INTEGRATED MANAGEMENT OF SHEATH BLIGHT DISEASE OF RICE

BY

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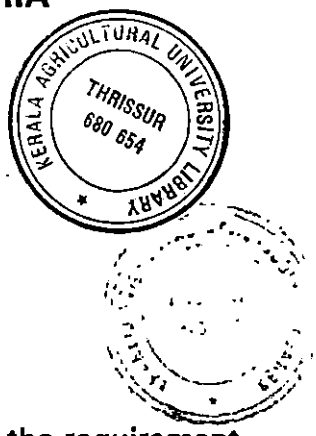

THESIS

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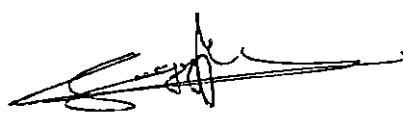
DECLARATION

I hereby declare this thesis entitled "Integrated management of sheath blight disease 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.



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CERTIFICATE

Certified that this thesis entitled "Integrated management of sheath blight disease of rice" is a record of research work done independently by Mr.Geogy Zacharia under my guidance and supervision and that it has not previously formed the basis for the award of any Degree, Fellowship or Associateship to him.

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INTRODUCTION

INTRODUCTION

Rice is the staple food of more than 60 per cent of the world's population. In India rice is the most important and extensively grown food crop, occupying about 40 million hectares. Eventhough India has the maximum area under rice among all the rice growing countries, its productivity is quite low and is much lower than the world average. The low yield in India is attributed to several reasons, viz., socio-economic conditions, drought, flood, lack of inputs and occurrence of pests and diseases.

In an attempt to increase rice production many important varieties have been developed. The disease situation has completely changed with the introduction of high yielding semi-dwarf varieties and associated new technology of crop production. So far over 30 fungal, 8 bacterial and 12 viral diseases are recorded on rice. Sheath blight disease caused by Rhizoctonia solani, once considered as a minor disease in the country, became the major limiting factor in successful rice cultivation in certain areas, including Kerala, in late sixties and early seventies. In Kerala the damage due to the disease is quite severe during the Kharif season. Losses due to this disease generally vary from 30 to 40 per cent and may be even 100 per cent in endemic areas. In spite of the heavy

losses, options for control are, however, somewhat limited as rice cultivars with high yield potential possess a low inherent resistance and chemical control is uneconomic. Hence, there is an urgent need for alternate methods of disease control. There is a large volume of research on sheath blight in rice little of which, however, deals with the microbial relationship in disease development.

The population of a plant pathogen present in soil at a given time (the quantity of inoculum) with the capacity of the environment to produce the disease on a host constitutes the inoculum potential. Unlike diseases of aerial parts, wherein one viable unit of inoculum is capable of initiating disease, in soil-borne diseases a certain minimum energy or mass of viable units of inoculum is required to cause infection. Hence, the concept of inoculum potential becomes more relevant in the case of soil-borne diseases.

Variable results were reported from different countries and within the country by different investigators on the sclerotial survival/viability in soil. Further, no uniform method has been adopted for the estimation of propagule population of R. solani and no effective selective medium has so far been developed.

Soil amendment, in general, encourages growth and multiplication of soil microflora and this can inactivate or kill the pathogen in soil by competition or antibiosis. Biocontrol employing species of Trichoderma has also been reported. However, the above has not so far been fully exploited for the management of the disease. With advances in our knowledge on the ecology of plant pathogens and antagonistic microorganisms, biological control has become a distinct possibility from a theoretical postulation.

Integrated disease management is the most effective strategy to be followed in which need based chemical measures are to be blended with cultural and biological measures as the above are never mutually exclusive. Attempts were made in the present investigation to determine the population dynamics of R. solani in soil, to identify a suitable biocontrol agent against the pathogen and to manage the disease by integrating different methods of cultural, biological and need based application of a systemic chemical. Standardisation of an appropriate methodology for precise estimation of R. solani from soil was the first task. This was followed by a laboratory assay of soil microorganisms which are antagonistic to R. solani and their identification. The efficiency of antagonists was screened in pot

trials and the best one was tested in an elaborate field trial in combination with organic and inorganic soil amendments and a systemic fungicide.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

2.1 SHEATH BLIGHT DISEASE

Sheath blight disease of rice was first described by Miyake (1910) from Japan under the name oriental sheath blight and leaf spot. He named the organism as Sclerotium irregulare. Later on, this disease has been reported from various rice growing countries of the world (Ou, 1985).

In India the occurrence of the disease was reported for the first time by Butler (1918). However, a detailed description of the fungus causing sheath blight disease of rice viz., Rhizoctonia solani Kühn was made by Paracer and Chahal (1963) from Punjab. Saksena and Chaubey (1972) reported a banded blight disease of rice from Uttar Pradesh. They observed that air borne basidiospores produced by the pathogen caused leaf blight with banded symptoms and spots on leaf sheath. The causal organism was identified as Thanatephorus cucumeris (Frank) Donk. Greatest loss in yield occurred when infection started during late vegetative or early reproductive phase (Hori, 1969; Lee and Rush, 1983).

In countries like Sri Lanka, China, Taiwan and Japan, sheath blight is considered to be of major importance and as far as the damage is considered it is next

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to blast (Gangopadhyay, 1983).

In Kerala, the incidence of the disease was first recorded by Prabhath (1971). The disease has gained much importance subsequent to wide cultivation of high yielding varieties (Menon, 1982). A survey of rice diseases in farmer's fields of major rice growing areas of Kerala viz., Palghat, Kole areas of Trichur and Kuttanad regions of Alleppey at monthly intervals for six continuous cropping seasons from 1977 onwards has shown that sheath blight was quite severe on all high yielding varieties. Eventhough the local varieties succumbed to infection, the pathogen did not travel to the inner whorls and hence plants were not killed outright (Rajan, 1983).

Plants are usually attacked at about the tillering stage, when leaf sheaths become discoloured at or above water level, or above soil level in upland conditions. The lesions are first pale greenish, grey ellipsoidal 2-3 cm long, with a narrow dark brown margin. The lesions afterwards coalesce and later become fawn or off-white with a brown or purplish-brown margin. The lesions are large, oblong or irregularly elongated and may appear on any part of the leaf sheath sometimes extending on to the leaf blade. Several such lesions can encircle the whole leaf or culm and cause death of plants (Gangopadhyay and Chakrabarti, 1982).

The causal organism is a fungus. Several names such as Hypochnus sasakii Shirai (from Japan), Rhizoctonia solani Kuhn (from China, Sri Lanka and Philippines), Corticium vagum Berk and Curt. (from India and West Germany), Corticium solani (Prill and Delacr.) Bourd. and Galz., Pellicularia filamentosa (Pat.) Rogers f. sasakii and P. sasakii (Shirai) S. Ito. According to recent studies, the imperfect stage of the fungus is known as Rhizoctonia solani and the perfect stage is called Thanatephorus cucumeris (Frank) Donk (Ou, 1972; Gangopadhyay, 1983).

2.2 Population dynamics of Rhizoctonia solani in paddy soil

2.2.1 Perpetuation of the pathogen

Soil-borne sclerotia left in the field from the previous crop and to a lesser degree mycelium in plant debris are the means of pathogen survival between crops and are the primary inocula (Kozaka, 1970; Hori and Anraku, 1971; Yamaguchi et al., 1971).

Prabath and associates (1974) and Roy (1976) observed that sclerotia remain viable up to 21 months in dry soil. However, in submerged soil the survival period is reduced to a maximum of three months.

Sclerotial population on undistributed Arkansas rice fields after a crop with high incidence of sheath

blight varied from high to low as depth increases (Lee, 1980). Densities in the surface zone 0-0.6 cm, varied from 216 to 701 sclerotia per litre of soil, with a viability range from 42.3-51 per cent. A linear correlation between the number of sclerotia of R. solani from soil and the incidence of sheath blight disease of rice at panicle initiation stage was worked out by Belmar and Jones (1985) and Belmar and associates (1987).

Under north Indian conditions, the pathogen produces basidiospores in abundance in nature on rice plants and also on weed hosts as thin powdery white layer over the surface of leaf sheath and leaf blade of affected parts. These basidiospores readily cause leaf and panicle infection leading to "banded blight" phase (Saksena and Chaubey, 1972; Saksena, 1985). Lakshmanan and associates (1982) also observed that the pathogen produces perfect state (Thanatephorus cucumeris) in straw from infected paddy fields of Kerala, which may also serve as source of inoculum.

2.2.2 Techniques of estimation of R. solani propagules from soil

The relationship between preplant inoculum density and disease intensity at critical growth stages of crop was designed to be studied by rapid and reliable methods to quantify R. solani propagules from infected soil. The

important techniques developed includes

(1) immersion and baiting techniques (Mueller and Durrell, 1957; Papavizas and Davey, 1962)

(2) the inoculum density method consisting of soil fractionation-floatation techniques (Naiki and Ui, 1977; Weinhold, 1977; Lee, 1980; Belmar et al., 1987) and direct plating of soil by weight (Ko and Hora, 1971) or in pellets (Henis et al., 1978) and

(3) the inoculum potential method by making use of susceptible host plants (Bouhot, 1979; Sneh et al., 1966).

2.2.2.1 Immersion and baiting techniques

Mueller and Durrell (1957) described the procedure of immersion tubes for isolation of R. solani from soil using nutrient agar. Martinson and Baker (1962) could easily isolate R. solani with such conventional laboratory medium in the immersion tubes as potato dextrose agar or Modified Richard's agar. Papavizas and Davey (1962) used mature dry stems such as buck wheat, cotton or bean for assaying R. solani population. Sneh and associates (1966) suggested that bean segment colonisation technique was superior to immersion tube method or plant debris particle isolation method. However, seedling infection was found to be the most reliable among four methods.

2.2.2.2 Soil fractionation and floatation techniques

The above technique was based on the principle that R. solani colonises organic debris or forms sclerotia which can be separated by sieving or floatation. Boosalis and Scharen (1959) described the floatation technique, wherein debris particles suspended were plated in water agar with pH adjusted and fortified with antibiotic in sugarbeet growing soils. Naiki and Ui (1977) determined the number of sclerotia by floatation sieving method using 2 per cent hydrogen peroxide in sugarbeet soils. Weinhold (1977) estimated R. solani population in cotton or potato soils by wet sieving through 0.35 mm mesh sieve and organic matter collected were dispersed in one per cent water agar for germination. Clark and associates (1978) described a procedure for the use of a semiautomatic elutriator to assay soils for R. solani. Lee (1980) modified the above method, in which all plant materials were suspended in water, sieved through 1.7 mm sieve placed over 0.6 mm sieve and were examined through dissecting microscope for R. solani sclerotia. Belmar and associates (1987) evolved a technique incorporating the methods viz., semiautomatic elutriator (Clark et al., 1978) and sieving (Lee, 1980). In this method, soil samples were processed as before, but a mass density separator was employed to reduce the time for

enumeration of extracted sclerotia by eliminating about 75 per cent of the lighter weight debris in the extracted samples.

2.2.2.3 Soil plating and pelleting on selective media

The use of selective media for R. solani was only recently perfected (Tsao, 1970). Ko and Hora (1971) devised a selective medium for the isolation of R. solani propagules. It consisted of 1.0 g of K_2HPO_4, 0.5 g of $MgSO_4$ 7 H_2O, 0.5 g of KCl, 0.01 g of $FeSO_4$ 7 H_2O, 0.2 g of $NaNO_3$ and 20 g of agar per litre (sterilized together at 121°C for 15 minutes) gallic acid 0.4 g, fenaminosulf (Dexon 70 WP) 0.6 g, chloramphenicol 0.05 g and streptomycin sulphate 0.05 g per litre were added after sterilization. Ferriss and Mitchell (1976) suggested modification of Ko and Hora medium, which consisted of 0.2 g of KNO_3, 30 g of agar, 0.09 g of metalaxyl (Redomil 2 E), 0.05 g of streptomycin sulphate and 0.05 g of chloramphenicol per litre. Plating on Ko and Hora medium is tedious and require many precisely and individually weighed samples for placing separately on agar medium. Henis and associates (1978) described a multiple-pellet-soil sampler in which soil pellets of predetermined weight were deposited them on smooth surface of an agar medium.

Gangopadhyay and Grover (1985) modified Ko and Hora medium by replacing fennaminosulf with fosetyl-al (Aliett 80 WP). Van Bruggan and Arneson (1986) compared wet sieving technique and soil pelleting technique, where pelleting method recovered more propagules of Rhizoctonia spp. from soil, but was found to be more costly and labour intensive. Trujillo and associates (1987) modified medium of Ferriss and Mitchel (1976). It consisted of 0.2 g of potassium nitrate, 30 g of agar, 947 ml of distilled water, 53 ml of 95 per cent ethyl alcohol, 0.38 ml of Metalaxyl 2E, 0.02 ml of prochloraze 40EC, 100 mg of tobramycin and 300 mg of streptomycin.

2.3 Chemical control of sheath blight disease

Chemical control of sheath blight has been attempted by different workers all over the world. But results from foliar fungicide application are often erratic, particularly in test with a large number of candidate fungicides (Lee and Rush, 1983).

In the past, nonsystemics were widely used. Recently the same are being replaced by potent systemic fungicides. Prabhat (1971) reported that Vitavax was superior to other fungicides against Corticium sasakii both in vitro and in vivo. The above observation has been confirmed by later workers (Mohan, 1977; Lakshmanan et al., 1980 and Gokulapalan, 1981).

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Chin (1977) recommended Carbendazim as the best fungicide for the control of sheath blight disease in Malaysia. Bhakthavatsalam and associates (1977) observed that Bavistin spray twice could check the disease significantly in field trials. Jaganathan and Kannaiyan (1978) also obtained good protection using Bavistin against sheath blight disease.

Effectiveness of Benlate, Difolatan, Captafol, Hinosan, Dithane Z-78 and Dithane M-45 against sheath blight disease was proved by Nair and Rajan (1978) and Rajan and associates (1979). Kannaiyan and Prasad (1979a) reported the effectiveness of Bavistin, Kitazin, Hinosan, Benlate and Demosan for the control of sheath blight in a pot trial. They also confirmed the effectiveness of these fungicides in field trials (Kannaiyan and Prasad, 1979b).

Kannaiyan and Prasad (1979c) found that Vitavax (Carboxin) completely inhibited the sclerotial germination. Kannaiyan and Prasad (1979d) have indicated that application of trace elements like borax, zinc sulphate, copper sulphate and ferrous sulphate at 0.05 per cent as two foliar sprays at ten days interval reduced the disease and increased the grain yield. Viswanathan and Mariappan (1980) found Carbendazim, Carboxin and Kitazin were effective as prophylactic and therapeutic spray in control of the disease.

Dev and Sathyarajan (1980) and Dev (1980) suggested Carbendazim to be the best foliar fungicide, eventhough it was less effective than a combination of Thiram and Hinosan as soil and foliar treatments, respectively. Carbendazim and Ediphenphos reduced the disease incidence in a pot trial conducted by Roy (1981). Reddy and his associates (1981) found that Carbendazim fungicides MBC, Derosal and Bavistin significantly checked R. solani development and increased the yields. Behera and associates (1982) conducted in vitro studies for the evaluation of fungicides in which Bavistin and Benlate were most effective. Kannaiyan and Prasad (1984) reported that fungicides Benlate, Kitazin, Hinosan, Demosan and Daconil were highly effective against the sheath blight disease and also increased the grain yield significantly. Kurono (1985) reported a new systemic fungicide Monocut in benzanilide group effectively controlled rice sheath blight disease. Dev and Mary (1986) recommended the use of Validamycin and Carbendazim for the control of sheath blight disease of rice. Importance of Validamycin was also emphasised by studies conducted by Devi and associates (1987).

Thangasamy and Rangaswamy (1989) found Carbendazim and Mancozeb sprayed at panicle initiation stage and 15 days afterwards effectively controlled the disease development.

2.4 Biological control of sheath blight

Garrett (1965) suggested that biological control can be brought about either by introduction or by augmentation in numbers of one or more species of controlling organisms, or by a change in environmental conditions designed to favour the multiplication and activity of such organisms, or by a combination of both procedures. He proposed two methods for biological control viz., inoculation of soil or plant tissues with antagonistic microorganisms and modification of the soil environment. The former can be achieved by direct introduction of antagonists mass cultured in the laboratory and the latter by applying various kinds of soil amendments.

2.4.1 Soil amendments

Role of soil amendments in the suppression of soil borne plant pathogens has been emphasized by several workers (Stover, 1962; Singh, 1968; Huber and Watson, 1970 and Singh et al., 1972). In sheath blight disease management programme also, soil amendment has a definite role.

Rajan and Menon (1975) reported that soil amended with punna cake, eluppa cake and rubber seed cake reduced the intensity of sheath blight of rice caused by Corticium sasakii. Rajan (1980) tested oil cakes of neem, marotti,

rubber and punna against sheath blight disease of rice and observed that the intensity of the disease has been reduced in amended plots. George (1981) also supported the above view. Kannaiyan and Prasad (1981a) observed reduced saprophytic survival of T. cucumeris with various oil cakes. Among different cakes neem cake was most effective. They also found effectiveness of neem cake, rice chaff, saw dust and farm yard manure in reducing seedling infection caused by R. solani (Kannaiyan and Prasad, 1981b). Naidu and John (1982) noticed that extract of neem oil cake could inhibit the growth of R. solani. Kannaiyan and Prasad (1983a) reported reduced seedling infection by neem cake, rice chaff, mustard cake, sawdust and farm yard manure. They also reported that soil incorporated with green manure viz., neem, sesbania, Glyricidia and sunhemp reduced the seedling infection (Kannaiyan and Prasad, 1983b). Lakshmanan and Nair (1984) found suppression of viability of sclerotia by neem cake and neem leaves in soil both in upland and flooded conditions.

Padmakumary and Balakrishnan (1987) observed reduced saprophytic activity of R. solani in soil amended with punna cake, neem cake, rice husk, saw dust, fish waste or groundnut shell. In a study on the effect of certain organic and inorganic amendments on the incidence and intensity of

sheath blight disease it was found that Glyricidia leaves, rice husk, gypsum and saw dust were better than others (Rajan and Alexander, 1987). Rajan and Alexander (1988a) tried several non-edible oil cakes viz., mahuva cake, marotti cake and neem cake. They suggested that punna cake is the most effective one in controlling the sheath blight disease. Effectiveness of neem cake, saw dust and mahuva cake was emphasised by Kannaiyan (1990) in reducing survival of the pathogen and control of the disease.

Inorganic amendments were also used in the management of soil borne diseases. Balasubramanian and Shanmugam (1986) established in inverse relationship between tissue calcium content and R. solani leaf blight intensity in black gram. Williamson and Dyce (1989) observed that the application of calcium cyanamide reduced the inoculum density of Plasmodiophora brassicae, the causal organism of the club root of crucifers. Alexander (1987) and Rajan and Alexander (1988a) could obtain significant disease reduction by the application of inorganic soil amendments viz., lime and gypsum on the intensity and incidence of sheath blight disease of rice.

2.4.2 Introduction of biocontrol agents

The direct application of biocontrol agent against a plant pathogen was first attempted by Hartley (1921),

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who inoculated forest nursery soil with thirteen fungi proved to be antagonistic to Rhizoctonia solani causal organism of damping off of pine seedlings. The specific role of different species of Trichoderma as biocontrol agent was first discussed by Weindling (1932).

