecting the rice plant. Most of these diseases have been reported from India and two Indian workers have comprehensively reviewed the studies on diseases of the rice plant (Gangopadhyay and Chakrabarti, 1983).

The most important foliage and sheath diseases affecting rice crop in Kerala includes sheath blight disease caused by Rhizoctonia solani Kuhn (Teleomorph. Thanatephorus cucumeris (Frank Donk) (Mahendra Prabhath, 1971) and the sheath rot disease of rice caused by Sarocladium oryzae Gams & Hawksworth (Nair and Sathyarajan, 1975).

II. MICROFLORA OF THE RICE FOLIAGE

Not much work has been done on the phylloplane microflora of the rice plant in India or abroad. A pioneering work in this aspect is that of Mishra and Srivastava (1971) who conducted an investigation into the fungal population of the phylloplane of rice plants in Gorakhpur, India. This study was undertaken to assess the qualitative and quantitative changes occurring in the phylloplane microflora with the ageing of rice plant. The fungi that frequented the green leaves included

Mucor hiemalis Wehmeyer

Syncephalastrum racemosum (Cohn.) Schroet

Rhizopus oryzae Went. & Geerlings

Rhizopus stolonifer (Ehrarb. ex Fr.) Lind

Choanephora cucurbitarum (Berk & Ray) Thaxt.

Chaetomium sp.

Aspergillus nidulans (Eidam) Wingate

A. flavus Link

A. niger Van Tiegh

A. sydowi (Bain and Sartoris) Thom & Church

A. fumigatus Fres

A. terreus Thom

Colletotrichum sp.

Curvularia lunata (Wakker) Boedijn

C. geniculata (Tracy & Earle) Boedijn

Phoma sp.

Trichoderma lignorum (Tode) Harz

Nigrospora sphaerica (Sacc.) Mason

Fusarium sp.

Jagadeesh and his co-workers studied the phylloplane microorganisms in relation to foliar diseases at CRRI, Cuttack, India (Jagadeesh et al., 1978). They found that the phylloplane bacteria (unidentified) reduced the intensity of blast, brown leaf spot and bacterial leaf blight. The phylloplane yeasts (unidentified) increased the intensity of blast and bacterial leaf blight while the phylloplane fungi, Aspergillus, Penicillium and Trichoderma reduced the intensity of blast and brown leaf spot.

Philip and Devadath (1980) found that the phylloplane fungal and bacterial flora varied on different cultivars with the age of the crop. There was no relation between the genetic background of the cultivars and the phylloplane microflora.

III. IMPORTANT SHEATH DISEASES OF RICE

A. Sheath Blight

Miyake (1910) first described a new disease of rice from Japan under the name oriental sheath blight and leaf spot and named the organism, Sclerotium irregulare, sp nov.

Subsequently the occurrence of this disease has been recorded from various rice growing countries of the world (Ou, 1985). Eventhough Butler (1918) mentioned about the occurrence of this disease in India, it was Paracer and Chahal (1963) who first described the sheath blight disease caused by Rhizoctonia solani Kuhn from Punjab in detail. This disease assumed serious proportions in the rice growing tracts of Kerala in the recent past (Mahendra Prabhath, 1971).

Saksena and Chaubey (1972) reported a banded blight disease of rice in North India where copious air-borne basidiospores caused leaf blight with banded symptoms and spots on leaf sheath. They indentified the causual organism as Thanatephorus cucumeris (Frank) Donk. This fungus causes a variety of diseases on a wide range of crops and an exhaustive review of the same is consolidated in a monograph (Parmeter, 1969).

Symptomatology

Miyake (1910) first described the symptoms of sheath blight disease in detail. According to him, the initial symptoms appeared as discoloured ellipsoidal spots on the leaves and sheath which measured upto 10 mm in length and 3 to 4 mm in breadth. These spots gradually got enlarged and turned grayish white with a blackish brown margin. Singh and Pavgi (1969) recorded the initial symptoms as oval to

irregular straw coloured lesions on the leaf tip and leaf sheath near the leaf base surrounded by a narrow reddish brown band. These lesions increased in size, coalesced and covered the leaf lamina completely. Kozaka (1970) described the lesions on the leaf sheath at first as greenish gray and ellipsoidal, 2 to 3 cm long or more gradually becoming white with a blackish brown margin. Ou (1985) described the spots as ellipsoid to ovoid, somewhat irregular, greenish gray varying from 1 to 3 cm long. The centre of the spot turns grayish white with a brown margin. Sclerotia are formed on or near these spots, but are easily detached.

Studies on various aspects of sheath blight disease including symptomatology, host range, physiology of parasitism, varietal reaction, survival of the pathogen and chemical control have been made in the Department of Plant Pathology, College of Agriculture, Vellayani (Mahendra Prabhath, 1971; Padmakumary, 1972; Muneera, 1973; Mathai, 1975; Radhakrishnan, 1975; Jagan Mohan, 1977; Lakshmanan, 1979; Gokulapalan, 1981; Babu George, 1981; Lakshmi, 1984; Lulu Das, 1986).

B. Sheath Rot

Sheath rot of rice caused by Sarocladium oryzae Gams & Hawksworth (= Acrocylindrium oryzae Saw.) was first described from Formosa by Sawada in 1922. Tasugi and Ikeda (1956) have

established its pathogenicity in rice plants and provided more cultural and physiological information about the pathogen.

The occurrence of this disease in India was first reported from Karnataka (Agnihothrudu, 1973) followed by reports from Tamil Nadu (Prabhakaran et al., 1974), Andhra Pradesh (Amin et al., 1974) and from Kerala (Nair and Sathyarajan, 1975).

The destructive nature of this disease has been recorded in the recent years. Attabhanyo and Rush (1973) reported sheath rot as a severe problem from USA. Prabhakaran et al. (1974) reported a yield loss of 85 per cent due to this disease from Annamalainagar.

Chakravarty and Biswas (1978) recorded a reduction of 79 per cent in the grain weight due to infection by Sarocladium oryzae. Estrada et al. (1984) reported an yield loss of 53 per cent from Philippines due to sheath rot infection.

Symptomatology

Tasugi and Ikeda (1956) reported that the fungus mainly attacked the uppermost leaf sheath resulting in rotting. The grayish brown lesions coalesce and form large irregular blotches. Ou (1985) observed that the rot occurred on the uppermost leaf sheath enclosing the panicle. The

lesions started as oblong spots with brown margin and gray centres. The young panicles remained within the sheath or only partially emerged. Abundant whitish growth could be noticed on the affected sheath. In Kerala, Nair and Sathyarajan (1975) described the symptoms of the disease in detail. They found that young spots appeared on the boot leaf sheath and turned whitish gray with a dark margin. In infected fields the panicles could be observed at various stages of emergence. A whitish powdery mass of fungal growth could be detected over the matured lesions inside the affected sheath.

Some basic work on the sheath rot pathogen, the etiology of the disease, control measures and effect of management practices on sheath rot disease have been carried out at the college of Agriculture, Vellayani (Balakrishnan, 1981; Krishnakumaran Nair, 1986; Nair et al., 1988).

IV PLANT PROTECTION CHEMICALS RECOMMENDED FOR RICE CULTURE IN KERALA

As early as in 1982, the Kerala Agricultural University had recommended the use of an assortment of pesticides for the control of pests and diseases affecting the rice crop, in the 'Package of Practices Recommendation' (Kerala Agricultural University, 1982). Some of the insecticides commonly recommended include hexachloro hexane,

carbaryl, carbofuran, dimethoate, fenitrothion, fenthion, malathion, monocrotophos, phenthoate, phorate, phosalone, phosphamidon and quinalphos.

Among the various chemcials recommended for control of rice diseases are zineb, mancozeb, captafol, edifenphos, Kitazin, carbendazim, carboxin and Aureofungin sol.

V EFFECT OF PLANT PROTECTION CHEMICALS ON IMPORTANT SHEATH PATHOGENS AND PHYLLOPLANE MICROFLORA

A. Effect of plant protection chemcials on sheath blight.

Chemical control of sheath blight has been attempted by different workers all over the world. Earlier, copper and mercury compounds were recommended (Hashioka and Saito, 1953). This was followed by the use of organo arsine compunds which were reported to be more effective (Hashioka, 1956, Kozaka, 1961). Several workers have reported the effectiveness of Hinosan in controlling the sheath blight disease of rice (Umeda, 1973; Yamaguchi, 1974; Mathai, 1975; Mukherjee, 1978; Kannaiyan and Prasad, 1979).

Hartzfield (1957) found that terrachlor was effective against sclerotial fungi. Benlate was found to be effective in reducing the intensity of sheath blight disease (Muneera, 1973; Jagan Mohan, 1977). Jagan Mohan (1977) and Lakshmanan (1979) have observed effective control of sheath blight under filed conditions in Kerala using Vitavax. Since the 1970s

benomyl has been widely tested and recommended as an effective fungicide for the control of sheath blight (Chien and Chu, 1973, Roy and Saikia, 1976; Kannaiyan and Prasad, 1976).

The efficacy of Bavistin in reducing tiller infection of sheath blight has been reported by Bhaktavalsalam et al. (1977). Kannaiyan and Prasad (1979) found that Bavistin, Kitazin, Hinosan, benlate, Demosan and thiabendazole gave significant control of sheath blight. Roy (1981) stated that sheath blight incidence was reduced under greenhouse conditions by carbendazim and edifenphos. Gokulapalan (1981) observed that the application of Vitavax with Furadan 3g significantly reduced the sheath blight incidence and intensity, rice root nematode infestation and increased the grain yield.

Rema Devi et al. (1987) highlighted the efficacy of different fungicides in the control of sheath blight of rice. The best curative and protective effects against this disease caused by R. solani were given by Validamycin.

Jones et al. (1987) evaluated the fungicides benomyl and propiconazole for controlling sheath blight of rice caused by R. solani. In the field trials, the application of propiconazole twice followed by benomyl significantly reduced disease severity and increased yields.

B. Effect of plant protection chemicals on sheath rot

Chinnaswamy et al. (1977) reported the efficacy of Bavistin followed by HMP-MBC, Aureofungin Sol and Hinosan reducing the infection by Sarocladium oryzae under field conditions in Kerala.

Kannaiyan (1979) reported from Tamil Nadu, the effective control of sheath rot using benomyl or chlorothalonil. Raina and Singh (1980) have suggested carbendazim 0.1 per cent for the effective control of sheath rot in the field. Raju and Singh (1981) observed that the fungicides carbendazim and benomyl could effectively check under field conditions, the incidence and intensity of sheath rot disease. Balakrishnan and Nair (1982) from Kerala have suggested the efficacy of Hinosan and Vitavax (carboxin) in reducing the incidence and intensity of sheath rot disease under field conditons. Lakshmanan (1984) from Tamil Nadu reported that a Calixin- Bavistin mixture (each at 100 g/ha) could effectively control sheath rot under field conditons.

Nair et al. (1988) have observed that the application of carboxin along with the top dressing of N and K as foliar sprays gave significant reduction in disease incidence and increased grain yields.

C. Effect of plant protection chemicals on phylloplane microflora

The greatest difficulty in biological control in the field is the maintenance of a sufficiently high population of antagonists in the environment. The extent of interference in the population of antagonistic microflora brought about by the application of pesticides is not clearly understood for most of the host-pesticide combinations. Chemicals used for disease and pest control are known to reduce the populations of phylloplane microorganisms (Andrews and Kenerley, 1978; Hislop, 1976).

Bainbridge and Dickinson (1972) found that the saprophytic phylloplane mycoflora on potato plants were more susceptible to captafol and maneb. Bacterial counts remained unaltered by these treatments. Jenkyn and Prew (1973) reported that the application of benomyl and thiophanate methyl decreased the population of mycelial fungi, mostly Cladosporium sp. Dickinson (1973) observed the reduction of leaf surface microflora on barley caused by seed treatment with ethirimol and regular spraying with zineb.

Warren (1974) found that benomyl sprays on oaks reduced the population of Aureobasidium pullulans de Bary Arnaud. Benomyl application was found to cause the development of an anomalous saprophytic microflora.

Dickinson and Wallace (1976) reported that repeated sprays of tridemorph had only minor effects on the phylloplane saprophytes but benomyl and zineb inhibited the development of many yeasts and filamentous fungi. Kuthubutheen and Pugh (1978) studied the effects of a few fungicides on the physiology of phylloplane fungi. At 50ppm concentration, thiram and verdasan caused inhibition of cellulose decomposition and starch hydrolysis in the strongly cellulolytic fungus, Trichoderma viride Pers ex Fr.

Mehan and Chohan (1981) found that sprays of the fungicide benlate greatly increased the phylloplane mycoflora of groundnut plants while sprays of mancozeb reduced the mycoflora population.

Fokkema and Nooij (1981) worked out the effect of a few fungicides on the microbial balance in the phyllosphere of cereal leaves. The fungicides like dithiocarbamates, captafol, benzimidazoles and tridemorph reduced the population of the phylloplane yeasts, Sporobolomyces spp., Cryptococcus sp., Aureobasidium pullulans and the mycelial fungus, Cladosporium spp. Other fungicides like thiophanate methyl, did not have any significant effect on the phylloplane microflora.

Papavizas et al. (1982) mutated Trichoderma spp. so that they became tolerant to benzimidazole fungicides.

Padmanabhan and Alexander (1982) found that fungicides like fenaminosulf, plantvax and demosan were found to favour the growth of Trichoderma spp. The fungicides ziram, agallol, Fytolan and Difolatan were highly inhibitory to Trichoderma spp.

VI. ANTAGONISM OF PHYLLOPLANE MICROORGANISMS TOWARDS FOLIAR/ SHEATH PATHOGENS OF THE RICE PLANT.

The phylloplane is usually inhabited by a variety of saprophytic and parasitic microgorganisms which interact among themselves and also with the living host plant. The first step in the indentification of the saprophytes antagonistic to plant pathogenic microbes is the in vitro screening of these microorganisms.

The antagonism of saprophytic fungi towards Helminthosporium diseases on the leves of cereals has been studied by Porter (1924) and Asare-Nyako (1967). Endo (1931; 32) studied the antagonism of several microorganisms towards Hypochnus centrifugus Tul. in vitro. These included 26 bacterial isolates and a few fungi. He found that several fungi including Aspergillus sp., Penicillium sp., Mucor sp., and a bacterium, Bacillus aroideae were strongly antagonistic towards Hypochnus centrifugus. Endo (1936) made elaborate studies on the anatgonism of several fungi towards Hypochnus sasakii Shirai. He observed the antagonistic effect of the

culture filtrates of Aspergillus niger van Tieghem, A. parasiticus Speare and A. tamarii Kita towards H. sasakii. In a comprehensive treatise on the sclerotial diseases of the rice plant, Endo (1937) discussed the antagonism of several microorganisms towards Corticium sasakii (Shirai) Matsumoto. He observed that a Bacillus sp., caused apical cell plasmolysis in the fungus. Many other workers have also documented the antagonistic effect of Bacillus sp., on R. solani (Hino, 1935; Olsen, 1965; Gokulapalan and Nair, 1984).

Tveit and Moore (1954) found that Chaetomium globosum Kunze and C. cochlioides Palliser were antagonistic to various fungi including Rhizoctonia sp.

Chandra et al. (1979) observed a number of bacterial isolates showing antifungal properties while making studies on the activities of nitrogen fixing organisms on the rice leaf. Fungi like Penicillium, Mucor, and Rhizoctonia were highly inhibited by these bacteria.

Sullia and Jayanthi (1979) found that bacteria and fungi isolated from the phylloplane of rice plants interact in the following manner. Drechslera oryzae (Breda de Haan) Subr. & Jain inhibited Curvularia lunata (Wakker) Boedijn, Alternaria tenuis Auct inhibited D. oryzae, Penicillium sp., inhibited C. lunata and both Xanthomonas oryzae (Uyeda & Ishiyama) Dowson and Pseudomonas sp., inhibited D. oryzae, C. lunata and A. tenuis.

Philip and Devadath (1980) found that two species of Aspergillus and one of Penicillium obtained from the phylloplane of rice plants were antagonistic to Xanthomonas oryzae in vitro.

Tschen and Kuo (1981) reported the inhibition and control of R. solani by Bacillus subtilis Cohn. by the production of antibiotics.

Bell et al. (1982) compared the in vitro interaction between seven isolates of Trichoderma harzianum Rifai and several pathogenic fungi and found a strong degree of antagonism towards R. solani. Turchetti (1982) reported the antagonism of a Bacillus sp. towards R. solani infecting Pinus nigra L. increasing the germination by controlling the pathogen.

Meshram and Jager (1983) observed that certain isolates of the free living, nitrogen-fixing bacterium, Azotobacter chroococcum Beijerinck exhibited antagonism against R. solani on agar plates. The degree of antagonism was found to vary strongly among the species and was found to be temperature dependant.

Rothrock and Gottlieb (1984) found that a soil actinomycete, Streptomyces hygroscopicus var. geldanus produced antibiotics having inhibitory action against Rhizoctonia solani.

Gokulapalan and Nair (1984) reported the inhibitory action of Trichoderma viride, Aspergillus niger, A. flavus and Rhizopus sp. on R. solani, the sheath pathogen affecting rice. Camprota (1985) tested 28 strains of Trichoderma against three strains of R. solani belonging to different anastomosis groups. he found that when compared with the action of volatile and non-volatile inhibitory substances, mycoparasitism was the best method for the destruction of hyphae of R. solani.

Islam and Nandi (1985) reported the antagonism of the common rice phylloplane inhabitant, Bacillus megaterium De Bary against the rice pathogens, Drechslera oryzae, Alternaria alternata (Fr.) Keissler and Fusarium roseum Link.

Turhan and Grossmann (1986) investigated the antatgonistic effects of some soil actinomycetes against a few soil borne plant pathogens. Of the 300 isolates tested, 17 per cent showed inhibition towards R. solani. Gupta et al. (1985) demonstrated the antagonism of Penicillum oxalicum Currie & Thom towards the sheath blight patogen.

VII STUDIES ON MYCOPARASITISM ON THE SHEATH BLIGHT PATHOGEN

All groups of fungi are known to be mycoparasitised (Lumsden, 1981). These are the necrotrophic or destructive

and the biotrophic type wherein balanced relationships occur between the host and the parasite (Barnett, 1963; Barnett and Binder, 1973).

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

Boosalis (1956) found that Penicillium vermiculatum could penetrate the hyphae of R.solani forming branches inside the host. Penetration and development of infection hyphae of Papulaspora sp. and Penicillium vermiculatum inside the hyphae of R.solani was noticed by Warren in 1948 and Boosalis in 1964. Dennis and Webster (1971) reported the penetration and coiling of several Trichoderma isolates on R.solani hyphae.

Gupta et al. (1979) found that Fusarium oxysporum parasitises R.solani causing coiling, penetration, lysis and chlamydospore formation inside the host.

The mycoparasitism of R.solani by Gliocladium virens leading to 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.

Arora and Dwivedi (1980) observed the penetration and coiling in and around the hyphae of R.solani by several species of Fusarium.

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 interacting with R.solani caused enzymatic digestion of the host cell walls. In response to invasion, the host produced a sheath matrix which encapsulated the penetrating hypha and the host cells became empty of cytoplasm.

The hyperparasitism of sclerotia of R.solani by Verticillium biguttatum Fr. has been reported by a group of workers from Netherlands (Velvis and Jager 1983; Jager and Velvis, 1984).

Chand and Logan (1984) reported the parasitism of R.solani by Penicillium cyclopium Westling, P.nigricans Bain, Gliocladium deliquescens Sopp. Fusarium culmorum (W.G. Smith) Sacc., F.moniliforme Sheldon, Epicoccum nigrum Link, Trichothecium roseum Link., Cylindrocarpon destructans (Zins.) Scholter and Cylindrocarpon olivaceum Cooke & Ellis.

Elad et al. (1984) found that the sclerotia of Sclerotium rolfsii Sacc. were parasitised by Trichoderma harzianum. The mycoparasite degraded the walls of sclerotial cells and the attacked cells lost their cytoplasmic contents.

Roy and Sayre (1984) conducted electron microscopic studies on the mycoparasitism of T.harzianum and T.viride on R.solani f.sp. sasakii. They observed that R.solani was parasitised by T.harzianum leading to the coiling of the mycoparasite on the mycohost and production of protruberances at certain points on the mycohost.

Wu et al. (1986) worked on the hyperparasitism of antagonistic species of Aspergillus, Penicillium and Trichoderma on anastomosis groups AG1 and AG4 of R.solani. There was no appreciable difference between the hyperparasitism on the two AG groups. Some isolates of T.harzianum could penetrate and erode the hyphae of R.solani besides coiling tightly around it. This was evident in natural field soils too.

Elad et al. (1987) observed that when the hyphae of the antagonist, T.harzianum approached those of R.solani they formed branches which grew directly towards the host. T.harzianum also produced hyphal coils over the interaction zone. Manibhushanrao et al. (1987) reported the mycoparasitism of Gliocladium virens and Trichoderma longibrachiatum Rifai on the rice sheath blight pathogen, R.solani.

Lewis and Papavizas (1987a) reported that the water extracts of young, actively growing hyphae of Trichoderma spp. and Glicocladium virens on bran medium, affected the growth of R.solani in liquid cultures. Leakage of compounds from mycelial mats of R. solani was induced after exposure to germling extracts of T.harzianum, T.hamatum or G.virens.

VIII BIOLOGICAL CONTROL OF RHIZOCTONIA SOLANI AND SAROCLADIUM ORYZAE

The surface of aerial plant parts provides a habitat for epiphytic microorganisms, many of which are capable of influencing the growth of plant pathogens in different ways. They may compete with the plant pathogens for the available nutrients, or directly attack them leading to different levels of mycoparasitism. These complex activities happening in the phylloplane have a profound influence on the course of

events in the infection of the host and is intimately related to the formulation of methods of disease control. Blakeman and Fokkema (1982) have exhaustively reviewed the potential for biological control of plant diseases in the phylloplane. As a microhabitat, the phylloplane exhibits strongly varying conditions in contrast with the rhizosphere. The irregular provision of surface water on leaves results in the intermittent growth of microorganisms, particularly bacteria and filamentous fungi.

The most exhaustively researched microorganism as a biocontrol agent can be considered to be Trichoderma spp., since the pioneering work of Weindling in 1932. He first demonstrated that T.viride was parasitic on and antagonistic to Rhizoctonia solani, an ubiquitous plant pathogen. The fungus was found to readily parasitise and kill the hyphae of R.solani.

Akai and Kuramoto (1968) found a 50 per cent reduction in infection by Cochliobolus miyabeanus (Ito and Kuribayashi) Drechsler on rice plants when the parasite was applied along with the phylloplane fungus Candida sp.

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 seeds 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 the rice sheath blight pathogen, R.solani emphasising its biological control using Trichoderma sp.

Biological control of R.solani affecting carnations was achieved by using the antagonistic fungus Trichoderma harzianum (Elad et al., 1981b).

Elad et al. (1981a) used T.harzianum for controlling R.solani causing black root rot of strawberries under field conditions. Disease control was improved when T.harzianum was applied after soil solarisation or fumigation with methyl bromide.

Chu and Wu (1980) found that species of Trichoderma, Penicillium and Aspergillus were efficient for the biological control of black scurf of potato caused by R.solani.

Tschen and Kuo (1981) reported that the coating of mung bean seeds with a culture of the bacterium, Bacillus megaterium could control the damping-off disease affecting the crop, caused by R.solani.

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

Chet et al. (1982) successfully controlled the soil and bulb borne pathogens of Iris, R.solani and Sclerotium rolfsii using Trichoderma 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). The damping off of snap beans caused by R.solani was effectively reduced by seed treatment using T.harzianum (Marshall, 1982).

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 under greenhouse conditions. Elad et al.

(1983) worked on different application techniques of a wheat bran culture of Trichoderma harzianum against R.solani, Sclerotium rolfsii, Pythium aphanidermatum (Eds.) Fritz, Aspergillus niger and Macrophomina phaseolina (Maubl.) Ashby. They found that the amount of Trichoderma preparation could be reduced by direct application of the biocontrol agent to the root zone of tomatoes. The broadcast application of the biocontrol agent was more effective than row application. They found that seed coating enabled the application of biocontrol agent at the most susceptible sites of the plant which was especially effective for controlling pre and post emergence diseases.

Nanda and Gangopadhyay (1983) while conducting studies on the survival of conidia of Bipolaris oryzae (Breda de Haan) Shoemaker isolated the bacterium Bacillus subtilis from soil and established its antagonistic effect on the fungus. They demonstrated that seed treatment with Bacillus subtilis enriched soil, followed by two foliar sprays with the bacterial suspension could prevent primary and secondary infection of brown spot disease at seedling and tillering stages.

Meshram and Jager (1983) found that an isolate of the free living, nitrogen-fixing organism, Azotobacter chroococcum effectively prevented the infection of potato

sprouts planted in a soil heavily infected with a pathogenic isolate of R. solani.

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 necrotrophic mycoparasite of R. solani which proved to be a valuable tool for the biological control of the soil borne plant pathogen. Van den Boogert and Jager (1983) reported that the addition of live mycelium of R. soalni to the soil markedly stimulated the growth of the hyperparasitic fungi Gliocladium roseum and Verticillium biguttatum.

Meshram (1984) reported that the inoculation of potato plants with Azotobacter chroococcum in combination with the mycoparasite Verticillium biguttatum effectively protected the sprouts against infection by R.solani. Van den Boogert and Jager (1984) found that inoculation of seed potatoes with Verticillium biguttatum, Gliocladium roseum , Trichoderma hamatum and Hormiactis fimicola Sacc.& March alone or in combination resulted in statistically significant reduction of infection of potato plants by R.solani.

Chand and Logan (1984) observed that Penicillium cyclopium Westling, Cylindrocarpon olivaceum Cooke & Ellis and Glicoladium roseum Link were the most effective fungi in

MATERIALS AND METHODS

-- [illegible] and were maintained on PDA by periodical sub-culturing. The identification of R.solani was confirmed

reducing the stem canker of potato caused by R.solani under controlled conditions.

Mew and Rosales (1984) reported the higher cellulolytic adequacy index of T.harzianum over R.solani thereby increasing its ability to decompose rice straw, thus affecting the survival of R.solani under natural conditions. They also found that two non-fluorescent bacterial isolates from the sclerotia of R.solani could reduce the incidence and severity of sheath blight significantly.

Venkatasubbaiah et al.(1984) found that Trichoderma harzianum was an effective biocontrol agent for R.solani, the incitant of collar rot of coffee seedlings.

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 preparation of Trichoderma and Gliocladium on populations of R.solani and the incidence of damping off in sugar beet. Mihuta and Rowe (1985) reported the fluid drilling of Trichoderma hamatum along with the seeds and a gel matrix 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.

Rabbinge et al. (1984) conducted comparative yield trials on winter wheat using phylloplane saprophytes, control of aphids and stimulatory effects on the phylloplane saprophytes. They found that the saprophytic fungi had no negative effects on yield and during one season there was a slightly positive effect on grain yield.

The biology, ecology and potential for biocontrol of Trichoderma and Gliocladium have been exhaustively reviewed by Papavizas (1985).

Parakhia and Vaishnav (1986) reported the biocontrol of Rhizoctonia batatícola (Taub.) Butler causing root rot of green gram using T.harzianum.

Sesan (1986) made studies on the conditions for mass culturing of the antagonists Trichothecium roseum Link and Gliocladium roseum Link for biological control of plant pathogens.

Sakthivel and Gnanamanickam (1986a,b) reported that bacterisation of rice plants with Pseudomonas fluorescens Migula reduced sheath rot infection.

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

Berberich (1987) reported the widespread use of biocontrol agents like Gliocladium and Trichoderma in the pellet form to achieve 75-95 per cent reduction of Rhizoctonia solani, a fungal pathogen affecting approximately 200 economically important crops.

MATERIALS AND METHODS

MATERIALS AND METHODS

I ISOLATION AND CULTURING OF FUNGAL PATHOGENS OF RICE LEAF SHEATH

Two of the important fungal pathogens causing serious diseases on leaf sheath of rice viz., Rhizoctonia solani Kuhn causing sheath blight and Sarocladium oryzae Gams & Hawksworth causing sheath rot were isolated from naturally infected rice plants collected from the paddy fields of the Instructional Farm, College of Agriculture, Vellayani. The sheath portions of infected plants showing characteristic symptoms of attack were cut into small bits, surface sterilised with 0.1 per cent mercuric chloride solution for two minutes and were repeatedly washed in three changes of sterile water. These bits were then planted over potato dextrose agar (PDA) (Appendix I) in sterile petri dishes and incubated under laboratory conditions (28±2°C) for 48 to 72h. The fungal growth starting from the inoculated bits were sub-cultured on to PDA slants. The isolates were purified by hyphal tip plating and were maintained on PDA by periodical sub-culturing. The identification of R.solani was confirmed by observing characters such as hyphal branching, septation of hyphae and sclerotial characters. The sheath rot causing fungus, Sarocladium oryzae was identified by observing the characters of the organism and conidial ontogeny on slide cultures (Riddel, 1950). The method for microscopic slide culture is detailed below.

Sterile plain agar medium was poured into sterilised petri dishes to a thickness of 2mm and after solidification, blocks of 6 mm square were cut out using a sterile needle. One such square was placed in the centre of a sterile microscope slide and all the four sides of the agar block were inoculated with small culture bits of the required isolate of the fungus. A cover slip was placed on top of the agar block and the slide was kept in a damp chamber (sterile petri dish with wet sterile filter paper in the bottom on which two glass rods were kept as support for the slide). The dish with the slide was then incubated at room temperature for two to three days. After this, the cover slip was lifted off gently, a drop of 95 per cent alcohol was placed in the centre and before drying, the cover slip was mounted using lactophenol cotton blue on another slide. The square of agar was removed from the original culture slide and another mount was prepared in a similar manner without any disturbance to the fungal growth on the slide. These slides were observed for the various morphological characters.