Endo (1973) suggested the possible use of Neurospora crassa; incidence of Thanatephorus cucumeris was markedly reduced by N. crassa in soil, while seedling growth was not affected. Roy (1977) observed the efficiency of Trichoderma viride as a biocontrol organism. When R. solani was grown in combination with T. viride the growth and sclerotial germination of the former became detrimental. However, when spores of T. viride were sprayed on aerial parts of rice plant before inoculation with R. solani, the disease could not be checked. Mew and his associates (1980) noticed that Trichoderma sp. coil around the sclerotium and makes it inactive.

Chet and Baker (1980) brought out the relationship between the suppressiveness of a soil type with the population density of T. harzianum of that soil. Significant reduction in the incidence of damping off of carnation by R. solani was obtained by Elad and his associates (1981) by soil application of T. harzianum preparation @ 150 g/ sq. metre. In a subsequent study seed coating with

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T. harzianum was found to reduce damping off caused by R. solani (Elad et al., 1982).

Gokulapalan and Nair (1984) suggested that Aspergillus niger and T. viride are the most effective antagonists in suppressing the linear growth of R. solani. Mew and Rosales (1984) opined that T. harzianum reduced the survival ability of R. solani by decomposing rice straw, the major substrate for survival. The potential use of A. niger and T. harzianum for biocontrol of collar rot of coffee caused by R. solani is quite promising (Venkatasubbaiah and Safeeulla, 1984; Venkatasubbaiah et al., 1984).

Mew and Rosales (1986) found seed bacterization with antagonists suppressed the sheath blight disease and protected the plant from infection. Das (1986) compared different antagonists of R. solani and suggested that T. viride to be the best. Hadwan and Khara (1987) observed that T. pseudokoningii isolated from rhizosphere of tomato seedlings parasitises R. solani and is a mycoparasite of R. solani. Control of damping off of cotton and sugarbeet in green house using alginate pellets of T. harzianum and T. hamatum is reported (Lewis and Papavizas, 1987). Increase in the germination of seedling and reduction in post emergence damping off due to R. solani in cotton was observed by seed pelleting with T. harzianum (Alagarsamy et al., 1987). Reduction in the incidence and severity of sheath

blight with a combination of soil amendments and T. auroviride was noticed by Manian and Paulsamy (1987). Many antagonistic fungi of R. solani can be isolated from native paddy soil. Manibhushanarao and associates (1987) isolated antagonistic fungi viz., Gliocladium virens and T. longibrachiatum from paddy soils of Kerala and Tamil Nadu. Alexander (1987) could also obtain antagonistic fungi viz., Trichoderma sp. Aspergillus sp. and Muror sp. from paddy fields of Kerala. Nagamani and Mew (1987) reported 13 species of Trichoderma antagonistic to R. solani from Philippines.

Superiority of T. viride and T. harzianum in reducing the survival of R. solani and sheath blight disease incidence was emphasised by Padmakumary and Balakrishnan (1988). Gokulapalan (1989) also endorsed the above view.

2.5 Integrated disease management

Baker and Cook (1974) remarked that "integrated control" is based on the fact that different methods work best at different times and places or under varying conditions. It offers the possibility of making up for the deficiencies of any single method.

Ohr and associates (1973) obtained control of Armillaria mellea by integrated effort by methyl bromide

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and antagonistic microorganisms like Trichoderma sp. They opined that controlling organism should be more tolerant to the chemical used than the pathogen or to have the ability to recover more rapidly from its effects. Henis and associates (1978) observed that addition of PCNB @ 4 g/g with T. harzianum inoculum had a significant effect on disease control and synergistic effect on the decrease in inoculum density of Rhizoctonia solani propagules. Harder and associates (1979) observed low concentration of PCNB improved control of damping off of bean, tomato and egg plant, when applied together with T. harzianum.

Papavizas and Lewis (1979) successfully tried integrated approach with cultural, chemical and biological components on damping off and hypocotyl rot of snap bean caused by Pythium spp. and R. solani. It included ploughing seed treatment and application of antagonists in the furrows. A similar study was undertaken on cucumber fruit rot caused by R. solani also in which greater reduction in disease was obtained by applying Trichoderma sp. (WT-6) in conjunction with ploughing than either component was used individually (Lewis and Papavizas, 1980).

Elad and associates (1980) reported integrated control of soil borne disease in potatoes by adopting optimal combination of physical, chemical and biological

means. They combined solar heating, fumigation with methyl bromide and T. harzianum, which improved the efficiency in control of Sclerotium rolfsii. Chet and associates (1982) found reduction in disease incidence up to 93 per cent and increase in yield by 35-41 per cent by applying T. harzianum along with soil treatment with PCNB or solarization against R. solani and S. rolfsii in Iris.

Kraft and Papavizas (1983) integrated host resistance, antagonists and fungicide combination to control soil borne diseases. Lifshitz and associates (1985) emphasised integration of chemical and biological control provides opportunities for enhancement and greater effectiveness in suppressing damping off induced by R. solani. They achieved control of damping off by both seed treatment with T. harzianum and soil mixing of Benodanil. Alagarsamy and Sivaprakasm (1988) observed reduced seedling mortality caused by Macrophomina phaseolina by seed pelleting with Carbendazim in combination with antagonists like T. viride and T. harzianum in pot culture.

Important milestone in integration of biological control with other control methods was the development of mutant genomes of antagonists which can tolerate chemicals and other control measures (Papavizas, 1985). Such studies were also made (Papavizas and Lewis, 1981; Papavizas et al.,

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1982; Papavizas and Lewis, 1983). They discussed further prospects of modifying Trichoderma genome to obtain biotypes that will tolerate fungicides for use in integrated pest management system.

Integrated approach on sheath blight disease caused by R. solani was emphasised by many workers (Kannaiyan, 1980; Lee and Rush, 1983; Belmar et al., 1987). Management of sheath blight disease with T. viride and some amendments in combination with Carbendazim was tried by Rajan and Alexander (1988b). They observed reduced incidence of the disease in plots receiving Glyricidia leaves, rice husk or lime as well as T. viride. Interactions between Glyricidia leaves and T. viride/Carbendazim and rice husk/lime with Carbendazim were significant.

Dath (1990) suggested management practices that could be integrated to minimise the incidence and severity of sheath blight. It included host resistance, field sanitation, need based plant protection measures and effective cultural practices which would be the answer for integrated management of the disease.

MATERIALS AND METHODS

MATERIALS AND METHODS

3.1 Isolation of the pathogen

Rhizoctonia solani (Kuhn) causing sheath blight of rice was isolated from naturally infected rice plants collected from the paddy fields of Cropping Systems Research Centre, Karamana. The sheath portions of affected parts were cut into small bits, surface sterilized 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 plated over Potato dextrose agar medium in sterile petri dishes and incubated under laboratory conditions (28 + 2°C) for 72 hours. The fungal growth starting from the inoculated bits were subcultured and transferred to potato dextrose agar slants. The isolates were purified by hyphal tip method of Rawlins (1933) and were maintained in potato dextrose slants by periodical subculturing. Morphological characters like hyphal branching, septation of hyphae and sclerotial characters confirmed the identity of R. solani.

The pathogenicity of the isolate thus obtained was proved by Koch's postulates. Sclerotia obtained in culture were placed in between leaf sheath of rice variety Jyothi grown in earthern pots. Humid conditions were provided

by placing moist cotton over it. Symptoms developed were compared with natural symptoms. Then the fungus was reisolated from portion showing typical sheath blight and confirmed as R. solani. This pure culture of the fungus was used throughout the study.

3.2 Population dynamics of Rhizoctonia solani in paddy soil

3.2.1 Comparison of floatation techniques

Principle behind this technique is that sclerotia of low specific gravity will float over the surface of water, thus getting separated from the heavier soil particles.

In floatation sieving method with glass cylinder, glass cylinder of 30 cm length with 7 cm diameter was used (Plate 1). It was placed over sieves of 10 mesh stacked over 60 mesh sieve. Weighed quantity of soil was added into the cylinder and was filled with water. Soil solution was stirred well. More water was poured into the cylinder so that floating debris along with sclerotia was allowed to spill out and get collected at bottom sieves. Large plant debris particles were screened by 10 mesh sieve so that sclerotia were collected at 60 mesh sieve. It was carefully washed into a tissue paper and was allowed to dry. After drying, the number of sclerotia was counted

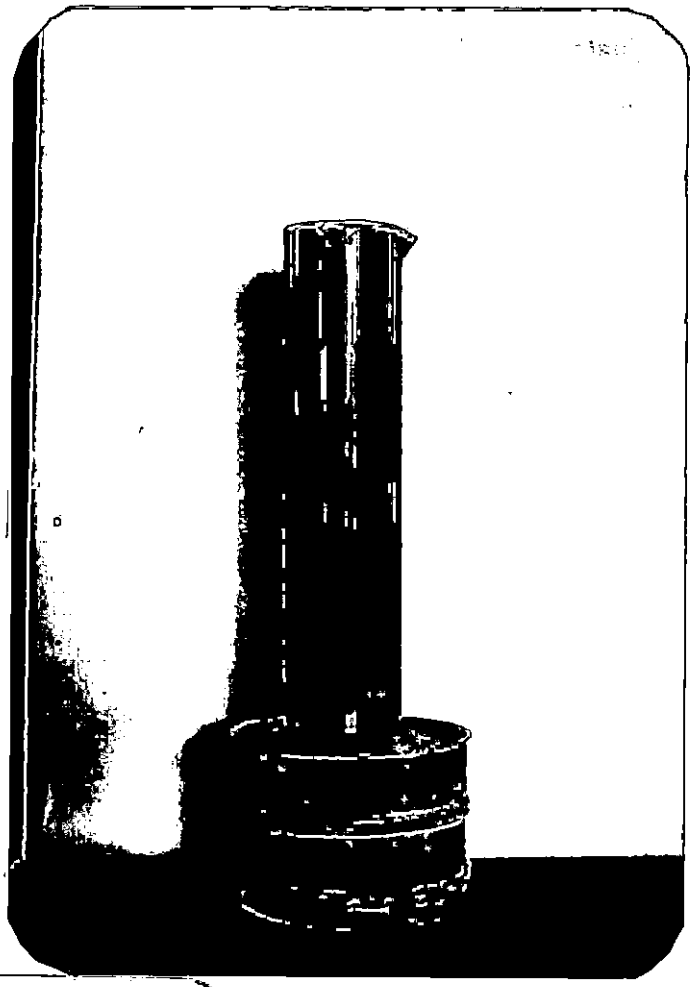

Plate 1. Floatation-sieving using glass cylinder

Plate 2. Floatation-sieving using Fenwick can

from the debris.

In floatation sieving with Fenwick can also the same principle is applied (Fenwick, 1940). Soil samples were introduced into the funnel after removing 20 mesh screen on the top of the funnel and water was poured (Plate 2). The floating debris were collected at the outlet, sclerotia and other plant debris were sieved through 10 mesh sieve placed over 60 mesh sieve. Sclerotia and other debris were washed out and screened as before.

In order to study the efficacy of these techniques different proportions of sclerotia were added and recovery percentage was recorded.

Design : Factorial CRD

Replication : Four

Treatment combination : 2 x 4

Factor A

a_0 Fenwick can

a_1 Glass cylinder

Factor B

b_0 5 sclerotia

b_1 10 "

b_2 15 "

b_3 20 "

Fifty g of powdered paddy soil samples were taken in 250 ml conical flasks. Each soil sample was mixed with different proportions of sclerotia viz., 5, 10, 15 and 20 respectively. Soil-sclerotial mixture were subjected to floatation sieving by Fenwick can and glass cylinder. For each proportion of sclerotia four replications were maintained. Recovery percentage was assessed and subjected to statistical analysis.

3.2.2 Modified floatation technique with glass cylinder

Floatation technique using glass cylinder was modified by increasing the specific gravity of water by dissolving different proportions of common salt (NaCl) viz., 0, 0.5, 1.0, 2.0, 3.0 and 5.0 per cent.

3.2.3 Comparison of selective media

This study was undertaken to compare two selective media viz., mineral antibiotic medium (MA medium) amended with fosetyl-al (Gangopadhyay and Grover, 1985) and MA medium amended with metalaxyl (composition of these media are given in Appendix I).

Design	:	Factorial CRD
Replication	:	Four
Treatment combination	:	2 x 2 x 4

Factor A : Selective media

a_0 : MA media with fosetyl-al

a_1 : MA media with metalaxyl

Factor B : Plating method

b_0 : Soil plating

b_1 : Soil pelleting

Factor C : Sclerotial proportion

c_0 : 5 sclerotia

c_1 : 10 "

c_2 : 15 "

c_3 : 20 "

Ten g of powdered sterile soil samples were taken in petri dishes. Soil samples were mixed with different proportion of sclerotia viz., 5, 10, 15 and 20 respectively. Both selective media were used for plating by two techniques viz., soil plating and soil pelleting.

Each treatment combination was replicated four times. In soil plate method 10 g of soil samples were divided into the 20 samples of 500 mg each and plated in twenty sterile petri dishes. Cooled molten selective medium was poured and swirled to disperse the soil particles.

In soil pellet method each soil sample was moistened and mixed well. Cooled molten selective medium was poured

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into sterile petri dishes and allowed to solidify. Soil pellets were transferred using a microspatula and entire samples (10 mg) were plated on solidified medium taken in 20 sterile dishes. After 24 to 48 hours observations were made on R. solani and percentage of germination was recorded.

3.2.4 Comparison of baiting technique

To compare the efficacy of baits viz., paddy stem and paddy straw a factorial experiment was laid out.

Design : Factorial CRD

Replication : Four

Treatment combination : 2 x 2 x 4

Factor A Baits

a_0 Paddy stem

a_1 Paddy straw

Factor B Sterility

b_0 Autoclaved

b_1 Non-autoclaved

Factor C Sclerotial proportion

c_0 5 Sclerotia

c_1 10 "

c_2 15 "

c_3 20 "

Fifty g of unsterilized paddy soil was taken in 250 ml conical flasks and mixed with different proportions of sclerotia viz., 5, 10, 15 and 20 respectively. Paddy stem pieces and paddy straw were used as baits and they were tried in autoclaved and non-autoclaved condition. Each treatment combination was replicated four times.

Sclerotial soil mixture was moistened and mixed with baits as per design. It was incubated for four days. Then baits were recovered and plated on MA medium amended with fosetyl-al for R. solani colonisation.

3.3 Isolation of soil fungi and testing their antagonism against R. solani

3.3.1 Isolation of the soil fungi

Isolation of soil fungi present in soil samples collected from the wet paddy soils of Cropping Systems Research Centre, Karamana were done at laboratory.

Methods used for isolation were dilution plate method (Waksman, 1922) and soil plate method (Warcup, 1950). In dilution plate method one g of each soil sample was placed in 250 ml conical flask containing 99 ml of sterile distilled water and the flasks were shaken by a mechanical shaker for 20 minutes. One ml of the suspension was pipetted from each flask while swirling and transferred to

99 ml of sterile water in 250 ml conical flasks. This dilution of 10^4 was used for isolating the fungi. The medium used was Martin's medium (Martin, 1950) (Peptone dextrose agar with rose bengal and streptomycin).

In soil plate method 100 mg of soil was transferred from each sample. A microspatula was used for transferring the microsample to petri dishes. Ten ml of melted and cooled Martin's medium was added and the soil particles were dispersed throughout the agar.

As soon as the fungal growth appeared in plates, the colonies were transferred to potato dextrose slants. Fungal cultures were purified by single spore isolation (Rawlins, 1933). Non-sporulating cultures were purified by hyphal tip method (Rawlins, 1933).

3.3.2 Antagonism of fungi to *R. solani*

Ten antagonistic reactions between *R. solani* and the species of fungi isolated in the above experiment were studied *in vitro* by dual culture technique (Skidmore and Dickinson, 1976). 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 potato dextrose agar in a petri dish and incubated. Four replications were kept for each combination. Observations were made on colony

Fig. 1. Layout of the field experiment conducted at CSRC, Karamana.

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diameter of the test fungus, inhibition zone if any and positive antagonistic interaction. Interaction types were assigned according to the method adopted by Purkayastha and Bhattacharya (1982). Interaction types were grouped into four categories.

A - Homogenous (free intermingling)

B - Over growth (R. solani - over grown by test fungus)

C - Cessation of growth at line of contact

D - Aversion (A clear zone of inhibition)

The fungi which showed positive antagonistic reactions were identified by sending cultures to Commonwealth Mycological Institute, Kew, Surrey, England.

3.3.3 Mass culturing of antagonistic fungi

Antagonistic fungi were mass cultured in rice bran medium (Elad et al., 1983). Mixtures of rice bran and tap water in the ratio 1:2 by weight were prepared in Erlenmayer flasks (500 ml) and autoclaved for one hour at a pressure of 1.2 kg/cm^2 for two successive days. Then the medium inoculated with test fungus was incubated at room temperature for 10 days. Rice bran medium was prepared in autoclavable polypropelene bags also. These fungal cultures were prepared and used for soil application.

Fig. 1. Layout of the field experiment conducted at CSRC, Karamana.

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3.4 Comparative efficacy of antagonistic fungi on intensity of sheath blight

A pot culture experiment was laid out at the College of Agriculture to assess the effect of antagonistic fungi on intensity of sheath blight disease.

Details of the trial are given below.

Layout - Completely Randomised Design

Variety - Jyothi

Replication - Four

Treatments - Six

A_1 Aspergillus niger Van Teigh

A_2 Penicillium pinophilum Hedgcock

A_3 Rhizopus oryzae Went and Prinsen Geerligs

A_4 Trichoderma harzianum Rifai

A_5 T. koningii Oudem

A_6 Control

The experiment was conducted in pots of size 22 x 26 cm uniformly filled with ten kg of wet land soil each, collected from the paddy field of the Instructional Farm, College of Agriculture, Vellayani. Fertilizers were added to these pots as per the Package of Practice Recommendations for rice (Kerala Agricultural University, 1989).

The different fungi were mass cultured as described earlier and were applied to soil @ 250 g/pot, seven days

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before transplanting. The soil was puddled thoroughly and twenty day old seedlings of rice variety Jyothi were transplanted into pots at the rate of three seedlings per clump. A week later the plants were inoculated with sclerotia of the pathogen and covered with moist cotton to provide sufficient humidity.

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

3.5 Temporal succession of R. solani propagules and antagonistic fungi with respect to sheath blight disease

Two pot experiments were laid out at the College of Agriculture, Vellayani to study the survival of R. solani propagules as well as antagonistic fungi at different periods of soil introduction.

One pot trial was carried out by applying the antagonistic fungi @ 250 g/pot one week after the addition of R. solani propagules and another trial by applying the antagonists one week before the addition of R. solani inoculum.

Details of the experiment are as follows.

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Layout - Completely Randomised Design

Treatments - Five

Replication - Four

Treatments

A_1 - *Aspergillus niger*

A_2 - *Penicillium pinophilum*

A_3 - *Trichoderma harzianum*

A_4 - *T. koningii*

A_5 - Control

The pots of size 22 x 26 cm were filled with ten kg of wet land soil collected from paddy field of the Instructional Farm, College of Agriculture, Vellayani. Fertilizers were added as per the Package of Practice Recommendations for rice (Kerala Agricultural University, 1989).