The pathogenicity of the isolates thus obtained was proved following Koch's postulates. Rice plants of the variety Jyothi were raised in earthern pots and inoculated by placing two uniform sized sclerotia of R.solani in between the leaf sheath and high humidity was provided by placing a

bit of moist cotton over it. The inoculated plants were kept under high humidity conditions for 48 to 72 h by giving periodical water sprays and covering with polythene bags. The fungus was then reisolated from the portions showing typical symptoms and maintained on PDA slants after purification by hyphal tip method. This pure culture of the fungus was used throughout the course of this study.

The sheath rot organism Sarocladium oryzae was cultured on paddy grains (Paromita Mukerjee et al., 1981) and these grains were used to inoculate healthy rice plants of variety Jyothi raised in earthen pots. A single grain with the inoculum was placed in between the sheath of the flag leaf and the unemerged panicle and a high percentage of humidity was maintained as in the earlier experiment. When the typical sheath rot symptons developed, the fungus was reisolated from the infected tissues and maintained on PDA. The pure culture of the sheath rot fungus thus obtained was used during the course of this study.

II EFFECT OF PLANT PROTECTION CHEMICALS ON THE PHYLLOPLANE MICROFLORA AND LEAF SHEATH PATHOGENS OF RICE.

A. Pot culture experiments

Experiments under pot culture conditions were conducted at the College of Agriculture, Vellayani, Kerala to study the effect of a few commonly plant protection chemicals

on the phylloplane microflora and the sheath blight and sheath rot diseases affecting the rice crop.

The details of the experiment were as follows.

Lay out - Completely rendomised design:

Variety - Jyothi and Karthika

Replication - Four

Treatment combinations - 12 x 2 = 24

The following commonly recommended plant protection chemicals for rice crop in Kerala were used for the study. (Kerala Agricultural University 1982).

Sl. No.	Common name	Chemical name	Dosage
1.	Carboxin Vitavax	5,6 dihydro-2-methyl-1,4 oxathiin-3-carboxanilide	500 g/ha
2	Edifenphos Hinosan	O ethyl S, S-diphenyl Phosphorodithioate	500 ml/ha
3	Mancozeb Dithane M-45	Manganese ethylene bis dithiocarbamate and Zinc ions	2kg/ha
4	Carbaryl Sevin	1-Napthyl-N Methyl carbamate	2.5kg/ha
5	Carbofuran Furadan	2,3-Dihydro 2,2 di methyl-7 benzofuranyl methyl carbamate	18 kg/ha

Treatment combinations

1. Control
2. Carboxin
3. Mancozeb
4. Edifenphos

5. Carbaryl
6. Carbaryl + Carboxin
7. Carbaryl + Mancozeb
8. Carbaryl + Edifenphos
9. Carbofuran
10. Carbofuran + Carboxin
11. Carbofuran + Mancozeb
12. Carbofuran + Edifenphos

The experiment was conducted in standard earthen pots uniformly filled with ten kg of wetland soil each, collected from the paddy fields of the Instructional Farm, College of Agriculture, Vellayani. Fertilisers were added to these pots as per the Package of Practices Recommendations for rice (Kerala Agricultural University, 1982). The soil was puddled thoroughly and twenty-day-old seedlings of the rice varieties, Jyothi and Karthika were transplanted in the pots at the rate of three seedling per clump. Three weeks later, the plants were inoculated with the sheath blight and sheath rot pathogens as described earlier. A week later, the plants were sprayed with the plant protection chemicals according to the schedule of treatments except carbofuran which was applied to the soil on the 25 day after transplanting, in the granular form. All the fungicides and carbaryl were once again sprayed on the 45 day after transplanting.

The phylloplane microflora were assessed one week after the application of the plant protection chemicals.

A leaf washing and dilution plate technique (Waksman, 1922) was used to study the qualitative and quantitative aspects of the microflora on the leaf surface. The leaf samples were collected from the experimental plants using sterile scissors and brought to the laboratory in fresh polythene bags. Every effort was made to avoid contamination in the field as well as in the laboratory. Each sample of ten leaves collected from each replicate hill was transferred aseptically to 250ml flasks containing 100 ml of sterile water and shaken for 20 min in a mechanical shaker to detach the propagules from the leaf surface. Samples of microflora were obtained by plating 0.5ml aliquots of leaf washings in 20ml of the respective agar medium in sterile petri dishes for each group of microorganisms. The media used were Rosebengal streptomycin agar (Appendix I) for fungi, nutrient agar (Appendix I) for bacteria, yeast extract malt extract agar (Appendix I) for yeasts, and Conn's glycerol asparaginate agar (Appexdix I) for actinomycetes. The petri dishes were incubated at 28 $\pm$ 2°C for 48 to 72 h in the case of bacteria, fungi and yeasts and for 10 to 14 days in the case of actinomycetes. After the incubation period, colony counts were made for each group of microorganisms. The microbial counts were expressed as number of colony forming (cfu) per cm^2 of leaf area by working out the average leaf area using an area meter (Li - 3000, LI - COR Ltd., Lincoln, U.S.A.).

The representative phylloplane microorganisms were maintained on their respective agar media slants for further studies. The fungal cultures were maintained on potato dextrose agar (PDA), the actinomycetes and bacteria on nutrient agar (NA) and the yeasts on yeast extract malt extract agar (YMA). Identification of fungi and bacteria was done tentatively referring to relevant literature and confirmed at the Commonwealth Agricultural Bureaux, International Mycological Institute, Kew, Surrey, England for fungi and at the Department of Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Trichur, Kerala for bacteria. The actinomycetes and yeasts were identified to the generic level by referring to relevant literature and following standard procedures, at the College of Agriculture, Vellyani.

The assessment of disease intensity was made following the procedure given in the "Standard Evaluation System for Rice Diseases." (IRRN, 1976) following the 0-9 scale. For sheath blight the assessment was made during three stages of crop growth, viz., active tillering stage, panicle initiation stage and 15 days prior to harvest. The percentage incidence was also recorded. Scoring for sheath rot disease was done at 15 days prior to the harvest of the crop. The percentage incidence of sheath rot was also recorded at this time.

The experiment was repeated twice and the pooled results were subjected to the analysis of variance test.

II B Field experiments

Considering the indications obtained from the pot culture experiments, field experiments were conducted at two localities, viz., Adoor (Pathanamthitta District), and Karamana (Trivandrum District), Kerala to study the effect of plant protection chemicals on foliar fungal pathogens and phylloplane microflora of rice at different localities.

B 1 Location I - Adoor

The details regarding the field experiment at Adoor were as follows:

Layout - Randomised block design

Variety - Jyothi

Season - Punja 1983 and Virippu 1984.

Replication - Three.

Treatment Combinations - Twelve

Plot size - 4.5 x $3m^2$

Location - Farmer's Field, Parakkode, Adoor, Pathanamthitta District, Kerala.

Treatment Combinations.

1 Control

2 Carboxin

3 Mancozeb

4 Edifenphos

5 Carbaryl

6 Carbaryl + Carboxin

7 Carbaryl + Mancozeb

8 Carbaryl + Edifenphos

9 Carbofuran

10 Carbofuran + Carboxin

11 Carbofuran + Mancozeb

12 Carbofuran + Edifenphos

Nursery

The seedlings of rice variety Jyothi were raised in a wet nursery in an area of $40m^2$. Twenty one day old seedlings were used for the experiments.

Main field

The crop was raised following Package of Practice Recommendations (Kerala Agricultural University, 1982).

Carbofuran was applied to the soil 25 days after transplanting. The fungicides and carbaryl were sprayed twice on the 30 and 45 day after transplanting of the crop. The observations on phylloplane microflora were made ten days after the fungicidal and insecticidal treatments, following the methodology already described. The observations on sheath blight incidence and intensity were recorded during the active tillering stage, panicle initiation stage and 15 days prior to harvest as described earlier. The observations on sheath rot incidence and intensity were made 15 days prior to harvest of the crop. After the harvest of the crop, the grain and straw yields of the crop were recorded plot wise.

The data obtained for the replicated experiments were pooled and analysis of variance was worked out. The effect of the commonly used plant protection chemicals on the frequently isolated phylloplane inhabitants of the rice plant was also worked out. The frequency of occurence of the phylloplane fungi on the treated leaves of the control plants was estimated as qualitative part of the microbial population studies. These values are presented as percentage increase or decrease over control.

B ii Location 2. Karamana

Field experiments were conducted at the Cropping Systems Research Centre (CSRC), Karamana, Trivandrum, Kerala to assess the effect of common plant protection chemicals on foliar fungal pathogens and phylloplane microflora of rice. The trials were conducted during the Punja Season of 1983 and Mundakan season of 1984. The details regarding these experiments are given below.

Layout	- Randomised block design
Variety	- Jyothi, Karthika
Season	- 1983 Punja, 1984 Mundakan
Replication	- 3
Plot size	- 4.5 x $3m^2$
Treatment Combinations	- 12 x 2 = 24
Location	-CSRC, Karamana, Trivandrum, Kerala.

The treatment combinations were the same as those of the earlier field trial, the only difference being that at this location one more variety, Karthika, was also included. This rice variety has been considered to be tolerant to sheath blight disease of rice. The crop was raised as per the Package of Practices Recomendations as described earlier. The application of plant protection chemicals and all the observations were recorded as in the previous field trial.

The estimation of disease incidence and yield loss due to sheath blight disease was worked out following Hashiba's method (Hashiba, 1984). According to this method, the whole disease incidence (D) at the maturing stage could be estimated by the relative height of the uppermost lesions to the plant height (X) and the percentage of affected hills (A) which can be calculated as

$D = (1.62X - 32.4)A/100$

The loss of fully ripened Kernel(L) owing to the incidence of sheath blight was worked out using the following equation

$L = (41.31X - 826.2)A/1000$

The data obtained during the two seasons were pooled and analysis of variance tests were done to interpret the results.

The qualitative assessment of the commonly occuring

rice phylloplane fungi was recorded for the rice varieties Jyothi and Karthika at this location and the percentage of increase or decrease over control was compared.

III IN VITRO STUDIES ON PHYLLOPLANE MICROORGANISMS OF RICE.

A. Fungi

The commonly occurring phylloplane fungi of rice plants obtained during the course of the previously detailed trails were maintained on PDA slants. A list of these fungi is presented in Table No.23a.

These fungi were grown on PDA in petri dishes and incubated at room temperature (28 $\pm$ 2°C) for 10 to 14 days. Observations were made on colony diameter, colony colour and pigmentation. The mycelial forms forming conidia were studied in detail using slide cultures (Riddel, 1950). Observations were made on the conidial dimensions and camera lucida drawings were made wherever possible. The enumerated characters were compared with those in relevant literature and tentative identification was made. This was later on confirmed as mentioned earlier.

B Bacteria

The bacterial cultures obtained from leaf washings and dilution plating were maintained on nutrient agar. Some of the preliminary tests for the indentification were conducted in this laboratory also. These included tests for

gram reaction, motility, oxygen requirement, colony characters, cell morphology and catalase test following standard laboratory procedures as under the Laboratory Manual of Microbiology (Seeley and Vandemark, 1970) and are outlined below.

1. Colony Characters

The colony characters of the phylloplane bacteria were studied by plating them on nutrient agar. The bacterial cultures were streaked on nutrient agar poured in petri dishes and incubated at 28 $\pm$ 2°C. After 48 to 72 h observations were made on the growth of the colonies and colony shape.

2. Gram staining'

The smear of the test bacterium was prepared and fixed on a clean microscopic slide. It was stained with crystal violet stain (Appendix II) for about 30 seconds after which it was rinsed off with water. The smear was then flooded with Gram's iodine (Appendix II) and allowed to react for 30 seconds after which it was again rinsed off with water. After this the preparation was decolorised with 95 per cent ethanol, rinsed with water and counterstained with safranin (Appendix II) for 30 seconds. The smear was then rinsed with water, blotted dry and examined under oil immersion objective of a student (Bausch and Lomb) microscope.

3. Motility

The motility of bacterial cultures was tested using the stab culture method. The stab cultures were carried out in a semi solid agar (Appendix VI). Test tubes containing sterile melted nutrient agar was cooled in an upright position for conducting this test. When the medium was cooled, it was inoculated by thrusting the inoculation needle containing the bacterial culture through the centre of the medium to the bottom of the tube. Incubation was done for six days at 28 $\pm$ 2°C unless positive results were obtained sooner. The motile organisms showed a diffuse zone of growth spreading from the line of inoculation.

4. Catalase Test

The bacterial cultures were inoculated in nutrient broth (Appendix I) and incubated at room temperature 28 $\pm$ 2°C for 24 to 48 h. A few drops of 3 per cent H_2O_2 were added to the broth cultures and the production of the oxygen bubbles and surface froth accumulation, if any, was observed.

5. Oxygen requirement

For this test, tubes containing deep agar (nutrient agar poured to a depth of 10 to 12 cm) was inoculated while in the fluid condition at about 45°C with an inoculum not too heavy to permit discrete colonies and rotated to mix the inoculm with the medium and cooled. After incubation at 28 $\pm$ 2°C for 24 to 48 h the strict aerobes were found to grow

upon the surface and the upper layers only while the strict anaerobes grew only in the depths of the medium.

C. Actinomycetes

The actinomycetes commonly isolated from the phylloplane of rice plants were maintained on nutrient agar slants. The cultures were inoculated on nutrient agar in petri dishes and incubated at room temperature 28 ± 2°C for 10 to 14 days after which observations were made on the soluble pigment production in the medium.

The method of Waksman (1919) was followed for the study of characters of the actinomycetes. A drop of melted nutrient agar medium was placed on a microscopic glass slide and allowed to cool to 45°C and inoculated with the actinomycete culture. The agar medium was then spread in a thin film on the slide and incubated in a sterile moist chamber prepared using a sterile petri dish containing a moistened filter paper at its bottom and a U shaped glass rod for support. The slides were incubated for 48 to 72 h at room temperature (28 ± 2°C). The preparations were then dry fixed using alcohol (95% ethanol) and stained using crystal violet (Appendix II). The entire colony with both vegetative and aerial mycelium could thus be observed.

D. Yeasts

Several yeasts isolated from the phylloplane of rice plants were studied for their important morophological and

cultural characters based on the methods formulated by Lodder (1974). The yeast cultures were maintained on yeast malt extract medium (YMA).

In order to study the cell morphology, the cells from a young, actively growing culture were inoculated into 30 ml of 2 per cent glucose-yeast extract peptone water (Appendix I) in 100 ml Erlenmeyer flasks. After 48 to 72 h incubation at 28 $\pm$ 2°C the culture was mounted in lactophenol cotton blue and examined microscopically. The length and width of cells were measured and the extreme values obtained from the measurement of fifty cells were recorded.

The colony colour was observed by plating the cultures on YMA which was freshly poured into sterile petri dishes and set aside for 24 to 48 h for allowing the surface to dry. A single streak was made near a side of the petridish and were examined after incubation for three to four days.

Mycelium production in yeast cultures was studied using the following method (Lodder 1974). A petri dish containing a U shaped glass rod support on which two glass slides are placed was sterilised by dry heat at 180°C for two hours. YMA was melted and poured into another sterile petri dish. The glass slides were removed from the glass rod with a flame sterilised pair of forceps, dipped into the agar and replaced on the glass rod support.

After solidification of agar on the slides the yeast was inoculated in three lines on each slide and a sterile cover slip was placed over the central portion of the lines. A few drops of sterile water were poured into the petri dish to prevent the agar from drying out. After 24 to 48 h the slides were taken out of the petri dish and the agar was wiped off the back of the slide and the areas of inoculation lines under and around the cover slip were studied.

The ballistospore production in the yeast cultures was also studied by means of slide cultures. For this purpose only one of the slides used in the preparation of the slide culture was covered with YMA. It was inoculated and placed upside down over the second slide with an U shaped glass rod in between. After incubation at 28 $\pm$ 2°C for 24 to 48 h, both the slides were microscopically examined. If there was ballistospore formation, the lower of the two slides showed the images of the inoculation lines on the upper slide. The discharged ballistospores fell on the bottom slide forming a mirror image.

IV STUDIES ON *IN VITRO* ANTAGONISM OF PHYLLOPLANE MICROORGANISMS AGAINST THE SHEATH BLIGHT PATHOGEN (R.SOLANI)

The fungi, bacteria, yeasts and actinomycetes isolated from the leaves of rice plants were paired separately on PDA medium to study colony interactions with *R.solani*. Methods

outlined by Skidmore and Dickinson (1976) were followed for studying interactions of R.solani with phylloplane fungi. Agar blocks (3 mm diameter) containing seven day old growth of mycelia of both R.solani and the fungi were placed 3.5 cm apart on PDA in a petri dish and incubated at 30°C for 12 days. Three replicates were maintained for each treatment. The paired cultures were examined at regular intervals for 12 days and the nature of the reactions was noted.

The method for testing *in vitro* antagonism of bacteria and yeasts against R.solani was adapted from similar studies by Utkhede and Rahe (1983) and Fokkema (1973) respectively. The test organisms were either singly streaked at a spacing of 3.5 cm from R.solani or streaked on either side of the centralised R.solani inoculum placed upon PDA in standard sized petri dishes (90 mm diameter). The paired cultures were examined after incubation at 28 ± 2°C for 48 to 72 h and the nature of reactions were noted.

The actinomycetes isolated from the phylloplane were tested for their antagonism towards R.solani using the cross streak assay method followed by Ahmed and Ahmed (1963). The actinomycete was streaked at a spacing of 3 cm from the test fungus inoculum placed on PDA in standard sized petri dishes (90 mm diameter) and incubated at room temperature (28 ± 2°C) for 48 to 98 h. Observations on colony interactions were then recorded.

V MYCOPARASITISM OF SELECTED PHYLLOPLANE FUNGI ON R.SOLANI

In order to study the mechanism of mycoparasitism of some of the rice phylloplane fungi on R.solani a dual culture technique of Dennis and Webster (1971) was used. In 90 mm sterile petri dishes, sterile PDA was poured and allowed to solidify. Sterilised 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 five mm diameter containing the mycelium of R.solani taken from an actively growing culture of the fungus was placed on one end of the petri dish and a five mm agar disc of the test fungus was placed two cm away from it. The plates were incubated at 28 $\pm$ 2°C for three to seven days. Direct observations were carried out after incubation period under a light microscope at the zone of hyphal contact. Microscopic observation for hyphal interaction was also made by cutting out one cm^2 portions of cellophane containing intermingling hyphal growth and mounting in glycerine. The different mechanisms of mycoparasitism exhibited by the efficient antagonists of R.solani were photomicrographed using an Olympus PM-6 Camera.

An attempt was also made to quantify the results of hyphal interaction. Interacting hyphae which coiled or penetrated or showed other types of mycoparasitic reactions with host hyphae were termed affected hyphae and their

percentage was calculated following the formula of Arora and Dwivedi (1980).

$$\text{Percent hyphal Interaction} = \frac{\text{No. of affected hyphae in a microscopic field}}{\text{Total no. of hyphae in a microscopic field}} \times 100$$

VI BIOASSAY OF COMMON PLANT PROTECTION CHEMICALS ON THE ANTAGONISTIC PHYLLOPLANE MYCOFLORA R.SOLANI

Seven day old cultures of the test fungi grown on PDA in petri dishes were used for this assay. The required concentration (500 and 1000 ppm) of the plant protection chemicals, viz., carbaryl, carboxin, edifenphos and mancozeb were prepared by adding appropriate quantities of the chemicals into the autoclaved (1.2 kg/cm^2 for 30 minutes) PDA cooled to 45°C. They were thoroughly mixed by gently swirling the flasks. The poisoned medium was poured aseptically into sterile petri dishes and five mm mycelial discs of the test fungi were placed in the centre of each dish. In the case of the control, non poisoned PDA was used and inoculated with the mycelial disc. The mean diameter of the radial growth of the test fungi was recorded after three days. The method adopted for this bioassay was modified version of the poisoned food technique described by Lilly and Barnett (1951).

VII EVALUATION OF THE EFFICACY OF FEW MYCOPARASITES OF R.SOLANI IN CHECKING THE SHEATH BLIGHT DISEASE

Pot culture trials were conducted at the College of

Agriculture, Vellayani to study the comparative efficacy of some of the phylloplane antagonists of R.solani in checking sheath blight of rice. Details regarding the trial are given below.

Design - Completely Randomised Design

Replication - Four

Treatments

1. Aspergillus aculeatus
2. Penicillium oxalicum
3. Trichoderma harzianum
4. Trichoderma viride
5. Carboxin
6. Control

Rice plants of variety Jyothi and Karthika were raised in earthen pots as described earlier. The fungi used included Trichoderma harzianum, T. viride, Penicillium oxalicum and Aspergillus aculeatus. These fungi were mass cultured on wheat bran as described under VIII. The mycoparasite inoculum was added to the soil in the experimental pots, fifteen days after planting of the crop. The fungicide, carboxin (2g/L) was sprayed on the 30 and 45 day after planting. The experimental plants were inoculated with the sclerotia of R.solani as described earlier, twenty days after planting.

Observations were made on the disease incidence, intensity, plant height and maximum height of sheath blight lesions on the plant. The trial was repeated once to confirm the results.

VIII EFFICACY OF DIFFERENT MYCOPARASITE INOCULA IN REDUCING SHEATH BLIGHT DISEASE

A study was conducted to find out the best type of mycoparasite inoculum suited for the biological control of sheath blight of rice. The different types of inocula used were spore suspension, mycelial suspension, rice bran culture and wheat bran culture. The spore suspension was prepared from ten-day old cultures of the test fungus Trichoderma harzianum on PDA in petri dishes. It was estimated to contain 10^7 colony forming units per ml (Bhat and Vaughan, 1962). The mycelial suspension was prepared by blending thoroughly the mycelial growth of Trichoderma harzianum on PDA in a waring blender and suspending the blended mycelium in the required quantity of sterile water (Chand and Logan, 1984). The mycelial growth of the fungus was prepared in 250 ml conical flasks containing sterile potato dextrose broth, inoculated with the fungus. The antagonistic isolate of T. harzianum was grown on wheat bran/rice bran, tap water (1:2) mixture which was autoclaved for 1 hour at a pressure of 1.2 Kg/cm^2 for two successive days, (Elad et al., 1983). Erlenmeyer flasks (500 ml)

containing these media inoculated with the test fungus were incubated at room temperature for 8 to 10 days.

The experimental plants were raised in earthen pots as described earlier. The rice variety used was Jyothi, a sheath blight susceptible variety. The rice bran/wheat bran preparations were added to the soil at the rate of 250 g/pot, fifteen days after transplanting. The experimental plants were inoculated with R.solani as described earlier, twenty days after planting. The treatments were replicated six times. The spraying of the spore suspension and mycelial suspension were done twice, the initial spray was given thirty days after planting and the next spray was given fifteen days later. Observations on the disease incidence and intensity were made 60 days after planting and the effect of different types of inoculum was assessed.

RESULTS

RESULTS

I FUNGAL PATHOGENS OF LEAF SHEATH OF RICE

A Sheath Blight Pathogen

Rhizoctonia solani Kuhn

The fungus was isolated and purified from naturally infected rice plants collected from the paddy fields of the Instructional Farm, College of Agriculture, Vellayani. The identity of the organism was studied and confirmed by microscopic examination of morphological characters and the pathogenicity was established following Koch's Postulates.

The disease causes spots on the leaf sheath. The spots were at first ellipsoid or ovoid, somewhat irregular, greenish gray varying from 1 to 3 cm in length. The centre of the spot became grayish white, with a brown margin. Sclerotia were formed on or near these spots, but were easily detached. In the field the spots were usually observed first near the water level. When conditions were favourable to the pathogen, they were later formed on the upper leaf sheath and on the leaf blades also. The presence of several large spots on a leaf sheath usually caused the death of the whole leaf, and in severe cases all the leaves of a plant were blighted in this way. Under hot, humid conditions, most of the leaves of an affected rice plant were killed by the infection.

B Sheath Rot Pathogen

Sarocladium oryzae Gams & Hawksworth

(= Acrocylindrium oryzae Saw.)

The fungus was isolated and purified from naturally infected rice plants collected from the paddy fields of the Instructional Farm, College of Agriculture, Vellayani. The identity of the organism was established by the study of morphological characters and the pathogenicity was confirmed following Koch's Postulates.

The sheath rot disease of rice was characterised by initiation of light purplish to brown oblong lesions on the sheath of the flag leaf. The young lesions were surrounded by a light yellow-brown halo, which on maturity turned dark brown with papery white or greyish white centre. The lesions were usually 0.5 to 2.5cm long and 0.5 to 1.5cm broad. The individual lesions coalesced together in advanced stages of infection and covered almost the entire sheath of the flag leaf. Often the panicles did not emerge leading to a choked appearance. The entire panicles remained choked within the flag leaf sheath and gradually rotted. Depending on the stage of infection of the plant, different stages of partially emerged panicles with discolored and fully or partially chaffy grains were noticed in the affected plants.

II EFFECT OF PLANT PROTECTION CHEMICALS ON PHYLLOPLANE MICROFLORA AND LEAF SHEATH PATHOGENS OF RICE

A Pot culture experiments

a Effect of plant protection chemicals on phylloplane microflora

The saprophytic mycoflora was found to be significantly higher in the leaves of rice variety Jyothi when compared with Karthika (Table 1). In general, the application of chemicals suppressed the mycoflora significantly on comparison with the control. The fungicide carboxin when applied in combination with insecticide, carbofuran did not cause any significant difference in the population of the phylloplane mycoflora. The fungicide edifenphos brought significant reduction in the phylloplane mycoflora of rice plants when applied by itself or in combination with the insecticides, carbaryl or carbofuran.

The bacterial population was also significantly higher on the leaves of rice variety Jyothi when compared with Karthika (Table 2). Initially, the fungicides brought about depressing effect on the phylloplane bacteria while the insecticide carbaryl caused an increase in the bacterial population on the phylloplane of both the rice varieties. Generally the bacterial counts were found to be higher during the second observation for all treatments. Carbofuran did

Table No.1 Effect of common plant protection chemicals on phylloplane mycoflora of rice at two stages of growth (cfu per cm^2 of leaf area).

Treatments	JYOTHI		KARTHIKA	
	Obs 1	Obs 2	Obs 1	Obs 2
Control	5.1240	6.4992	2.9334	5.2307
Carboxin	4.0626	4.8277	3.0261	3.3117
Mancozeb	3.3093	4.3395	3.4139	3.7035
Edifenphos	4.0789	4.0192	3.3357	4.8287
Carbaryl	3.8443	5.1144	3.3503	5.0831
Carbaryl + Carboxin	3.8074	4.2449	3.5249	5.9171
Carbaryl + mancozeb	3.5014	3.5678	3.7056	2.8650
Carbaryl + edifenphos	4.5796	5.2773	3.3156	3.5706
Carbofuran	4.7893	4.2773	2.9908	5.7391
Carbofuran + carboxin	4.3612	5.6813	3.3286	5.1044
Carbofuran + mancozeb	0.2653	4.9171	3.1312	3.7394
Carbofuran + edifenphos	3.3797	3.1125	3.8585	4.8565
C.D. (5%)	0.9034	0.8872	0.9034	0.8872

(Values after $\sqrt{x}$ transformation)

Obs 1 37 days after planting

Obs 2 52 days after planting

Table No.2 Effect of common plant protection chemicals on population of bacteria on phylloplane of rice at two stages of growth (cfu per cm^2 of leaf surface).

Treatments	JYOTHI		KARTHIKA	
	Obs 1	Obs 2	Obs 1	Obs 2
Control	15.2321	13 0860	10.4086	11.8113
Carboxin	6.2023	13.1384	4.3961	12.1325
Mancozeb	8.0631	15.0863	4.9397	15.0247
Edifenphos	14.9667	14.8451	9.6361	16.2824
Carbaryl	17.1873	17.7115	12.0549	14.6201
Carbaryl + carboxin	7.2458	12.7513	8.8801	12.3249
Carbaryl + mancozeb	8.5018	7.3511	6.5815	6.6851
Carbaryl + edifenphos	16.9411	15.1395	6.3918	12.0550
Carbofuran	14.6487	12.1793	11.1957	9.2319
Carbofuran + carboxin	8.8801	11.8499	9.9666	7.1555
Carbofuran + mancozeb	7.5815	7.4847	8.4099	6.4875
Carbofuran + edifenphos	14.4266	17.4484	7.3915	15.4848
C.D. (5%)	1.1470	1.7845	1.1470	1.7845

(Values after $\sqrt{x}$ transformation)

Obs 1 37 days after planting

Obs 2 52 days after planting

not have any effect on the bacterial population on the phylloplane, when applied alone.

The population of yeasts on the phylloplane of rice plants did not show any significant difference between the two varieties, Jyothi and Karthika (Table 3). Carboxin by itself did not cause any significant change in the population of phylloplane yeasts in the rice variety Jyothi while they were suppressed by the application of the fungicide in the rice variety, Karthika. Of the two insecticides, carbaryl caused considerable reduction of yeasts during both he observations on both the varieties, while carbofuran did not have any significant effect on the phylloplane yeasts.

b Effect of plant protection chemicals on incidence and intensity of sheath blight.

The intensity of sheath blight disease of rice was found to be significantly lower in the variety Karthika when compared with Jyothi while the percentage of incidence of sheath blight did not register any significant difference (Table 4,5). The incidence and intensity of sheath blight were found to be significantly reduced by the application of carboxin or edifenphos. The effect of these fungicides was found to be enhanced by their combined application with the insecticides carbaryl or carbofuran. The disease incidence and intensity registered an increasing trend as the crop

Table No. 3 Effect of common plant protection chemicals on phylloplane flora of yeasts on rice at two stages of growth (c fu per cm^2 of leaf area).

TREATMENTS	JYOTHI		KARTHIKA	
	Obs 1	Obs 2	Obs 1	Obs 2
Control	4.3070	4.5440	4.5907	4.2319
Carboxin	5.2694	4.1617	3.5178	3.6497
Mancozeb	4.5403	3.8508	3.1999	3.2526
Edifenphos	3.1459	3.1732	3.4503	3.3141
Carbaryl	3.0500	3.4983	3.6591	3.5933
Carbaryl + carboxin	3.7047	3.1856	4.5097	4.2319
Carbaryl + mancozeb	3.1436	4.3703	3.8852	3.9465
Carbaryl + edifenphos	3.3954	4.0820	2.8788	2.9465
Carbofuran	4.3261	4.6733	4.6519	4.0470
Carbofuran + carboxin	2.4793	2.9753	2.9776	3.1005
Carbofuran + mancozeb	2.3911	3.4483	3.9081	2.8333
Carbofuran + edifenphos	2.5403	2.7035	2.9964	3.4952
C.D. (5%)	0.3932	0.3957	0.3932	0.3957

(Values after $\sqrt{x}$ transformation)

Obs 1 37 days after planting

Obs 2 52 days after planting

Table No.4 Effect of common plant protection chemicals on intensity of sheath blight at three stages of growth of the rice plant.

TREATMENTS	JYOTHI			KARTHIKA		
	AT	PI	15 DPH	AT	PI	15 DPH
Control	3.1666	4.8333	6.4103	1.7767	1.8333	3.2223
Carboxin	1.1110	0.5623	0.5556	0.2223	0.2136	0.3691
Mancozeb	2.1667	1.7780	2.7913	0.7767	0.7913	1.5856
Edifenphos	1.4443	0.7290	1.0136	0.5690	0.2223	0.5691
Carbaryl	2.2220	1.9440	3.6667	0.5023	1.0066	1.3223
Carbaryl + carboxin	1.3330	0.3956	0.6176	0.1556	0.3956	0.1443
Carbaryl + mancozeb	2.6667	1.8333	2.4446	0.5136	0.8956	1.5566
Carbaryl + edifenphos	1.7770	0.5690	0.8473	0.2223	0.3956	0.1567
Carbofuran	2.7760	2.7910	2.2780	1.2776	0.6110	0.8443
Carbofuran + carboxin	1.2223	0.2776	0.5690	0.1556	0.3956	0.1443
Carbofuran + mancozeb	2.7913	0.9770	2.5556	0.6733	1.0066	1.4467
Carbofuran + edifenphos	1.2776	0.7776	0.5223	0.1756	0.3843	0.1333
C.D. (5%)	0.6257	0.7787	0.5611	0.6257	0.7787	0.5611

AT Active tillering

PI Panicle Initiation

DPH Days prior to harvest

Table No.5 Effect of common plant protection chemicals on the percentage incidence of sheath blight at three stages of growth of the rice plant.

TREATMENTS	JYOTHI			KARTHIKA		
	AT	PI	15 DPH	AT	PI	15 DPH
Control	46.3390	54.2444	58.4275	44.9819	44.9819	54.7318
Carboxin	23.1928	24.5040	18.9473	27.8069	31.5284	31.5284
Mancozeb	38.4939	34.7724	24.5040	28.8446	41.5284	45.2500
Edifenphos	31.5284	31.5284	38.4939	24.5631	31.5284	37.8069
Carbaryl	44.9819	48.2258	58.1913	31.6474	41.6424	48.4939
Carbaryl + carboxin	21.6774	22.0660	19.0295	19.2095	21.6284	29.2964
Carbaryl + mancozeb	39.9939	51.4698	38.0164	31.5284	35.2500	41.7379
Carbaryl + edifenphos	27.8069	28.2257	27.8069	25.3689	24.0854	34.7724
Carbofuran	38.5284	41.7379	51.4698	34.6474	38.4939	38.4939
Carbofuran + carboxin	21.6474	22.0660	27.8069	21.6474	23.3849	19.2905
Carbofuran + mancozeb	38.4939	51.4698	55.1913	31.5284	37.8669	38.4939
Carbofuran + edifenphos	21.6474	19.2095	21.6474	19.2095	24.0854	24.5040
C.D. (5%)	8.9320	9.4171	10.7100	8.9320	9.4171	10.7100

(Values after angular transformation)

AT Active tillering

PI Pancile initiation

DPH Days prior to harvest

advanced from the active tillering stage to the harvesting stage in the case of control plants. The percentage incidence of sheath blight did not increase appreciably as the crop aged, with the application of carboxin by itself or in combination with carbaryl or carbofuran. The intensity of sheath blight was reduced as the crop aged from active tillering to panicle initiation stage by the application of carboxin with or without carbaryl or carbofuran indicating the therapeutic effect of carboxin.

c Effect of plant protection chemicals on incidence and intensity of sheath rot.

The rice variety Karthika was found to be significantly tolerant to sheath rot disease in comparison with the rice variety Jyothi (Table 6). Carboxin or edifenphos applied in combination with the insecticide carbofuran could reduce the incidence and intensity of sheath rot significantly. The application of these fungicides alone could cause significant reduction in the incidence and intensity of sheath rot disease when compared with the untreated control.

Table No.6 Effect of common plant protection chemicals on percentage incidence and intensity of sheath rot disease of rice.

TREATMENTS	JYOTHI		KARTHIKA	
	Intensity	Incidence	Intensity	Incidence
Control	4.556	(68.1913)	2.5556	(60.7859)
Carboxin	0.6223	(19.2095)	0.1443	(19.2095)
Mancozeb	1.3336	(27.8069)	0.8899	(33.7724)
Edifenphos	0.7223	(31.5284)	0.4430	(21.6474)
Carbaryl	2.5556	(38.0164)	1.6670	(44.9819)
Carbaryl + carboxin	0.7223	(21.1444)	0.4890	(21.6474)
Carbaryl + mancozeb	1.3333	(34.7722)	0.8333	(34.9819)
Carbaryl + edifenphos	0.9743	(27.8069)	0.5433	(24.6474)
Carbofuran	2.2223	(38.4936)	0.9443	(34.7729)
Carbofuran + carboxin	0.2223	(19.2095)	0.1443	(19.2095)
Carbofuran + mancozeb	1.5556	(27.8094)	0.6553	(38.4939)
Carbofuran + edifenphos	0.4556	(19.2095)	0.1443	(19.2095)
C.D. (5%)	0.3099	6.8723	0.3099	6.8723

Values in parentheses after angular transformation

B Field Experiments

1 Location I - Adoor

a Effect of plant protection chemicals on phylloplane microflora

The fungicides, mancozeb and edifenphos could cause considerable reduction in the population of phylloplane mycoflora (Table 7). This suppressive effect on the phylloplane fungi was often enhanced by the combined application of the fungicides with the insecticides carbaryl or carbofuran. Carboxin when applied alone did not cause any significant change in the phylloplane mycoflora but when the fungicide was applied in combination with carbofuran significant increase in the phylloplane mycoflora was noted during the second post treatment observation. The plants were found to harbour more phylloplane fungi as they aged.

The fungicides, edifenphos and mancozeb were found to exhibit significant suppressive effect on the phylloplane bacteria when applied alone or in combination with the insecticides carbaryl or carbofuran (Table 7). The insecticides carbaryl and carbofuran by themselves reduced the phylloplane bacteria. Carboxin was found to cause no suppressive effect on the phylloplane bacteria after the initial spray, leading to a significant increase in the

Table No.7 Effect of plant protection chemicals on phylloplane microflora of rice (Location I).

TREATMENTS	Post treatment 1		Post treatment 2	
	Fungi	Bacteria	Fungi	Bacteria
Control	8.7715	19.3608	9.2528	19.6023
Carboxin	7.2825	16.2518	7.4132	22.4220
Mancozeb	6.4073	8.7769	6.2843	9.6613
Edifenphos	6.8734	4.1578	6.1090	11.6126
Carbaryl	5.4770	12.0692	7.2266	16.7699
Carbaryl + carboxin	9.4735	10.8850	8.8184	13.7106
Carbaryl + mancozeb	5.3604	11.2791	5.2688	16.7460
Carbaryl + edifenphos	5.0275	9.9059	6.6185	10.8016
Carbofuran	6.7102	11.8552	5.6194	18.5375
Carbofuran + carboxin	8.9165	18.6812	12.7172	16.5044
Carbofuran + mancozeb	4.1872	12.8003	6.5672	11.3933
Carbofuran + edifenphos	4.4268	10.1306	4.0022	11.1908
C.D (5%)	1.8463	3.5018	2.1182	2.1969

(Values after $\sqrt{x}$ transformation)

population of the phylloplane bacteria after the second spray. The population of phylloplane bacteria did not vary considerably with the age of the plant.

b Effect of plant protection chemicals on incidence and intensity of sheath blight disease.

The incidence and intensity of sheath blight disease were found to increase considerably with the age of the plants (Table 8,9). The fungicides, carboxin and edifenphos were found to be significantly superior to mancozeb in reducing the incidence and intensity of sheath blight at all the three stages at which observations were made. The insecticides carbaryl and carbofuran could cause significant reduction in the incidence and intensity of sheath blight disease, though not comparable with the effect of the fungicides. Carboxin and edifenphos when applied in combination with the insecticides carbaryl or carbofuran were found to be effective in reducing the incidence and intensity of sheath blight significantly. Mancozeb could also reduce the disease incidence and intensity considerably over control though this was not comparable with the effect of carboxin or edifenphos. In the plots treated with carboxin there was a decrease in the incidence of sheath blight at the panicle initiation stage indicating the therapeutic value of the treatment.

Table No.8 Effect of common plant protection chemicals on the intensity of sheath blight disease in rice (Location I)

	AT	PI	15 DPH
Control	2.4223	3.2870	4.5507
Carboxin	0.3120	0.3253	0.6307
Mancozeb	1.6027	1.3070	1 9140
Edifenphos	0.5260	0.4180	1.1587
Carbaryl	1.6313	2.0320	3.1417
Carbaryl + carboxin	0.4030	0.4943	0.7567
Carbaryl + mancozeb	0.5120	0.9623	1.9600
Carbaryl + edifenphos	0.5340	0.5280	1.4080
Carbofuran	0.5193	1.4303	2.0287
Carbofuran + carboxin	0.1367	0.6673	0.7817
Carbofuran + mancozeb	1.0682	0.8637	2.5800
Carbofuran + edifenphos	0.1693	0.7423	0.5373
C.D. (5%)	0.3237	0.3575	0.4192

AT Active tillering

PI Panicle initiation

DPH Days prior to harvest

Table No.9 Effect of plant protection chemicals on the percentage incidence of sheath blight disease in rice (Location I).

	AT	PI	15 DPH
Control	56.4219	62.0709	65.8207
Carboxin	23.0804	15.2751	20.7696
Mancozeb	36.8075	33.6150	45.1382
Edifenphos	23.1039	22.4966	31.8015
Carbaryl	30.6147	33.8801	35.3434
Carbaryl + carboxin	20.7964	19.3691	19.2142
Carbaryl + mancozeb	36.1517	39.6253	31.2153
Carbaryl + edifenphos	25.9780	23.2343	25.5378
Carbofuran	42.7464	50.3300	45.5125
Carbofuran + carboxin	27.8800	16.6210	38.3304
Carbofuran + mancozeb	40.2309	28.9124	55.9713
Carbofuran + edifenphos	28.6397	22.4966	47.7732
C.D. (5%)	5.1663	6.2782	5.4405

(Values after angular transformation)

c Effect of plant protection chemicals on grain and straw yield.

The maximum grain and straw yields were obtained from plots treated with carboxin alone or carboxin in combination with carbaryl or carbofuran (Table 10). Though not comparable with carboxin, the fungicides edifenphos and mancozeb also gave significantly higher grain and straw yields when applied in combination with the insecticide carbofuran. The insecticide carbaryl was found to be significantly inferior to carbofuran in increasing grain and straw yields. The grain and straw yield obtained in plots treated with carbaryl was comparable with that obtained in control plots.

d Effect of plant protection chemicals on phylloplane mycoflora.

Carboxin could bring about substantial increase in the population of some of the phylloplane antagonists of Rhizoctonia solani viz., Aspergillus flavus, Chaetomium globosum, Gliocladium virens, Trichoderma harzianum and Trichoderma viride (Table 11). This fungicide had a depressing influence on only a few leaf surface saprophytes and had no effect at all on others. The high efficacy of this fungicide can be attributed to its ability to increase the antagonistic mycoflora. Carboxin caused a reduction in the population of the pink yeast, Sporobolomyces sp.

Table No.10 Effect of plant protection chemicals on grain and straw yield of rice in kg/ha (Location I)

	Grain	Straw
Control	2425	8825
Carboxin	4250	13300
Mancozeb	2200	11875
Edifenphos	3775	11975
Carbaryl	2450	10925
Carbaryl + carboxin	4075	14175
Carbaryl + mancozeb	2258	9200
Carbaryl + edifenphos	3600	9750
Carbofuran	3750	11250
Carbofuran + carboxin	4350	14975
Carbofuran + mancozeb	3400	10005
Carbofuran + edifenphos	3933	10450
C.D. (5%)	295	500

Table No.11 Effect of common plant protection chemicals on phylloplane mycoflora of rice - population fluctuations expressed as percent increase/decrease over control.

Organism	Carboxin	Mancozeb	Edifenphos	Carbaryl	Carbofuran
Aspergillus niger	C	C	-66.87	+21.22	C
Aspergillus aculeatus	C	C	-66.67	+22.00	C
Aspergillus flavus	+9.00	C	-88.88	C	C
Cladosporium oxysporum	-10.00	C	C	+91.00	+82.00
Chaetomium globosum	+72.00	-27.7	-100.00	+60.00	+28.89
Fusarium chlamydosporum	+35.30	-65.56	-77.50	+76.40	+61.12
Gliocladium virens	+37.50	-55.82	-33.33	+75.0	C
Penicillium oxalicum	C	-22.32	-42.44	+30.00	+36.66
Penicillium islandicum	+6.75	-32.14	-41.11	+28.00	+47.14
Trichoderma harzianum	+64.00	-88.88	-64.00	+40.00	+18.88
Trichoderma viride	+66.67	-100.00	-86.00	+83.33	+50.00
Sporobolomyces roseus	-20.00	-66.67	-88.87	+23.66	C

C indicates no change in population.

Edifenphos and mancozeb had strongly depressing effect on the non-target phylloplane mycoflora of rice. These fungicides were highly deleterious to the phylloplane antagonists of Rhizoctonia solani. The insecticides carbaryl and carbofuran had a stimulatory effect on the phylloplane saprophytes of rice.

e. Effect of plant protection chemicals on incidence and intensity of sheath rot disease of rice.

Carboxin was found to be the best fungicide for controlling the incidence and intensity of sheath rot disease of rice when applied in combination with the insecticide, carbofuran (Table 11a). The individual effects of carboxin and edifenphos were on par with respect to the incidence of sheath rot. Mancozeb was not at all effective in reducing the incidence and intensity of sheath rot disease.

There was no significant effect for the two seasons on the different factors studied.

Table 11a. Effect of common plant protection chemicals on percentage incidence and intensity of sheath rot disease of rice (Location I)

	Incidence	Intensity
Control	47.9245	2.5660
Carboxin	20.5514	0.4430
Mancozeb	42.8428	2.3360
Edifenphos	28.0607	0.8833
Carbaryl	40.6384	1.4133
Carbaryl + carboxin	22.6761	0.7834
Carbaryl + mancozeb	29.8394	2.0783
Carbaryl + edifenphos	24.3584	1.5427
Carbofuran	28.1598	1.0263
Carbofuran + carboxin	18.6761	0.3624
Carbofuran + mancozeb	31.4848	1.1123
Carbofuran + edifenphos	26.1432	0.8733
C.D. (5%)	6.5670	0.6412

B. 2 Location II - Karamana.

a. Effect of plant protection chemicals on phylloplane microflora

The population of fungal saprophytes on the leaf surface of the two rice varieties, Jyothi and Karthika were found to vary significantly (Table 12). The variety Jyothi was found to harbour a significantly higher population than the variety Karthika. The fungicides were found to have a suppressive effect on the saprophytic fungi but the insecticides did not have any effect on the population of the phylloplane mycoflora of rice initially. Though carbaryl and carbofuran by themselves did not have any effect on the phylloplane mycoflora initially, during the second observation both the insecticides were found to reduce the population of phylloplane mycoflora on both the rice varieties. Edifenphos and mancozeb were found to reduce the phylloplane mycoflora significantly when applied alone or in combination with the insecticides carbaryl or carbofuran. However, the fungicide carboxin was found to cause an enhancement in the population of phylloplane mycoflora when applied alone or in combination with carbofuran. When

Table No.12 Effect of common plant protection chemicals on the phylloplane mycoflora of rice at two stages of growth (cfu/Cm^2 of leaf area) (Location II).

TREATMENTS	JYOTHI		KARTHIKA	
	Obs 1	Obs 2	Obs 1	Obs 2
Control	10.3386	10.1286	8.6947	8.9859
Carboxin	8.0898	11.0019	8.3306	8.3548
Mancozeb	5.3930	3.4712	4.0994	5.9632
Edifenphos	5.7560	5.1620	2.9591	4.8588
Carbaryl	8,8573	4.7716	7.4261	4.2748
Carbaryl + carboxin	4.8762	7.9299	5.8975	3.7690
Carbaryl + mancozeb	4.3995	4.7532	5.4390	5.6599
Carbaryl + edifenphos	7.4098	6.5852	5.0646	4.6333
Carbofuran	8.3863	7.3271	6.8161	7.8727
carbofuran + carboxin	7.6408	13.7090	7.9706	9.0443
Carbofuran + mancozeb	6.0363	5.4914	3.9917	5.0385
Carbofuran + edifenphos	7.5508	4.0099	4.3035	2.9580
C.D. (5%)	1.8406	1.8763	1.8406	1.8763

(Values after $\sqrt{x}$ transformation)

Obs 1 37 days after planting

Obs 2 52 days after planting

carboxin was sprayed along with the insecticide carbaryl, significant reduction of the phylloplane mycoflora resulted.

The population of phylloplane bacteria was also found to be significantly higher in the case of the rice variety Jyothi than in the variety Karthika (Table 13). The fungicide carboxin was found to have no deleterious effect on the phylloplane bacteria, while edifenphos and mancozeb were found to depress the phylloplane bacteria significantly. The fungicides edifenphos and mancozeb were found to exert a depressive effect on the phylloplane bacteria of rice variety Jyothi when applied alone or in combination with the insecticide carbaryl.

The phylloplane yeasts were found to flourish significantly better on the leaf surface of the rice variety Jyothi (Table 14). The fungicide, carboxin was found to be the least harmful to the phylloplane yeasts, while edifenphos was found to reduce the phylloplane yeasts considerably when applied alone or in combination with carbaryl or carbofuran. The effect of mancozeb on the phylloplane yeasts was found to be erratic.

b. Effect of plant protection chemicals on incidence and intensity of sheath blight.

The rice variety Karthika was found to be significantly tolerant to the incidence and intensity of

Table No.13 Effect of common plant protection chemicals on phylloplane bacteria at two stages (cfu / cm^2 of leaf area) of growth (Location II).

TREATMENT	JYOTHI		KARTHIKA	
	Obs 1	Obs 2	Obs 1	Obs 2
Control	22.2536	17.7872	14.0177	16.6129
Carboxin	19.1774	20.9939	16.4770	16.3051
Mancozeb	13.8869	11.5219	13.4992	16.4066
Edifenphos	13.4226	17.0611	15.6926	16.2380
Carbaryl	12.0797	14.9307	19.0273	14.6806
Carbaryl + carboxin	13.3859	15.7262	14.5952	15.2798
Carbaryl + mancozeb	13.1656	12.8902	12.6184	14.7361
Carbaryl + edifenphos	13.5135	15.0763	18.3986	14.5439
Carbofuran	17.3968	14.9066	12.0178	11.7343
Carbofuran + carboxin	15.5062	17.7419	10.8314	10.8445
Carbofuran + mancozeb	15.9095	13.2964	8.6458	12.7137
Carbofuran + edifenphos	16.6345	21.8290	15.0721	11.7762
C.D. (5%)	6.2165	4.1293	6.2165	4.1293

Values after $\sqrt{x}$ transformation

Obs 1 37 days after planting

Obs 2 52 days after planting

Table No.14 Effect of common plant protection chemicals on phylloplane yeasts of rice at two stages of growth (cfu / cm^2 of leaf area) (Location II).

TREATMENTS	JYOTHI		KARTHIKA	
	Obs 1	Obs 2	Obs 1	Obs 2
Control	4.8571	7.0742	2.7667	4.7857
Carboxin	3.4478	5.9155	3.4503	8.7924
Mancozeb	3.2455	4.2821	2.6664	4.1712
Edifenphos	2.9511	3.4381	2.9033	5.1779
Carbaryl	3.1531	7.1678	3.5137	3.1306
Carbaryl + carboxin	5.2001	9.1393	5.9105	8.8781
Carbaryl + mancozeb	3.4292	6.0663	3.9663	6.7576
Carbaryl + edifenphos	3.3420	4.0067	2.6254	3.4242
Carbofuran	4.7569	5.3350	3.9663	3.6506
Carbofuran + carboxin	3.3406	8.5208	2.8406	7.9703
Carbofuran + mancozeb	1.7345	5.3080	3.2575	4.3630
Carbofuran + edifenphos	2.2410	4.2131	4.5137	4.2763
C.D.(5%)	0.7347	1.5966	0.7347	1.5966

Values after $\sqrt{x}$ transformation

Obs 1 37 days after planting

Obs 2 52 days after planting

sheath blight, when compared with the variety Jyothi (Table 15, 16). The fungicide carboxin alone or in combination with carbaryl or carbofuran could reduce the incidence and intensity of sheath blight in the susceptible rice variety Jyothi. Mancozeb and edifenphos also could bring about significant reduction in the incidence and intensity of sheath blight. The insecticides carbaryl and carbofuran could also bring about some reduction in the incidence and intensity of sheath blight which was comparable with the effect of the fungicide mancozeb.

The application of carboxin alone or in combination with carbaryl or carbofuran was found to cause considerable reduction in the percentage incidence of sheath blight indicating its therapeutic value in disease control.

c Effect of plant protection chemicals on incidence and intensity of sheath rot

The rice variety Karthika was found to be significantly tolerant to sheath rot disease also when compared with the variety Jyothi, (Table 17). Carboxin was found to be efficient in reducing the incidence of sheath rot. The fungicides brought about significant decrease in sheath rot intensity over control. The insecticides carbaryl and carbofuran could also significantly reduce the incidence and intensity of sheath rot when compared with the control. This effect of the insecticides can be attributed to their

Table No.15 Effect of common plant protection chemicals on the intensity of sheath blight disease (Location II).

TREATMENTS	JYOTHI		KARTHIKA	
	PI	15 DPH	PI	15 DPH
Control	2.6136	5.2026	1.6936	2.3530
Carboxin	0.8113	1.2330	0.2573	0.1996
Mancozeb	0.6436	2.3436	0.6336	0.6956
Edifenphos	0.9540	1.7540	0.3020	0.5333
Carbaryl	0.6486	3.7320	0.2950	0.4796
Carbaryl + carboxin	0.8260	0.7706	0.1573	0.5093
Carbaryl + mancozeb	0.6140	3.3763	0.4586	0.4143
Carbaryl + edifenphos	0.8466	1.3493	0.2876	0.2453
Carbofuran	1.9916	3.2776	0.6313	0.8703
Carbofuran + carboxin	0.8583	1.1490	0.2160	0.2460
Carbofuran + mancozeb	0.4366	3.9590	0.4573	0.4436
Carbofuran + edifenphos	0.8290	2.7720	0.1947	0.7093
C.D (5%)	0.5337	1.2015	0.5337	1.2015

PI Panicle initiation

DPH Days prior to harvest

Table No.16 Effect of common plant protection chemicals on percentage incidence of sheath blight disease (Location II).

TREATMENTS	JYOTHI		KARTHIKA	
	PI	15 DPH	PI	15 DPH
Control	56.0343	63.6268	26.3784	34.6028
Carboxin	22.6753	25.8185	16.2314	12.6005
Mancozeb	48.3574	48.7782	19.9243	26.0849
Edifenphos	32.8876	38.1747	14.6817	21.2497
Carbaryl	43.4706	56.7151	24.3291	22.1500
Carbaryl + carboxin	19.0138	28.4237	15.6005	17.1949
Carbaryl + mancozeb	31.7800	53.6206	19.2145	32.4237
Carbaryl + edifenphos	31.5744	40.1528	12.4413	16.9382
Carbofuran	28.4392	45.7290	18.2544	28.0112
Carbofuran + carboxin	21.6803	30.5705	10.6188	14.3385
Carbofuran + mancozeb	41.3064	55.6477	20.2291	24.1568
Carbofuran + edifenphos	22.9440	32.2963	19.3140	23.5339
C.D.(5%)	5.7032	12.2915	5.7032	12.2915

(Values after angular transformation

PI Panicle initiation

DPH Days prior to harvest

Table No.17 Effect of common plant protection chemicals on percentage incidence and intensity of sheath rot disease of rice (Location II).

TREATMENTS	JYOTHI		KARTHIKA	
	INTENSITY	INCIDENCE	INTENSITY	INCIDENCE
Control	3.8516	(59.1735)	1.1113	(27.8270)
Carboxin	0.2273	(23.1044)	0.2990	(16.5516)
Mancozeb	0.3956	(35.8860)	0.6770	(28.0212)
Edifenphos	0.6173	(31.7118)	0.5733	(30.8508)
Carbaryl	2.8030	(41.5781)	0.5876	(20.9383)
Carbaryl + carboxin	0.3633	(18.2565)	0.5020	(18.4538)
Carbaryl + mancozeb	1.9866	(28.8319)	0.6700	(28.9765)
Carbaryl + edifenphos	0.8243	(29.3584)	0.6896	(29.3896)
Carbofuran	2.8406	(41.8676)	0.9616	(23.4722)
Carbofuran + carboxin	0.5823	(26.2861)	0.2383	(15.6299)
Carbofuran + mancozeb	1.4613	(33.2827)	0.7510	(25.5988)
Carbofuran + edifenphos	0.5030	(34.0781)	0.4360	(26.9457)
C.D. (5%)	0.4781	6.5966	0.4781	6.5966

Values in parentheses after angular transformation

role in reducing stem borer infestation, a predisposing factor for sheath rot infection.

d Effect of plant protection chemicals on the whole disease incidence (D) and total yield loss (L)

The whole disease incidence (D) which constitutes a sum total of disease intensity in a field was found to vary with seasons. With respect to whole disease incidence and yield loss, the rice variety Karthika was found to be significantly tolerant than the rice variety Jyothi during the first season (Table 18).

The best treatment leading to significant reduction in yield loss and disease incidence was found to be the application of carboxin along with the insecticide carbofuran during the first crop season.

During second crop season there was no significant difference between the two varieties with respect to the total disease incidence and the yield loss (Table 19). The application of carboxin alone or in combination with carbaryl or carbofuran could reduce the whole disease incidence and yield loss significantly over other treatments. The fungicide edifenphos and mancozeb could also bring about significant reduction in the total disease incidence and yield loss though not comparable with the fungicide carboxin.

Summing up the findings during the two seasons it can be surmised that the application of carboxin along with

Table No.18 Effect of common plant protection chemicals on the whole disease incidence (D) and total yield loss (L) in kg /10 acres (Location II - Season I).

TREATMENTS	JYOTHI		KARTHIKA	
	D	L	D	L
Control	62.3986	160.2473	13.8633	62.0813
Carboxin	12.6150	35.1523	14.7656	32.7400
Mancozeb	21.8703	62.6850	16.7516	59.6473
Edifenphos	29.6670	79.5626	13.8846	39.8406
Carbaryl	21.7700	54.9933	11.2140	29.5190
Carbaryl + carboxin	8.8033	23.5283	4.3530	13.4050
Carbaryl + mancozeb	15.0426	38.4316	13.3160	38.9336
Carbaryl + edifenphos	32.7166	70.0350	11.5870	34.4426
Carbofuran	12.6096	38.0273	13.2996	39.2113
Carbofuran + carboxin	4.4190	13.2576	4.6443	13.9490
Carbofuran + mancozeb	33.6460	93.5976	14.5450	22.0013
Carbofuran + edifenphos	22.0273	60.1626	15.0970	45.6770
C.D. (5%)	9.8813	25.5021	9.8813	25.5021

Table No.19 Effect of common plant protection chemicals on the whole disease incidence (D) and total yield loss in Kg/10 acres (L) (Location II - Season II).