In the first pot experiment antagonistic fungi, mass cultured as described earlier, were incorporated into soil @ 250 g/pot initially. Later *Rhizoctonia solani* grown in Sand maize meal medium was incorporated into the soil @ 250 g/pot, one week after addition of antagonistic fungi.

In the second pot experiment antagonistic fungi were introduced into the soil, one week before addition of *R. solani* inoculum.

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In both pot experiments one week gap was given before transplanting for the establishment of antagonists/ R. solani. The soil was puddled thoroughly and twenty day old seedlings of the rice variety Jyothi were transplanted into the pots at the rate of three seedlings per clump. Disease incidence was estimated by observing all the hills and noting the percentage of infection. The intensity of disease was assessed by scoring all the infected hills based on the Standard Evaluation System for Rice Diseases (IRRI, 1976).

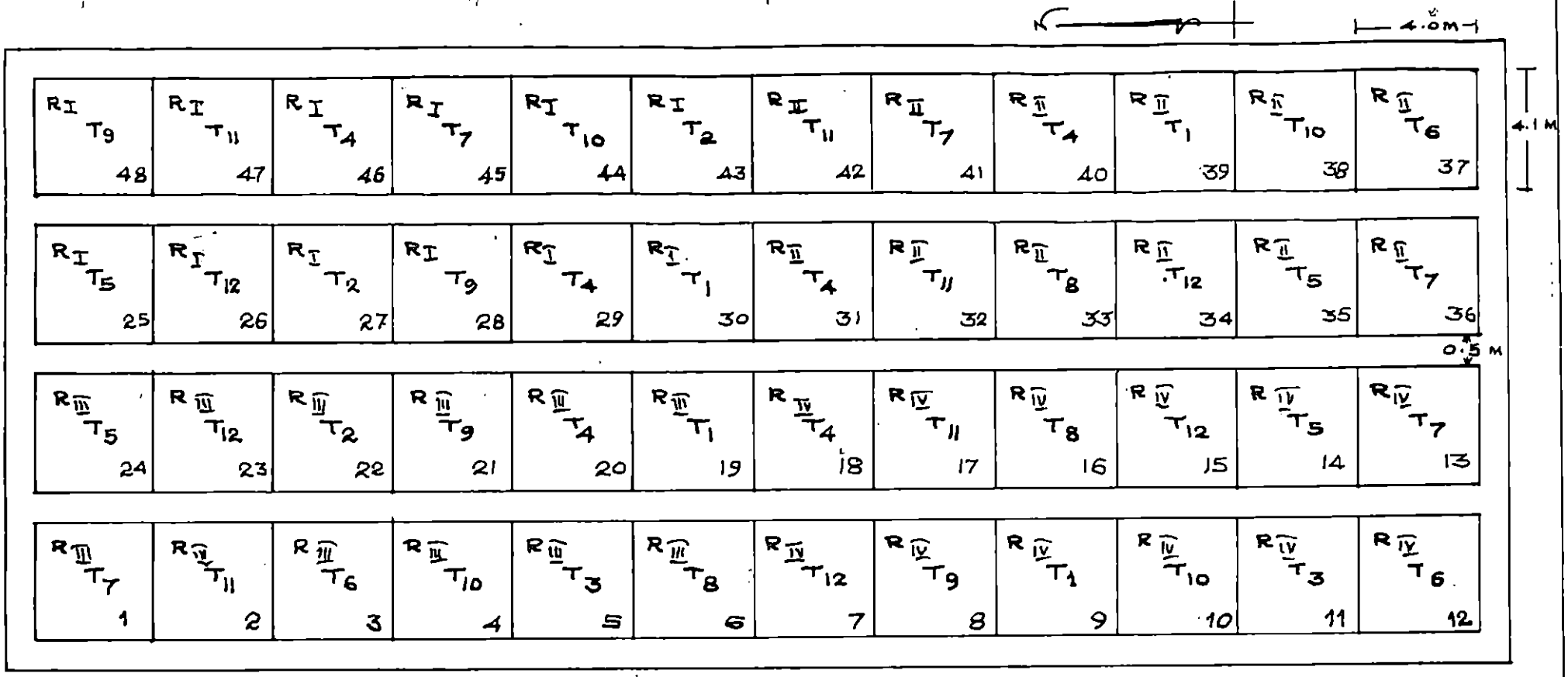
3.6 Field evaluation of soil amendments, plant protection chemicals and antagonistic organisms in management of sheath blight disease of rice

Field experiment was conducted at Cropping Systems Research Centre (CSRC), Karamana, Thiruvanandapuram to study the management aspects of sheath blight disease involving soil amendments plant protection chemicals and antagonistic microorganisms.

The antagonistic fungus Trichoderma harzianum used in field trial was selected from pot trial conducted at College of Agriculture, Vellayani.

The details of the experiment were as follows (Fig. 1).

Fig. 1. Layout of the field experiment conducted at CSRC, Karamana.



LAYOUT PLAN

DESIGN ~ ~ ~ ~ RBD
TREATMENTS ~ ~ 12
REPLICATION ~ ~ FOUR

SOIL AMENDMENTS

NEEM CAKE ~ ~ M_1
LIME ~ ~ ~ ~ M_2
CONTROL ~ ~ M_3

TREATMENT COMBINATION

T_1 ~ ~ $M_1 m_1$	T_5 ~ ~ $M_2 m_1$	T_9 ~ ~ $M_3 m_1$
T_2 ~ ~ $M_1 m_2$	T_6 ~ ~ $M_2 m_2$	T_{10} ~ ~ $M_3 m_2$
T_3 ~ ~ $M_1 m_3$	T_7 ~ ~ $M_2 m_3$	T_{11} ~ ~ $M_3 m_3$
T_4 ~ ~ $M_1 m_4$	T_8 ~ ~ $M_2 m_4$	T_{12} ~ ~ $M_3 m_4$

ANTAGONIST, FUNGICIDE AND COMBINATIONS

BAVISTIN ~ ~ m_1
BIO CONTROL AGENT ~ ~ m_2
BAVISTIN + BIOCONTROL AGENT ~ ~ m_3
CONTROL ~ ~ m_4

FIG: 1

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Layout	-	Randomised Block Design
Variety	-	Jyothi
Treatments	-	12
Replication	-	Four
Spacing	-	10 x 15 cm
Plot size	-	4 x 4.1 m^2

Treatment combination

1. M_1m_1	5. M_2m_1	9. M_3m_1
2. M_1m_2	6. M_2m_2	10. M_3m_2
3. M_1m_3	7. M_2m_3	11. M_3m_3
4. M_1m_4	8. M_2m_4	12. M_3m_4

Soil amendments

Neem cake	M_1
Lime	M_2
Control	M_3

Antagonists, fungicide and combinations

Bavistin	m_1
Biocontrol agent	m_2
Bavistin + Biocontrol agent	m_3
Control	m_4

3.6.1 Nursery

The seedlings of the rice variety Jyothi were raised in a wet nursery as per Package of Practices Recommendations of rice (Kerala Agricultural University, 1989).

3.6.2 Main field

The crop was raised following the Package of Practices Recommendations for rice (Kerala Agricultural University, 1989). The fertilizer recommendations for the high yielding short duration rice varieties (70:35:35 kg NPK/ha) were followed. The dose of neem cake was fixed depending upon its nitrogen content. Half of the recommended dose of N were applied through neem cake. Extra P and K required were supplied through superphosphate and muriate of potash respectively. Rest of the N and K were applied at the time of panicle initiation stage as urea and muriate of potash. Control plots also received the normal dose of NPK per hectare.

Lime was applied as @ 1200 kg/ha in two splits, half at the time of planting and the rest at the time of panicle initiation stage. R. solani inoculum was applied to each plot one week before applying amendments. Lime and neem cake were applied two weeks before transplanting. Twenty one day old Jyothi seedlings were transplanted with

a spacing of 10 x 15 cm. T. harzianum and the fungicide Bavistin were applied when initial symptoms of the disease at the stage of panicle initiation. The fungicide Bavistin was sprayed as @ 0.1 per cent. T. harzianum mass cultured in polypropelene bags was applied @ 1 kg per plot.

3.6.3 Intensity and incidence of sheath blight disease

Observations on the incidence of disease was recorded at tillering stage, panicle initiation stage, booting stage, milk stage and mature grain stage. Disease incidence was recorded by observing 40 hills from four rows selected at random in each plot and counting the number of infected tillers.

Disease intensity was also recorded at tillering stage, panicle initiation stage, booting stage, milk stage, and mature grain stage. It was scored on 25 randomly selected hills per plot according to Standard Evaluation System for Rice (IRRI, 1976) (Plate 3).

Score	Description
0	No incidence of disease.
1	Lesions limited to lower ¼th of leaf sheath area.
3	Lesions present in lower ½ of the leaf sheath.
5	Lesions present on more than ½ of leaf sheath area slight infection on lower 3rd and 4th leaves.

Plate 3. Sheath blight infection grades (IRRI, 1976)

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7 Lesions present on more than ¾ of leaf sheath. Severe infection on lower leaves and slight infection on upper leaves (flag and 2nd leaf).

9 Lesions reaching top of tillers severe infection on all leaves.

3.6.4 Estimation of soil fungi, bacteria and actinomycetes

Soil samples were collected during different periods viz., before addition of amendments, planting, panicle initiation, booting and mature grain stages from each plot. Soil samples air dried in shade were pooled and utilized for the assay of total fungi, bacteria and actinomycetes (Waksman, 1922).

One g of soil from each treatment was taken in 250 ml conical flask containing 99 ml of sterile water. The contents of the flask were shaken by keeping in a shaker for 20 minutes. One ml of this soil solution was transferred using a sterile pipette into another conical flask containing 99 ml sterile water, so that the dilution becomes 10^{-4}. This again was shaken well and one ml of this was transferred into another conical flask containing 99 ml sterile water so as to bring the dilution to 10^{-6}.

In order to assess the population of fungi, one ml of the 10^{-4} dilution was transferred into sterile petri

plate and Rose bengal streptomycin agar was added. For estimating the bacterial population one ml from the 10^{-6} dilution was pipetted and soil extract agar was added. Population of actinomycetes were also assessed from 10^{-6} dilution and using Kenknights and Munuier's medium (Subba Rao, 1986).

3.6.5 Estimation of population of R. solani propagules in paddy soil

Population of R. solani was estimated in two methods, one was by estimating R. solani propagules by selective medium (Gangopadhyay and Grover, 1985) and other was by estimating the sclerotial population in the soil by floatation, sieving using glass cylinder. For assaying the population, soil samples were collected at different periods viz., before addition of amendments, planting, panicle initiation, booting and mature grain stages.

3.6.6 Growth and yield attributes

The sample plants were maintained in each treatment and biometric observations were recorded. Growth parameters viz., height of the plant, total number of tillers, number of productive tillers were recorded. Yield observations like grain yield, straw yield and number of grain per panicle were made.

3.6.7 Soil analysis

Soil samples were taken from the experimental area after the experiment. The air dried soil samples were analysed for available nitrogen, available phosphorus and available potash contents. Available nitrogen content was estimated by alkaline potassium permanganate method (Subbaiah and Asiija, 1956). Available phosphorus content was estimated by Bray's colorimetric method (Jackson, 1973) and available potassium and calcium was estimated by ammonium acetate method (Jackson, 1973). Trace elements viz., Ca, Mg and Zn were estimated by atomic adsorption spectrophotometry (Jackson, 1973).

3.6.8 Ecpnomics of cultivation

The economics of cultivation was worked out based on the following assumption.

Cost of neem cake per kg	Rs. 2.40
Cost of lime per kg	Rs. 1.20
Cost of Bavistin per 100 g	Rs.54.00
Cost of biocontrol agent media per kg	Rs. 3.60
Application charges of Bavistin/biocontrol agent per hectare	Rs.212.5

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Cost of cultivation of paddy per hectare excluding cost of treatments	Rs. 6500.00
Price of 1 kg of paddy	Rs. 3.00
Price of 1 kg of straw	Rs. 1.00

The net income and return per rupee invested were calculated as follows:

Net income (Rs./ha) = Gross income - Cost of cultivation

Average return per rupee invested ($\frac{X}{Y}$ Rs.) = $\frac{\text{Gross income}}{\text{Cost of cultivation}}$

(Rate of turn over)

RESULTS

RESULTS

4.1 Isolation of sheath blight pathogen

The fungus Rhizoctonia solani Kuhn was isolated and purified from the paddy fields of Cropping System Research Centre, Karamana, Trivandrum. The identity of the organism was confirmed by studying its morphological characters and pathogenicity.

4.2 Population dynamics of R. solani in paddy soil

4.2.1 Comparison of floatation techniques

Floatation-sieving techniques using Fenwick can and glass cylinder were compared (Table 1). In the laboratory trial using different proportions of sclerotia, the two techniques were found to be statistically on par. However, the percentage of recovery of sclerotia was slightly better in Fenwick can method (94.07%) than glass cylinder method (91.96%).

4.2.1.2 Modification of floatation sieving using different concentrations of salt

Floatation sieving was further modified by adding salt. As the salt concentration increased, specific gravity of the water also increased, which in turn increased the sclerotial recovery. It is seen that 2 per cent salt

Table 1

Floatation techniques and sclerotial proportion in relation to recovery of *Rhizoctonia solani*

Floatation technique	Sclerotial proportion				Mean
	5	10	15	20	
Fenwick can	88.40(70.06)*	97.44(80.77)	92.31(73.87)	98.15(82.15)	94.07(76.71)
Glass cylinder	88.40(70.06)	96.21(78.74)	91.71(73.23)	91.51(73.03)	91.96(73.77)
Mean	88.40(70.06)	96.83(79.75)	92.01(73.55)	94.83(77.59)	

* Letters in the parenthesis indicate the transformed values

Critical difference

for Techniques NS

for Sclerotial proportion NS

for Interaction x Sclerotial proportion NS

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solution gave significantly higher recovery than lower concentrations. However, beyond 2 per cent there was no significant increase in the recovery (Table 2).

4.2.2 Comparison of selective media

Among selective media, mineral antibiotic medium (MA medium) with fosetyl-al was found to be better than MA medium with metalaxyl at various proportions of sclerotia (Table 3). In both selective media, as the sclerotial proportion increased percentage of germination of R. solani was decreased.

Soil pellet technique was found to be superior to soil plate technique in both the selective media. In soil plate technique, both the selective media were on par, whereas in soil pellet technique MA medium amended with fosetyl-al was better.

4.2.3 Comparison of baiting technique

A comparative study on baiting technique using paddy stem and straw bits showed no significant difference between the two in colonisation of R. solani (Table 4). However, as the number of sclerotia increased from five to 20, there was a general increase in colonisation by the fungus both in stem as well as in straw bits, even-though the difference between ten and fifteen sclerotia

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Table 2

Modified floatation technique in relation to recovery of Rhizoctonia solani

Salt concentration (percentages)	Recovery percentage
0.0	81.23 (63.93)*
0.5	80.95 (64.10)
1.0	85.36 (67.47)
2.0	97.44 (80.77)
3.0	98.15 (82.15)
5.0	98.74 (83.52)
CD (0.05)	11.86

* Letters in the parenthesis indicate the transformed values

Table 3

Selective media, plating technique and sclerotial proportion in relation to recovery of *Rhizoctonia solani*

Selective media	Sclerotial proportion (number)				Plating technique		Mean
	5	10	15	20	Soil plate	Soil pellet	
Mineral antibiotic medium with Fosetyl-al	77.5* (67.19)**	72.50 (58.85)	74.16 (59.68)	65.63 (55.40)	58.96 (50.29)	85.93 (70.26)	72.45 (62.28)
Mineral antibiotic medium with Metalaxyl	57.5 (49.45)	75.0 (60.88)	75.84 (61.34)	61.25 (51.58)	58.13 (49.72)	76.67 (61.90)	67.40 (55.81)
Mean	67.50 (58.32)	73.75 (59.86)	75.0 (60.51)	63.44 (53.49)	58.55 (50.01)	81.3 (66.08)	

* Percentage germination

** Letters in the parenthesis indicate the transformed values

Critical difference

Selective media	2.95
Selerotial proportion	4.17
Selective media x Sclerotial proportion	5.90
Plating technique	2.95
Selective media x plating technique	4.17

Table 4

Baiting technique, sterilization and sclerotial proportion in relation to recovery of Rhizoctonia solani

Baiting technique	Sclerotial proportion (number)				Sterilisation		Mean
	5	10	15	20	Autoclaved	Nonautoclaved	
Paddy stem bits	26.87* (31.35**)	56.25 (48.64)	55.62 (48.25)	58.95 (50.18)	52.18 (46.43)	46.56 (42.77)	49.37 (44.60)
Paddy straw bits	25.62 (29.80)	46.88 (43.13)	58.13 (49.78)	68.13 (56.26)	59.68 (50.82)	40.00 (38.67)	49.84 (44.75)
Mean	26.25 (30.58)	51.56 (45.89)	56.87 (49.01)	63.73 (53.22)	55.93 (48.63)	43.28 (40.72)	

* Percentage colonisation

** Letters in the parenthesis indicate the transformed values

Critical difference

baits	NS
Sclerotial proportion	4.17
Sterilisation	1.62
Baits x sclerotial proportion	3.24
Baits x Sterilisation	4.17

was not significant. In paddy straw bits, as the number of sclerotia increased percentage of colonisation also increased. But an increasing trend was not observed in paddy straw bits.

In general, autoclaved baits gave better recovery, than non-autoclaved ones. Among autoclaved baits, paddy straw bits were superior than stem bits whereas in non-autoclaved baits it was just the reverse.

4.3 Laboratory studies of antagonism of R. solani and soil fungi

4.3.1 Isolation of soil fungi

Thirtyfour different soil fungi were isolated by soil dilution plate and soil plate methods. The above fungal cultures were purified by single spore isolation/ hyphal tip method and were maintained in Potato dextrose agar slants.

4.3.2 Antagonism of different fungi isolated to R. solani

The above fungi isolated from soil were tested for antagonistic reaction against R. solani. Thirteen fungi showing positive antagonism against R. solani were grouped into four categories as described in the previous chapter (Table 5).

Table 5

Antagonistic reaction of soil fungi against *Rhizoctonia solani*

Name of fungus	4th day		8th day		12th day		16th day	
	Type of interaction	Colony diameter (mm)	Type of interaction	Colony diameter (mm)	Type of interaction	Colony diameter (mm)	Type of interaction	Colony diameter (mm)
1. *Aspergillus aculeatus* Iizuka	A	40	A	50	B	60	B	60
	A	40	A	54	B	60	B	60
	A	40	A	60	B	60	B	60
2. *A. flavus* Link	A	30	A	40	B	50	B	60
	A	30	A	50	B	60	B	60
	A	30	A	40	B	50	B	70
3. *A. niger* Van Teigh	B	40	B	70	B	90	-	-
	B	40	B	70	B	90	-	-
	B	40	B	70	B	90	-	-
4. *A. terreus* Thom	-	20	-	30	C	50	B	80
	-	20	-	40	C	50	B	70
	-	20	-	40	C	50	B	80
5. *Chaetomium globosum* Kunze	-	30	A	40	A	60	A	60
	-	30	A	40	A	60	A	60
	-	30	A	40	A	60	A	60
6. *Mucor* sp.	-	30	C	44	C	44	-	-
	-	40	C	44	C	44	-	-
	-	36	C	44	C	44	-	-
7. *Penicillium oxalicum* Currie and Thom	D	30	D	30	D	40	D	40
	D	30	D	30	D	50	D	50
	D	30	D	30	D	50	D	50

Table 5 (Contd.)