TREATMENTS	JYOTHI		KARTHIKA	
	D	L	D	L
Control	35.7286	77.6846	29.8413	63.1803
Carboxin	8.4987	22.7973	8.8096	39.0833
Mancozeb	26.3653	49.2830	18.4710	42.4670
Edifenphos	15.6163	36.3136	17.3123	34.1073
Carbaryl	38.4460	33.9980	29.6863	33.4383
Carbaryl + carboxin	14.1930	22.4193	8.4213	19.9303
Carbaryl + mancozeb	24.4853	32.3420	18.1716	29.4576
Carbaryl + edifenphos	18.5480	31.8006	21.3023	33.4386
Carbofuran	32.6400	59.1986	29.5945	37.7090
Carbofuran + carboxin	8.6126	19.9303	9.5945	18.0626
Carbofuran + mancozeb	18.3486	38.0190	14.5980	32.2490
Carbofuran + edifenphos	20.5460	44.8473	15.4790	32.5923
C.D. (5%)	6.3786	9.7690	6.3786	9.7690

carbofuran could reduce the total disease incidence and yield loss significantly.

e Effect of plant protection chemicals on grain and straw yield

There was no significant difference between the two varieties with respect to grain yield, but straw yield was found to be significantly higher in the variety Karthika (Table 20). The application of the fungicide carboxin alone or along with the insecticide carbaryl or carbofuran gave significantly higher grain and straw yields for both the varieties. The fungicide edifenphos also was found to increase the grain and straw yield considerably when applied together with the insecticide carbofuran. The insecticides by themselves did not have any bearing on the grain and straw yield.

During field trials at this location it was found that there was no significant effect for the two seasons on the factors studied, excepting on the 'D' and 'L' values.

f Effect of plant protection chemicals on the phylloplane mycoflora

The fungicides edifenphos and mancozeb were found to have a highly depressing effect on the phylloplane mycoflora of rice variety Jyothi (Table 21). Most of the mycelial forms and phylloplane yeasts were found to be suppressed by the application of these two fungicides. The fungicide

Table No.20 Effect of common plant protection chemicals on grain and straw yield (kg /ha).

TREATMENTS	JYOTHI		KARTHIKA	
	Grain	Straw	Grain	Straw
Control	4692	15271	4219	16798
Carboxin	5469	17770	5039	20269
Mancozeb	4692	15896	4345	17353
Edifenphos	4692	16660	4456	17215
Carbaryl	4695	15063	4192	18464
Carbaryl + carboxin	5358	17493	5048	18603
Carbaryl + mancozeb	4414	16382	4165	19714
Carbaryl + edifenphos	4664	15827	4970	19019
Carbofuran	4866	16660	4650	15965
Carbofuran + carboxin	5581	18659	5098	20200
Carbofuran + mancozeb	4845	16798	4414	19019
Carbofuran + edifenphos	5172	17423	5664	20963
C.D. (5 %)	589.4	1882.6	589.4	1882.6

Table No.21 Effect of plant protection chemicals on phylloplane mycoflora of rice (variety Jyothi) population fluctuations expressed as per cent increase/decrease over control.

Organism	Carboxin	Mancozeb	Edifenphos	Carbaryl	Carbofuran
Aspergillus niger	C	-60.00	-70.00	+40.00	C
Aspergillus flavus	+9.00	-66.66	-90.00	+20.00	C
Aspergillus versicolor	C	-66.66	-70.00	+12.00	C
Aspergillus ustus	+2.00	-50.00	-65.00	+24.00	+2.00
Cladosporium oxysporum	-10.00	-20.00	-42.40	+36.20	+22.00
Fusarium tricinctum	+35.72	-56.25	-75.00	+76.48	+75.00
Gliocladium virens	+47.50	-50.40	-44.44	+46.00	+50.00
Mucor hiemalis	+37.50	-22.22	-10.12	+34.50	+47.80
Nigrospora sphaerica	-31.25	-83.34	-72.73	+71.43	+26.39
Trichoderma viride	+66.67	-100.00	-88.87	+50.00	+28.00
Cryptococcus spp.	-35.50	-97.56	-100.00	-43.9	+43.88
R.solani	-100.00	+33.33	-100.00	+83.33	+50.00

C indicates no change in population.

carboxin was found to enhance most of the phylloplane antagonists of Rhizoctonia solani in the variety Jyothi. R. solani was completely inhibited by carboxin and edifenphos while mancozeb, carbaryl and carbofuran caused increases in the population of the sheath blight pathogen. The phylloplane yeast, Cryptococcus sp. and the mycelial forms, Cladosporium oxysporum and Nigrospora sphaerica were found to be restricted by the application of carboxin while the population of A. niger and A. versicolor remained unchanged. Among the different species of Trichoderma commonly encountered on the phylloplane of rice plants, T. viride was the one most frequently isolated from the rice variety Jyothi. The insecticides carbaryl and carbofuran had an enhancing influence on the phylloplane mycoflora of the variety Jyothi.

In the rice variety Karthika, several species of the phylloplane antagonist Trichoderma were frequently isolated including T.viride, T. hamatum and T.harzianum (Table 22). Populations of all these three species of Trichoderma were found to be enhanced by the application of the fungicide carboxin. The application of carboxin generally caused an enhancement of the phylloplane mycoflora of the rice variety Karthika. The fungicides edifenphos and mancozeb were found to restrict severely the phylloplane fungi, sometimes bringing about a complete elimination of these fungi from the phylloplane. Both the insecticides were found to have an

Table No.22 Effect of plant protection chemicals on the phylloplane mycoflora of rice plants of variety Karthika, population fluctuations expressed as per cent increase/decrease over control.

Organism	Carboxin	Mancozeb	Edifenphos	Carbaryl	Carbofuran
Aspergillus aculeatus	+9.00	-50.00	-48.87	+40.00	+27.00
Aspergillus flavus	+11.00	-67.77	-50.00	+23.00	+25.00
Aspergillus wentii	+3.50	-75.00	-88.88	+24.00	+28.00
Chaetomium dolichotrichum	+37.75	-88.88	-76.60	+22.00	+12.00
Cladosporium cladosporioides	C	-11.67	-20.00	+80.00	+85.72
Gliocladium virens	+55.00	-75.00	-88.88	C	C
Gliomastix murorum	-20.55	-88.88	-66.67	+2.00	C
Hendersonula toruloidea	-9.00	-44.57	-70.88	+21.00	+22.00
Trichoderma harzianum	+67.77	-88.88	-100.00	+27.00	+67.00
Trichoderma hamatum	+70.00	-100.00	-100.00	+22.00	+47.77
Trichoderma viride	+66.67	-87.80	-75.00	+12.00	+21.00
Tritirachium oryzae	C	-61.67	-47.68	+7.00	+16.77

C indicates no change in population.

enhancing effect on the phylloplane mycoflora of the rice variety Karthika.

III IN VITRO STUDIES ON PHYLLOPLANE MICROORGANISMS OF RICE

The microorganisms isolated and identified from the phylloplane of rice plants are described below.

A Fungi

The following fungi were isolated from the phylloplane of rice plants (Table 23.a). They are described below.

1. Aspergillus aculeatus Iizuka (Plate 1)

Colonies reaching 5-8 cm diameter in ten days, characteristically brownish black. Conidia globose and echinulate, sterigmata are uniseriate.

2. Aspergillus flavus Link ex Gray.

Colonies reaching 3-7 cm diameter in ten days, characteristically yellow green. Conidia globose to sub globose, finely roughened to echinulate.

3. Aspergillus niger van Tieghem (Plate 1)

Colony diameter reaching 2.5-5cm in ten days, typically black, powdery. Conidiophores arising from long, broad, thick walled brownish sometimes branched foot cells, conidia irregularly roughened.

4. Aspergillus ustus (Bain) Thom & Church (Plate 1)

Colonies spreading broadly reaching 4.5-6 cm in ten

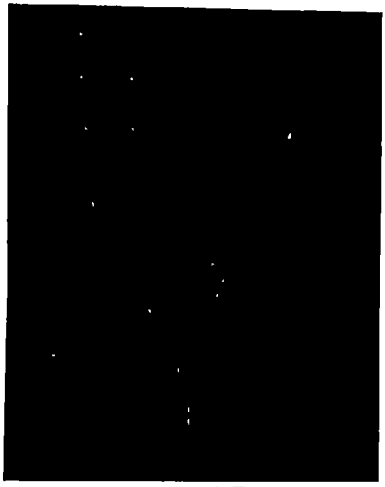
Table No.23 a. Fungi isolated from the phylloplane of rice.

1. Aspergillus aculeatus Iizul a
2 Aspergillus flavus Link ex Fr.
3 A. niger van Tieghem
4 A. ustus (Bain) Thom & Church
5 A. versicolor (Vuill.) Tiraboschi
6 A. wentii Wehmer
7 Chaetomium globosum Kunze
8 Chaetomium dolichotrichum L. Ames
9 Cladosporium cladosporiodies (Fres.) de Vries
10 Cladosporium oxysporum Berk. & M.A. Curtis
11 Curvularia affinis Boedijn
12 Curvularia lunata (Wakker) Boedijn
13 Cylindrocarpon destructans (Zinssm) Scholten
14 Fusarium chlamydosporum Wollenw. & Reinking
15 Fusarium tricinctum (Corda) Sacc.
16 Gliocladium virens Miller, Giddens & Foster
17 Gliomastix murorum (Corda) S. Hughes
18 Hendersonula toruloidea Nattrass
19 Mucor hiemalis Wehmer
20 Myrothecium verrucaria (Alb. & Schw. : Fr) Ditm. Fr.
21 Nigrospora sphaerica (Sacc) Mason
22 Penicillium funiculosum Thom
23 Penicillium islandicum Sopp
24 Penicillium oxalicum Currie & Thom
25 Trichoderma hamatum (Bonord) Bain
26 Trichoderma harzianum Rifai
27 Trichoderma koningii Oudem
28 Trichoderma viride Pers. ex Fr.
29 Rhizopus stolonifer (Ehrenb, ex Link) Lind
30 Tritirachium oryzae (Vincens) de Hoog.

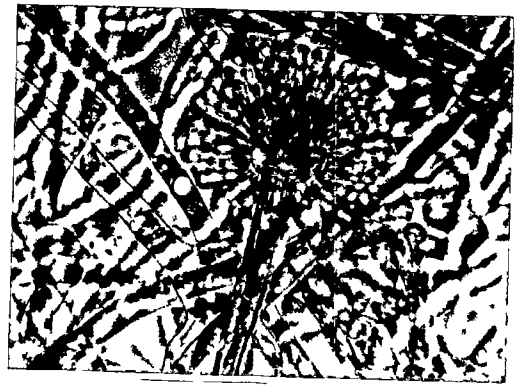
Aspergillus spp. isolated from the phyllosphere of rice

A. aculeatus

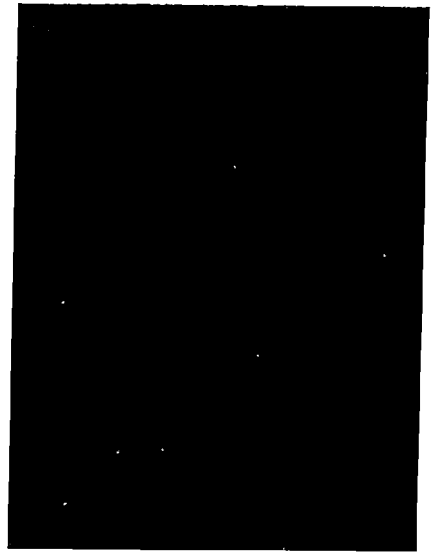
A. niger



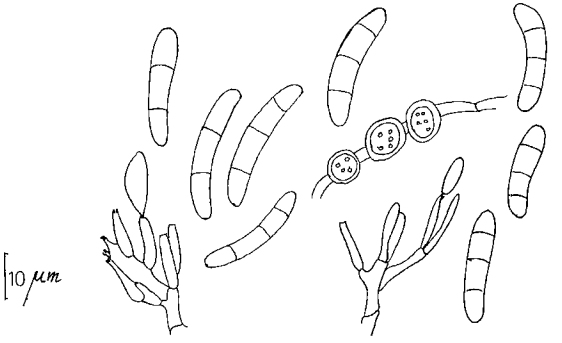
A. versicolor



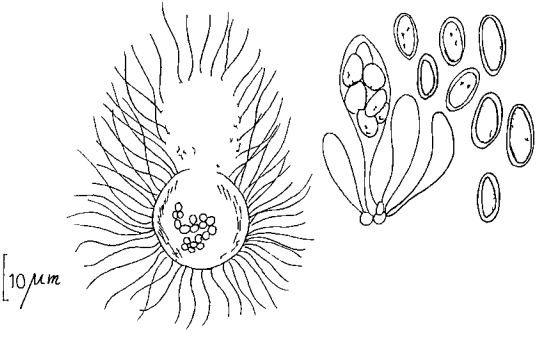
A. ustus



A. wentii

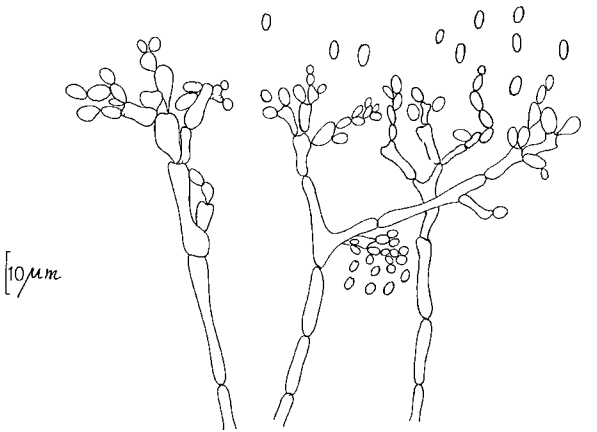


Cylindrocarpon destructans



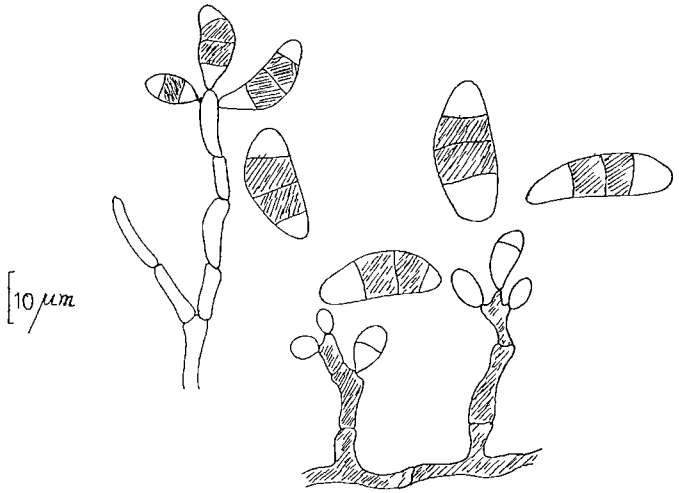
Chaetomium globosum

Fig 1

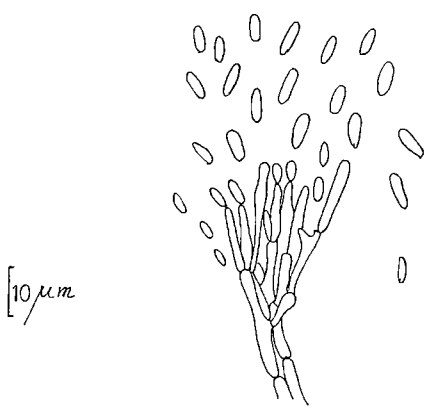


Cladosporium cladosporioides

Fig. 2

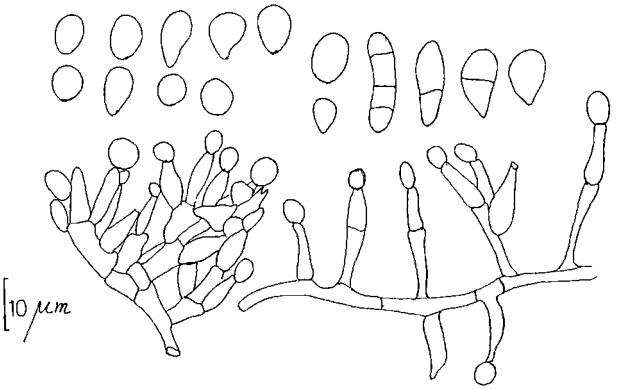


Curvularia affinis

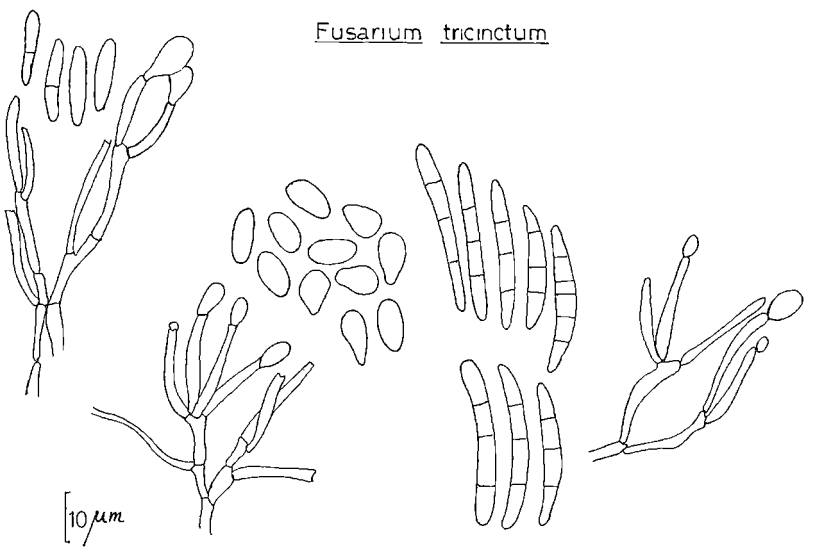


Myrothecium verrucaria

Fig 3

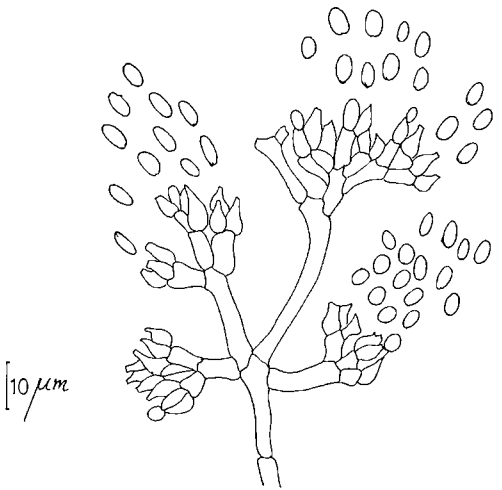


Fusarium tricinctum



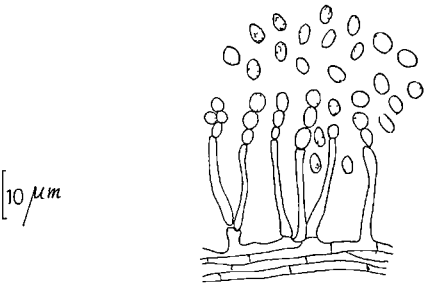
Fusarium chlamydosporum

Fig 4

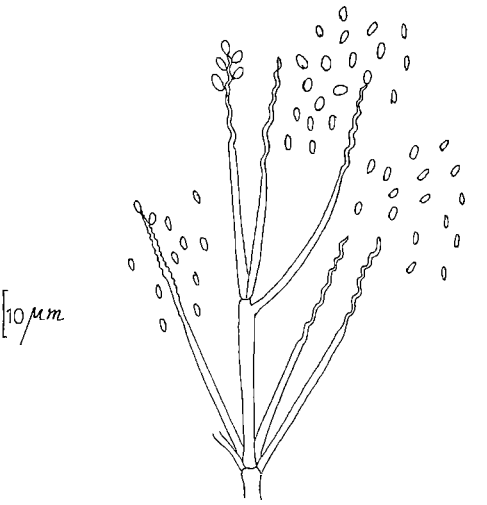


Gliocladium virens

Fig 5

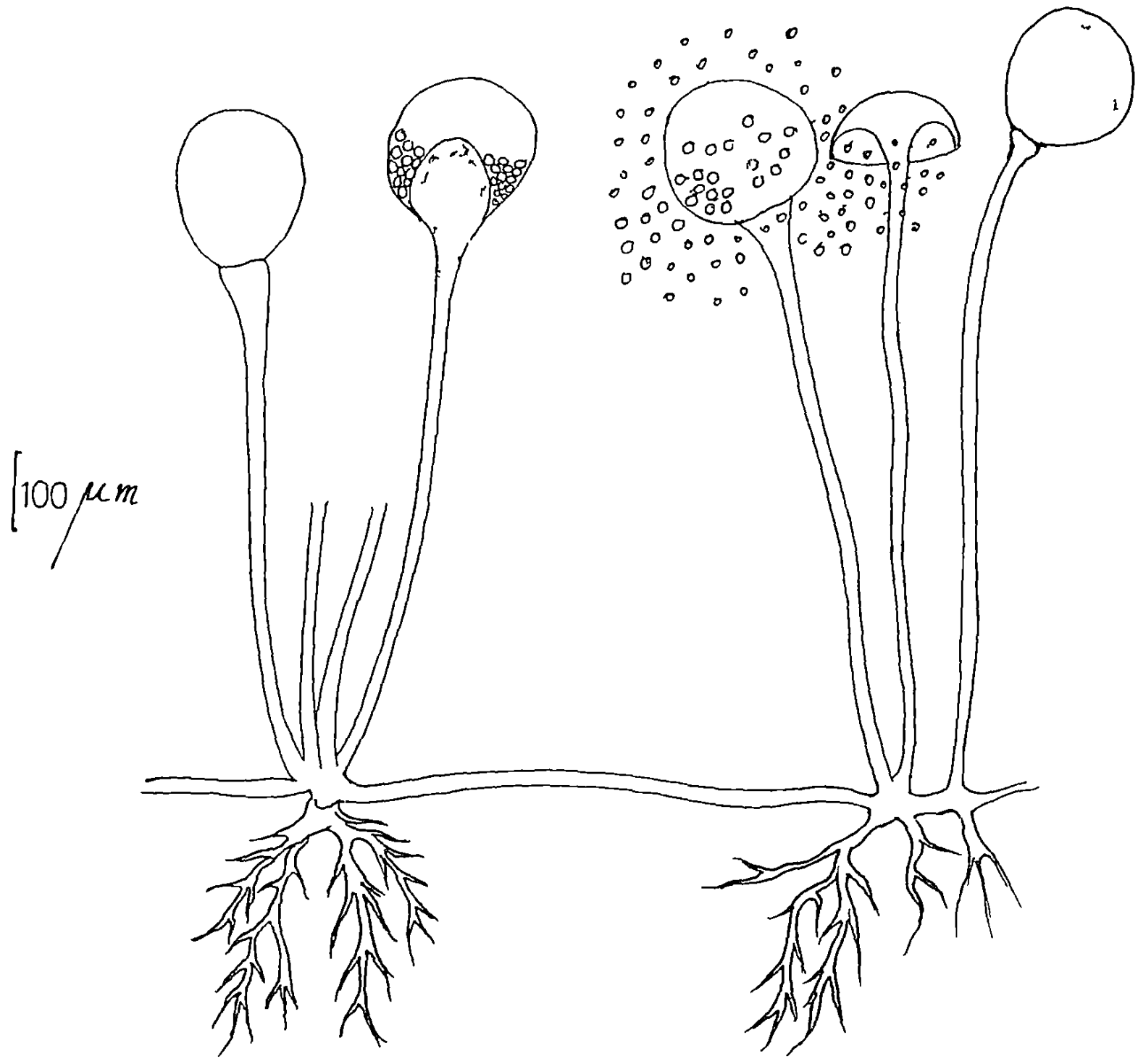


Gliomastix murorum



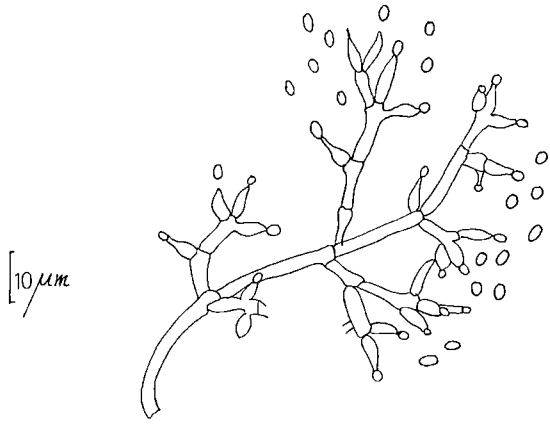
Tritirachium oryzae

Fig 6

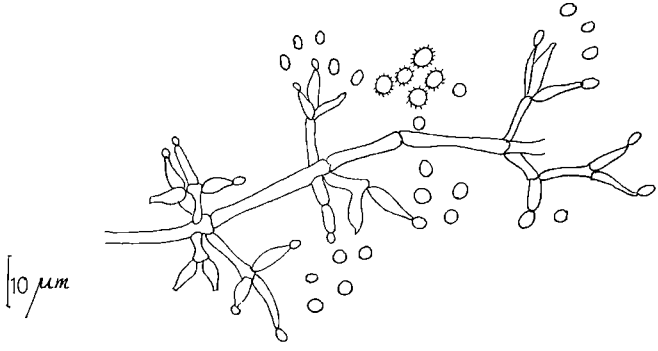


Rhizopus stolonifer

Fig 7

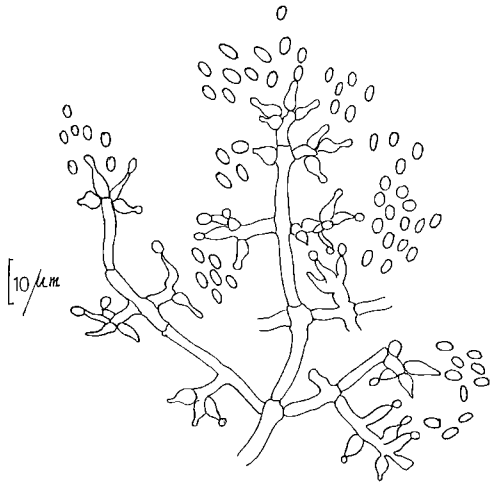


Trichoderma hamatum



Trichoderma viride

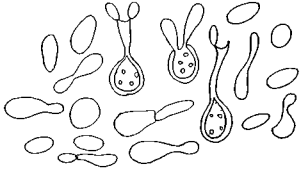
Fig 8



Trichoderma harzianum

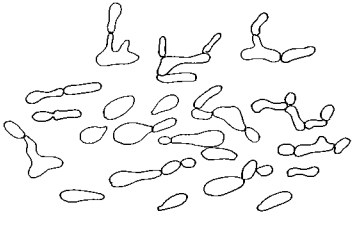
Fig 9

[10 µm



Sporidiobolus sp

[10 µm



Sporobolomyces

Fig 10

days. Conidial heads globose; conidia globose, roughened. Hyaline hulle cells typically present and scattered throughout the colony.

5 Aspergillus versicolor (Vuill) Tiraboschi (Plate 1)

Colonies reaching 2-3cm diameter in ten days. Variable in colour, ochre or orange yellow with exudate and reverse of equally variable colour. Globose hulle cells present.

6 Aspergillus wentii Wehmer

Colonies reaching 2 to 3.5cm diameter in 10 days. One of the tallest Aspergillus sp., conidiophores 1 to 2 mm long. Vesicles globose, conidia ellipsoidal, verrucose.

7 Chaetomium globosum Kunze (Fig.1)

Colonies reaching 4.5 to 5.5 cm diameter in ten days. Ascomata dark brown to black, globose to subglobose. Ascospores are lemon shaped (4 to 6 μm diameter).

8 Chaetomium dolichotrichum L.Ames.

Colonies reaching 2.5 to 3.5 cm diameter in ten days. Ascomata inky black, globose to subglobose. Mycelium cottony, thick and fluffy. Ascospores oval shaped (4.5 to 6.5 μm diameter).

9 Cladosporium cladosporioides (Fres.) Vries. (Fig.2)

Colonies reaching 3 to 4 cm diameter in ten days, olivaceous green. Conidia ellipsoidal to lemon shaped, smooth walled (2 to 11 x 2 to 5 μm).

10 Cladosporium oxysporum Berk & M.A. Curtis

Colonies reaching 3 to 6 cm diameter in ten days greyish brown. Conidia lemon shaped variable smooth walled (5 to 30 x 3 to 6 μm).

11 Curvularia affinis (Boedijn (Fig.3)

Colonies effuse, grey reaching 3 to 5 cm diameter in ten days. Mycelium immersed in the medium. Conidia formed solitary, with three or more transverse septa. Hilum protruberant (27 to 39 x 8 to 13 μm).

12 Curvularia lunata (Wakker) Boedijn

Colonies effuse, grey reaching 4 to 6 cm in diameter in ten days. Mycelium immersed in the medium. Conidia formed solitary with three or more transverse septa. (18 to 32 x 9 to 15 μm).

13 Cylindrocarpon destructans (Zinssm) Scholten (Fig.1)

Colonies reaching 4 to 5 cm diameter in seven days on PDA, aerial mycelium whitish to cream, floccose. Conidiophores branched or consisting of solitary phialides. Conidia are uniform in shape with 0 to 3 septate, 15-35 x 2.4-4.5 μm. Chlamydospores intercalary or terminal, brownish and warted 9-12 μm diameter.

14 Fusarium chlamydosporum Wollenw & Reinking (Fig.4)

Colonies fast growing reaching 4 to 6 cm in four days. Aerial mycelium abundantly developed, intensely pink or red. Microconidia accumulate in dry heads (8.5 to 10 x 2.5 to

3.0 μm). Macroconidia rarely produced. Chlamydospores numerous, intercalary, often roughened.

15 Fusarium tricinctum (Corda) Sacc. (Fig.4)

Colonies reaching 3.2 to 4.0 cm diameter in four days. Aerial mycelium forming a complete cushion, red-purple. Microconidia scattered (8 to 11 x 4.5 to 7.5 μm) rarely two celled. Macroconidia produced only in sporodachia, curved 3 to 5 septate (24 to 50 x 3.3 to 4.5 μm). Chlamydospores not common.

16 Gliocladium virens Miller, Giddens & Foster (Fig.5)

Colonies very fast growing reaching 5 to 8 cm diameter in five days. Phialides appressed bearing one large drop of green conidia, on each whorl. Conidia short-ellipsoidal smooth walled (4.5 to 6 x 3.5 to 4 μm).

17 Gliomastix murorum (Corda) Hughes (Fig.6)

Colonies reaching 1.8 to 2.8 diameter in ten days, olivaceous-black, mostly strongly tufted and powdery, reverse often brown. Conidia ellipsoidal, olivaceous black, coarsely warted (3.4 to 5.7 x 2.0 to 3.7 μm).

18 Hendersonula toruloidea Natrass

Colonies effuse, dark blackish brown reaching 2 to 3 cm diameter in ten days. Conidia catenate, simple and non septate (6 to 15 x 5 to 10 μm).

19 Mucor hiemalis Wehmer

Colonies 15 to 20 mm high, buff, reverse pale.

Sporangiophores 12 to 14 μm wide. Chlamydospores absent, heterothallic. Sporangia globose (50 to 70 μm).

20 Myrothecium verrucaria (Alb & Schw.)Ditm ex Steudel.(Fig.3)

Colonies reaching 4.0 to 5.0 cm diameter in 14 days. Mycelium white to rosy buff forming 3 to 6 conidiophores in a whorl (10.5 to 14.5 x 1.5 to 2.0 μ). Conidia broadly fusiform, apical end pointed (6 to 10 x 2 to 4.5 μm).

21 Nigrospora sphaerica (Sacc.) Mason

Colonies spreading broadly reaching 5.5 to 8.0 cm diameter in ten days. Grayish, cottony, reverse olivaeous black. Conidia black, oval (14 to 20 μm).

22 Penicillium funiculosum Thom

Broadly spreading colonies reaching 4.5 to 5.5 cm diameter in ten days. Sporulating areas yellow green. Colonies funiculose, tufted. Reverse orange brown. Conidia ellipsoidal to subglobose (2.5 to 3.5 x 2.0 to 2.5 μm).

23 Pencillium islandicum Sopp.

Colonies reaching 2.5 to 3.5 cm in ten days. Reverse orange brown to red, conidial areas dark green. Conidia ellipsoidal, smooth and thick walled (3.0 to 3.5 x 2.5 to 30 μm).

24. Pencillium oxalicum Currie & Thom

Colonies spreading reaching 3.5 to 5.0 cm diameter in ten days, dull green, reverse uncoloured. Conidia ellipsoidal, smooth walled (4.5 to 6.5 x 3 to 4 μm).

25 Rhizopus stolonifer (Ehrenb. ex Link) Lind. (Fig. 7)

Colonies very fast growing, often over 2cm high, grey brown. Stolons hyaline to brown 13 to 20μm wide, abundantly branched rhizoids. Sporangiophores in whorls, 1.5 to 3 mm tall. Sporangia black 100 to 200μm in diameter. Sporangiospores subglobose (5.5 to 12 x 4.5 to 10μm).

26 Trichoderma hamatum (Bonord.) Bain (Fig.8)

Colonies reaching over 7 cm diameter in five days. Forms greyish green pustules on the surface. Conidia, short, cylindrical green (3.4 to 4.2 x 2.5 to 3μm).

27 Trichoderma harzianum Rifai (Fig.9)

Colonies reaching over 9 cm diameter in five days. Colonies light green. Conidia sub globose to short oval (2.8 to 3.2 x 2.5 to 2.8μm).

28 Trichoderma koningii Oudem

Colonies reaching 3 to 5 cm diameter in five days. Reverse pale yellow in colour. Phialides arise in clusters. Conidia smooth walled, short, cylindrical with a truncate base (3 to 4.8 x 1.9 to 2.8μm).

29 Trichoderma viride Pers. ex Gray (Fig.8)

Colonies reaching 4.5 to 7.5 cm diameter in five days. Conidiophores have short branches. Phialides in divergent groups, slender. Conidia globose (3.6 to 4.5μm) in diameter, surface roughened.

30 Tritirachium oryzae (Vincens) de Hoog (Fig.6)

Colonies slow growing reaching 2.5 to 3 cms diameter in 14 days. Mycelium in tufts, dark pink. Conidia hyaline, cylindrical (2.5 to 3.5 x 2.0 to 3.0μm).

B Bacteria

The bacteria isolated and identified from the rice phylloplane are described in Table 23b.

C Actinomycetes

A few actinomycetes isolated from the phylloplane of rice plants are described below (Table 23c).

A-1 Streptomyces

Gram positive colonies appeared as encrustation. Sporophores flexuous. The reverse of colonies were dark gray in colour. Produced gray pigment.

A-2 Streptomyces

Gram positive colonies appeared as appressed circular mats, 1.5 to 2 cm in diameter. The sporophores were open loop shaped. The reverse of colonies were deep violet coloured. Produced deep violet coloured pigment.

A-3 Streptomyces

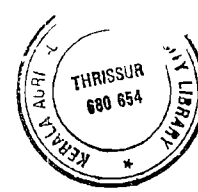
Gram positive. Colonies appeared as dark brown powdery masses. The sporophores were in the form of closed spirals. the reverse of the colonies were colourless. No pigment production was observed.

Table No. 23b Characters of bacteria isolated from the phylloplane of rice plants.

Sl.No.	Acc.No.	Genus	Gram reaction	Motility	Oxygen requirement	Growth	Cell morphology	Colony form	Catalase test
1	B1	Alcaligenes	-	motile	aerobic	moderate	rods	filiform	++
2	B3	Bacillus	+	motile	aerobic	moderate	rods	beaded	++
3	B4	Bacillus	+	motile	aerobic	scanty	rods	filiform	+
4	B5	Bacillus	+	motile	aerobic	moderate	rods	filiform	++
5	B8	Acinetobacter	-	non motile	aerobic	scanty	rods	filiform	++
6	B9	Rothia	+	non motile	aerobic	moderate	cocci	effuse	++
7	B10	Xanthomonas	-	motile	aerobic	scanty	rods	effuse	++
8	B12	Bacillus subtilis	+	motile	aerobic	moderate	rods	arborescent	++
9	B16	Bacillus	+	motile	aerobic	moderate	rods	filiform	++
10	B18	Alcaligenes	-	motile	aerobic	scanty	cocci	filiform	+
12	B20	Chromobacterium	-	motile	aerobic	moderate	rods	beaded	+
13	PAB1	Propionibacterium	+	non motile	aerobic	moderate	rods	beaded	++

Table No.23 c Characters of Actinomycetes isolated from the phylloplane of rice.

Sl.No.	Acc No.	Genus	Sporophore type	soluble pigment production
1	A-1	Streptomyces	flexuous	gray
2	A-2	Streptomyces	open-loop	deep violet
3	A-3	Streptomyces	closed spiral	deep brown



D Yeasts

The following four genera of yeasts were isolated and identified from the phylloplane of rice plants (Table 23d).

1 Bullera Derx

Reproduces by budding and by the formation of symmetrical ovoidal ballistospores which develop in an oblique position at the tips of the aerial sterigmata. Mycelium or pseudomycelium not formed. Cells are spherical to oval (5 to 7μm). Growth on malt agar, cream coloured to slightly yellowish.

2 Cryptococcus Kutzing emend. Phaff et Spencer

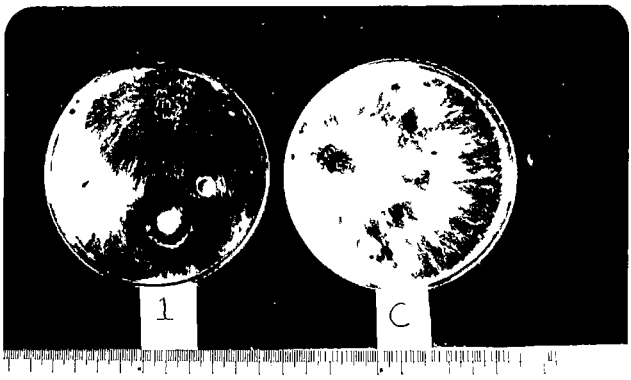
Reproduces asexually by budding (3.5 to 8.8 x 5.5 to 10.2μm) vegetative cell elongate, amoeboid or polymorphic. Ballistospores or ascospores not formed. Dark brown pigments produced.

3 Sporobolomyces Kluyver et Van Niel (Fig.10)

Reproduces by budding and by production of assymetrical kidney shaped ballistospores formed on aerial sterigmata. The growth on YMA was salmon pink. Vegetative cells ovoidal to elongate (3 to 5 x 4 to 7μm).

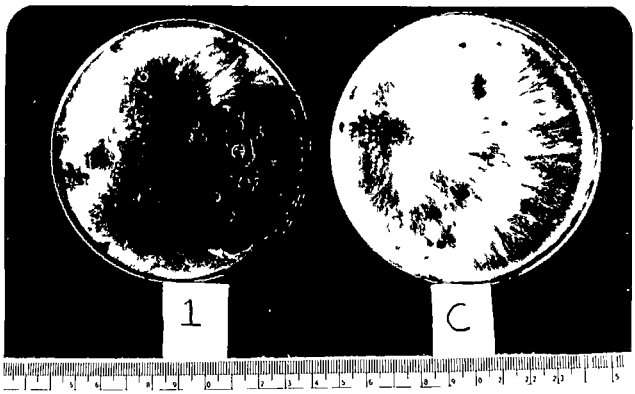
4 Sporidiobolus Nyland (Fig.10)

Reproduces by budding and by means of assymetrical ballistospores borne at the tips of aerial sterigmata. Ballistospores reproduce by budding. Sparse mycelial growth seen. Cultures pinkish in colour. Cells elongate (3 to 4 x 8 to 11μm).

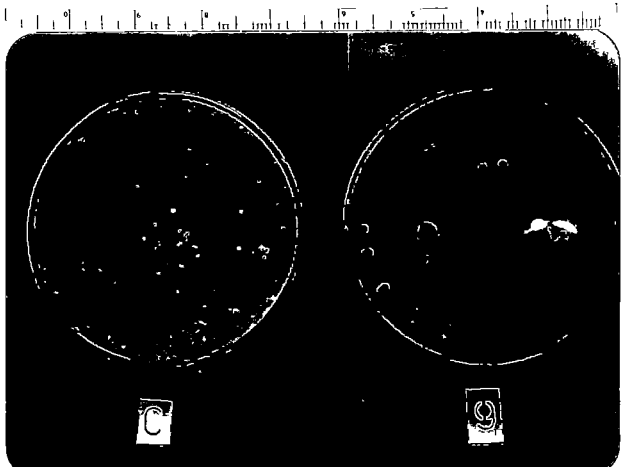


Trichoderma harzianum

C - *R. solani*



1. *Penicillium oxalicum*



Chaetomium globosum

Table No. 23d. Characters of yeasts isolated from the phylloplane of rice plants.

Sl.No	Acc.No	Genus	Colony colour	Spore type	colony shape
1	Y1	Sporidiobolus	light pink	ballistospore	large, fluid
2	Y-2	Sporobolomyces	dark pink	ballistospore	restricted, fluid
3	Y-3	Cryptococcus	cream	no ballisto- spore ascospore	large, fluid
4	Y-4	Cryptococcus	brown	do ,,	restricted, fluid
5	Y-5	Sporobolomyces	dark pink	ballistospore	rounded, fluid
6	Y-6	Bullera	creamy yellow	ballistospore	large, fluid.

IV STUDIES ON IN VITRO ANTAGONISM OF PHYLLOPLANE MICROORGANISMS AGAINST THE SHEATH BLIGHT PATHOGEN

A i Fungi

When the different phylloplane fungi were paired with R.solani, many of them were found to overgrow the test fungus causing a smothering effect. eg., Aspergillus spp., Penicillium spp., Rhizopus stolonifer, Mucor hiemalis (Table 24, plate No.2). Some of the fungi intermingled freely with R.solani and grew together eg., Curvularia spp., Cladosporium oxysporum, Cylindrocarpon destructans, Hendersonula toruloidea, Myrothecium verrucaria and Nigrospora sphaerica (Table 24 Plate 3). Some of the fungi were found to have a cessation of growth at the point of contact with the test organism, eg. Aspergillus versicolor, A.wentii, Chaetomium dolichotrichum, Gliomastix murorum, Penicillium oxalicum, Fusarium tricinctum and Tritirachium oryzae (Table 24 Plate 4). The fungi which emerged as the potential antagonists of R.solani caused a clear zone of inhibition between the paired cultures (Plate 5). This included T.harzianum, T.viride and Chaetomium globosum. The Trichoderma spp. completely overgrew and parasitised R.solani after seven days (Plate 5).

A ii Mycoparasitism of selected phylloplane fungi on R.solani

Out of the different fungi tested for the mechanism of parasitism, Trichoderma harzianum and T.viride proved to be

Table No. 24 Reaction of the phylloplane fungi of rice to the sheath blight pathogen, *Rhizoctonia solani*.

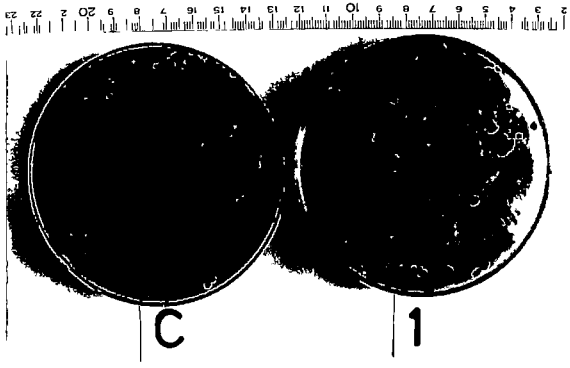
Pairing of culture	Type of Reaction
R. solani with	
Aspergillus aculeatus	B
A. flavus	B
A. niger	B
A. ustus	B
A. versicolor	C
A. wentii	C
Chaetomium globosum	D
C. dolichotrichum	C
Cladosporium cladosporiodies	B
Cladosporium oxysporum	A
Curvularia affinis	A
Cylindrocarpon destructans	A
Fusarium tricinctum	C
F. chlamydosporum	B
Gliocladium virens	B
Gliomastix murorum	C
Hendersonula toruloidea	A
Mucor hiemalis	B
Myrothecium verrucaria	A
Nigrospora sphaerica	A
Penicillium funiculosum	B
P. islandicum	B
P. oxalicum	C
Rhizopus stolonifer	B
Trichoderma hamatum	B
T. harzianum	D
T. koningii	B
T. viride	D
Tritirachium oryzae	C

A. Homogenous : Free intermingling between pairing organisms.

B. Overgrowth : *R.solani* over grown by the test organism.

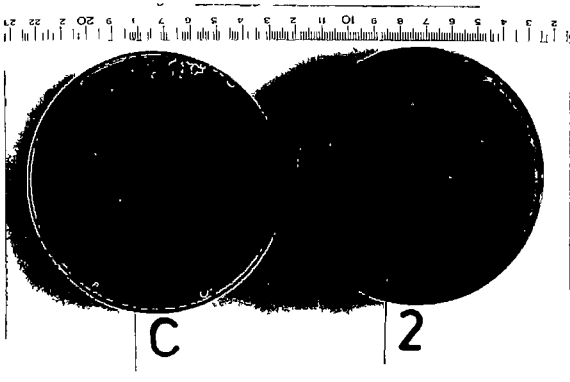
C. Cessation of growth at the line of contact of the cultures.

D. Aversion: A clear zone of inhibition was observed between the two organisms.

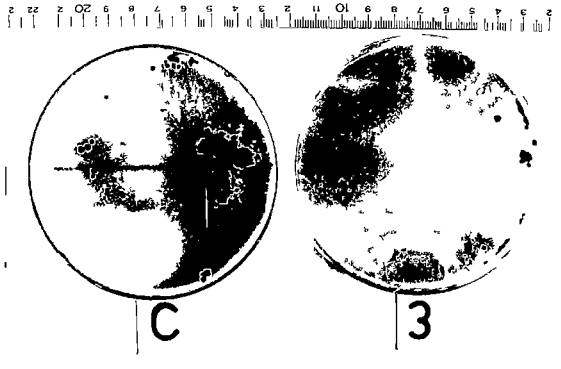


1 Penicillum islandicum

C-R. solani

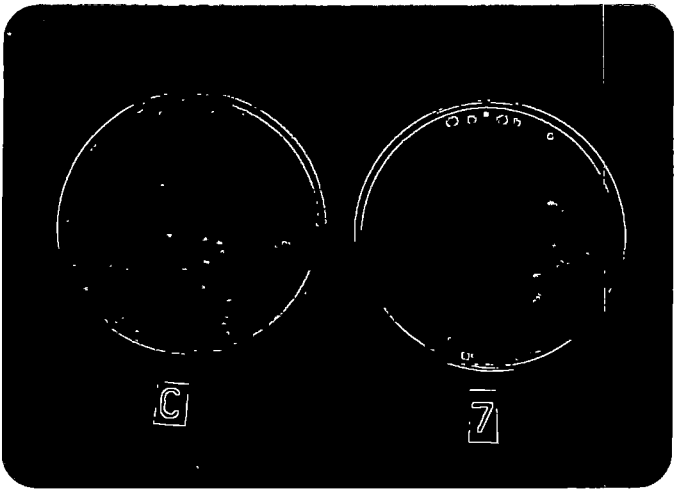


2. Rhizopus stolonifer



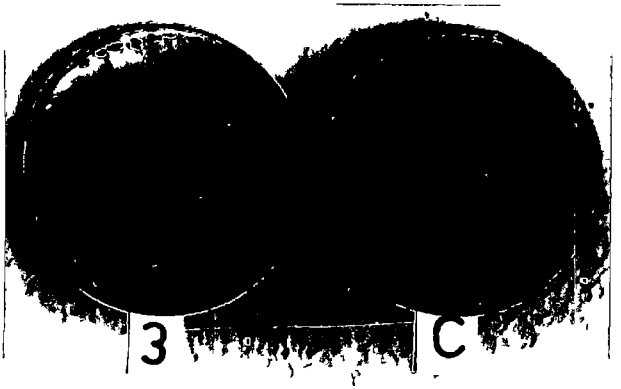
3. Aspergillus niger

Plate 3. Free intermingling of phylloplane fungi with R. solani

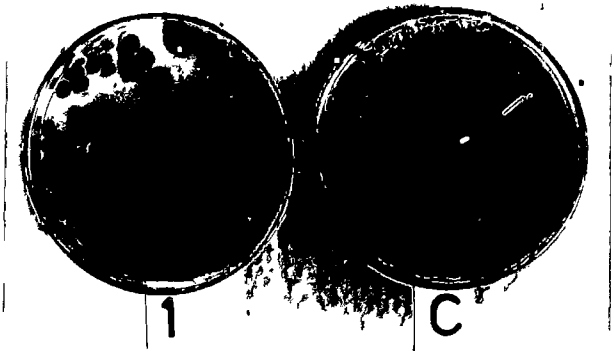


Cladosporium oxysporum

C-R. solani

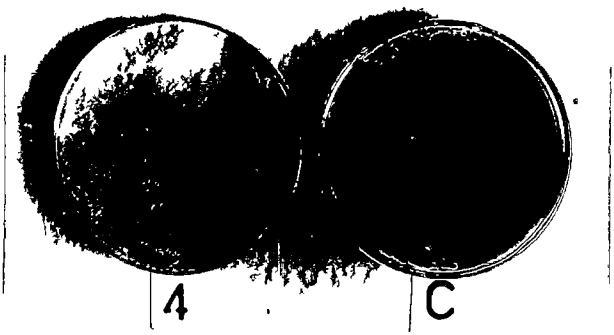


3. Myrothecium verrucaria

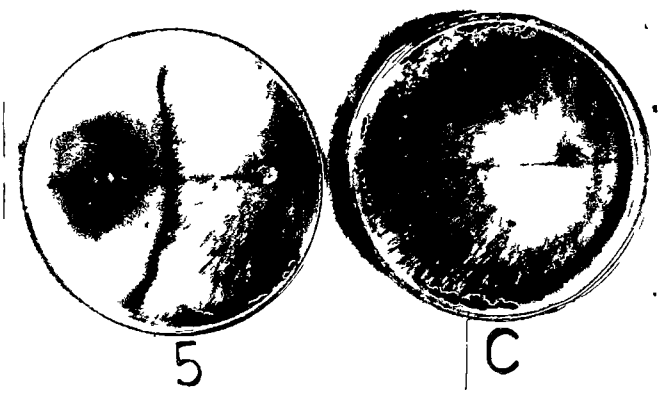


1. *Aspergillus versicolor*

C-*R. solani*

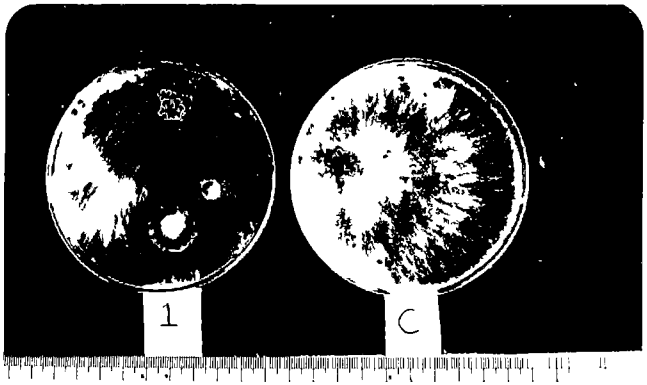


4. *Trichothecium oryzae*



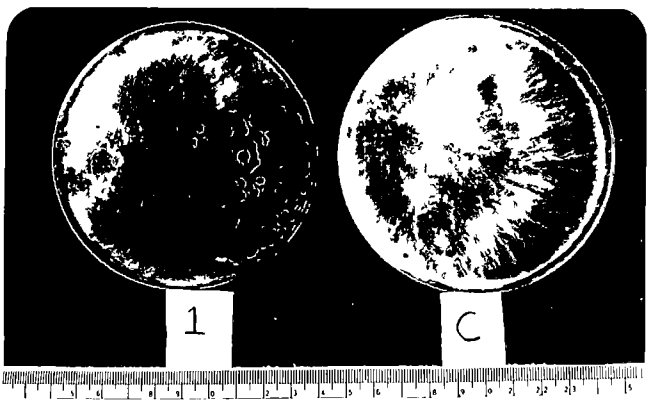
5. *Fusarium tricinctum*

Plate 5. Clear zone of inhibition between paired cultures

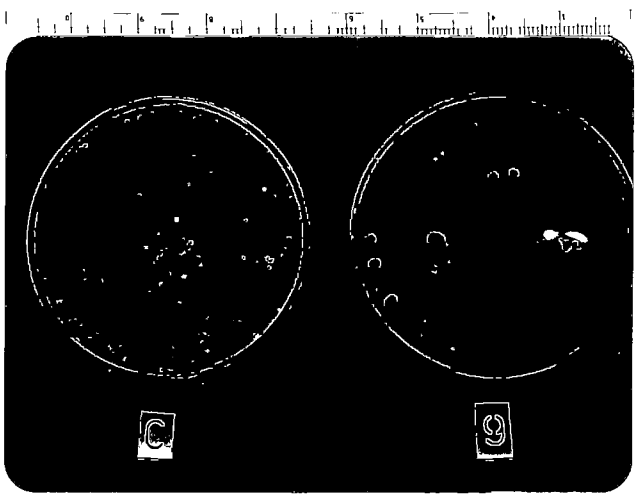


Trichoderma harzianum

C - *R. solani*

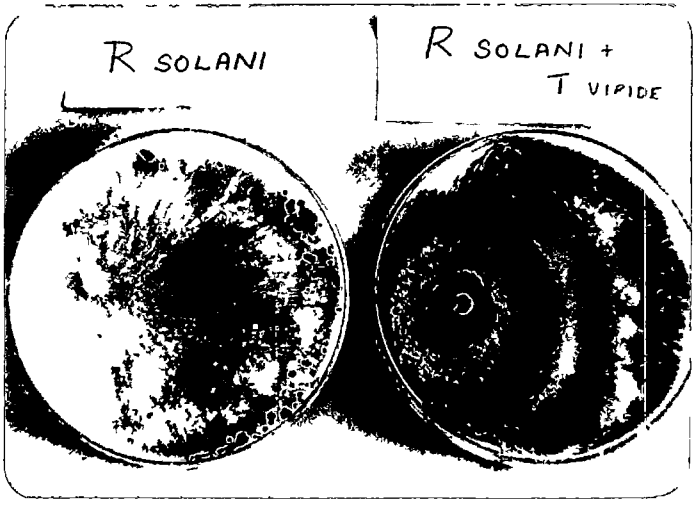


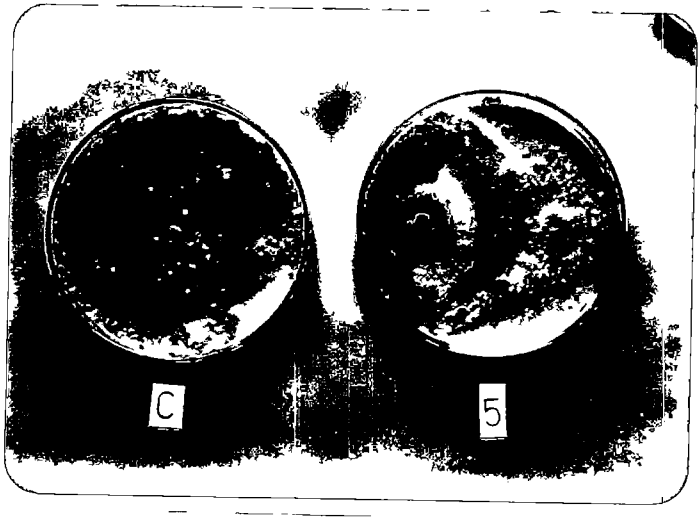
1. *Penicillium oxalicum*



Chaetomium globosum

Plate.5. Contd. Complete overgrowth





Trichoderma harzianum

the most efficient parasites of R.solani (Table 25 Plate 6a). These fungi were found to cause excessive granulation, vacuolation and finally disintegration of the host hyphae. They were also found to coil around and penentrate the hyphae of R.solani leading to disintegration and death (Plate 6a). The hyphae of R.solani were found to split entirely at the septal plates leading to cellular leakage. The mycoparasites were found to grow and sporulate profusely on R.solani (Plate 6b). The two species of Aspergillus, viz., A.aculeatus and A.versicolor caused granulation and vacuolation of the host hyphae and were found to coil around it but there were no signs of hyphal penetration. Trichoderma hamatum, T.koningii and Gliocladium virens were found to cause granulation and vacuolation of host hyphae followed by coiling and penetration but to a lesser degree than that exhibited by T.viride or T.harzianum (Plate 6c). The fungus Chaetomium globosum only caused the granulation and vacuolation of the host hyphae without any hyphal coiling or penetration. The fungus Fusarium tricinctum did not have any specialised mycoparasitic action on R.solani.

B Bacteria

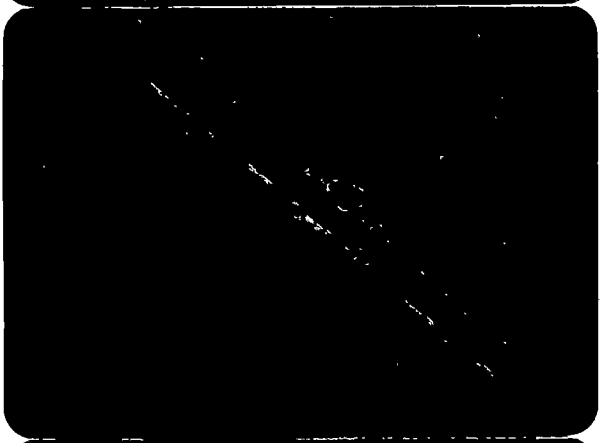
Some of the bacterial isolates from the phylloplane of rice showed clear cut antagonism against R.solani in vitro. In such cases of complete aversion, a clear zone of inhibition became visible demarkating the test fungus and the bacterium. Several bacterial genera including Alcaligenes, Bacillus

Table No.25. Mycoparasitic reactions of selected phylloplane fungi on *Rhizoctonia solani*.