Name of soil fungus	4th day		8th day		12th day		16th day	
	Type of interaction	Colony diameter (mm)	Type of interaction	Colony diameter (mm)	Type of interaction	Colony diameter (mm)	Type of interaction	Colony diameter (mm)
8. Penicillium pinophilum Hedgecock	B	50	B	70	B	90	-	-
	B	50	B	70	B	90	-	-
	B	50	B	70	B	90	-	-
9. P. roseopurpurem Dierekx	-	20	-	40	C	50	C	50
	-	20	-	40	C	50	C	50
	-	20	-	40	C	50	C	50
10. Rhizopus oryzae Went and Prinson Geerlings	B	90	-	-	-	-	-	-
	B	90	-	-	-	-	-	-
	B	90	-	-	-	-	-	-
11. Talaromyces stipitatus (Thom) C.R. Benjamin	-	40	B	70	B	80	B	90
	-	50	B	70	B	80	B	90
	-	40	B	70	B	80	B	90
12. Trichoderma harzianum Rifai	B	60	B	90	-	-	-	-
	B	60	B	90	-	-	-	-
	B	60	B	90	-	-	-	-
13. T. koningii Oudem	B	60	B	90	-	-	-	-
	B	60	B	90	-	-	-	-
	B	60	B	90	-	-	-	-

A - Homogenous (Free intermingling)

B - Overgrowth (R. solani overgrown by test fungus)

C - Cessation of growth at line of contact

D - Aversion (A clear zone of inhibition)

Their identity was confirmed by Commonwealth Mycological Institute, Kew, Surrey, England as Aspergillus aculeatus, A. flavus, A. niger, A. terreus, Chaetomium globosum, Mucor sp., Penicillium roseopurpureum, P. oxalicum, P. pinophilum, Rhizopus oryzae, Talaromyces stipitatus, Trichoderma harzianum and T. koningii.

The type of interaction varied very much among different fungi. When paired with R. solani, Trichoderma spp., Rhizopus oryzae, Penicillium pinophilum, Talaromyces stipitatus and Aspergillus spp. smothered the growth of Rhizoctonia solani. Among these fungi Rhizopus oryzae attained maximum colony diameter of 90 mm in first observation (fourth day) itself, whereas Trichoderma spp. on second observation only (eighth day) (Plate 4, 5 and 6). Growth of A. niger and P. pinophilum was slower than R. oryzae or Trichoderma spp., as they covered petriplates reaching 90 mm colony diameter only on third observation (twelth day) (Plate 7 and 8). During earlier observations they measured 40 and 50 mm (fourth day) and 70 mm (eighth day) respectively. However, A. terreus, C. globosum, Mucor sp., P. roseopurpurem and Talaromyces stipitatus did not show any interaction by first or second observation due to small colony diameter. Slow growing fungi viz., A. aculeatus, A. flavus (Plate 9), A. terreus (Plate 10)