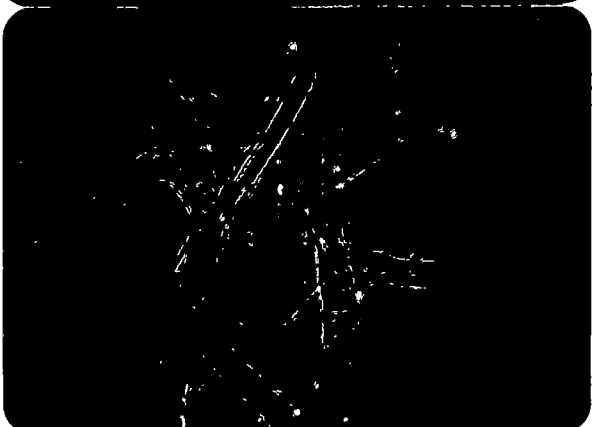
Sl.No.	Phylloplane Antagonists	Type of interaction*	Per cent hyphal interaction	
			Coiling	Penetration
1	*Aspergillus aculeatus*	+	33.33	0
2	*A. versicolor*	+	12.50	0
3	*Chaetomium globosum*	++	0	0
4	*Fusarium tricinctum*	+	0	0
5	*Gliocladium virens*	+	30.00	20.00
6	*Trichoderma hamatum*	+	10.00	7.50
7	*T. harzianum*	+++	44.25	33.33
8	*T. koningii*	++	6.67	11.87
9	*T. viride*	+++	40.00	35.00

* granulation, vacuolation and disintegration

Appressorium formation

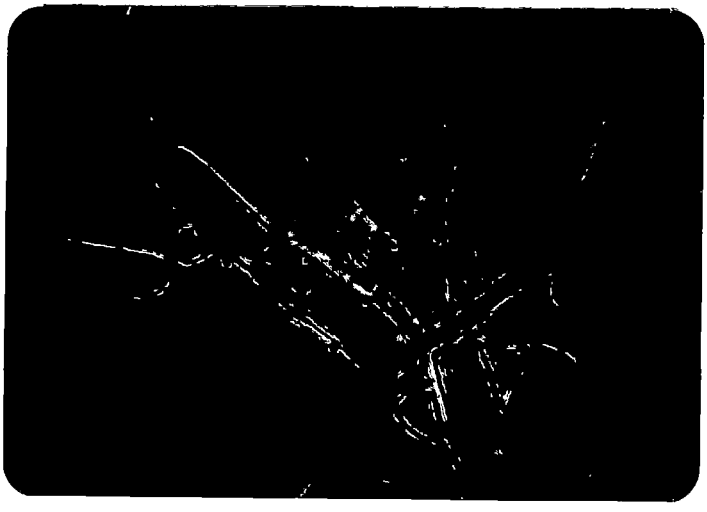


Coiling

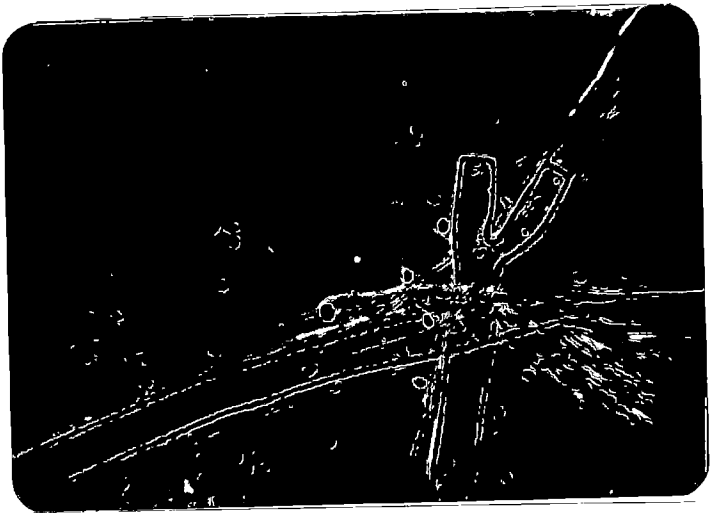


Granulation

Penetration-Internal Growth



Disintegration

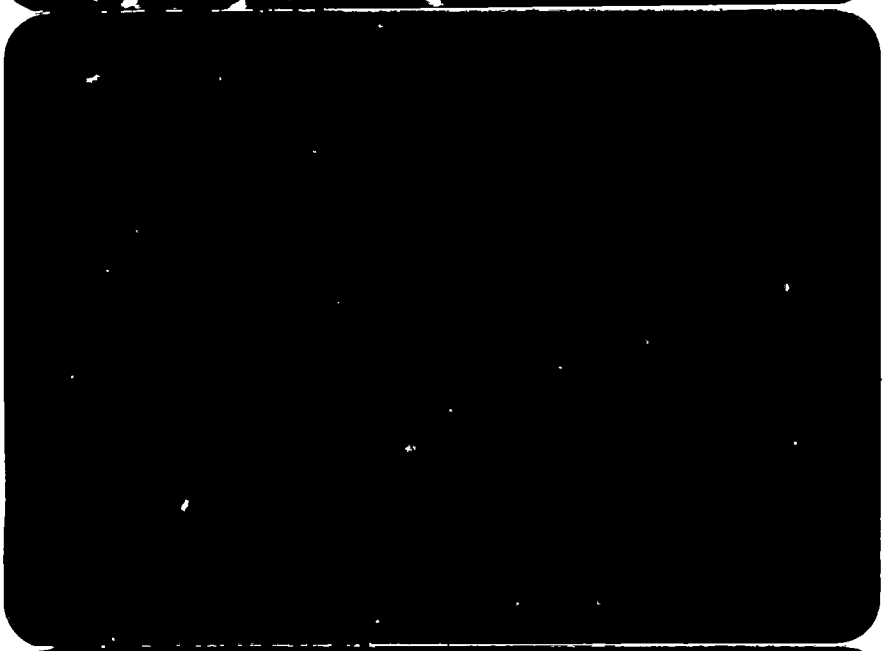


Penicillium oxalicum

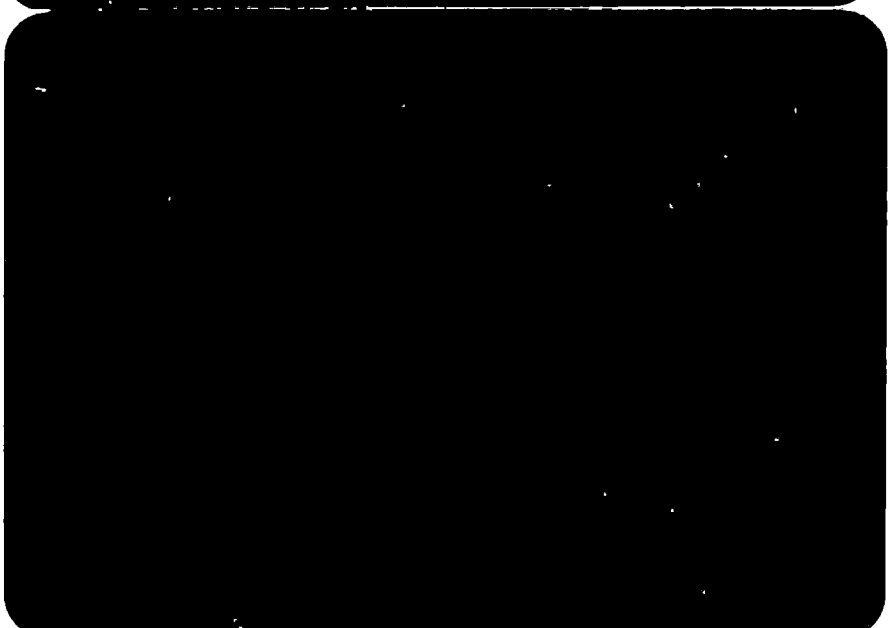
Trichoderma harzianum

Trichoderma viride

G. virens



T. hamatum



T. koningii

spp., Chromobacterium, Propionibacterium, and Rothia showed different degrees of aversion towards R.solani in vitro (Table 26, Plate 7a). One bacterial genus, Acinetobacter overgrew R.solani, thus suppressing its growth completely. Some of the bacterial isolates tested did not have any antagonism against R.solani in vitro eg. Bacillus spp., and Xanthomonas.

C Actinomycetes

Three species of Streptomyces isolated from the phylloplane of rice plants were tested for their antagonism towards R.solani in vitro. None of the three actinomycetes tested showed any antagonistic action against R.solani.

Yeasts

Six isolates of yeasts isolated from the phylloplane o of rice plants were tested in vitro for their reaction towards R.solani. Four of these exhibited inhibitory action against R.solani (Table 27 Plate 7b). The basidiomycetous yeasts Sporobolomyces sp. and Cryptococcus sp. exhibited well defined aversion towards R.solani in vitro. Two other yeasts frequently isolated from the phylloplane of rice plants, viz., Sporidiobolus sp. ad Bullera sp. did not exhibit any antagonistic action towards R.solani in vitro.

Table No. 26 *In vitro* effect of a few phyllopane bacteria on *Rhizoctonia solani*.

Acc.No.	Type of interaction	Inhibition zone in mm
B_1	Aversion	16.66
B_3	Aversion	8.67
B_4	Aversion	6.67
B_5	Aversion	3.33
B_8	Complete overgrowth	-
B_9	Aversion	15.66
B_{10}	No antagonism	-
B_{12}	Aversion	11.11
B_{16}	No antagonism	-
B_{18}	Aversion	13.50
B_{19}	No antagonism	-
B_{20}	No antagonism	-
PAB_1	Aversion	16.17

Table No. 27. *In vitro* effect of few phylloplane yeasts on *Rhizoctonia solani*

Yeast	Type of Antagonism	Inhibition zone (mm)
Cryptococcus sp	Aversion	24.40
Cryptococcus sp	Aversion	28.33
Bullera sp	No antagonism	--
Sporobolomyces sp	Aversion	21.00
Sporobolomyces sp	Aversion	25.50
Sporidiobolus sp	No antagonism	--

1. Acinetobacter

2. Alcaligenes

3. Bacillus sp.

4. Chromobacterium

5. Propionibacterium

c Control

1. Sporobolomyces

2. Control

3. Cryptococcus

Plate 7a. Inhibition of R. solani by several phylloplane bacteria

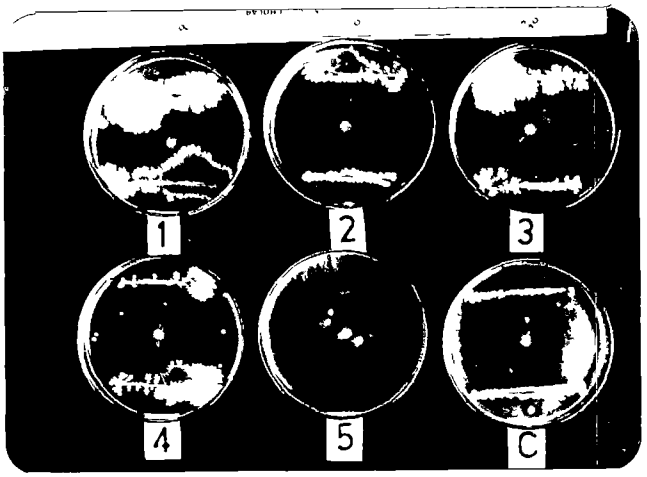
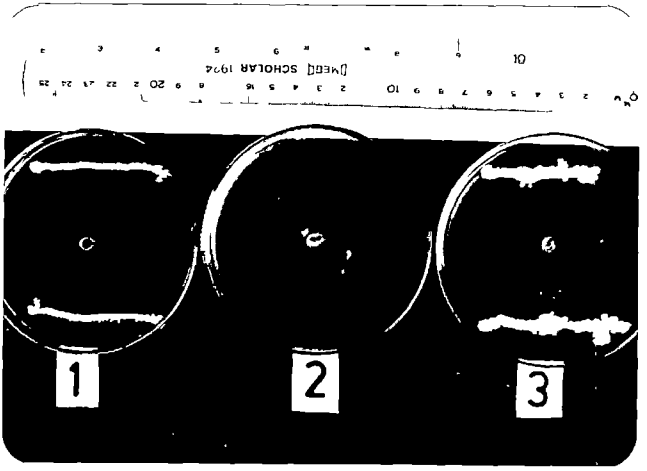


Plate 7b. Inhibition of R. solani by a few phylloplane yeasts



V BIOASSAY OF PLANT PROTECTION CHEMICALS ON THE ANTAGONISTIC PHYLLOPLANE MYCOFLORA AND R.SOLANI

All the fungicides and insecticides at both the levels tested restricted the colony diameter of R.solani significantly over control in vitro (Table 28 Plate 8). The fungicide edifenphos was found to restrict the colony diameter of R.solani significantly at both the levels tested. However, the fungicide, carboxin was found to have significant inhibitory action on the phylloplane antagonists only at 1000ppm while at 500ppm this fungicide did not have any inhibitory action on antagonistic fungi. At the higher dose of 1000ppm, the fungicide carboxin caused 55 to 70% inhibition of the phylloplane antagonists of R.solani. The insecticide carbaryl was found to restrict the colony diameter of R.solani in vitro. The fungicide carboxin thus emerges as a relatively safe fungicide to control R.solani, causing minimum disturbance to the natural balance of phylloplane mycoflora of rice.

VI EFFICACY OF DIFFERENT TYPES OF MYCOPARASITE INOCULA IN REDUCING SHEATH BLIGHT DISEASE

When the mycoparasites were applied as different types of inocula it was found that rice bran and wheat bran cultures were more efficient than other treatments in reducing the incidence and intensity of sheath blight of rice (Table 29).

Table No.28 Effect of common plant protection chemicals on R. solani and its phylloplane antagonists (Percentage inhibition)

	R.solani	T.viride	T.harzianum
Carboxin 500 ppm	90.00	0.0	0.0
Carboxin 1000 ppm	90.00	57.52	69 60
Mancozeb 500 ppm	66.49	0.0	0.0
Mancozeb 1000 ppm	90.00	41.17	48.77
Edifenphos 500 ppm	90.00	57.43	62.95
Edifenphos 1000 ppm	90.00	63.14	90.00
Carbaryl 500 ppm	90.00	44.54	68.73
Carbaryl 1000 ppm	90.00	46.91	75.27
Control	0.0	0.0	0.0
C.D. (5%)	2.69	2.69	2.69

(Values after angular transformation)

1 - Trichoderma viride

2 - T.harzianum

3 - R. solani

4 - 1000 ppm on T. viride

5 - 1000 ppm on T. harzianum

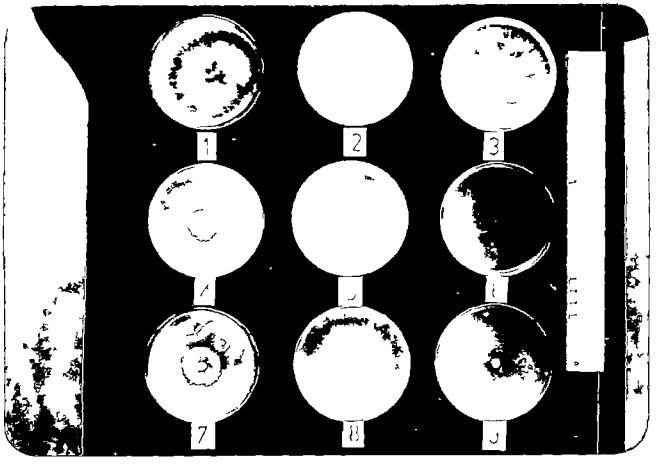
6 - 1000 ppm on R. solani

7 - 500 ppm on T. viride

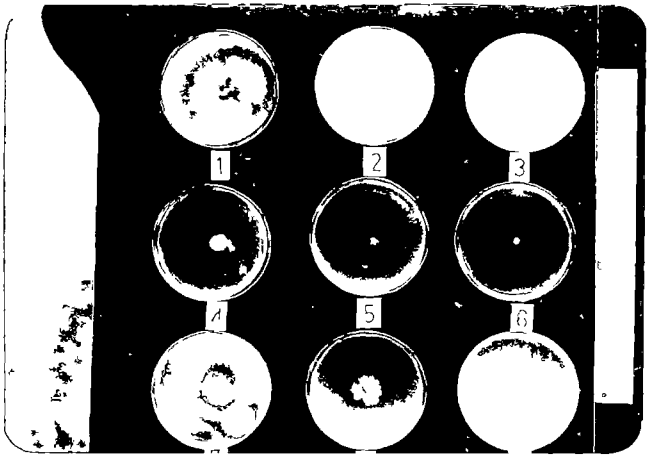
8 - 500 ppm on T. harzianum

9 - 500 ppm on R. solani

Plate 8. Effect of plant protection chemicals on *R. solani* and its phylloplane angtagonists



Carboxin



Carbaryl

1 - *Trichoderma viride*

2 - *T.harzianum*

3 - *R. solani*

4 - 1000 ppm on *T. viride*

5 - 1000 ppm on *T. harzianum*

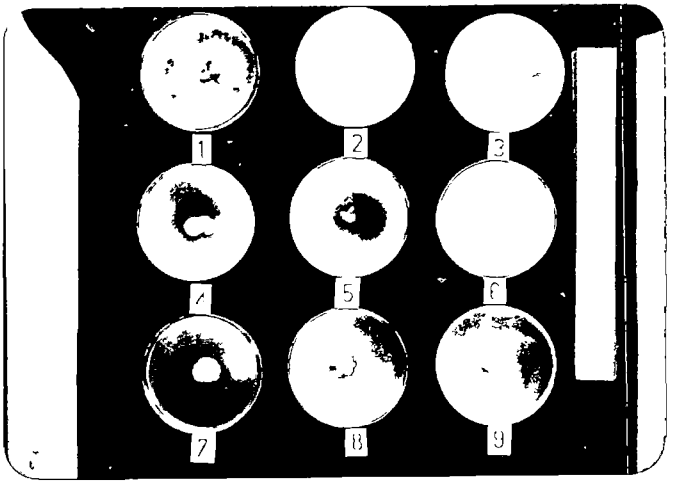
6 - 1000 ppm on *R. solani*

7 - 500 ppm on *T. viride*

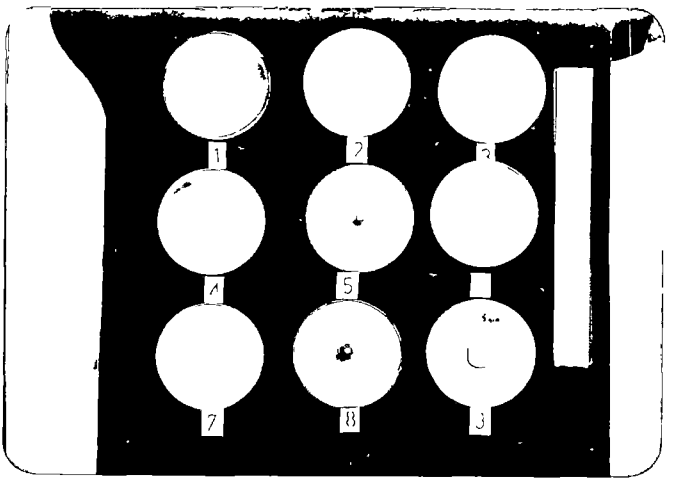
8 - 500 ppm on *T. harzianum*

9 - 500 ppm on *R. solani*

Plate 8. contd.



Edifenphos



Mancozeb

Table No. 29. Efficacy of different types of mycoparasite inocula in reducing sheath blight of rice.

Treatments	Disease Incidence(%)	Disease Intensity
Spore suspension	67.80	5.65
Mycelial suspension	48.19	3.62
Wheat bran culture	23.11	1.73
Rice bran culture	27.14	1.89
Control	71.47	6.51
CD (5%)	8.750	0.851

The application of mycoparasites as mycelial suspension could also bring about reduction in disease incidence and intensity when compared with the control. The application of spore suspension of the mycoparasite could not cause any reduction in the incidence and intensity of sheath blight disease. The results indicate that the rice/wheat bran cultures of the mycoparasites could bring about significant reduction in the incidence and intensity of sheath blight disease.

VII EVALUATION OF THE EFFICACY OF A FEW MYCOPARASITES OF R.SOLANI IN CHECKING THE SHEATH BLIGHT DISEASE

The incidence and intensity of sheath blight were found to be significantly lower in the rice variety Karthika (Table 30). All the mycoparasites tried could bring about significant reduction in the disease incidence and intensity of sheath blight of rice when compared with the control (Plate 9). However the effect of these mycoparasites in bringing about reduction in disease incidence and intensity was not comparable with the effect of the fungicide carboxin. Carboxin was found to be the best among the treatments for reduction of sheath blight disease incidence and intensity.

The whole disease incidence and yield loss due to sheath blight were significantly reduced by the application of fungicide carboxin. The effect of the mycoparasites, T.viride, T.harzianum and P.oxalicum on whole disease incidence was comparable with that of carboxin (Table 31).

Table No.30 Efficacy of a few mycoparasites of Rhizoctonia solani in checking sheath blight disease.

	Disease Incidence %		Disease Intensity	
	Jyothi	Karthika	Jyothi	Karthika
Aspergillus aculeatus	49.875	40.151	0.383	0.333
Penicillium oxalicum	48.816	34.133	0.233	0.267
Trichoderma harzianum	49.782	38.229	0.283	0.250
T.viride	52.858	41.025	0.410	0.267
Carboxin	25.295	22.586	0.150	0.033
Control	68.636	55.830	1.000	0.833
C.D.	5.0883	5.0883	0.146	0.146

Table No.31 Effect of mycoparasites on whole disease incidence (D) and yield loss (L) due to sheath blight disease of rice.

	D VALUES		L VALUES	
	JYOTHI	KARTHIKA	JYOTHI	KARTHIKA
Aspergillus aculeatus	3.863	0.852	2.111	2.040
Penicillium oxalicum	3.203	1.165	2.350	1.892
Trichoderma harzianum	2.513	1.255	2.122	1.993
Trichoderma viride	2.893	0.525	2.173	2.122
Carboxin	1.497	0.147	1.375	1.340
Control	17.424	12.461	4.820	4.331
C.D. (5%)	2.1602	2.1602	0.1714	0.1714

Plate 9. Biological control of sheath blight using mycoparasites of *R. solani*

1 - Control

2a - Carboxin

2b - *Trichoderma harzianum*

2c - *Penicillium oxalicum*

2
1
2a
2b
2c
CUCKOO

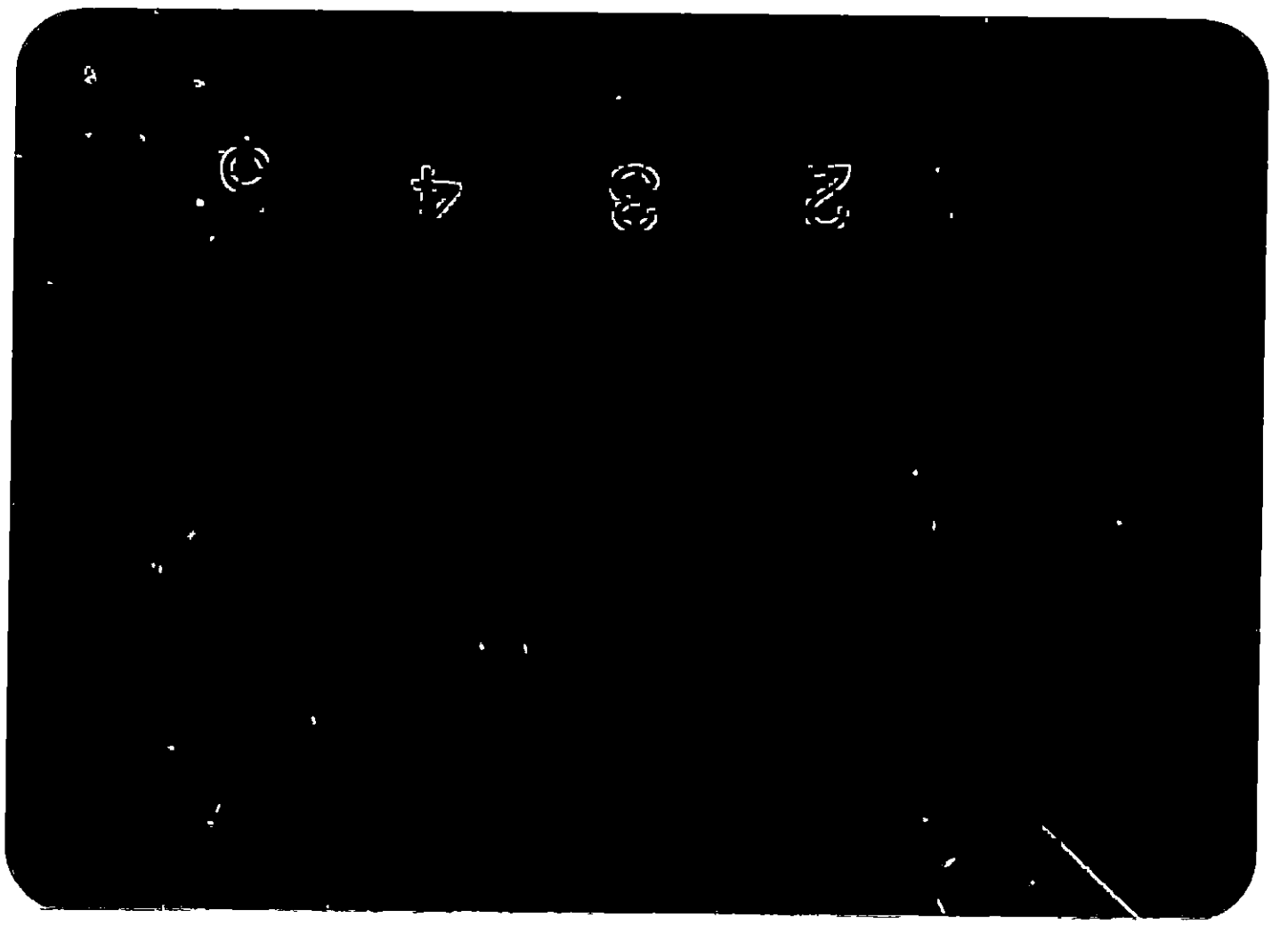
1 - Control

2 - *Trichoderma viride*

3 - *Trichoderma harzianum*

4 - *Penicillium oxalicum*

5 - Carboxin



The mycoparasites could also bring about significant reduction in the whole disease intensity and yield loss when compared with the control. The results reveal that the mycoparasites can also be used as one of the factors in the integrated management of the sheath blight disease.

VIII FUNGAL SUCCESSION ON THE RICE PHYLLOPLANE

The fungal succession on the rice phylloplane was found to vary considerably with the age of the rice plant. As the plants aged the fungi involved in the senescence were found to increase considerably. During the active tillering stage, the population of the phylloplane antagonists of R.solani was relatively low or absent (Table 32). There was a gradual increase in the population of these fungi at the panicle initiation stage of the crop. The level of these antagonists increased further more as the crop reached the harvesting stage. As the plants aged, the phylloplane saprophytes including Cladosporium cladosporioides, C.oxysporum, Curvularia lunata C.affinis, Fusarium chlamydosporum, F.tricinctum and Nigrospora sphaerica were found to register an increasing trend. This indicates the probable role of these leaf surface saprophytes in the senescence of rice plants.

Table 32: Fungal succession on the rice phylloplane at three stages of the crop growth (cfu per cm^2 of leaf)

	Active tillering stage	Panicle initiation stage	15 days prior to harvest
Aspergillus aculeatus	0	21	12
Aspergillus flavus	0	26	21
Aspergillus niger	0	16	8
Cladosporium cladosporiodies	1	9	27
Cladosporium oxysporum	2	7	41
Curvularia affinis	3	6	11
Curvularia lunata	0	2	7
Fusarium chlamydosporum	6	8	21
Fusarium tricinctum	4	7	28
Gliocladium virens	9	18	7
Hendersonula toruloidea	1	3	3
Myrothecium verrucaria	1	4	6
Mucor hiemalis	0	7	5
Nigrospora sphaerica	3	7	11
Penicillium funiculosum	0	12	18
Trichoderma harzianum	8	11	13
T. viride	4	16	21
Cryptococcus sp.	41	72	103
Sporobolomyces sp.	52	36	71

cfu - colony forming units.

DISCUSSION

DISCUSSION

Of the various maladies affecting the rice plant, diseases caused by fungi form a significant part. Among these fungal diseases apart from the sporadic incidence of blast disease, those which are causing havoc to rice cultivation in Kerala, include the sheath blight disease caused by Rhizoctonia solani Kuhn and the sheath rot disease caused by Sarocladium oryzae Gams & Hawksworth. For comabting these diseases, an arrary of chemicals have been suggested to the growers in the 'Package of practices recommendations' published by the Kerala Agricultural University (Kerala Agricultural University, 1982). The effects of these chemicals on the non-target microflora of the rice plants are little understood.

Baker and Cook (1974) have opined that "the biological world is a vast interacting network of living population in a state of dynamic equilibrium reflecting changes in their physical environment and their relations to each other". The leaf and plant surface harbour a complex array of microorganisms, composed of saprophytes and parasites. When plant protection chemicals are introduced 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 effects of some of the commonly used plant protection chemicals on the phylloplane microflora of rice and the important diseases affecting the leaf sheath were assessed under pot culture conditions followed by field trials at two localities in Kerala.

The pot culture studies conducted at the College of Agriculture, Vellayani, revealed that the population of saprophytic microflora was significantly higher in the rice variety Jyothi, than in the variety Karthika. Among the fungicides, carboxin when applied by itself or in combination with the insecticide carbofuran did not cause significant change in the phylloplane microflora whereas the fungicide edifenphos when applied by itself or in combination with insecticides brought about significant reduction in the mycoflora of rice. Many of the plant protection chemicals are known to reduce the population of phylloplane microflora (Hislop, 1976; Andrews and Kenerley, 1978).

Under field conditions also at the CSRC Karamana, the rice variety Jyothi was found to harbour a significantly higher population than the rice variety, Karthika. At both the locations, the fungicides edifenphos and mancozeb were found to reduce the phylloplane microflora significantly.

The fungicide carboxin was found to be the least deleterious to the non-target organisms at both the locations tested. Many workers have reported the suppressing effect of dithiocarbamate fungicides on the phylloplane microflora (Bainbridge and Dickinson, 1972; Dickinson, 1973; Dickinson and Wallace, 1976; Kuthubutheen and Pugh, 1978; Mehan and Chohan, 1981; Fokkema and Nooij, 1981).

The changes in phylloplane microflora of different rice varieties due to ageing has been recorded in India by Philip and Devadath (1980). Leben (1965) and Vosnyakovaskaya and Khudyakov (1960) indicated that in a given ecological situation the majority of the saprophytic mycoflora on a variety of host species will be identical. The results of the present study contradict these findings in that the rice variety Jyothi was found to harbour more leaf surface microorganisms than the rice variety Karthika. This difference is attributed to the differences in the plant characters such as the erect nature of leaves and wider leaf blades in the case of the variety Karthika.

Carboxin was found to stimulate several phylloplane antagonists of R. solani including Aspergillus flavus, Chaetomium spp., Gliocladium virens, Trichoderma harzianum and T. viride at both the locations. So also the two insecticides carbaryl and carbofuran were found to enhance

the phylloplane microflora at both the localities. The phylloplane yeasts were found to be inhibited by all the three fungicides used, edifenphos, mancozeb and carboxin and the insecticide carbaryl at the CSRC, Karamana.