Plate 4. Antagonism of Rhizopus oryzae and Rhizoctonia solani

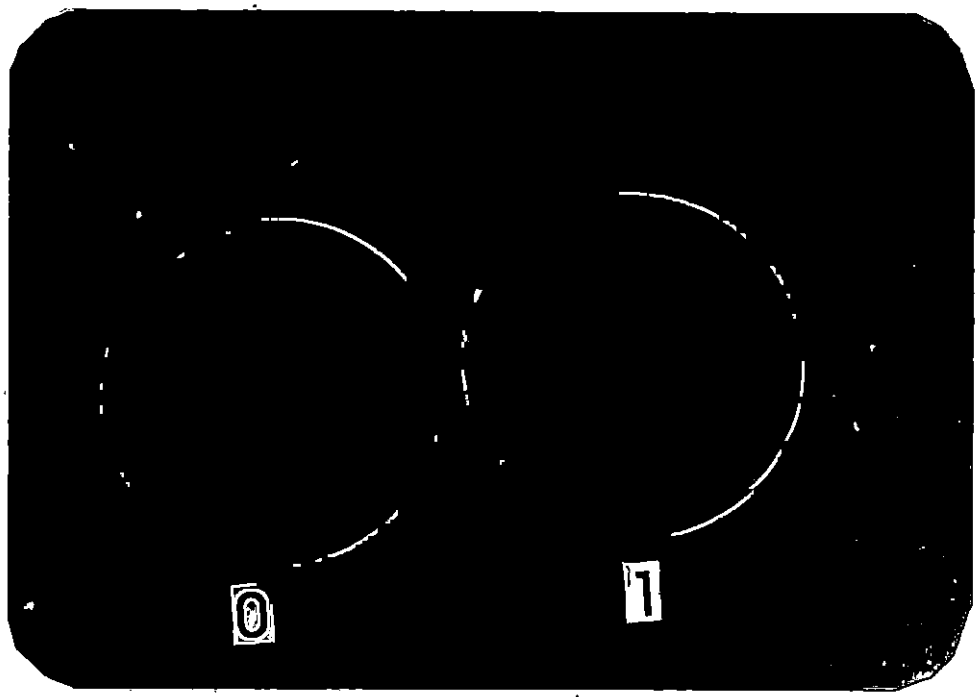


Plate 5. Antagonism of Trichoderma harzianum and R. solani

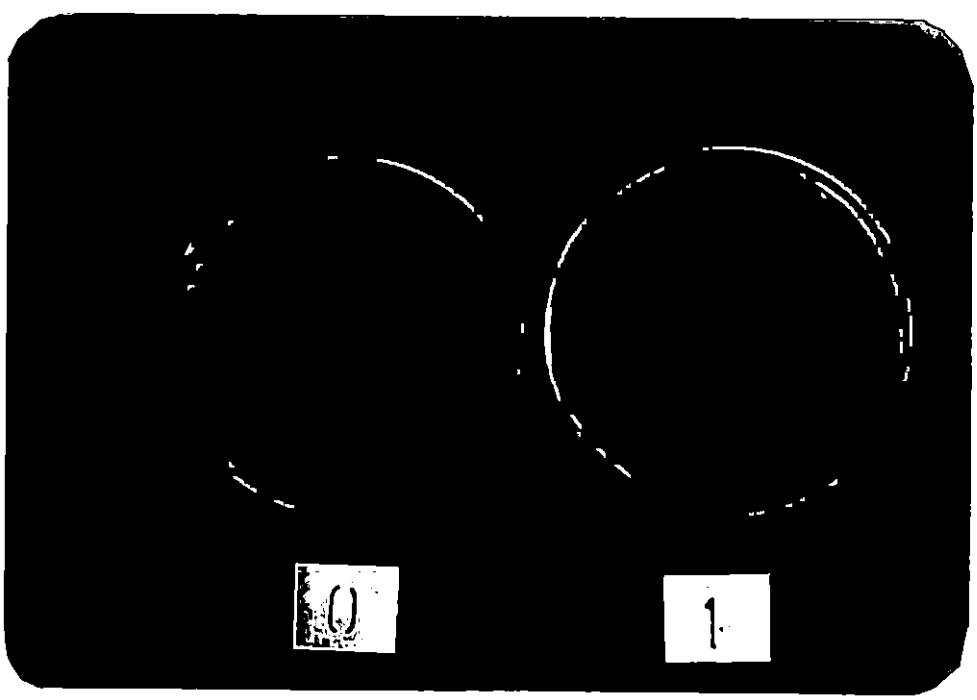


Plate 6. Antagonism of T. koningii and R. solani

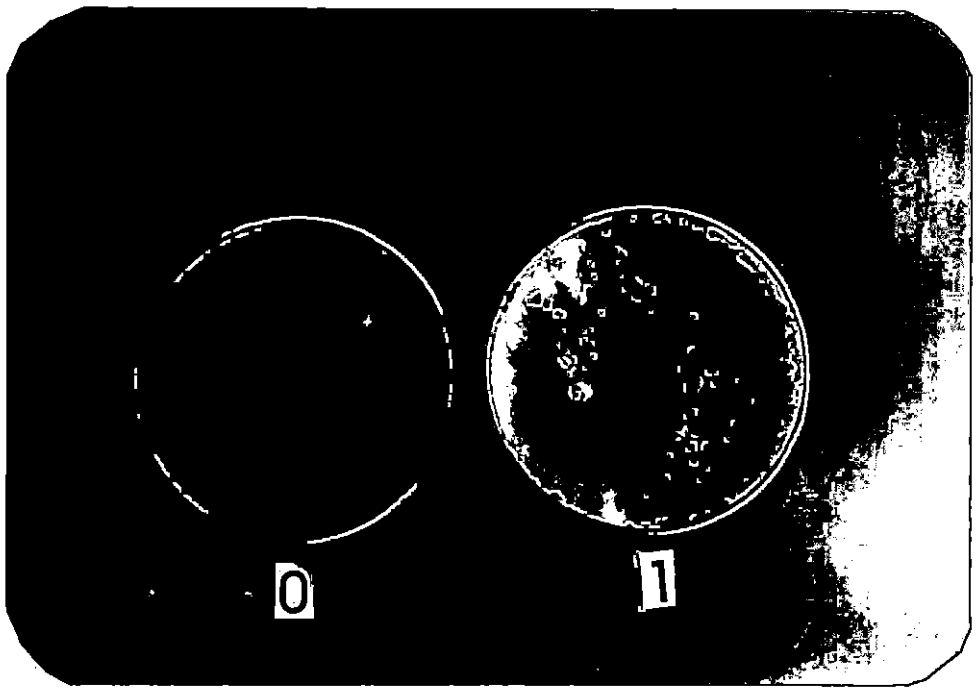


Plate 7. Antagonism of Aspergillus niger and R. solani

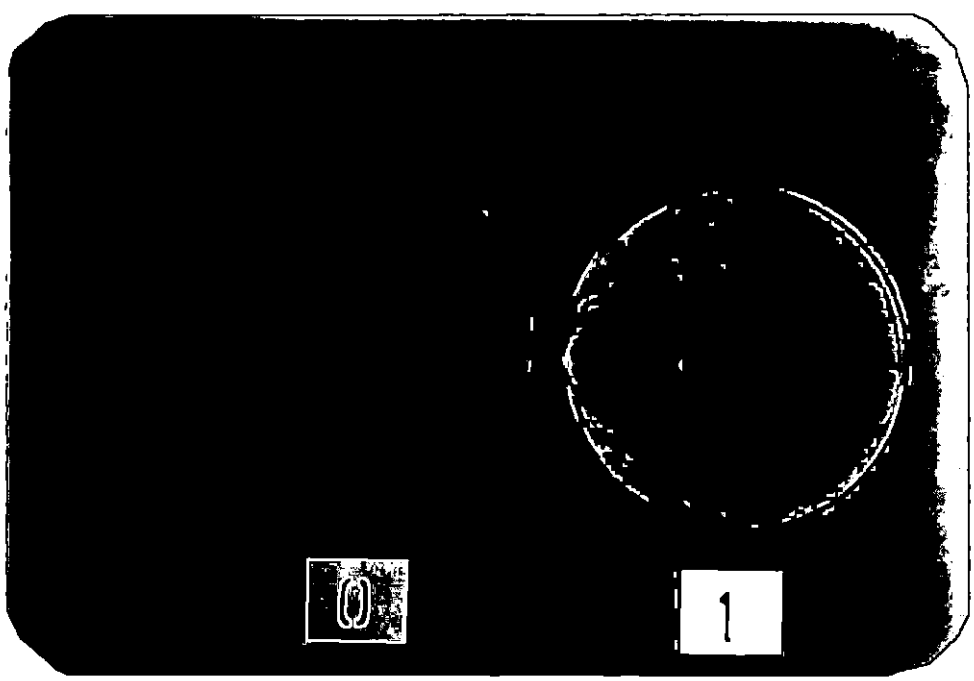


Plate 8. Antagonism of Penicillium pinophilum and R. solani

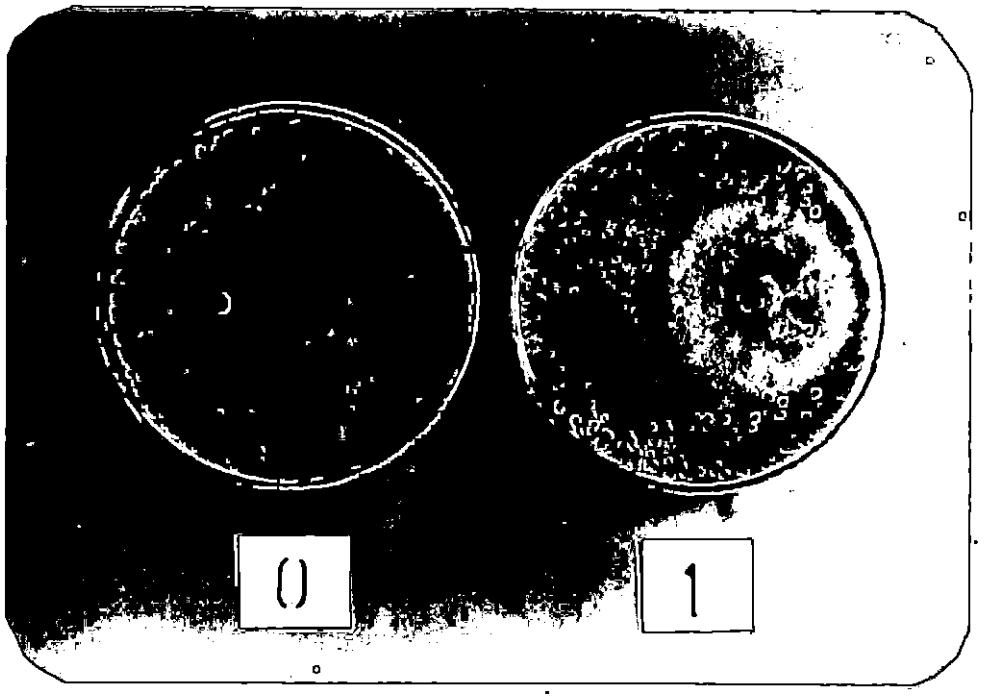


Plate 9. Antagonism of A. flavus and R. solani

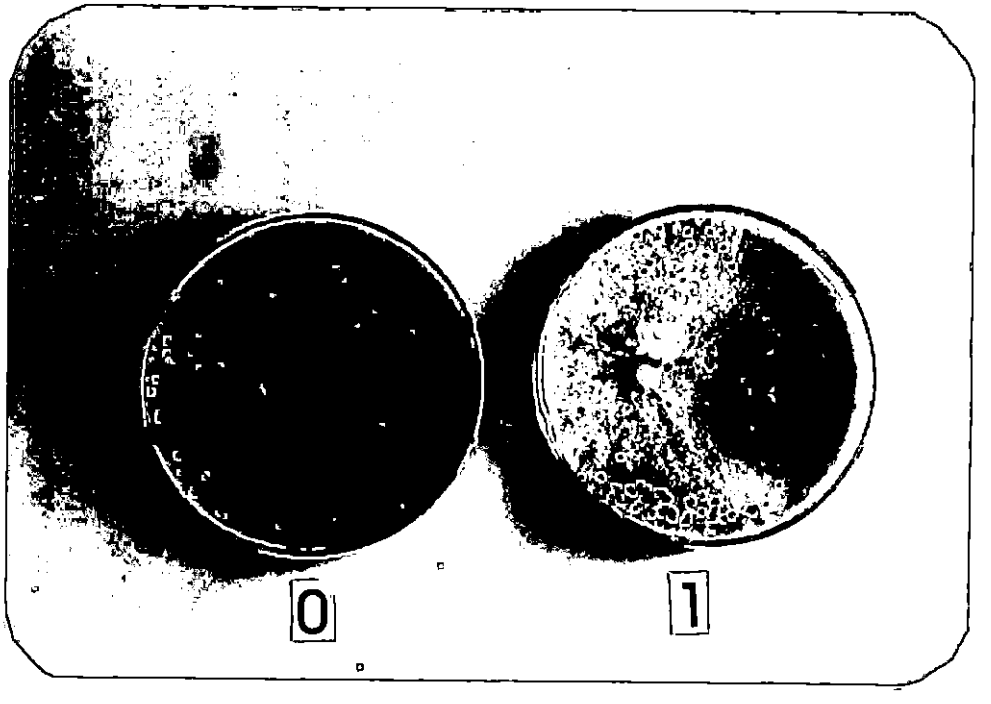


Plate 10. Antagonism of A. terrus and R. solani

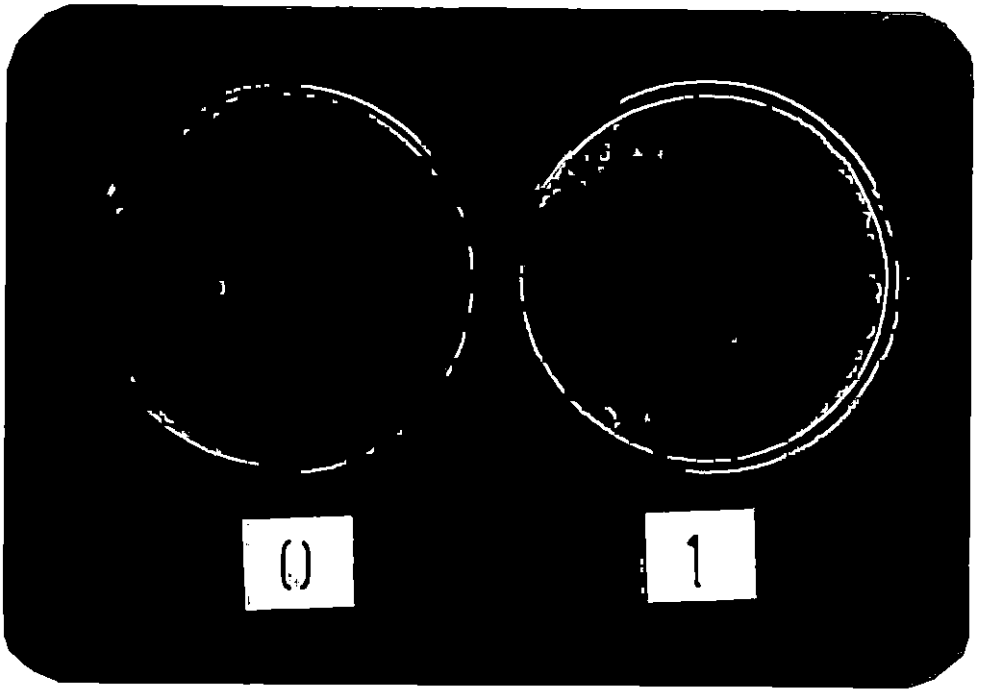


Plate 11. Antagonism of Talaromyces stipitatus and R. solani

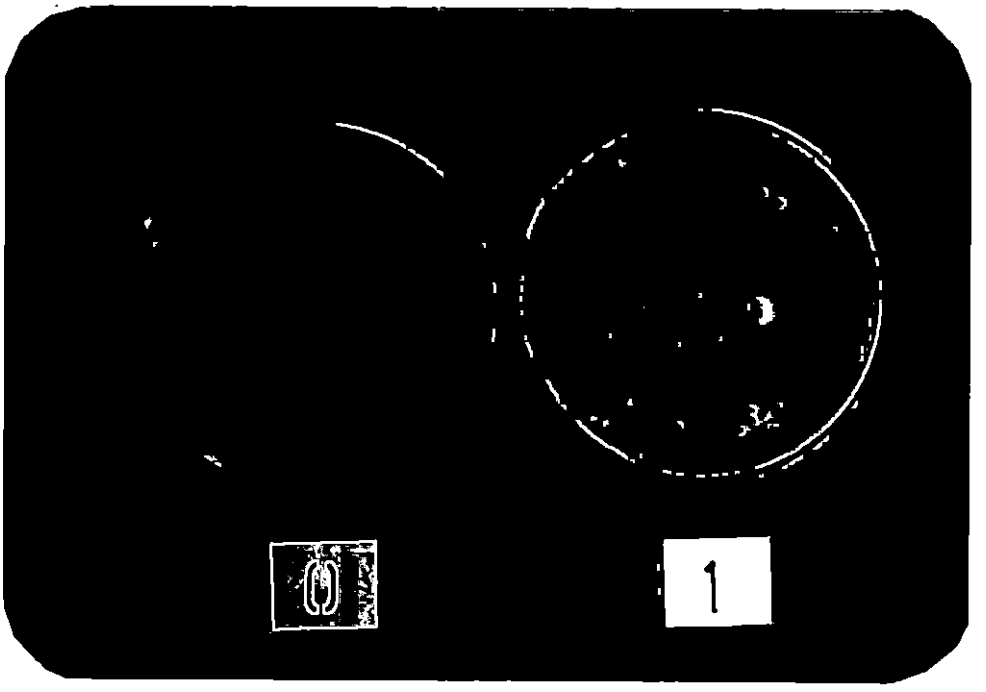


Plate 12. Antagonism of Mucor sp. and R. solani

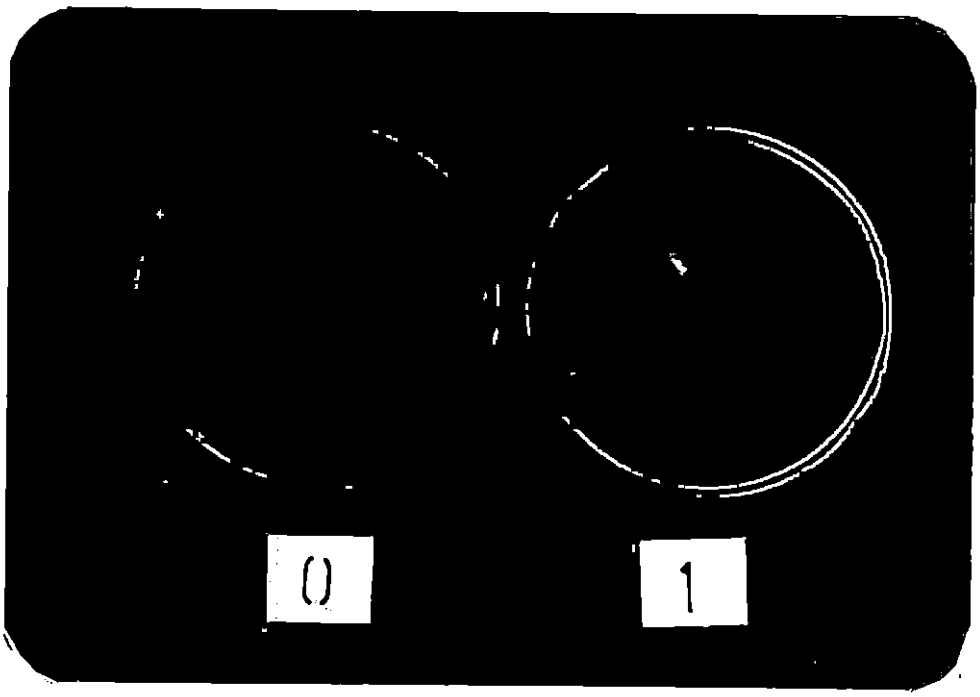
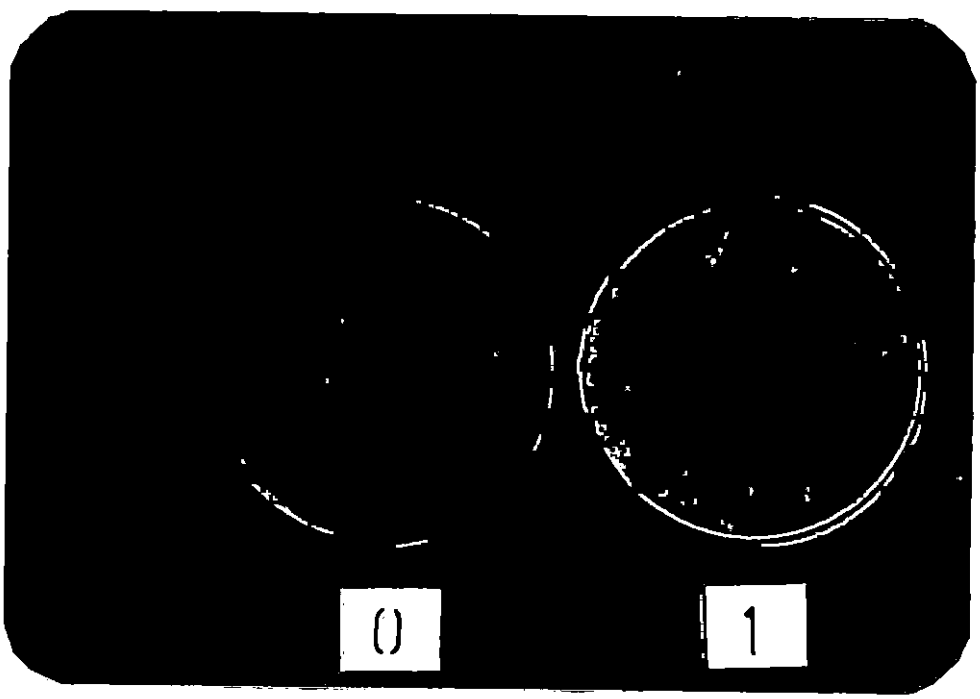


Plate 13. Antagonism of P. oxalicum and R. solani



and T. stipitatus (Plate 11) reached colony diameter of 60, 63, 73 and 90 mm (average), respectively as on final observation (16th day). For Mucor sp., cessation of growth at the point of contact was observed on eighth day, after which it ceased to grow (Plate 12). In P. roseopurpureum this interaction was observed on 3rd observation (twelth day). A. flavus and A. aculeatus showed homogenous intermingling on second observation and overgrowing in third and fourth observations (Table 5).

Chaetomium globosum intermingled freely with R. solani and grew together. A clear zone of inhibition of eight mm was observed between R. solani and P. oxalicum (Plate 13).

4.4 Pot studies

4.4.1 Effect of antagonistic fungi on the intensity of sheath blight

During tillering stage all the antagonistic fungi tested, except Rhizopus oryzae and A. niger reduced the intensity of sheath blight significantly (Table 6). Trichoderma harzianum, T. koningii and Penicillium pinophilum were found to be the best treatments.

At stem elongation stage also, R. oryzae and A. niger were not effective as they were on par with untreated

Table 6

Effect of antagonistic soil fungi on the intensity of sheath blight disease of rice

Treatments	Disease intensity (0-9 scale)			
	Tillering	Stem elongation	Booting	Filling stage
Aspergillus niger	1.00 (1.41)* ab**	1.50 (1.58) ab	2.16 (1.77) bc	2.10 (1.76) b
Penicillium pinophilum	0.30 (1.14) bc	1.25 (1.50) b	1.66 (1.63) cd	2.21 (1.79) b
Rhizopus oryzae	2.30 (1.51) a	2.00 (1.73) a	3.33 (2.08) ab	3.67 (2.14) a
Trichoderma harzianum	0.00 (1.00) bc	0.75 (1.32) b	0.75 (1.32) d	1.05 (1.43) c
T. koningii	0.30 (1.14) bc	1.25 (1.50) b	1.20 (1.48) cd	1.20 (1.48) c
Control	1.80 (1.67) a	2.25 (1.80) a	3.66 (2.15) a	4.00 (2.23) a
CD (0.05)	0.36	0.28	0.37	0.20

* Letters in the parenthesis indicate the transformed values

** Means followed by a common letter are not significantly different

control. The remaining treatments were superior to the above and were equal in their efficiency. During booting stage results were similar to tillering stage and R. oryzae was least effective and on par with untreated control. A. niger was better than the above but inferior to T. koningii, T. harzianum and P. pinophilum. During last observation made in filling stage, T. harzianum and T. koningii were found to be best. In this case also, as in the earlier stages, R. oryzae was the least effective treatment.

The overall effect suggests that Trichoderma spp. tried was the most effective fungus in reducing the intensity of sheath blight disease. A. niger and P. pinophilum were intermediary in their antagonistic action, while R. oryzae was least effective (Table 6).

4.4.2 Temporal succession of R. solani propagules and antagonistic fungi with respect to sheath blight disease

4.4.2.1 Addition of antagonists one week before inoculation of R. solani

During tillering stage least intensity of sheath blight was observed in treatment T. harzianum. T. koningii ranked second. A. niger and P. pinophilum were on par with untreated control (Table 7).

Table 7

Effect of antagonistic soil fungi on the incidence and intensity of sheath blight disease of rice (Addition of antagonists one week before inoculation of *Rhizoctonia solani*)

Antagonistic fungi	Intensity of sheath blight (0-9 scale)			Incidence of sheath blight (percentage)		
	Tillering	Stem elongation	Booting	Tillering	Stem elongation	Booting
Aspergillus niger	2.41 (1.84)* ab**	2.94 (1.98) ab	3.72 (2.17) ab	39.06 (38.06)	45.97 (42.67) bc	52.87 (46.62) b
Penicillium pinophilum	2.39 (1.84) ab	2.77 (1.94) ab	3.25 (2.06) bc	46.36 (42.89)	53.42 (46.94) b	56.78 (48.87) b
Trichoderma harzianum	0.73 (1.31) c	1.20 (1.48) c	1.93 (1.71) d	22.01 (27.96)	42.06 (40.41) c	49.21 (44.52) b
T. koningii	1.67 (1.63) b	2.02 (1.73) b	2.89 (1.97) c	39.58 (38.96)	46.90 (43.26) bc	53.72 (47.11) b
Control	2.62 (1.90) a	3.45 (2.10) a	4.33 (2.30) a	56.68 (48.81)	62.62 (52.28) a	70.55 (57.11) a
CD (0.05)	0.28	0.26	0.19	NS	4.93	5.40

* Letters in the parenthesis indicate the transformed values

** Means followed by a common letter are not significantly different

At stem elongation stage also, A. niger and P. pinophilum were not effective as they were on par with untreated control. T. harzianum and T. koningii were effective. At booting stage T. harzianum was found to be the most effective treatment. Other effective treatments were T. koningii and P. pinophilum. With respect to incidence of sheath blight at tillering stage none of the treatments showed any difference with control. At stem elongation stage T. harzianum, T. koningii and A. niger were superior to P. pinophilum, eventhough it was better than untreated control. During booting stage all the treatments were superior to untreated control, with no difference among treatments. The overall analysis indicates that the antagonistic fungi when added, before pathogen inoculation, reduced the disease intensity better than disease incidence. Among different fungi two species of Trichoderma were more effective than A. niger or P. pinophilum.

4.4.2.2 Addition of antagonists one week after inoculation of R. solani

During tillering stage treatments, T. harzianum and T. koningii were superior in reducing the intensity of sheath blight. Other treatments viz., A. niger and P. pinophilum were on par with untreated control (Table 8).

Table 8

Effect of antagonistic soil fungi on the intensity and incidence of sheath blight disease of rice (Addition of antagonists one week after inoculation of *Rhizoctonia solani*)

Antagonistic fungi	Intensity of sheath blight (0-9 scale)			Incidence of sheath blight (percentage)		
	Tillering	Stem elongation	Booting	Tillering	Stem elongation	Booting
Aspergillus niger	2.34 (1.82)* abc**	3.14 (2.03) b	3.62 (2.14) b	43.00 (40.95) b	48.75 (44.25) b	57.71 (49.41) b
Penicillium pinophilum	2.53 (1.87) ab	2.97 (1.99) b	3.31 (2.07) bc	48.92 (44.36) ab	54.09 (47.32) ab	60.77 (50.84) b
Trichoderma harzianum	1.03 (1.42) d	1.40 (1.54) c	1.83 (1.68) d	20.62 (26.99) c	38.26 (38.19) c	43.58 (41.29) c
T. koningii	1.49 (1.57) cd	1.81 (1.67) c	2.92 (1.98) c	39.98 (39.20) b	46.06 (42.72) bc	54.56 (47.59) b
Control	3.19 (2.04) a	4.29 (2.30) a	5.07 (2.46) a	55.26 (48.00) a	62.40 (52.16) a	72.49 (58.33) a
CD (0.05)	0.29	0.19	0.12	6.50	5.74	5.30

* Letters in the parenthesis indicate the transformed values

** Means followed by a common letter are not significantly different

During stem elongation stage also least disease incidence was in T. harzianum or T. koningii. P. pinophilum and A. niger were better than untreated control. At booting stage T. harzianum was found to be the most effective treatment.

Disease incidence at tillering stage was minimum in T. harzianum. Other treatments viz., T. koningii, A. niger and P. pinophilum were better than untreated control (Table 8). During stem elongation stage T. harzianum and T. koningii were better than other treatments eventhough all were superior to untreated control. In booting stage, T. harzianum was having least disease incidence. Other antagonistic fungi viz., T. koningii, A. niger and P. pinophilum were on par and better than untreated control. Overall performance revealed that when antagonists were added, after pathogen inoculation, the different fungi were found to reduce both intensity and incidence of sheath blight. Among them T. harzianum was the best (Table 8).

4.5 Field experiment

Effect of soil amendment, plant protection chemical and antagonistic fungi on the intensity and incidence of sheath blight disease of rice, population of R. solani and soil saprophytes and growth and yield attributes

The results of the field experiment conducted in

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Kharif season at Cropping Systems Research Centre, Karamana, Thiruvanandapuram during 1989, with combination of neem cake and lime along with systemic fungicide, Carbendazim and biocontrol agent, T. harzianum are presented (Tables 9 to 19).

4.5.1 Disease intensity

Observations on disease intensity were recorded at six different stages viz., tillering, stem elongation, booting, flowering, filling and mature grain stage (Table 9).

During early stages viz., tillering and stem elongation, plots amended with neem cake or lime alone or the incombination of fungicide/biocontrol agent had significantly lower disease severity than untreated control or fungicide/biocontrol agent alone (Table 9). During booting stage neem cake alone as well as in combination with biocontrol agent, Carbendazim or their combination and lime in combination with biocontrol agent, Carbendazim or their combinations had less disease intensity than other treatments or untreated control. Among the above treatment combinations of neem cake with biocontrol agent/ Carbendazim or lime with biocontrol agent with or without Carbendazim were better than remaining treatments.

During flowering and filling stages neem cake in combination with Carbendazim with or without biocontrol

Table 9

Effect of amendments, biocontrol agent and fungicide on the intensity of sheath blight disease of rice

Treatment	Intensity of sheath blight at different growth stages of rice (0 - 9 scale)					
	Tillering	Stem elongation	Booting	Flowering	Filling	Mature grain
1. Neem cake	0.05 (1.02)* b**	0.10 (1.04) d	0.89 (1.37) bc	1.24 (1.50) cd	1.98 (1.73) cd	2.27 (1.81) cd
2. Neem cake + Carbendazim	0.00 (1.00) b	0.11 (1.05) d	0.54 (1.23) cd	0.98 (1.40) de	1.62 (1.61) cde	1.98 (1.73) cd
3. Neemcake + bio-control agent	0.01 (1.00) b	0.12 (1.05) d	0.46 (1.20) cd	1.26 (1.50) cd	1.64 (1.67) cd	2.15 (1.77) cd
4. Neemcake + Carbendazim + biocontrol agent	0.02 (1.00) b	0.10 (1.04) d	0.29 (1.13) d	0.63 (1.27) e	0.93 (1.38) e	1.16 (1.45) e
5. Lime	0.02 (1.00) b	0.11 (1.05) d	1.25 (1.50) ab	1.67 (1.63) bc	2.15 (1.77) bcd	2.82 (1.95) bc
6. Lime + Carbendazim	0.11 (1.05) b	0.23 (1.11) cd	0.90 (1.38) bc	1.35 (1.53) bcd	1.80 (1.67) cd	2.20 (1.79) cd
7. Lime + bio-control agent	0.06 (1.02) b	0.20 (1.09) d	0.64 (1.26) cd	1.32 (1.52) cd	1.97 (1.72) cd	2.[illegible] (1.88) bcd
8. Lime + Carbendazim + biocontrol agent	0.01 (1.00) b	0.28 (1.12) bcd	0.64 (1.26) cd	1.17 (1.47) cde	1.31 (1.50) de	1.74 (1.65) de
9. Carbendazim	0.38 (1.16) a	0.59 (1.26) ab	1.46 (1.56) ab	1.79 (1.67) bc	2.52 (1.87) bc	2.94 (1.98) bc
10. Bicontrol agent	0.36 (1.16) a	0.54 (1.24) abc	1.53 (1.58) ab	2.00 (1.73) b	3.03 (2.00) ab	3.40 (2.09) ab
11. Carbendazim + biocontrol agent	0.34 (1.15) a	0.60 (1.26) ab	1.37 (1.53) ab	1.72 (1.64) bc	1.99 (1.72) cd	2.43 (1.85) bcd
12. Control	0.44 (1.20) a	0.73 (1.31) a	1.93 (1.71) a	2.97 (1.99) a	3.98 (2.23) a	4.27 (2.29) a
CD (0.05)	0.10	0.14	0.23	0.20	0.26	0.26

* Letters in the parenthesis indicate the transformed values

** Means followed by a common letter are not significantly different

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agent had least disease intensity. However, all the treatments were better than control. During the last observation viz., mature grain stage treatments lime/neem cake in combination with biocontrol agent and Carbendazim had the least disease intensity. During this stage all treatments, except biocontrol agent alone, were better than untreated control (Table 9).

The overall picture emphasized that neem cake and lime were useful as soil amendments in reducing the intensity of sheath blight disease. Among them, neem cake was especially good in combination with a systemic fungicide (Carbendazim) and biocontrol agent (T. harzianum).

4.5.2 Disease incidence

Disease incidence recorded at different stages of the crop is presented (Table 10).

During tillering stage, neem cake or lime alone or in combination with biocontrol agent/Carbendazim was significant. During stem elongation stage neem cake alone or in combination with Carbendazim and biocontrol agent or lime in combination with Carbendazim with or without biocontrol agent were better than other treatments. At booting stage, neem cake/lime with all combinations of Carbendazim or biocontrol agent recorded least disease

Table 10

Effect of amendments, biocontrol agent and fungicide on the incidence of sheath blight disease of rice

Treatments	Incidence of sheath blight (percentage of infected tillers) at different growth stages of rice					
	Tillering	Stem elongation	Booting	Flowering	Filling	Mature grain
Neem cake	0.48(3.97)* b**	2.41(8.42) b	21.04(27.29) abc	26.62(31.39) cd	30.89(34.11) cd	36.39(37.67) cde
Neem cake + Carbendazim	0.60(4.00) b	2.50(9.09) b	12.45(20.65) cd	20.95(27.22) de	24.45(29.62) de	29.28(32.74) de
Neem cake + biocontrol agent	0.16(2.27) b	2.82(9.68) b	14.70(22.53) cd	21.04(27.29) de	26.86(31.20) cde	34.48(35.94) de
Neem cake + Carbendazim + biocontrol agent	0.32(3.22) b	1.83(7.77) b	8.08(16.50) d	15.85(23.45) e	20.11(26.03) e	25.14(30.08) e
Lime	0.63(4.57) b	4.08(10.99) b	24.45(29.69) abc	28.29(32.11) cd	33.29(35.11) cd	38.43(38.30) cde
Lime + Carbendazim	0.92(5.50) b	3.51(10.79) b	17.14(24.44) bcd	24.08(29.37) cde	26.74(30.82) cde	34.59(36.01) de
Lime + biocontrol agent	1.83(7.77) b	4.68(12.49) ab	18.29(25.31) bcd	26.33(30.85) cd	27.36(31.52) cde	35.20(36.37) de
Lime + Carbendazim + biocontrol agent	0.63(4.55) b	3.51(10.79) b	12.98(21.11) cd	19.12(26.21) de	25.29(30.11) de	33.43(35.30) de
Carbendazim	8.54(16.98) a	5.61(13.69) ab	24.05(29.35) abc	43.21(41.08) ab	47.89(43.77) ab	50.34(45.17) abc
Biocontrol agent	7.49(16.00) a	8.71(17.15) ab	31.05(33.04) ab	45.18(42.21) a	52.85(46.61) ab	56.47(48.69) ab
Carbendazim + biocontrol agent	7.69(16.09) a	7.83(16.23) a	24.16(29.72) abc	32.26(34.51) bc	38.26(38.19) bc	43.64(41.32) bcd
Control	7.91(17.94) a	12.95(20.64) a	33.28(35.21) a	52.64(46.49) a	56.64(48.44) a	64.26(53.26) a
CD (0.05)	5.08	9.65	9.76	6.57	7.41	8.58

* Letters in the parenthesis indicate the transformed values

** Means followed by a common letter are not significantly different

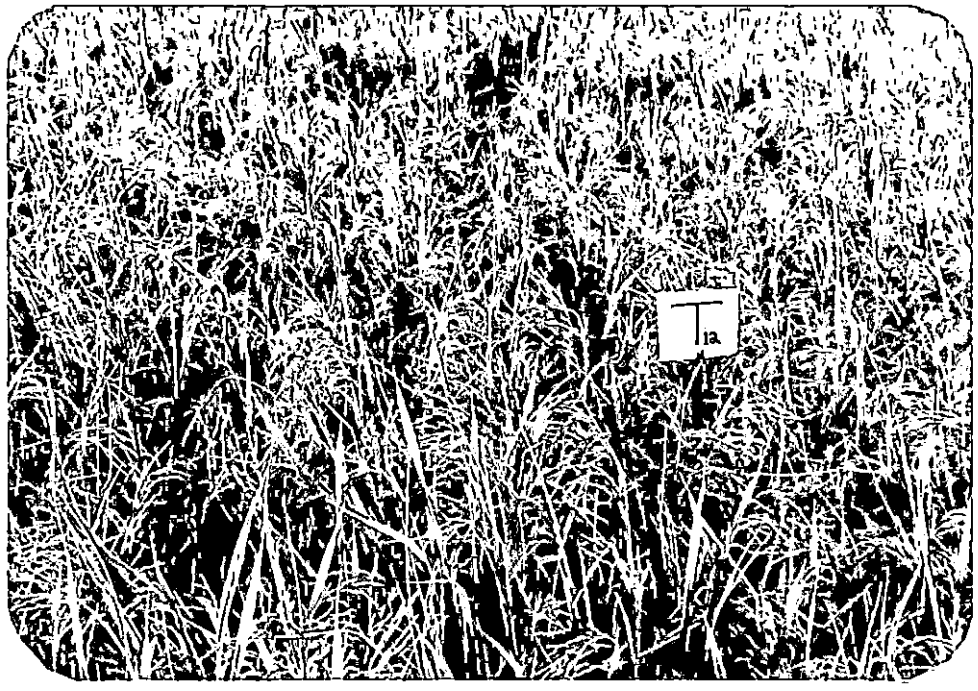
incidence. During flowering stage, Carbendazim or bio-control agent were on par with untreated control. All combinations of neem cake with Carbendazim or biocontrol agent and of lime in combination with Carbendazim with or without biocontrol agent were the best treatments. During filling stage, all combinations of neem cake or lime with Carbendazim or biocontrol agent were superior. Carbendazim or biocontrol agent alone were on par with untreated control as in flowering stage. During last stage viz., mature grain, neem cake or lime alone or in combinations with biocontrol agent or Carbendazim were the best treatments (Table 10). The overall effect suggested that both neem cake and lime reduced disease incidence or intensity. Neem cake/lime in combination with systemic fungicide (Carbendazim) or biocontrol agent (T. harzianum) alone and neem cake with Carbendazim were the most effective combinations (Plate 14 and 15).

4.5.3 Population of soil saprophyte and R. solani

Population of the pathogen as well as total fungi, bacteria and actinomycetes under different treatments at different growth stages of the crop with their deviations from original population and their correlations are presented (Tables 11, 12, 13, 14, 15 and 16).

Plate 14. Treatment plots applied with neem cake, biocontrol agent and Carbendazim

Plate 15. Control



66

4.5.3.1 Rhizoctonia solani propagules in soil

The population of the pathogen did not vary during early observations viz., just before addition of amendments and planting stage of the crop (Table 11) (Fig. 2).

During stem elongation stage all treatments of neem cake or lime alone or in combinations with Carbendazim or biocontrol agent had lower pathogen population than untreated control. In booting and mature grain stages all the treatments had lower pathogen population than control. However, in mature grain stage, neem cake in combination with biocontrol agent with or without Carbendazim as well as lime in combination with biocontrol agent with or without Carbendazim or biocontrol agent alone were better than other treatments.

During planting, there was no remarkable change in population of pathogen in any of the treatments. During stem elongation stage, reduction was more prominent in neem cake alone or in combinations with other treatments. During booting and mature grain stages neem cake or lime in combination with biocontrol agent had greater reduction in pathogen population.

4.5.3.2 Sclerotial population of R. solani in soil

An estimation of the sclerotia present in soil

Table 11

Number of propagules of *R. solani* per 10 g dry soil treated with amendments, biocontrol agent and fungicides at different growth stages of rice

Treatments	*Rhizoctonia solani*					Percentage deviation			
	Before addition of amendments	Planting	Stem elongation	Booting stage	Mature grain stage	Planting	Stem elongation	Booting	Mature grain
Neem cake	8.63 (3.10)*	7.35 (2.89)	5.09 (2.47) d**	5.92 (2.63) b	7.29 (2.87) b	-6	-20	-15	-7
Neem cake + Carbendazim	7.21 (2.87)	7.23 (2.87)	5.29 (2.50) d	5.89 (2.62) b	6.98 (2.82) b	0	-13	-9	-2
Neem cake + biocontrol agent	8.79 (3.13)	8.35 (3.06)	5.55 (2.56) d	4.58 (2.36) b	4.21 (2.28) c	-2	-18	-24	-27
Neem cake + Carbendazim + biocontrol agent	7.93 (2.99)	7.67 (2.95)	5.59 (2.57) d	5.26 (2.52) b	5.29 (2.51) bc	+2	-14	-15	-17
Lime	8.23 (3.04)	8.82 (3.13)	5.66 (2.58) cd	5.27 (2.50) b	6.98 (2.82) b	+6	-15	-17	-7
Lime + Carbendazim	8.34 (3.04)	9.24 (3.20)	6.81 (2.79) bcd	6.42 (2.72) b	6.81 (2.85) b	+5	-9	-10	-6
Lime + biocontrol agent	8.69 (3.11)	9.12 (3.18)	6.61 (2.76) bcd	4.89 (2.43) b	4.49 (2.36) c	+2	-11	-15	-22
Lime + Carbendazim + biocontrol agent	8.79 (3.13)	9.87 (3.30)	6.50 (2.74) bc	5.13 (2.48) b	5.56 (2.56) bc	+5	-12	-22	-18
Carbendazim	8.54 (3.07)	10.67 (3.62)	9.29 (3.21) abc	6.55 (2.75) b	6.98 (2.82) b	+10	+4	-11	-6
Biocontrol agent	8.63 (3.10)	9.85 (3.29)	8.35 (3.06) abc	6.32 (2.71) b	5.59 (2.57) b	+6	-1	-12	-17
Carbendazim + biocontrol agent	8.74 (3.12)	10.87 (3.45)	10.63 (3.41) a	6.21 (2.69) b	5.86 (2.62) bc	+10	+9	-14	-16
Control	8.94 (3.15)	10.31 (3.36)	10.67 (3.47) a	11.29 (3.51) a	12.30 (3.65) a	+7	+8	+12	+16
	NS	NS	0.48	0.52	0.45				

* Letters in the parenthesis indicate the transformed values

** Means followed by a common letter are not significantly different

Fig. 2. Effect of soil amendments, biocontrol agent and fungicide on the number of R. solani propagules per g at different growth stages of rice

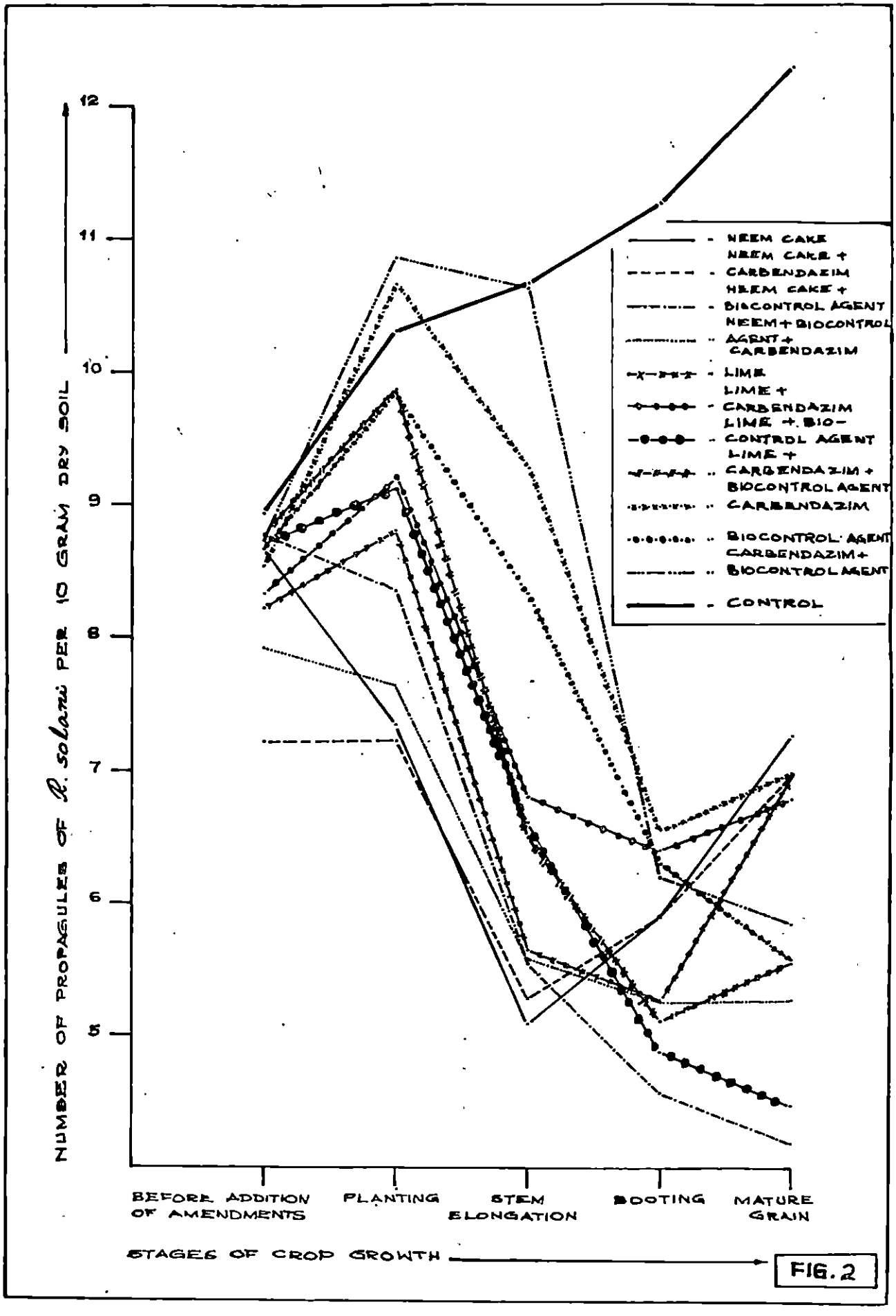

NUMBER OF PROPAGULES OF R. solani PER 10 GRAM DRY SOIL
12
11
10
9
8
7
6
5
NEEM CAKE
NEEM CAKE + CARBENDAZIM
NEEM CAKE + BIOCONTROL AGENT
NEEM + BIOCONTROL AGENT + CARBENDAZIM
LIME
LIME + CARBENDAZIM
LIME + BIO-CONTROL AGENT
LIME + CARBENDAZIM + BIOCONTROL AGENT
CARBENDAZIM
BIOCONTROL AGENT CARBENDAZIM +
BIOCONTROL AGENT
CONTROL
BEFORE ADDITION OF AMENDMENTS
PLANTING
STEM ELONGATION
BOOTING
MATURE GRAIN
STAGES OF CROP GROWTH

FIG. 2

during early periods of crop growth showed that there was no difference among treatments (Table 12) (Fig. 3). However, during later stages viz., booting and mature grain stage number of sclerotia became less in certain plots. In booting stage neem cake/lime with all combinations of Carbendazim or biocontrol agent were the best treatments. During last stage, all the treatments were effective, among which neem cake with biocontrol agent with or without Carbendazim, lime with Carbendazim or biocontrol agent as well as combination of biocontrol agent with Carbendazim were better.

At planting stage slight decline in the sclerotial population was noticed in plots amended with neem cake followed by lime. A similar trend was observed in stem elongation stage also. However, in booting and mature grain stages, increase in sclerotia was observed in neem cake or lime alone. In the final analysis at harvest, all treatments, except neem cake, Carbendazim and biocontrol agent combination, showed an increasing trend. The percentage increase of sclerotia was maximum in untreated control (60%). This was followed by lime (32%) or neem cake (28%) alone.

4.5.3.3 Total fungi

Populations of total fungi in different stages of

Table 12

Number of sclerotia of R. solani per 50 g dry soil treated with amendments, biocontrol agent and fungicides at different growth stages of rice

Treatment	Sclerotia of R. solani					Percentage deviation			
	Before addition of amendments	Planting	Stem elongation	Booting stage	Mature grain stage	Planting stage	Stem elongation	Booting stage	Mature grain stage
Neem cake	0.50 (1.22)*	0.37 (1.17)	0.22 (1.10)	1.00 (1.41) bc**	1.36 (1.52) bc	-4	-10	+16	+25
Neem cake + Carbendazim	0.47 (1.21)	0.21 (1.10)	0.00 (1.00)	0.42 (1.09) d	1.20 (1.44) bc	-8	-17	+1	+19
Neem cake + biocontrol agent	0.39 (1.18)	0.20 (1.10)	0.22 (1.10)	0.98 (1.14) cd	0.83 (1.34) cd	-7	-7	-3	+13
Neem cake + Carbendazim + biocontrol agent	0.60 (1.26)	0.32 (1.15)	0.00 (1.00)	0.46 (1.20) cd	0.52 (1.23) d	-9	-20	-5	-2
Lime	0.42 (1.19)	0.29 (1.14)	0.22 (1.10)	1.23 (1.44) ab	1.47 (1.57) b	-5	-8	+25	+32
Lime + Carbendazim	0.52 (1.23)	0.33 (1.15)	0.22 (1.10)	0.46 (1.21) cd	0.93 (1.39) bcd	-5	-8	+1	+15
Lime + biocontrol agent	0.43 (1.20)	0.31 (1.14)	0.22 (1.10)	0.46 (1.21) cd	0.93 (1.39) bcd	-2	-8	+1	+15
Lime + Carbendazim + biocontrol agent	0.42 (1.19)	0.36 (1.17)	0.22 (1.10)	0.46 (1.21) cd	1.00 (1.41) bcd	-2	-8	+1	+18
Carbendazim	0.40 (1.15)	0.42 (1.19)	0.46 (1.20)	0.93 (1.39) bc	1.20 (1.44) bc	0	+2	+18	+19
Biocontrol agent	0.49 (1.22)	0.47 (1.21)	0.46 (1.20)	0.85 (1.36) bc	0.98 (1.39) bcd	1	-2	+11	+18
Carbendazim + biocontrol agent	0.52 (1.23)	0.48 (1.22)	0.46 (1.20)	1.23 (1.49) ab	1.23 (1.49) bc	1	-2	+21	+21
Control	0.47 (1.21)	0.47 (1.21)	0.46 (1.20)	1.96 (1.71) a	2.75 (1.93) a	0	+1	+41	+60
CD (0.05)	NS	NS	NS	0.27	0.20				

* Letters in the parenthesis indicate the transformed values

** Means followed by a common letter are not significantly different

Fig. 3. Effect of soil amendment, biocontrol agent and fungicide on the sclerotial population of *R. solani* per 50 g at different growth stages of rice.