The incidence of phylloplane yeasts at Adoor during the field trial was found to be erratic during both the seasons. This can be attributed to the dry climatic conditions prevalent in this rice growing tract, endemic for sheath blight disease.

An increase in the phylloplane microflora of groundnut plants by the application of the systemic fungicide benlate has been recorded by Mehan and Chohan (1981). Although benomyl is known as a wide spectrum fungicide (Warren, 1974) unlike the dithiocarbamates and other wide spectrum fungicides, the recovery of the microbial populations was observed even after repeated sprays with this fungicide (Dickinson and Wallace, 1976). Fokkema and Nooij (1981) observed that the fungicide oxycarboxin did not have any significant effect on the phylloplane microflora on cereal leaves.

The intensity of sheath blight and sheath rot was found to be significantly lower in the rice variety Karthika compared with the variety Jyothi. Varietal differences in susceptibility to these sheath diseases have been reported by many workers. (Hashioka, 1951; Mahendra Prabhath, 1971).

Under pot culure conditions and during the course of field trials, edifenphos and carboxin emerged as the most effective fungicides for reducing the incidence and intensity of sheath blight and sheath rot of rice.

At the CSRC Karamana, carboxin was found to surpass other fungicidal treatments in reducing incidence and intensity of sheath blight. The fungicides edifenphos and carboxin were found to be more efficient in checking both the diseases when applied in combination with the insecticides carbaryl or carbofuran. The increased efficacy of these fungicides when applied along with the insecticides has been documented. (Gokulapalan, 1981). This can be attributed to the control of infestation by insects and nematodes, which could aggravate the infection caused by fungi.

The efficacy of edifenphos and carboxin for the control of sheath blight and sheath rot diseases of rice has been reported by many workers and it is a routinely recommended fungicide for rice disease control. (Jagan Mohan, 1977; Lakshmanan, 1980; Mathai, 1975). The increased efficiency of the fungicide carboxin in reducing sheath blight can be owing to its ability to foster an increased level of the antagonistic microflora on the phylloplane thereby boosting the naturally occurring biological control. The greater

effectiveness of carboxin may also be attributed to the production of phytoalexins induced by colonisation of leaves by non-pathogenic fungi. Phylloplane saprophytes have been suggested as being involved in the production of phytoalexins and possibly changing the reaction of the host plants to pathogens (Blakeman, 1973; Mehan, 1978; Sinha, 1965). The grain and straw yields were found to have significantly increased at both the locations during both the seasons by the application of carbofuran. The reduction in disease intensity and insect damage can be attributed to be causes for the increased yields.

Following the method devised by Hashiba (1984), the experimental plants at CSRC Karamana were scored to assess the total disease incidence and yield loss due to sheath blight disease. The total disease incidence and the yield loss due to sheath blight were found to be significantly lesser in the variety Karthika when compared with Jyothi, during the first crop season. However, during the second season, there was no significant difference between the two varieties with respect to D and L values and during both the seasons, carboxin alone or in combination with carbofuran was found to reduce the D and L values considerably.

The naturally occurring microflora on the phylloplane of rice plants were assessed following standard procedures.

Among the fungi isolated and described, excepting for Aspergillus flavus, A. niger, Curvularia lunata, Mucor hiemalis, Nigrospora sphaerica and Rhizopus stolonifer, all the rest are new reports from the rice phylloplane. Between the two rice varieties, Karthika was found to harbour two species of Trichoderma, viz., T. hamatum and T. harzianum in addition to T. viride. A relatively rare species of Aspergillus, A aculeatus was also found to be prevalent on the phylloplane of the rice variety Karthika. All these fungi are efficient antagonists of R. solani, which can be the reason for the reduction both in disease incidence and intensity in this variety.

The bacteria isolated from the phylloplane of rice plants included several species of Bacillus, Alcaligenes sp., Chromobacterium sp., Acinetobacter sp., Propionibacterium sp. and Rothia sp. Philip and Devadath (1980) have done some preliminary work on the bacterial flora in the rice phylloplane. However, no bacterial species has been identified and reported. Islam and Nandi (1985) have reported the presence of Bacillus megaterium as a common inhabitant of the phylloplane. Apart from a few gram negative species, gram positive bacteria were found in abundance in the phylloplane of rice plants. The populations of phylloplane bacteria were significantly higher in the rice variety Jyothi than in Karthika.

Phylloplane yeasts have been reported on the phylloplane of rice plants by Jagadeesh and his co-workers from CRRI, Cuttack, (Jagadeesh, et al., 1976). This forms an isolated report of a few unidentified yeasts from the rice phylloplane. During the course of the present study, four different genera of basidiomycetous yeasts including the pink yeasts, Sporobolomyces and Sporidiobolus, the cream yeast Bullera and the brown yeast, Cryptococcus were found to occur frequently on the phylloplane of rice plants. This forms the first world report of this group of microorganisms from the phylloplane of rice plants.Last (1955) reported the presence of basidiomycetous yeasts on the phylloplane of cereals.

The different types of Streptomyces were occasionally isolated from the phylloplane of rice plants. These were not found in large numbers nor were they frequently isolated. The phylloplane has not been considered to be a suitable ecological niche for actinomycetes (Di Menna, 1962).

When the different leaf surface microorganisms were tested for their antagonism towards Rhizoctonia solani, several microbes were found to exhibit varying degrees of antagonism towards the test fungus. Both the zygomycetous fungi, Rhizopus stolonifer and Mucor hiemalis were found to have a smothering effect on the test fungus. Very early reports are present regarding the antagonistic action of these zygomycetous fungi on R. solani (Endo, 1931,32).

Some of the phylloplane fungi were found to cause cessation of growth of the test fungus, R.solani at the point of contact of the colonies. These include Aspergillus versicolor, A. wentii, Chaetomium dolichotrichum, Gliomastix murorum, Penicillium oxalicum, Fusarium tricinctum and Tritirachium oryzae. This type of reaction by these organisms on R. solani has not been reported as yet. The potential biocontrol agents of R. solani, viz., Trichoderma harzianum, T. viride and Chaetomium globosum caused a clear zone of inhibition between the paired cultures. These fungi completely overgrew R.solani parasitising it within a period of seven days. Many workers have reported the in vitro antagonism of these fungi against R. solani (Endo, 1936; Tveit and Moore, 1954; Bell et al., 1982; Gokulapalan and Nair, 1984).

Some of the bacterial isolates from the phylloplane of rice plants showed a high degree of in vitro antagonism towards R. solani. These included Alcaligenes, Bacillus spp., Chromobacterium, Propionibacterium and Rothia. The bacterial genus, Acinetobacter completely overgrew R. solani, suppressing its growth completely. The in vitro antagonism of several Bacillus species has been documented by many workers. (Endo, 1931, 1937; Hino, 1935; Olsen, 1965; Tschen and Kuo, 1981; Turchetti, 1982; Gokulapalan and Nair, 1984; Islam and Nandi, 1985).

There are recent reports regarding the antagonistic action of several Streptomyces spp. towards R. solani (Rothrock and Gottlieb, 1984; Turhan and Grossmann, 1986). None of the three types of Streptomyces isolated from the phylloplane exihibited antagonism towards R. solani.

Of the six isolates of phylloplane yeasts tested for their antagonism towards R. solani, four exhibited inhibitory action against the test fungus. The basidiomycetous yeasts, Sporobolomyces sp. and Cryptococcus sp. exhibited strong aversion towards R. solani. This type of interaction between R. solani and phylloplane yeasts of rice forms a new report.

When the mycoparasitism of several fungi towards R.solani was tested, it was found that Trichoderma harzianum and T.viride could efficiently parasitise R.solani hyphae. These fungi could cause granulation, vacuolation and ultimately the disintegration of host hyphae. These fungi would coil around hyphae of R.solani and penetrate the same before causing its lysis. The mycoparasitism of T.harzianum and T.viride towards R.solani has been reported by many workers. (Lewis and Papavizas, 1980; Elad et al., 1983; 1984; 1987; Roy and Sayre, 1984; Wu et al., 1986; Lewis and Papavizas, 1987b). The complete disintegration of hyphae at the septal plates forms a new record, hitherto not recorded for this host - parasite combination. The phylloplane fungi, Gliocladium virens, T.hamatum and T.koningii could also cause

granulation and vacuolation of host hyphae followed by coiling and penetration but to a lesser extent than in the earlier cases. The mycoparasitism of R.solani by G.virens and T.hamatum, has already been documented (Tu, 1980; Tu and Vaartaja, 1981; Chet et al., 1981; Elad et al., 1982; 1983; Lewis and Papavizas, 1987).

When four different plant protection chemicals employed in the present study were assayed for their action on R.solani, T.viride and T.harzianum, the fungicides carboxin and edifenphos, restricted the colony diameter of R.solani at both the levels tried. Edifenphos was found to be suppressive towards the phylloplane antagonists at both the concentrations tried while carboxin was not inhibitory to the phylloplane antagonists at the lower dose tried. This indicates that the use of carboxin at 500 ppm can cause reduction in the inoculum of R.solani without causing any supressive effect on its natural antagonists. Padmanabhan and Alexander (1982) demonstrated that while fungicides like plantvax and Demosan favour the growth of Trichoderma spp., other fungicides including ziram, agallol and fytolan were highly inhibitory.

Trials under pot culture conditions were conducted to assess the efficacy of a few phylloplane antagonists, Aspergillus aculeatus, Penicillium oxalicum, Trichoderma harzianum and T.viride to control sheath blight disease.

These fungi were found to reduce the disease incidence and intensity significantly. However, carboxin proved to be the best treatment in controlling the disease. An integrated method of controlling sheath blight disease of rice using carboxin along with the phylloplane antagonists can thus prove very fruitful.

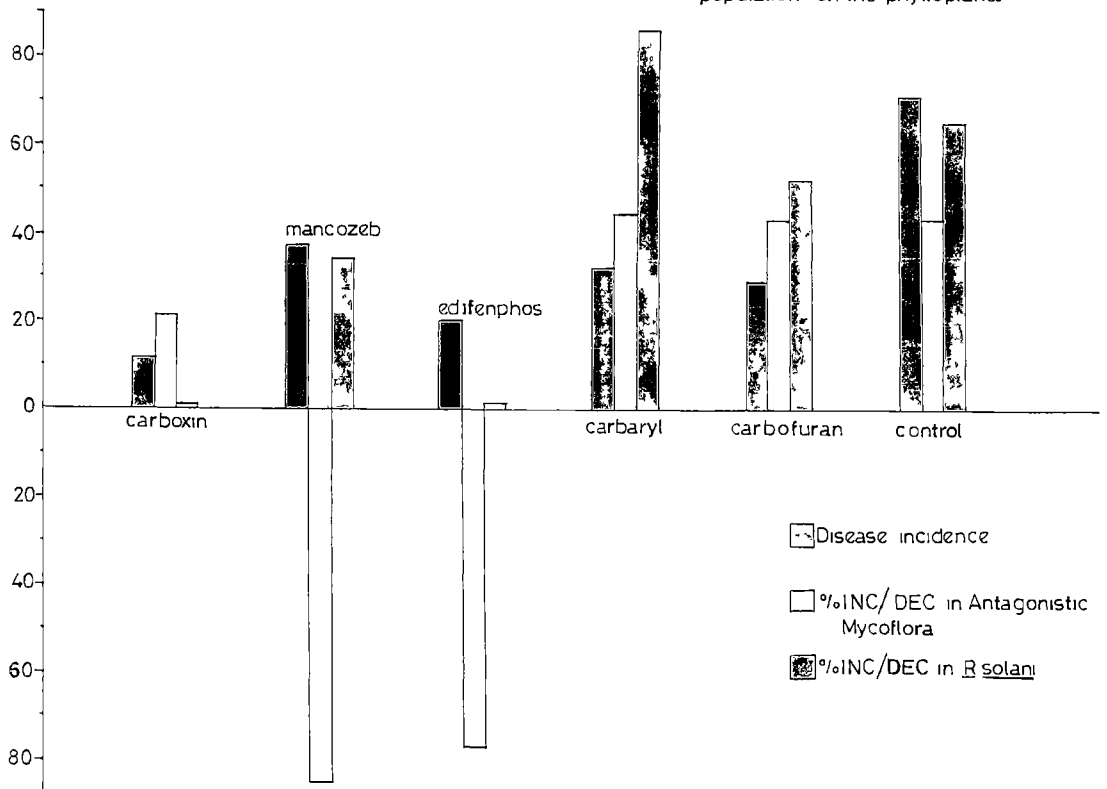
The biological control of R.solani using various microorganisms have been reported by many workers from all over the world (Weindling, 1932; 1934; Hadar et al., 1978; Tu and Chang, 1981; Chu and Wu, 1980; Chet and Elad, 1982; Velvis and Jager, 1984; Mew and Rosales, 1984; Lewis and Papavizas, 1987a).

When different types of inocula of the antagonistic organism were tested, wheat or rice bran cultures ofTrichoderma sp. were found to be efficient for controlling sheath blight disease. The use of wheat bran as a carrier material for this biocontrol agent has been well documented (Hadar et al., 1978; Chet and Elad, 1982; Elad et al., 1983; Lewis and Papavizas, 1987; Mukhopadhyay, 1987).

The concept that emerges out of the results of this study is briefly as follows (Fig. 11)

1. Indiscriminate use of pesticides on the rice plants can upset the natural balance of microflora on their aerial surface leading to a flare up of the various diseases.

Fig 11 Effect of pesticides on incidence of sheath blight, antagonistic phylloplane mycoflora and R. solani population on the phylloplane.



2. There are several resident microbes on the phylloplane of rice plants which offer them natural protection against the sheath blight pathogen.

3. These antagonists can be isolated, identified and mass multiplied and applied in the fields in conjunction with safe pesticides for an integrated method of disease control.

4. As far as possible only those pesticides which cause the least environmental disturbances or those which may enhance the multiplication of the natural antagonistic flora should be utilised.

SUMMARY

SUMMARY

The sheath blight disease caused by Rhizoctonia solani and the sheath rot disease caused by Sarocladium oryzae are two of the important diseases affecting the rice crop in Kerala. The present investigation was taken up to assess the effect of the commonly used plant protection chemicals on these important pathogens and the phylloplane microflora of rice. Emphasis was made on developing a strategy for biological control of the sheath blight disease, which is often not satisfactorily controlled by the application of fungicides.

Pot culture trials were conducted during three seasons to assess the effect of the plant protection chemicals on the fungal pathogens and phylloplane microflora of rice. Of the fungicides tested, carboxin was found to be the best for reduction of incidence and intensity of sheath blight and sheath rot disease affecting rice. This effect of carboxin was found to be enhanced when it was applied in combination with carbofuran. The phylloplane microflora was the least disturbed by the application of carboxin.

When field trials were conducted at Adoor and Karamana, the fungicide carboxin emerged as the best

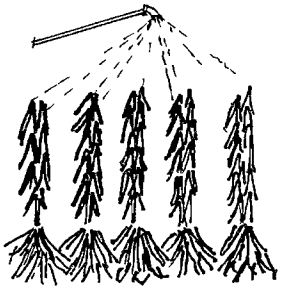
treatment for control of sheath blight and sheath rot at both the locations. The total disease incidence and yield loss were also significantly reduced by the application of carboxin or edifenphos along with carbofuran. The rice variety Karthika was found to be significantly tolerant to sheath blight and sheath rot diseases when compared with rice variety, Jyothi. The phylloplane microflora was not deleteriously affected by the application of carboxin. In some instances, the population of phylloplane fungi antagonistic to R. solani such as Trichoderma harzianum, T. viride, Penicillium oxalicum and Aspergillus aculeatus were found to be enhanced by the application of carboxin.

In an in vitro trial conducted to assess the effect of a few plant protection chemicals on the phylloplane antagonists of R. solani, it was found that at 500ppm, carboxin and mancozeb did not inhibit the mycelial growth of the antagonists.

Several microorganisms isolated from the rice phylloplane were found to exhibit in vitro antagonism towards R. solani. These include Aspergillus niger, A. aculeatus, A. versicolor, A. ustus, Chaetomium globosum, Mucor hiemalis, Penicillium oxalicum, P. funiculosum, Rhizopus stolonifer Trichoderma harzianum, T. viride, several bacteria and a few basidiomycetous yeasts.

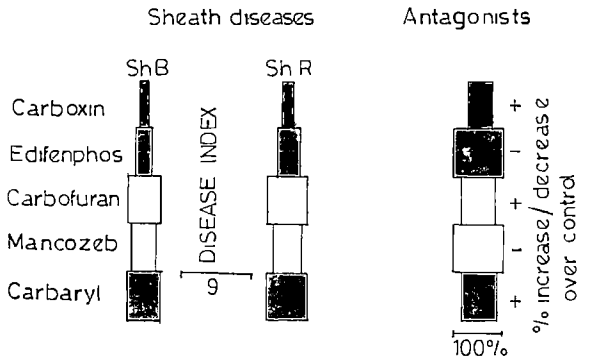
Fig 12 SELECTED BIOCIDES NATURAL PHYLLOPLANE MYCOFLORA AND THEIR INTERACTION IN THE MANAGEMENT OF RICE SHEATH DISEASES

BIOCIDES IN RICE CULTURE



- Control of pests & diseases
- Affects antagonists
- Persists in soil

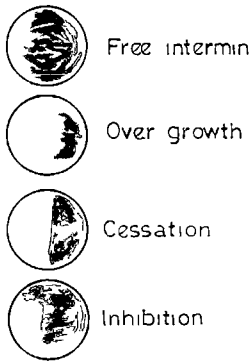
SELECTED BIOCIDES ON



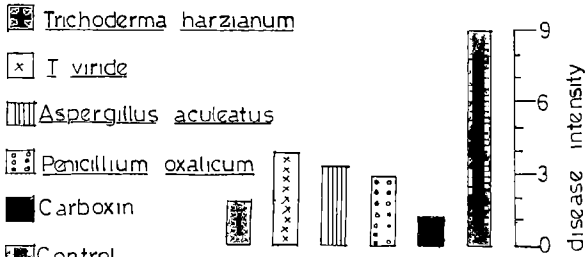
INTEGRATED MANAGEMENT OF SHEATH BLIGHT

MECHANISM

In vitro antagonism of phylloplane fungi



Selected efficent mycoparasites against Sh.B



When the mechanism of mycoparasitism of selected phylloplane antagonists on R. solani was studied, it was observed that Trichoderma harizianum, T. viride and Penicillium oxalicum could cause hyphal coiling and penetration of R. solani, leading to its disintegration.

Of the different types of inocula tested for the multiplication of the mycoparasites of R. solani, rice bran or wheat bran cultures were found to be the efficient ones for the control of the sheath blight of rice. When the efficacy of the phylloplane antagonists in controlling sheath blight was worked out it was found that the antagonistic fungi, Trichoderma harzianum, T. viride, Penicillium oxalicum and Aspergillus aculeatus could bring about significant reduction of sheath blight disease of rice, though this effect was not comparable with that of the fungicide carboxin.

REFERENCE

REFERENCES

Agnihothrudu,V. (1973). Acrocylindrium oryzae Sawada, Sheath rot on paddy. Kavaka 1: 69-71.

Ahmed, N.and Ahmed, Q.A. (1953). Selection of antagonistic soil microbes against a few fungal pathogens of Piper betle L. Mycopath.et.Mycol.Appl.21: 331-340.

Akai, S. and Kuramoto, T.(1968). Microorganisms existing on leaves of rice plants and the occurrence of brown leaf spot. Ann. Phytopathol.Soc. Japan 34 : 313-316.

Amin, K.S., Sharma, B.D. and Das, C.R. (1974). Occurrence in India of sheath rot caused by Acrocylindrium. Plant Dis.Reptr. 58 : 358-360.

Andrews, J.H. and Kenerley, C.M. (1978). The effect of a pesticide programme on non-target epiphytic microbial populations of apple leaves. Can.J.Microbiol. 24 : 1058-72.

Arora, D.K. and Dwivedi, R.S. (1980). Mycoparasitism of Fusarium sp.on Rhizoctonia solani Kuhn.Plant & Soil 55 : 43-53.

Asare-Nyako, A.(1967). The role of the leaf microflora on epidemiology of the northern leaf blight of corn.Diss.Abstr. 27-B : 4206-4207.

*Attabhanyo,A. and Rush, M.C. (1973). Sheath rot of rice in Louisiana. Proc. 2nd Int.Cong. Plant Pathol. Minneapolis. Minn. pp.5-12.

Babu George (1981). The role of organic amendments on the control of sheath blight of rice. M.SC. (Ag.) Thesis, Kerala Agricultural University. pp.79.

Bainbridge, A. and Dickinson, C.H. (1972). Effect of fungicides on the microflora of potato leaves. Trans.Br. mycol.Soc.59 : 31-41.

Baker, K.F. and Cook, R.J. (1974). Biological control of plant pathogens. W.H. Freeman and Co., San Francisco. pp.433.

Balakrishnan, B.(1981). Symptomatology, etiology, and control of sheath rot disease of rice caused by Acrocylindrium oryzae. M.Sc.(Ag.) Thesis, Kerala Agricultural University. pp.97.

Balakrishnan, B. and Nair, M.C. (1982). Chemical control of sheath rot disease of rice in Kerala. Indian J. Mycol. & Plant Pathol. 12 : 129-130.

Barnett, H.L. (1963). The nature of mycoparasitism by fungi. Ann.Rev.Microbiol. 17 : 1-44.

Barnett, H.L. and Binder, F.L. (1973). The fungal host parasite relationship. Ann. Rev. Phytopathol. 11 : 273-292.

Bell, D.K., Wells, H.D. and Marrwam, C.K. (1982). In vitro antagonism of Trichoderma species against six fungal pathogens. Phytopathology 72 : 379-382.

Berberich, S. (1987). Biological defense for many U.S. Crops. Agri.Res. U.S.A., March 1987. pp.6-7.

Bhakthavalsalam, G., Reddy, A.P.K and John, J.T. (1977). Chemical control of sheath blight of rice. Pesticides 11 (12) : 13-16.

Bhat, D.D. and Vaughan, E.K. (1962). Preliminary investigations on biological control of gray mold (Botrytis cinerea) of strawberries. Plant Dis. Reptr. 46 : 342-345.

Blakeman, J.P.(1973). The chemical environment of leaf surfaces with special reference to spore germination of pathogenic fungi. Pestic.Sci. 4: 575-588.

Blakeman, J.P. and Fokkema, N.J. (1982). Potential for biological control of plant diseases on the phylloplane. Ann.Rev, Phytopathol. 20 : 167-192.

Boosalis, M.G.(1956). Effect of soil temperature and green manure amendment of unsterilised soil on parasitism of Rhizoctonia solani by Penicillium vermiculatum and Trichoderma sp. Phytopathology 46 : 473-478

Boosalis, M.G. (1964). Hyperparasitism. Ann.Rev.Phytopathol. 2 : 363-376.

Butler, E.J. (1918). Fungi and diseases in plants. Spink and Co., Calcutta. pp.547.

*Camprota, P.(1985). Antagonism in vitro of Trichoderma spp. to Rhizoctonia solani Kuhn. Agronomie 5:613-620.

Chakravarty, D.K. and Biswas, S. (1978). Estimation of yield loss in rice affected by sheath rot. Plant Dis.Reptr. 62 : 226-231.

Chand, T.and Logan, C. (1984). Antagonists and parasites of Rhizoctonia solani and their efficacy in reducing stem canker of potato under controlled conditions. Trans. Br. mycol.Soc.83 : 107-112.

Chandra, A.K., Pati, B.R. and Gupta, S.K. (1979). Antifungal activities of the phyllosphere organisms. Curr.Sci. 48 : 522-525.

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

Chet, I. and Elad Y. (1982). Prevention of plant infection by biological means. Le Colloques de I'INRA, Bordeaux, France 21-26 March, 1982, 195-204.

Chet, I., Elad.Y., Kalfon, A., Hadar, Y.and Katan, J.(1982). Integrated control of soil borne and bulb borne pathogens in Iris. Phytoparasitica 10: 229-236.

Chet, I., Harman, G.E. and Baker, R. (1981). Trichoderma hamatum: its hyphal interactions with Rhizoctonia solani and Pythium spp. Microb.Ecol. 7 : 29-38.

*Chien, C.C. and Chu, C.L. (1973). Studies on the control of rice blast and sheath blight of rice with benlate. J. Taiwan Agric. Res. 22 : 41-46.

Chinnaswamy, R., Nair,M.C. and Menon,M.R.(1977). Comparative efficacy of fungicides in sheath rot control. Abstract of paper presented at Golden Jubilee Symposium on RRS, Pattambi, December 1977, KAU pp. 41-42.

*Chu, F.F. andWu, W.S.(1980). Biological and chemical control of potato black scurf. Plant Prot. Bull. Taiwan 22 : 269-286.

Dennis, C. and Webster, I.(1977). Antagonistic properties of species groups of Trichoderma III.Hyphal interaction. Trans. Br. mycol. Soc. 57 : 630-637.

Dickinson, C.H.(1973). Effects of Ethirimol and Zineb on Phylloplane microflora of Barley. Trans. Br. mycol. Soc. 60 : 423-431.

Dickinson, C.H. and Wallace, B.(1976). Effect of late applications of foliar fungicides on activity of microorganisms on winter wheat flag leaves. Trans. Br.mycol.Soc.67 : 103-112.

Di Menna, M.E.(1962). The antibiotic relationship of some yeasts from soil and leaves. J.Gen.Microbiol 27 : 249-257.

Elad, Y., Barak, R. and Chet, I. (1984). Parasitism of Sclerotium rolfsii by Trichoderma harzianum. Soil Biol. Biochem.16:381-386.

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

Elad,Y., Chet, I. and Henis, Y.(1981a). Biological control of Rhizoctonia solani in strawberry fields by Trichoderma harzianum. Plant & Soil 60: 245-254.

Elad, Y., Hadar, Y. and Chet, I. (1983). The potential of Trichoderma harzianum as a bicontrol agent under field conditions. 24th Colloquium, SFP, Bordeaux, France, 26-28th May 1983, 305-310.

Elad, Y., Hadar, Y., Chet I. and Henis, Y.(1981b). Biological control of Rhizoctonia solani by Trichoderma harzianum in carnation. Plant Dis. 65 : 675-677.

Elad, Y., Kalfon, A. and Chet, I.(1982). Control of Rhizoctonia solani in cotton by seed coating with Trichoderma spp. spores. Plant & Soil 66 : 279-281.

Elad, Y., Sadowsky, Z. and Chet, I. (1987). Scanning electron microscopical observations of early stages of interactions of Trichoderma harzianum and Rhizoctonia solani. Trans. Br. mycol. Soc. 88: 259-263.

Endo, S. (1931). Studies on the antagonism of microorganisms. I. Growth of Hypochnus centrifugus Tul., as influenced by the antagonistic action of other microorganisms. Bull. Miyazaki Coll. Agric. 3: 95-119.

Endo, S. (1932). Studies on antagonism of microorganisms. II. Growth of H.sasakii Shirai as influenced by the antagonistic action of other microorganisms. Bull.Miyazaki. Coll. Agric. 4: 133-158.

Endo, S. (1936). Studies on the antagonism of microorganisms. V. Pathogenicity of Hypochnus sasakii Shirai, H.centrifugus Tul. and Sclerotium oryzae sativae Saw., as influenced by the antagonistic action of the filtrates of certain fungous antagonists. Bull. Miyazaki Coll. Agric. 7: 61-75.

Endo, S. (1937). Physiological studies on the causal fungi of Sclerotium diseases of rice plant with special reference to some factors controlling the occurrence of the diseases. Bull.Miyazaki Coll. Agric. 11: 55-218

Estrada, B.A., Torres, C.Q. and Bonman, J.M (1984). Effect of sheath rot on some yield components. IRRN 9 (2): 14.

Fokkema, N.J.(1973). The role of saprophytic fungi in antagonism against Drechslera sorokiniana on agar plates and on rye leaves with pollen. Physiol.Plant Pathol. 3: 195-205.

Fokkema, N.J. and De Nooij M.P. (1981). The effect of fungicides on the microbial balance in the phyllosphere. EPPO Bull. 11: 303-310.

Gangopadhyay, S. and Chakrabarti, N.K. (1983). Current concepts on Fungal Diseases of rice. Today & Tomorrow's Printers and Publishers, New Delhi. pp. 349.

Gokulapalan, C. (1981). Role of the rice root nematode (Hirschmanniella oryzae) in the incidence of sheath blight disease of rice in Kerala, M.Sc. (Ag.) Thesis, Kerala Agricultural University. pp. 92.

Gokulapalan, C. and Nair, M.C. (1984). Antagonism of few fungi and bacteria against Rhizoctonia solani Kuhn. Ind. J. Microbiol. 24: 57-58.

*Gupta, A.K., Aggarwal, A. and Mehrotra, R.S. (1985). In vitro studies on antagonistic microorganisms against Sclerotium oryzae Catt. Geobios. 12: 3-5.

Gupta, R.C., Upadhyay, R.S. and Rai, B. (1979). Biological control of damping off with wheat bran culture of Trichoderma harzianum. Phytopathology 69: 147-151.

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

Harman, G.E., Chet, I. and Baker R. (1980). Trichoderma hamatum effects on seedling disease induced in radish and pea by Pythium sp. or Rhizoctonia solani. Phytopathology 70: 1167-1172.

*Hartzfield, F.G. (1957). Terrachlor, a new fungicide. Agric. Chemie. 21: 31-33.

Hashiba, T. (1984). Estimating method of severity and yield loss by rice sheath blight disease. Bull. Hokoriku Natn. Agric. Exp. Stn. 25: 115-164.