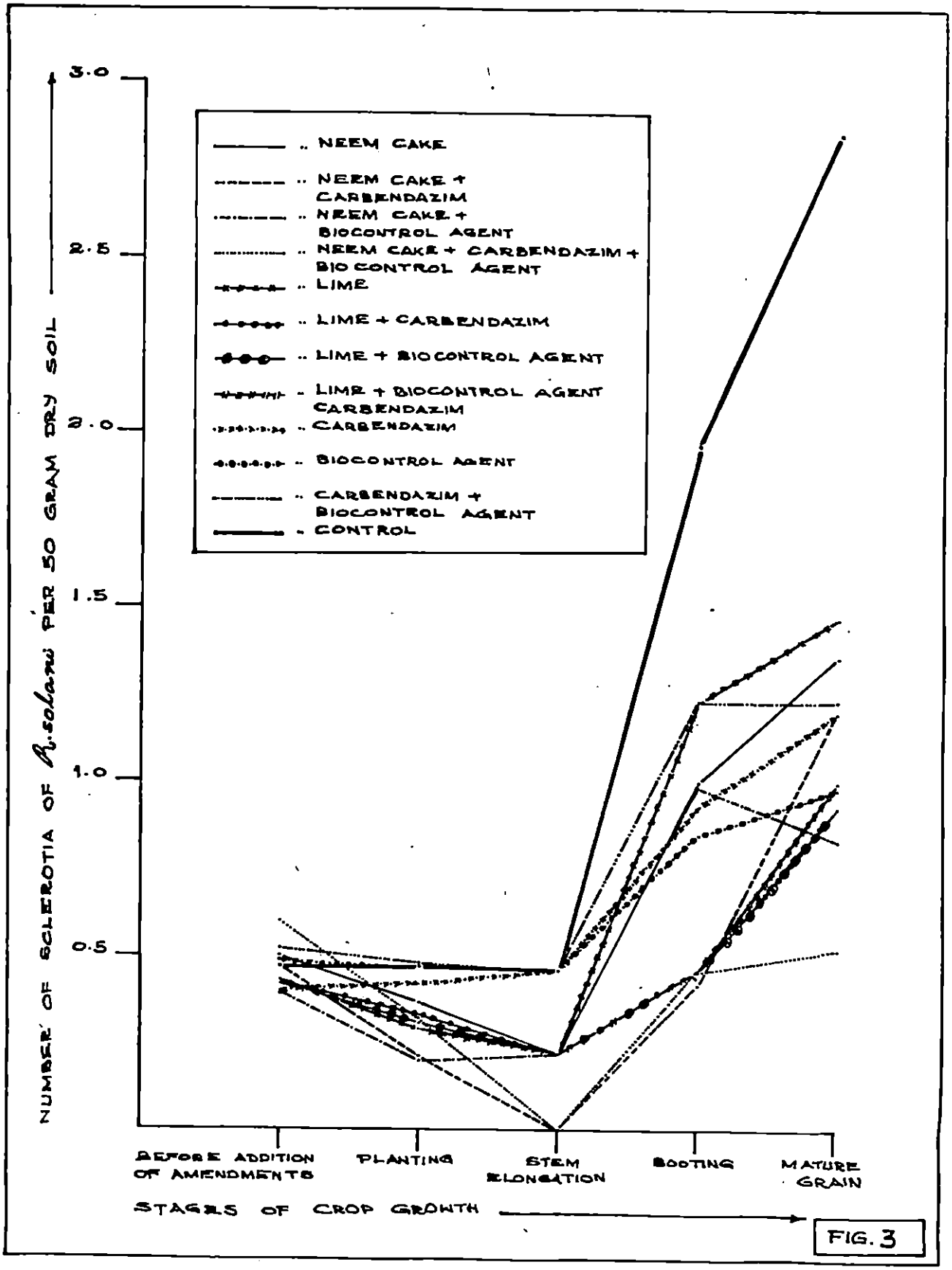

NEEM CAKE
NEEM CAKE + CARBENDAZIM
NEEM CAKE + BIOCONTROL AGENT
NEEM CAKE + CARBENDAZIM + BIO CONTROL AGENT
LIME
LIME + CARBENDAZIM
LIME + BIOCONTROL AGENT
LIME + BIOCONTROL AGENT CARBENDAZIM
CARBENDAZIM
BIOCONTROL AGENT
CARBENDAZIM + BIOCONTROL AGENT
CONTROL
NUMBER OF SCLEROTIA OF R. solani PER 50 GRAM DRY SOIL
3.0
2.5
2.0
1.5
1.0
0.5
BEFORE ADDITION OF AMENDMENTS
PLANTING
STEM ELONGATION
BOOTING
MATURE GRAIN
STAGES OF CROP GROWTH

FIG. 3

70

the crop growth are presented (Table 13). During planting stage, highest fungal population was observed in plots amended with lime alone or in combination with Carbendazim or biocontrol agent. During stem elongation stage, plots of neem cake alone or in combinations as well as lime with biocontrol agent had maximum fungal population. At booting stage, neem cake in all combinations of Carbendazim and lime in combination with biocontrol agent with or without Carbendazim and biocontrol agent without amendment had highest number of total fungi. Neem cake alone was also better than untreated control. During the last observation made in mature grain stage, maximum fungal population was in treatment in which a combined application of neem cake with biocontrol agent with or without Carbendazim and in biocontrol agent was added. Other treatments which had more population than untreated control were neem cake with Carbendazim, lime with biocontrol agent and combination of biocontrol agent and combination of biocontrol agent and Carbendazim (Table 13, Fig. 4).

Stimulation of fungi was more in neem cake alone or in combinations and lime with biocontrol agent during stem elongation stage. During booting stage there was a remarkable improvement in populations in all treatments, except untreated control and lime alone. Highest stimulation was in plots of biocontrol agent especially in

Table 13

Population of total fungi per g dry soil treated with amendments, biocontrol agent and fungicides at different growth stages of rice

Treatments	Total fungi					Percentage deviation			
	Before addition of amendments	Planting	Stem elongation	Booting	Mature grain	Planting	Stem elongation	Booting	Mature grain
Neem cake	14.56 (3.94)*	15.25 (4.03)cd**	21.99 (4.79) a	22.62 (4.86)bcd	19.25 (4.50) ef	-2	+21	+23	+14
Neem cake + Carbendazim	15.23 (4.03)	15.95 (4.12)bcd	21.65 (4.75) ab	24.63 (5.06)abc	24.61 (5.06) bc	+2	+18	+26	+26
Neem cake + biocontrol agent	14.00 (3.87)	15.29 (4.04) cd	19.99 (4.58)abc	26.64 (5.25) a	29.62 (5.53) a	+4	+18	+36	+42
Neem cake + Carbendazim + biocontrol agent	13.22 (3.77)	14.98 (4.00) d	20.93 (4.68)abc	24.22 (5.02) a	29.28 (5.50) a	+6	+24	+33	+45
Lime	15.57 (4.07)	18.31 (4.59) a	18.65 (4.43)bcd	19.61 (4.54) d	17.96 (4.35) f	+12	+8	+11	+6
Lime + Carbendazim	15.10 (4.01)	17.63 (4.32)abc	17.84 (4.34)cde	21.26 (4.71)cde	17.96 (4.35) f	+8	+8	+17	+8
Lime + biocontrol agent	14.92 (4.00)	17.94 (4.35) ab	17.11 (4.73) ab	25.26 (5.12) a	24.55 (5.05) b	+8	+18	+28	+26
Lime + Carbendazim + biocontrol agent	15.23 (4.03)	17.99 (4.36) ab	18.31 (4.40) dc	23.54 (4.95) ab	22.52 (4.85)cde	+8	+10	+22	+20
Carbendazim	13.12 (3.81)	14.29 (3.91) d	13.99 (3.87) f	19.66 (4.54) de	20.63 (4.65)def	+2	+2	+20	+22
Biocontrol agent	13.93 (3.86)	15.63 (4.08) bc	15.93 (4.11) def	24.59 (5.06) ab	26.66 (5.25)ab	+6	+6	+31	+36
Carbendazim + biocontrol agent	14.71 (3.96)	15.62 (4.07) bc	16.11 (4.13) def	24.66 (5.06) ab	23.99 (4.99) bcd	+3	+4	+27	+26
Control	14.92 (4.00)	14.98 (4.00) d	14.96 (3.99) ef	18.66 (4.43) de	19.97 (4.57) ef	0	0	+11	+14
CD (0.05+	NS	0.30	0.35	0.38	0.38				

* Letters in the parenthesis indicate the transformed values

** Means followed by a common letter are not significantly different

Fig. 4. Effect of soil amendment, biocontrol agent and fungicide on the population of total fungi per g (10^{-4}) at different growth stages of rice.

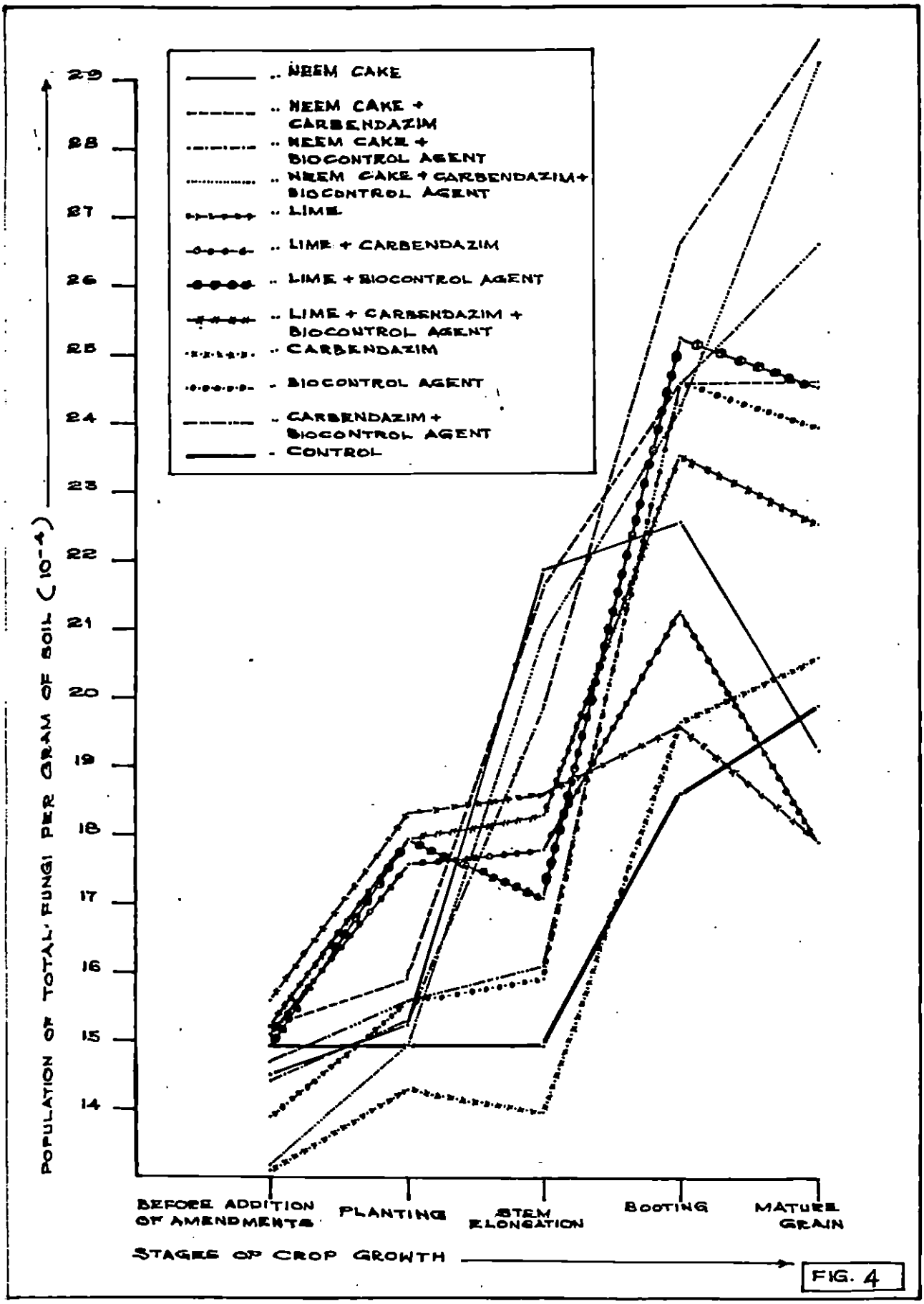

.. NEEM CAKE
.. NEEM CAKE + CARBENDAZIM
.. NEEM CAKE + BIOCONTROL AGENT
.. NEEM CAKE + CARBENDAZIM + BIOCONTROL AGENT
.. LIME
.. LIME + CARBENDAZIM
.. LIME + BIOCONTROL AGENT
.. LIME + CARBENDAZIM + BIOCONTROL AGENT
.. CARBENDAZIM
- BIOCONTROL AGENT
- CARBENDAZIM + BIOCONTROL AGENT
- CONTROL
POPULATION OF TOTAL FUNGI PER GRAM OF SOIL (10⁻⁴)
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
BEFORE ADDITION OF AMENDMENTS
PLANTING
STEM ELONGATION
BOOTING
MATURE GRAIN
STAGES OF CROP GROWTH

FIG. 4

combination with neem cake. The same trend was noticed in mature grain stage also.

4.5.3.4 Soil bacterial population

During planting, a higher bacterial population was observed over untreated control in neem cake or lime alone or in combinations. During stem elongation stage, neem cake alone or its combinations had highest population of bacteria, eventhough neem cake alone or in combination with biocontrol agent were on par with untreated control. In booting stage neem cake with all combinations of Carbendazim or biocontrol agent had high bacterial population but the treatment neem cake with biocontrol agent was on par with untreated control. At mature grain stage, neem cake in combination with Carbendazim with or without biocontrol agent had highest bacterial population (Table 14, Fig. 5).

During planting, stimulation of bacterial population was greater in plots of neem cake alone or its combinations and also lime with Carbendazim. Highest bacterial stimulation, in general, occurred during stem elongation stage. Among treatments, neem cake in combination with Carbendazim and biocontrol agent had the greatest stimulation. In booting and maturity grain stages also, neem cake combined with biocontrol agent and Carbendazim had high bacterial population (Table 14, Fig. 5).

Table 14

Population of bacteria per g dry soil treated with amendments, biocontrol agents and fungicides at different growth stages of rice

Treatments	Bacteria					Percentage deviation			
	Before addition of amendments	Planting	Stem elongation	Booting	Mature grain	Planting	Stem elongation	Booting	Mature grain
Neem cake	8.23 (3.04)*	13.33 (3.79)ab**	20.59 (4.64)abc	15.58 (4.07)bcd	12.25 (3.64)bcd	+25	+52	+34	+20
Neem cake + Carbendazim	8.71 (3.12)	13.25 (3.77)ab	21.24 (4.71)ab	17.61 (4.31)ab	13.59 (3.82)ab	+21	+51	+32	+22
Neem cake + biocontrol agent	7.21 (2.87)	14.97 (4.00)a	20.57 (4.64)abc	16.55 (4.18)abc	12.89 (3.72) bc	+39	+62	+46	+11
Neem cake + Carbendazim + biocontrol agent	9.10 (3.18)	13.99 (3.87)ab	24.00 (5.00)a	21.95 (4.79)a	17.84 (4.34)a	+22	+75	+51	+36
Lime	9.24 (3.2)	12.63 (3.69)ab	16.61 (4.20)bcd	11.61 (3.55)def	8.64 (3.10)de	+15	+31	+11	-3
Lime + Carbendazim	8.38 (3.06)	14.99 (4.00)a	16.61 (4.20)bcd	13.59 (3.82)bcdef	9.94 (3.30)cde	+31	+37	+25	+8
Lime + biocontrol agent	9.00 (3.18)	11.98 (3.60)b	17.29 (4.28)bcd	14.60 (3.94)bcde	10.57 (3.40)cde	+13	+35	+24	+7
Lime + Carbendazim + biocontrol agent	9.37 (3.22)	12.30 (3.65)b	17.61 (4.31)bcd	14.60 (3.94)bcde	10.57 (3.40)cde	+13	+34	+22	+6
Carbendazim	7.12 (2.85)	7.98 (3.00)c	14.28 (3.90)d	10.96 (3.45)ef	8.95 (3.15)cde	+5	+37	+21	+11
Biocontrol agent	7.82 (2.97)	9.29 (3.21)c	15.36 (4.04)cd	9.41 (3.22)f	7.92 (2.98)e	+8	+36	+8	0
Carbendazim + biocontrol agent	8.31 (3.05)	9.33 (3.21)c	15.93 (4.12)bcd	11.91 (3.57)bcdef	9.90 (3.30)cde	+5	+35	+17	+8
Control	8.65 (3.11)	8.66 (3.11)c	15.60 (4.07)cd	12.25 (3.64)cdef	10.96 (3.45)cde	0	+31	+17	+11
CD (0.05)	NS	0.33	0.60	0.60	0.58				

* Letters in the parenthesis indicate the transformed values

** Means followed by a common letter are not significantly different

Fig. 5. Effect of soil amendment, biocontrol agent and fungicide on the population of bacteria per g (10^{-6}) at different growth stages of rice.

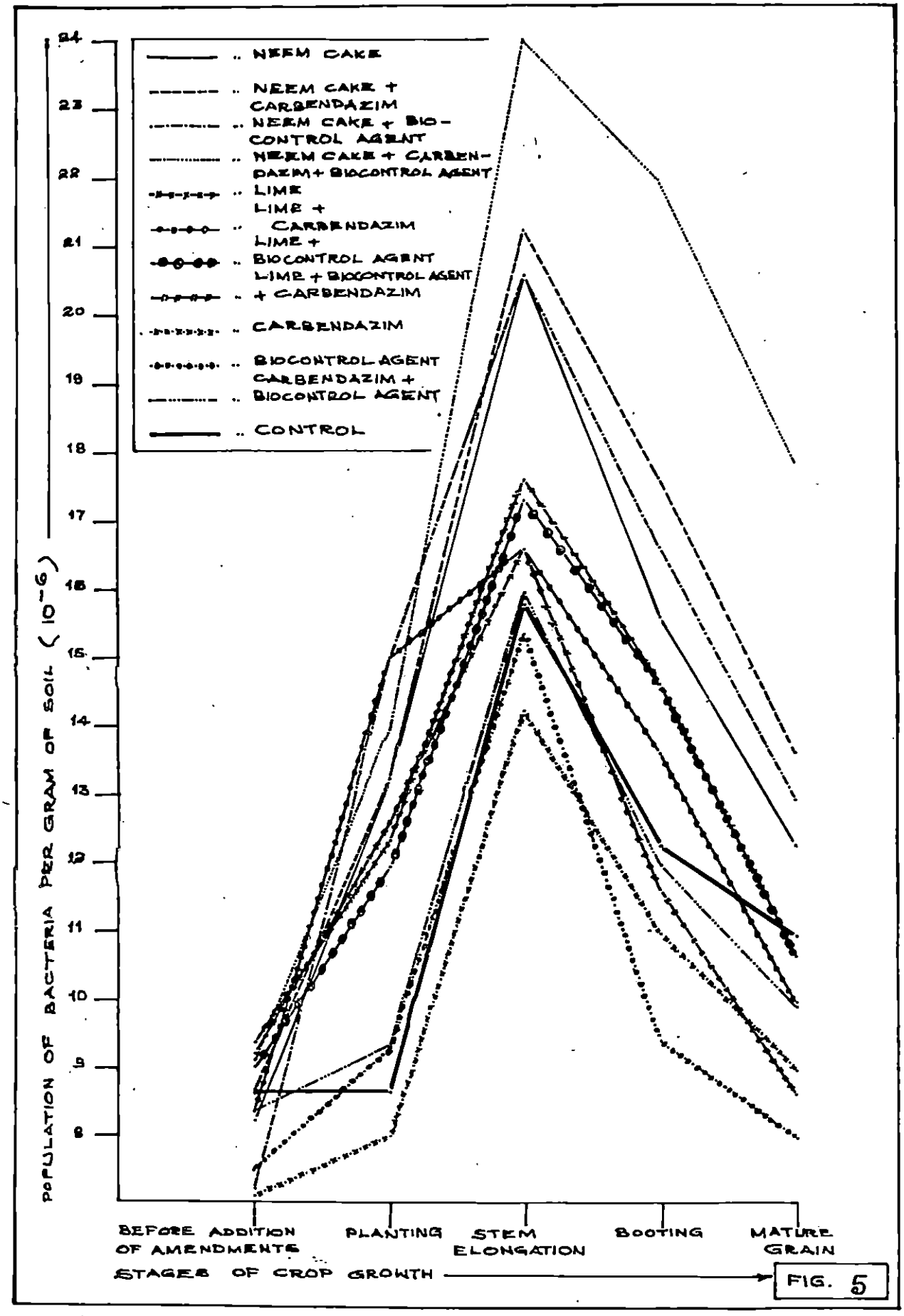

NEEM CAKE
NEEM CAKE + CARBENDAZIM
NEEM CAKE + BIO-CONTROL AGENT
NEEM CAKE + CARBEN-DAZIM + BIOCONTROL AGENT
LIME
LIME + CARBENDAZIM
LIME + BIOCONTROL AGENT
LIME + BIOCONTROL AGENT + CARBENDAZIM
CARBENDAZIM
BIOCONTROL AGENT
CARBENDAZIM + BIOCONTROL AGENT
CONTROL
POPULATION OF BACTERIA PER GRAM OF SOIL (10⁻⁶)
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
9
8
BEFORE ADDITION OF AMENDMENTS
PLANTING
STEM ELONGATION
BOOTING
MATURE GRAIN
STAGES OF CROP GROWTH

FIG. 5

4.5.3.5 Soil actinomycetes

The soil actinomycetes population did not vary among different treatments in different growth stages of the rice crop. However, a general increase in population occurred from stem elongation onwards (Table 15, Fig. 6).