*Hashioka, Y. (1951). Varietal resistance of rice to sheath blight and the sclerotial diseases. Jap. J. Breed. 1: 21-26.

*Hashioka, Y. (1956). Prevalence and fungicidal control of rice sheath rot. Agric. & Hort., Tokyo 31: 953-957

*Hashioka, Y and Saito, T. (1953). Phytopharmacology of the Rice diseases I. In vitro tests on application of the dust fungicides to the important pathogenic fungi. Res.Bull.Coll.Agric.Gifu. 2: 12-18.

Hino, I, (1935). Antagonistic action of soil microbes with special reference to plant hygiene. Trans. third Int. Congr. Soil Sci. 1 : 173-174.

Hislop, E.C. (1976) Some effects of fungicides and other agrochemicals on the microbiology of the aerial surface of plants. In Microbiology of Aerial Plant Surfaces. (ed. Dickinson, C.H. & Preece, T.F.) pp. 41-74. London: Academic Press.

*Islam, K.Z. and Nandi, B. (1985). Inhibition of some fungal pathogens of host phylloplane by Bacillus megaterium. Z. Pflanzenkrankh. Pflanzenshutz 92: 233-240

IRRN (1976). Standard evaluation system for Rice Diseases. Los Banos, Laguna, Philippines, pp.64.

Jagadeesh, K.M., Lakshminarayana, C.S. and Mathur, S.C. (1978). Studies on phylloplane microorganisms of rice in relation to foliar diseases. Paper presented at the 3rd International Congress of Plant Pathology, Munchen 16-23rd August, 1978.

Jagan Mohan, K.P. (1977). Studies on the control of Sheath blight of rice caused by Corticium sasaki (Shirai) Matsumoto. M.Sc. (Ag.) Thesis, Kerala Agricultural University, pp. 53.

Jager, G. and Velvis, H. (1984). Biological control of Rhizoctonia solani on potatoes by antagonists. 2. Sprout protection against soil borne Rhizoctonia solani through seed inoculation with Verticillium biguttatum. Neth. J. Plant Path. 90: 29-33.

Jager, G., Ten Hoopen, A. and Velvis, H. (1979). Hyperparasites of Rhizoctonia solani in Dutch Potato fields Neth. J. Plant Path. 85: 253-268.

Jenkyn, J.F. and Prew, R.D. (1973). Activity of six fungicides against cereal foliage and root diseases. Ann.appl.Biol. 75: 241-252.

Jones, R.K. Belmar, S.B. and Jeger, M.J. (1987). Evaluation of benomyl and propiconazole for controlling sheath blight of rice cause by Rhizoctonia solani.Plant Dis. 71: 222-225.

Kannaiyan,S. (1979). Chemical control of sheath rot disease of rice. IRRN : 4 (3) : 14-15.

*Kannaiyan, S.and Prasad, N.N. (1976). Efficacy of certain fungicides on the control of sheath blight. IRRN 1 : 19.

*Kannaiyan, S.and Prasad, N.N. (1979). Effect of foliar spray of certain fungicides in the control of sheath blight disease of rice Res. Bull. Macco Agric. Digest 4: 3-6

Kerala Agricultural University (1982). Package of Practices Recommendations. pp. 199.

*Kozaka, T. (1961). Ecological studies on sheath blight of rice plant caused by Pellicularia sasakii (Shirai) S. Ito and its chemical control. Chugoku Agric. Res. 20 : 1-133.

Kozaka,T. (1970). Pellicularia sheath blight of rice plants and its control. Jap. Agric. Res. Q. 5: 12-16.

Krishnakumaran Nair, B. (1986). Edpidemiology and control of sheath rot disease of rice. M.Sc. (Ag.) Thesis, Kerala Agricultural University. pp. 109.

Kuthubutheen, A.J. and Pugh, C.J.F (1978). Effects of fungicides on physiology of phylloplane fungi. Tras. Br.mycol. Soc.71 : 261-269.

Lakshmanan, P. (1979). Studies on sheath blight of rice with special reference to the survival of the causal organism and control of the disease. M.Sc.(Ag.) Thesis, Kerala University. pp. 98.

Lakshmanan, P. (1984). Effective control of sheath rot of paddy. IRRN : 9(5) : 14.

Lakshmi, T.R. (1984). Evaluation of various herbicides on the control of sheath blight (Rhizoctonia solani Kuhn) on rice. (M.Sc. (Ag.) Thesis, Kerala Agricultural University. pp. 108.

Last, F.T. (1955). Seasonal incidence of Sporobolomyces on cereal leaves. Trans.Br. mycol. Soc. 38: 221-239.

Leben, C. (1965). Epiphytic microorganisms in relation to plant disease. Ann. Rev. Phytopathol. 3 : 209-230.

Lewis, J.A. and Papavizas, G.C. (1980). Integrated control of Rhizoctonia solani fruit rot of cucumber. Phytopathology 70 : 85-89.

Lewis, J.A. and Papavizas, G.C. (1985). Effect of mycelial preparations of Trichoderma and Gliocladium on populations of Rhizoctonia solani and the incidence of damping off. Phytopathology 75 : 812-817.

Lewis, J.A. and Papavizas. G.C. (1987a). Permeability changes in the hyphae of Rhizoctonia solani induced by germling preparation of Trichoderma and Gliocladium. Phytopathology 77: 699-703.

Lewis, J.A. and Papavizas, G.C. (1987b). Reduction of inoculum of Rhizoctonia solani in soil by germlings of Trichoderma hamatum. Soil Biol. Biochem. 19 : 195-201.

Lifhitz, R., Lifhitz, S.and Baker, R. (1985). Decrease in incidence of Rhizoctonia pre-emergence damping off by use of integrated chemical and biological control. Plant Dis. 69: 431-434.

Lilly, V.G. and Barnett, H.L. (1951). Physiology of the fungi. Mc Graw Hill Book Co. Inc. New York pp.464.

Lodder, J. (1974). The yeasts - A taxonomic study. North Holland Publishing Company Amsterdam - Oxford. pp, 34-113.

Lulu Das, (1986). Effect of application of plant protection chemicals on the survival of Rhizoctonia solani. Ph.D Thesis, Kerala Agricultural University, pp.108

*Lumsden, R.D. (1981). Mycoparasitism. Mycol.Ser.2 : 295.

Mahendra Prabhath, C.A. (1971). Studies on sheath blight of rice caused by Corticium sasakii (Shirai) Matsumoto. M.Sc.(Ag.) Thesis, University of Kerala. pp.80.

Manibhushanrao, K., Sreenivasaprasad, S. and Baby, U.I.(1987). Susceptibility of rice sheath blight pathogen Rhizoctonia solani to mycoparasites from rice field soils. Abstr. of paper presented in workshop on Biological control of Plant Diseases. TNAU Coimbatore 10-12 March pp.11-12.

Marshall, D.S. (1982). Effect of Trichoderma harzianum seed treatment and Rhizoctonia solani inoculum concentration and damping off on snap bean in acidic soils. Plant Dis. 66 : 788-789.

Mathai, G. (1975). Studies on the effect of fungicides and silica in the control of sheath blight of rice caused by Corticium sasakii (Shirai) Matsumoto. M.Sc.(Ag.) Thesis, Kerala Agricultural University.pp.58.

Mehan, V.K. (1978). Induction of resistance with non-pathogens and chemicals against 'tikka' disease of groundnut caused by Cercospora arachidicola and Cercosporidium personatum. Ph.D. Thesis, Punjab Agricultural University, Ludhiana, India.

Mehan, V.K. and Chohan, J.S.(1981). Effect of fungicides on leaf spot pathogens and the phylloplane mycoflora of groundnut Trans.Br.mycol. Soc 76 : 361-366.

Meshram, S.U.(1984). Suppressive effect of Azotobacter chroococcum on Rhizoctonia solani infestation of potatoes. Neth.J. Plant Path. **90** : 127-132.

Meshram, S.U. and Jager, G.(1983). Antagonism of Azotobacter chroococcum isolates to Rhizoctonia solani.Neth.J.Plant.Path. 89: 191-197.

Mew, T.W. and Rosales, A.M.(1984). Relationship of soil microorganisms to rice sheath blight development in irrigated and dryland cultures. In Soil borne Crop diseases in Asia. (Ed.Jan Bay-Petersen) pp.147-158. Taiwan : ASPAC.

*Mihuta, L.J. and Rowe, R.C.(1985). Potential biological control for fungus disease of radish. Ohio Report **70** : 9-11.

Mishra, R.R. and Srivastava, V.B.(1971). Leaf surface fungi of Oryza sativa Linn. Mycopathol. et Mycol. appl.**44** : 289-294.

*Miyake, I, (1910). Studien uber elie pilze der Reispflanze in Japan.J. Coll. Agric. Tokyo **2**: 237-276.

Mukherjee, N.(1978). Sheath blight of rice (Thanatephorus cucumeris) and its control possibilities. Pesticides **12** : 39-40.

Mukhopadhyay,A.N. (1987). Biocontrol efficacy of Trichoderma spp. in controlling soil borne diseases. Abstract of paper presented at workshop on Biological control of plant diseases, TNAU, Coimbatore 10-12, March pp.29.

Muneera, V.K.(1973). Studies on the control of sheath blight of rice, M.Sc.(Ag.) Thesis, Kerala Agricultural University, pp.66.

Nair, B.K., Balakrishnan, B. and Nair.M.C.(1988). Managing rice sheath rot disease in Kerala, India. IRRN 13 : 20-21.

Nair, M.C. and Sathyarajan, P.K.(1975). Sheath rot of rice. Agric. Res.J. Kerala 13 : 105-106.

Nanda, H.P. and Gangopadhyay, S.(1983). Control of Rice Helminthosporiose with Bacillus subtilis antagonistic towards Bipolaris oryzae. Int. J. Trop. Plant. Dis. 1 : 25-30.

Olsen, C.M. (1985). Antagonistic effects of microorganisms on Rhizoctonia in soil. Diss. Abstr. 25 : 3783-3784.

Ou, S.H. (1972). Rice Diseases Commonwealth Mycological Institute, Kew, Survey. pp.368.

Ou, S.H.(1985). Rice Diseases (2nd ed.) Commonwealth Mycological Institute, Kew, Survey.pp.380.

Padmakumary, G.(1972). Studies on some biochemical changes in paddy plants infected with Corticium sasakii (Shirai) Matsumoto. M.Sc.(Ag.)Thesis, Kerala Agricultural University.pp.61.

Padmanabhan, P. and Alexander, K.C.(1982). Studies on sugarcane seedling root rot in seed bed nurseries. Pestology 6 (9) : 9-12.

Padwick, W.G.(1950). Manual of Rice Diseases. Commonwealth Mycological Institute, Kew, Surrey. pp.198.

Papavizas, G.C.(1985). Trichoderma and Gliocladium: Biology and potential for biocontrol. Ann. Rev. Phytopathol. 23 : 23-54.

Papavizas, G.C., Lewis, J.A. and Abd-El Moity, T.H.(1982). Evaluation of new biotypes of Trichoderma harzianum for tolerance of benomyl and enhanced biocontrol capabilities. Phytopathology 72 : 126-132.

Paracer, C.S.and Chahal, D.S.(1963). Sheath blight of rice caused by Rhizoctonia solani Kuhn, a new record in India. Curr. Sci. 32: 328-329.

Parakhia, A.M. and Vaishnav, M.U.(1986). Bio-control of Rhizoctonia bataticola. Ind. Phytopath. 39 : 239-440.

Parmeter, Jr.(1969). Rhizoctonia solani Biology and Pathology Univ. California Press, Berkeley. pp.225.

Paromita Mukerjee, Singh, B.B. and Rahman, F. (1981). Testing of indigenous germplasm of rice against sheath rot by artificial inoculation. Indian Phytopath. 34 287-290.

Philip, R. and Devadath, S.(1980). Phylloplane fungal and bacterial flora of bacterial blight tolerant and susceptible races. Oryza 17: 34-37.

Porter, C.L. (1924). Concerning the characters of certain fungi as exhibited by their growth in the presence of other fungi. Amer. J. Bot. 11: 168-188

Prabhakaran, J., Ragunathan, V. and Prasad, N.H.(1974). Occurrence of sheath rot disease of rice caused by Acrocylindrium oryzae Sawada. Annamalai Univ.Agric. Res.Ann. 5: 182-183.

Pugh, G.J.F. and Van Emben, J.H.(1969). Cellulose decomposition fungi in polder soils and their possible influence on pathogenic fungi. Neth.J. Plant Pathl. 75 : 287-295.

Rabbinge, R.,Brouwer,A., Fokkema, N.J., Sinke,J. and Stomph, T.J. (1984). Effects of the saprophytic leaf mycoflora on the growth and productivity of winter wheat.Neth.J. Plant Path. 90 : 181-197.

Radhakrishnan,T.C. (1975). Studies on the control of sheath blight of rice caused by Corticium sasakii (Shirai) Matsumoto. M.Sc.(Ag.) Thesis, Kerala Agricultural University. pp.56.

Raina, G.L. and Singh G. (1980). Sheath rot out break in the Punjab. IRRN 5 (1): 16.

Raju, C.A. and Singh, R.A.(1981). Studies on sheath rot of rice II Chemical control. Pesticides. 15 (3): 26-28.

Rema Devi, L., Paul,T.S. and Gokulapalan, C.(1987). Efficacy of different fungicides in the control of sheath blight of rice. Indian J. Plant Prot.15: 69-70.

Riddel, R.W.(1950). Slide cultures. Mycologia 42 : 265-270.

Rothrock, C.S.and Gottlieb, D.(1984). Role of antibiosis in antagonism of Streptomyces hygroscopicus var. geldanus to Rhizoctonia solani in soil. Can.J. Microbiol.30: 1440-1447

Roy, A.K.(1981). Efficacy of a few fungicides on the control of sheath blight o rice.J. Res.Assam Agric. Univ.2 : 177-181.

Roy, A.K. and Saikia, U.N.(1976). Chemical control of sheath blight of rice. Indian Phytopath. 29 : 354-356.

Roy, A.K. andSayre, R.M.(1984). Electron microscopical studies of Trichoderma harzianum and T. viride and mycoparasitic activity of the former on Rhizoctonia solani f.sp. sasakii. Ind. Phytopath. 37 : 710-712.

Saksena, H.K. and Chaubey, R.D.(1972). Banded blight disease of paddy. Paper presented in the All India Coordinated Rice Research Project Workshop, October, 1972, Hyderabad, India.

Sakthivel, N.and Gnanamanickam,S.S.(1986a). Bacterisation of rice with Pseudomonas fluorescens reduces sheath rot infection. IRRN 11 : 17-18.

Sakthivel, N. and Gnanamanickam, S.S.(1986b). Toxicity of Pseudomonas fluorescens towards rice sheath rot pathogen, Acrocylindrium oryzae. Curr. Sci. 55 : 106-107.

*Sawada, K.(1922). Descriptive catalogue of Formosan fungi. II.Rep.Govt. Res. Inst. Dept. Agric.Formosa No.2 pp.135.

Seeley, H.W. and Vandemark, P.J.(1970). Microbes in Action-A laboratory manual of microbiology. D.B. Taraporewala sons & Co. Private Ltd., Bombay pp.189.

*Sesan, T.(1986). New contributions to the study of biology of Trichothecium roseum Link and Gliocladium roseum Bain. Studii Cercet. Biol. Veg. 38 : 78-82.

Singh, R.A. and Pavgi, M.S.(1969). Oriental sheath and leaf spot of Rice. Plant Dis. Reptr. 53 : 444-445

Sinha, S.(1965). Microbiological complex of the phyllosphere and control. Ind. Phytopath. 18 : 1-20.

Skidmore, A.M. and Dickinson, C.H.(1976). Colony interactions and hyphal interference between Septoria nodorum and phylloplane fungi. Trans. Br. mycol. Soc. 66 : 57-64

Sportelli, M., Nipoti, P. and D'Ercole, N.(1983). Biological control of certain fungal diseases of tomatoes grown in green houses. Informatore Fitopatologico 33 : 35-38.

Strashnov, Y., Elad,Y., Sivan, A. and Chet,I. (1985). Integrated control of Rhizoctonia solani by methyl bromide and Trichoderma harzianum. Plant Pathol. 34: 146-151.

Sullia, S.B. and Jayanthi, N.R.(1979). Antagonism between leaf surface organisms of rice. Curr.Sci. 48 : 266-267.

*Tasugi, H. and Ikeda, Y.(1956).Studies on the sheath rot of rice plant caused by Acrocylindrium oryzae Saw. Bull. Nat. Inst.Agric. Sci. Tokyo Series C. 6 : 151-161.

*Tschen, J.S.M. and Kuo, W.L.(1981). Antibiotic inhibition and control of Rhizoctonia solani by Bacillus subtilis. Plant Prot. Bull. Taiwan 27 : 85-103.

*Tu,C.and Chang, Y.C.(1981). Ecology of rice sheath blight pathogen. Rhizoctonia solani Ag-1 with reference on the biological control with Trichoderma sp. Res. Bull. Tainan Dist. Agrl. Develpt. Stn. 15 : 1-24.

Tu, J.C.(1980). Gliocladium virens, a destructive mycoparasite of Sclerotinia sclerotiorum Phytopathology 70 : 670 - 674.

Tu, J.C. and Vaartaja, O.(1981). The effect of hyperparasite (Gliocladium virens) on Rhizoctonia solani and Rhizoctonia root rot of white beans. Can.J. Bot. 59: 22-27.

*Turchetti, T.(1982). Antagonism of some Bacillus species to Rhizoctonia solani Kuhn isolate and its effect on the germination of Pinus nigra Arn. seed. European J.For. Path. 12 : 36-41

Turhan, G.and Grossmann, F. (1986). Investigation of a great number of actinomycetes on their antagonistic effects against soil borne plant pathogens by an improved method. J. Phytopath. 116 : 238-243.

Tveit, M and Moore, M.B.(1954). Isolates of Chaetomium sp. that protect oats from Helminthosporium victoriae. Phytopathology 44 : 686-689.

*Umeda, V.(1973). Hinosan, a fungicide for control of rice blast. Japan Pestic. Informn. 17: 25-28.

Utkhede, R.S. and Rahe, J.E.(1983). Interactions of antagonist and pathogen in Biological control of Onion White Rot. Phytopathology 73 : 890-893.

Van den Boogert, P.H.J.F. and Jager, G.(1983). Accumulation of hyperparasites of Rhizoctonia solani by addition of live mycelium of Rhizoctonia solani to soil. Neth. J. Plant Path. 89 : 223-228.

Van den Boogert, P.H.J.F., and Jager, G.(1984). Biological control of Rhizoctonia solani on potatoes by antagonists. 3. Incoculation of seed potatoes with different fungi. Neth. J. Plant Path. 90: 117-126

Velvis, H. and Jager, G. (1983). Biological control of Rhizoctonia solani on potatoes by antagonists. 1. Preliminary experiments with Verticillium biguttatum, a sclerotium inhabiting fungus. Neth. J. Plant Path. 89: 113-123.

Venkatasubbiah, P., Safeeulla, K.M. and Somasekhar, R.K. (1984). Efficacy of Trichoderma harzianum as a biocontrol agent for Rhizoctonia solani the incitant of collar-rot of coffee seedlings. Proc. Ind. Natn. Sci. Acad. Part B 50: 525-529.

*Vosnyakovskaya, Y.M. and Khudyakov, Y.P. (1960). Species compsition of the epiphytic microflora of the livig plants. Microbiologiya 28: 97-103.

*Waksman, S.A., (1919). Cultural studies of species of Actinomyces. Soil. Sci. 8: 71.

Waksman, S.A., (1922). A method for counting the number of fungi in soil. J.Bac eriol. 1: 339-341.

Warren, L.W. (1948). An undescribed species of Papulaspora parasitic on Rhizoctonia solani Kuhn. Mycologia 40: 391-401.

Warren, R.C. (1974). Differential effects of fungicides on phylloplane fungi isolated from oak. Trans. Br. mycol. Soc. 62: 215-218.

Weindling, R. (1932). Trichoderma lignorum as a parasite of other soil fungi. Phytopathology 22: 837-845.

Weindling, R.(1934). Studies on a lethal principle effective in the parasitic action of Trichoderma lignorum on Rhizoctonia solani and other soil fungi. Phytopathology 24: 1153-1179.

*Wu, W.S. (1980). Biological and chemcial seed treatments of soybeans (Trichoderma pseudokoningii and Trichoderma harzianum). Mem. Coll. Agric. Natn. Taiwan Univ. 20: 1-16.

*Wu, W.S., Liu, S.D. Chang, Y.C. and Tschen, J. (1986). Hyperparasitic relationships between antagonists and Rhizoctonia solani. Plant Prot. Bull. Taiwan 28: 91-100.

*Yamaguchi, T. (1974). Control of rice diseases by fine granular formulation Japan Pestic. Informn. 19: 9-13.

* original not seen.

APPENDICES

APPENDIX - I

Potato dextrose agar

Potato - 200 g

Dextrose - 20 g

Agar - 20 g

Distilled water - IL

Rosebengal streptomycin agar

Dextrose - 10 g

Peptone - 5 g

Potassium dihydrogen phosphate - 1 g

Magnesium sulphate - 0.5 g

Rosebengal - 1 part in 30,000 parts of the medium

Agar - 20 g

Distilled water - IL

Nutrient agar

Beef extract - 1 g

Yeast extract - 2 g

Peptone - 5 g

Sodium chloride - 5 g

Agar - 15 g

Distilled water - IL

Adjust pH to 7.2 - 7.4

Yeast extract - Malt extract agar

Yeast extract - 4 g

Malt extract - 10 g

Glucose - 4 g

Agar - 17 g

Distilled water - IL

Adjust pH to 7.3 - 7.4

Conn's glycerol asparaginate agar

Glycerol - 10.0 g

Sodium asparaginate - 1.0 g

Dipotassium phosphate - 1 g

Agar - 20 g

Distilled water - IL

Adjust to pH 7.0

Nutrient broth

Beef extract - 1 g

Yeast extract - 2 g

Peptone - 5 g

Sodium chloride - 5 g

Distilled water - IL

Adjust pH to 7.2 - 7.4

Semi solid agar

Beef extract - 1 g

Yeast extract - 2 g

Peptone - 5 g

Sodium chloride - 5 g

Agar - 7 g

Distilled water - IL

Adjust pH to 7.2 - 7.4

Glucose - Yeast extract peptone water

Glucose - 2 g

Yeast extract - 2 g

Peptone - 2 g

Water - IL

APPENDIX - II

Crystal violet

One volume saturated alcohol solution of crystal violet in four volumes of one per cent aqueous ammonium oxalate.

Gram's iodine

Iodine crystals - 1 g

Potassium iodide - 2 g

Distilled water - 300 ml

Safranin

Ten ml saturated solution of safranin in 100 ml distilled water.

APPENDIX III

Abstract of Anova - Pot Culture Studies (MSS Values)

Source	df	Table 1 Obs 1	Table 1 Obs 2	Table 2 Obs 1	Table 2 Obs 2	Table 3 Obs 1	Table 3 Obs 2	Table 4 AT	Table 4 PI	Table 4 15 DPH	Table 5 AT	Table 5 PI	Table 5 15 DPH	Table 6 Int	Table 6 Inc
Treatment	23	2.66	1.60	42.02	34.91	2.19	0.91	2.48	3.87	5.83	22.60	28.01	48.10	3.52	9.12
A	2	8.18	2.10	69.24	66.18	7.92	2.16	15.21	6.72	10.76	6.16	16.57	18.30	3.28	7.29
B	3	3.17	4.38	4.34	3.88	4.44	2.37	0.65	0.77	2.23	5.98	30.39	12.45	0.39	3.23
C	1	0.37	2.52	0.59	1.09	0.20	0.73	1.07	1.11	6.10	2.20	3.90	3.83	4.90	4.89
A x B	6	2.50	1.95	14.13	3.37	0.54	0.24	1.81	1.67	2.73	7.80	1.33	6.05	0.19	1.17
B x C	3	3.72	3.01	20.27	7.14	0.86	0.90	0.97	0.98	2.06	1.84	4.40	3.66	0.55	3.26
A x C	2	0.81	0.64	2.77	8.06	2.41	0.26	0.34	0.67	2.80	1.78	1.25	2.48	0.41	4.35
A x B x C	6	1.18	1.32	18.53	23.32	2.55	0.69	1.51	1.12	2.78	9.68	1.74	2.14	0.35	1.51
Error	48	0.60	0.42	0.97	2.35	0.11	0.11	1.95	0.78	0.23	0.90	0.65	0.23	1.11	0.52

Location II Karamana

Abstract of Anova - Field Trials (MSS Values)

Source	df	Table 12 obs 1	Table 12 obs 2	Table 13 obs 2	Table 14 obs 1	Table 14 obs 2	Table 15 PI	Table 15 15 DPH	Table 16 PI	Table 16 15DPH	Table 17 INT	Table 17 INC	Table 18 D	Table 18 L	Table 20 Grain	Table 20 Straw
Replication	2	0.83	1.32	1.53	0.97	0.15	2.36	1.11	0.14	5.25	2.24	0.29	7.61	3.58	1.84	0.38	0.58
Treatment	23	10.09	8.43	4.17	2.63	3.88	15.31	4.15	1.11	62.50	41.51	0.46	26.71	5.35	3.57	1.71	10.56
A	3	14.35	9.21	3.05	4.18	3.10	6.49	0.95	4.15	27.91	21.03	1.17	59.07	8.11	3.42	2.46	29.39
B	2	3.67	5.04	3.91	6.26	13.30	4.77	5.08	0.95	31.30	21.27	0.32	26.42	3.97	2.03	0.67	4.91
C	1	2.19	2.94	11.31	5.09	2.87	5.81	0.56	5.08	38.88	24.81	0.14	11.26	10.86	0.99	0.15	15.31
A x B	6	5.96	10.55	5.42	2.31	0.97	5.16	0.56	0.56	33.40	10.07	0.17	11.90	6.82	5.73	0.75	5.23
B x C	2	7.37	6.90	2.61	2.29	5.82	1.40	0.61	0.56	24.31	20.93	0.31	4.18	3.05	1.80	0.48	2.80
A x C	3	6.87	1.41	5.46	1.90	0.51	7.81	0.52	0.61	33.44	21.98	0.70	2.97	5.37	0.49	0.67	1.01
A x B x C	6	1.65	7.68	2.53	0.64	2.67	7.28	0.14	0.62	9.52	9.27	0.63	1.81	5.49	0.30	0.34	3.11
Error		1.82	1.81	1.99	0.87	0.24	1.66	0.12	0.14	7.71	3.67	0.36	6.14	0.50	0.33	0.28	4.26

Abstract of ANOVA For Table 28

Source	df	MSS
Treatments	23	44.96
A	3	99.20
B	2	91.13
C	1	33.79
A x B	6	10.31
B x C	2	41.92
A x C	3	64.22
A x B x C	6	76.03
Error	95	7.27

Abstract of Anova for Tables 30 & 31

Source	df	Incidence	Intensity	D	L
Replication	2	4.44	3.88	1.93	0.11
Treatments	11	50.99	8.66	0.21	5.72
A	5	20.27	6.85	7.66	2.15
B	1	13.98	2.24	0.66	15.81
A x B	5	6.39	7.72	0.26	7.27
Error	24	1.82	0.98	0.15	0.10

EFFECT OF PLANT PROTECTION CHEMICALS ON FOLIAR PATHOGENS AND PHYLLOPLANE MICROFLORA OF RICE

By

C. GOKULAPALAN M.Sc. (Ag.)

ABSTRACT OF A THESIS

Submitted in partial fulfilment of the requirement

for the degree

DOCTOR OF PHILOSOPHY

Faculty of Agriculture

Kerala Agricultural University

Department of Plant Pathology

COLLEGE OF AGRICULTURE

Vellayani, Trivandrum

1989

ABSTRACT

The present investigation was undertaken to assess the effects of plant protection chemicals on the important fungal pathogens and phylloplane microflora of rice. An attempt has been made to identify potential biological control agents to combat sheath blight disease causing havoc to rice cultivation in Kerala.

Both under pot culture conditions and during the course of field trials at two locations viz., Adoor and Karamana, Kerala, the fungicide carboxin was found to be the best treatment for reducing the incidence and intensity of sheath blight and sheath rot diseases of rice. The fungicide was found to be the least harmful to the epiphytic microflora of the rice plant. In some instances the population of phylloplane antagonists of R. solani, including Trichoderma harzianum, T. viride, Penicillium oxalicum and Aspergillus aculeatus were found to be enhanced by the application of carboxin.

The total disease incidence and yield loss were significantly reduced by the application of edifenphos. The rice variety Karthika was found to be significantly tolerant to sheath blight and sheath rot compared with the rice variety Jyothi.

The fungicides carboxin and mancozeb at 500 ppm did not inhibit the growth of the phylloplane antagonists of R.solani viz., Trichoderma harzianum and T. viride under in vitro conditions.

Several microorganisms isolated from the rice phylloplane were found to exhibit in vitro antagonism towards R. solani. These include Aspergillus aculeatus, A.niger, Chaetomium globosum, Penicillium oxalicum, Trichoderma harzianum, T. viride, several bacteria and a few basidiomycetous yeasts.

The phylloplane antagonists, Trichoderma harzianum, T. viride and Penicillium oxalicum were found to readily parasitise R.solani hyphae leading to coiling, penetration followed by disintegration and death of the mycohost.

When these antagonists were cultured on bran and tried for their efficacy as potential biocontrol agents of R.solani, it was found that these fungi could significantly reduce the incidence and intensity of sheath blight of rice, though this was not comparable with the effect of the fungicide carboxin.