4.5.3.6 Correlation of soil saprophytes with R. solani propagules at different stages of the crop

Partial correlation coefficients between populations of R. solani, total fungi, bacteria and actinomycetes worked out during different growth stages are presented (Table 16). Results indicated that highly significant negative correlation exist between population of fungi and R. solani during stem elongation stage. Significant negative correlations also exist between bacteria and R. solani and between actinomycetes and R. solani at this stage.

Significant negative correlation between total fungi and R. solani was observed in booting stage also (Table 16). The above results indicate that there is a corresponding reduction in the population of R. solani due to increase in population of total fungi, bacteria and actinomycetes in stem elongation stage and due to increase in fungal population in booting stage.

Table 15

Population of actinomycetes per g dry soil treated with amendments, biocontrol agent and fungicide at different growth stages of rice

Treatments	Actinomycetes					Percentage deviation			
	Before addition of amendments	Planting	Stem elongation	Booting	Mature grain	Planting	Panicle initiation	Booting	Mature grain
Neem cake	6.85 (2.61)*	7.88 (2.97)	12.28 (3.64)	11.58 (3.54)	11.61 (3.55)	+14	+39	+36	+36
Neem cake + Carbendazim	5.31 (2.30)	7.29 (2.87)	8.98 (3.15)	10.30 (3.36)	12.33 (3.65)	+25	+37	+46	+59
Neem cake + biocontrol agent	5.72 (2.39)	8.29 (3.04)	9.66 (3.26)	10.63 (3.41)	10.96 (3.45)	+27	+36	+43	+44
Neem cake + Carbendazim + biocontrol agent	6.21 (2.49)	8.29 (3.04)	11.30 (3.50)	10.94 (3.45)	12.28 (3.64)	+23	+41	+39	+46
Lime	5.43 (2.33)	6.92 (2.81)	9.98 (3.31)	9.98 (3.31)	10.63 (3.41)	+21	+42	+42	+46
Lime + Carbendazim	5.69 (2.40)	6.23 (2.68)	9.63 (3.26)	10.99 (3.46)	10.87 (3.44)	+12	+36	+44	+43
Lime + biocontrol agent	5.27 (2.30)	5.90 (2.62)	8.98 (3.15)	10.63 (3.41)	10.31 (3.36)	+14	+37	+48	+46
Lime + Carbendazim + biocontrol agent	5.53 (2.35)	6.92 (2.81)	9.95 (3.30)	9.63 (3.26)	10.63 (3.41)	+20	+40	+39	+45
Carbendazim	5.41 (2.33)	5.98 (2.64)	8.55 (3.09)	8.29 (3.04)	11.26 (3.50)	+13	+33	+30	+50
Biocontrol agent	5.72 (2.39)	6.61 (2.75)	10.30 (3.36)	9.94 (3.30)	9.30 (3.20)	+15	+41	+38	+34
Carbendazim + biocontrol agent	5.33 (2.31)	5.59 (2.36)	8.25 (3.04)	8.98 (3.15)	9.98 (3.31)	+3	+32	+37	+43
Control	5.76 (2.4)	5.56 (2.56)	8.33 (3.05)	8.98 (3.15)	10.63 (3.41)	+7	+27	+31	+42
CD (0.05)	NS	NS	NS	NS	NS				

* Letters in the parenthesis indicate the transformed values

Fig. 6. Effect of soil amendment, biocontrol agent and fungicide on the population of actinomycetes per g (10^{-6}) at different growth stages of rice.

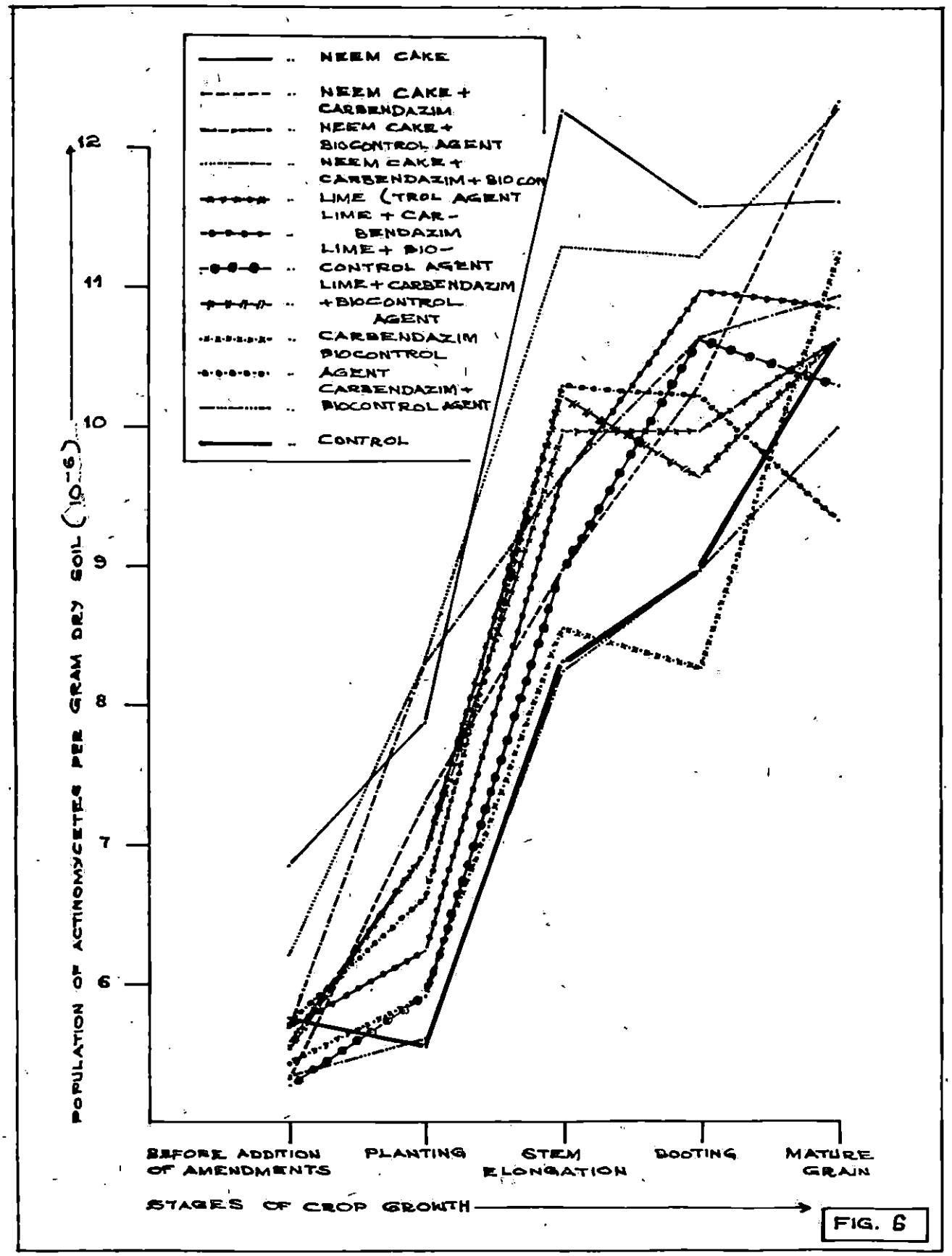

NEEM CAKE
NEEM CAKE + CARBENDAZIM
NEEM CAKE + BIOCONTROL AGENT
NEEM CAKE + CARBENDAZIM + BIOCONTROL AGENT
LIME
LIME + CARBENDAZIM
LIME + BIOCONTROL AGENT
LIME + CARBENDAZIM + BIOCONTROL AGENT
CARBENDAZIM
BIOCONTROL AGENT
CARBENDAZIM + BIOCONTROL AGENT
CONTROL
POPULATION OF ACTINOMYCETES PER GRAM DRY SOIL (10⁻⁶)
12
11
10
9
8
7
6
BEFORE ADDITION OF AMENDMENTS
PLANTING
STEM ELONGATION
BOOTING
MATURE GRAIN
STAGES OF CROP GROWTH

FIG. 6

Table 16

Correlation of microorganisms with respect to R. solani propagules at different stages of the crop

Microorganism	Partial correlation coefficient at			
	Planting stage	Stem elongation	Booting	Mature grain stage
Actinomycetes	-0.2543	-0.4028*	-0.1305	0.0272
Bacteria	-0.2237	-0.3315*	-0.1873	-0.1882
Fungi	-0.3012	-0.5456**	-0.4022*	-0.2030

*r at 0.05 : 0.3246

**r at 0.01 : 0.4182

4.5.4 Chemical analysis of soil

Results of the chemical analysis of soil taken from different treatment plots are presented (Table 17).

There was no remarkable change in soil reaction or in available major nutrient levels in different treatments. However, changes in magnesium and calcium levels were observed. There was a drastic reduction in the level of magnesium in all plots, including untreated control after experimentation. However, in plots amended with lime, there was a definite increase in calcium concentration (Table 17).

4.5.5 Growth and yield attributes

Effect of amending soil with neem cake or lime alone or in combination with Carbendazim and biocontrol agent T. harzianum on growth parameters and yield attributes of rice crop are presented (Table 18). Biometric characters observed viz., plant height, total and productive tillers did not vary among treatments (Table 18). However, grains per panicle and yields of grain and straw differed significantly (Table 18).

Maximum grains per panicle was noticed in plot amended with neem cake, in which Carbendazim and biocontrol agent were applied. All the treated plots had significantly

Table 17

pH and major nutrient content of soil as influenced by amendments, biocontrol agent and fungicide

Treatments	pH	Nutrient percentages			Available			
		N	P_2O_5	K_2O	Calcium (ppm)	Magnesium (ppm)	Zinc (ppm)	Iron (ppm)
Before experiment	4.50	0.012	8.720	31.27	0.226	0.284	0.012	0.103
After experiment								
Neem	4.67	0.015	8.987	29.76	0.274 b*	0.242 a	0.017	0.107
Neem + Carbendazim	4.50	0.015	10.007	38.33	0.284 b	0.226 a	0.012	0.119
Neem + biocontrol agent	4.37	0.021	9.012	35.33	0.254 b	0.262 a	0.011	0.110
Neem + Carbendazim + biocontrol agent	4.33	0.018	11.123	29.76	0.274 b	0.185 a	0.013	0.119
Lime	5.32	0.019	9.074	36.73	0.753 a	0.188 a	0.014	0.107
Lime + Carbendazim	5.23	0.013	11.237	31.23	0.778 a	0.173 b	0.009	0.103
Lime + biocontrol agent	5.37	0.013	10.931	35.33	0.817 a	0.183 a	0.011	0.106
Lime + Carbendazim + biocontrol agent	5.33	0.020	10.554	32.24	0.731 a	0.193 a	0.012	0.107
Carbendazim	4.61	0.013	10.234	35.38	0.312 b	0.127 b	0.012	0.105
Biocontrol agent	4.47	0.018	11.107	28.35	0.273 b	0.111 b	0.013	0.110
Carbendazim + biocontrol agent	4.59	0.015	9.833	33.39	0.223 b	0.136 b	0.011	0.109
Control	4.79	0.013	8.923	31.12	0.226 b	0.129 b	0.013	0.110
CD (0.05)	NS	NS	NS	NS	0.234	0.103	NS	NS

* Means followed by a common letter are not significantly different

Table 18

Effect of amendments, biocontrol agent and fungicide on growth and yield of rice crop

Treatments	Plant height	Total number of tillers/m^2	Number of productive tillers/m^2	Grain/panicle	Grain yield (kg per hectare)	Straw yield (kg per hectare)
Neem cake	75.75	465	414	44.35 c*	3787.500 e	6575.000 a
Neem cake + Carbendazim	75.75	437	391	47.40 bc	4275.000 ab	6559.000 a
Neem cake + biocontrol agent	77.40	386	350	53.80 ab	3775.000 e	5700.000 d
Neem cake + Carbendazim + biocontrol agent	77.40	440	380	56.90 a	4350.000 a	6664.370 a
Lime	80.00	410	385	49.55 abc	3954.500 d	6074.300 c
Lime + Carbendazim	78.50	428	337	47.85 bc	4064.000 cd	6250.000 bc
Lime + biocontrol agent	80.50	367	323	48.73 abc	3954.500 d	6132.000 c
Lime + Carbendazim + biocontrol agent	80.00	366	373	45.55 c	4223.750 b	6337.25 b
Carbendazim	76.37	418	367	46.25 c	3990.500 d	5632.500 d
Biocontrol agent	80.00	470	423	53.70 b	3590.500 f	5632.500 d
Carbendazim + biocontrol agent	78.75	390	350	45.25 c	4168.250 bc	5825.600 d
Control	78.30	433	390	47.45 b	3157.500 g	5200.000 e
CD (0.05)	NS	NS	NS	8.17	126.16	201.98

* Means followed by a common letter are not significantly different

higher yields of grain and straw than untreated control. Highest grain yield was observed in plots of neem cake with Carbendazim with or without biocontrol agent. Highest straw yield was noticed in plots amended with neem cake alone or in combination with Carbendazim with or without biocontrol agent (Table 18).

4.5.6 Economics of cultivation

Economic feasibility of different treatments calculated from cost of cultivation and returns from output on per hectare basis is presented (Table 19). Cost of production excluding treatment was found to be Rs.6500/- per hectare.

Neem cake with Carbendazim biocontrol agent combination incurred maximum cost of cultivation of Rs.10,281/- per hectare. Lime with Carbendazim biocontrol agent combination ranked second (Rs.9,840/- /hectare). Minimum cost of cultivation occurred in lime alone which was followed by Carbendazim alone or biocontrol alone barring absolute control.

Considering price per kg of grain as three rupees and that of straw as one rupee per kg, highest total value outturn was in neem cake with Carbendazim biocontrol agent combination (Rs.19,714.37) followed by neem cake with

Table 19

Economics of amendments, biocontrol agent and fungicide in cultivation of rice crop

Treatments	Cost of production excluding treatments (Rs./ha)	Additional cost of the treatments (Rs./ha)	Total cost of production Y (Rs.)	Grain yield (kg/ha)	Straw yield (kg/ha)	Value X (Rs.)	Net profit (X - Y) Rs.	Average returns per rupee invested (Rate of turn over) ($\frac{X}{Y}$ Rs.)
Neem cake	6500.00	1500.00	8000.00	3787.500	6575.250	16937.75	8937.75	2.12
Neem cake + Carbendazim	6500.00	2656.00	9156.00	4275.000	6559.000	19484.00	9528.00	2.13
Neem cake + biocontrol agent	6500.00	2625.00	9125.00	3775.000	5700.000	17025.00	7900.00	1.87
Neem cake + Carbendazim + biocontrol agent	6500.00	3781.00	10281.00	4350.000	6664.370	19714.37	9433.37	1.92
Lime	6500.00	1068.00	7568.00	3854.500	6074.300	17637.80	10069.00	2.33
Lime + Carbendazim	6500.00	2213.00	8713.00	4064.000	6250.000	18442.00	9729.00	2.12
Lime + biocontrol agent	6500.00	2193.00	8693.00	3954.500	6132.000	17995.50	9302.50	2.07
Lime + Carbendazim + biocontrol agent	6500.00	3349.00	9849.00	4223.750	6337.250	19008.50	9159.50	1.93
Carbendazim	6500.00	1156.00	7656.00	3990.500	5632.500	17604.00	9948.00	2.30
Biocontrol agent	6500.00	1125.00	7625.00	3590.500	5632.500	16404.00	8779.00	2.15
Carbendazim + biocontrol agent	6500.00	2281.00	8781.00	4166.200	5625.600	18330.20	9549.20	2.09
Control	6500.00	-	6500.00	3057.500	5200.00	14372.50	7872.50	2.21

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Carbendazim (Rs.19,484/-) and lime with Carbendazim biocontrol agent combination (Rs.19,008.50).

Net profit of Rs.10,069/- per hectare was obtained in plots of lime alone. This was followed by Carbendazim alone (Rs.9,948.00). Next in the order of merit was lime Carbendazim combination (Rs.9,729/-) and neem cake Carbendazim combination (Rs.9,528/-). In absolute control it was only Rs.7,872.50 per hectare.

Calculation on average returns per rupee invested or rate of turnover showed that lime alone was having highest value of 2.33. This was followed by Carbendazim alone with 2.30. However, in other treatments the average returns per rupee invested was lower than that of untreated control (2.21).

DISCUSSION

DISCUSSION

Sheath blight disease of rice caused by Rhizoctonia solani Kuhn (Thanatephorus cucumeris (Frank) Donk), occurs throughout the temperate and tropical rice growing areas of the world. Intensive rice cultivation with modern rice varieties and higher inputs has resulted in severe incidence of the disease. Development of an effective management strategy against this disastrous disease is of utmost importance in order to keep pace in rice production with the ever increasing human population.

For an effective management of any plant disease a knowledge of its inoculum potential is an essential prelude. Garret (1960) regarded inoculum potential as a form of potential energy and as the product of the quantity of inoculum present (the intensity factor) and the capacity of the environment to produce disease in a host of given susceptibility (the capacity factor). Various measurements have been used by different workers for measuring inoculum potential. Papavizas and Davey (1959) showed that frequency of isolation of Rhizoctonia solani from buck wheat stem pieces used as a bait in soil increased with higher inoculum densities.

A positive correlation between sclerotial number of R. solani and sheath blight severity has been reported

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(Lee, 1979; Ou, 1985). Belmar and associates (1987) suggested that an improvement is required for estimating the inoculum potential of R. solani in soil, taking into consideration its economic importance. Under the present investigation, laboratory studies were carried out to improve selective methods and media which were employed for the estimation of inoculum potential of R. solani in soil.

Methods for estimating the sclerotial distribution in the field were investigated by Yamaguchi and associates (1971) and Hori and Anraku (1971). These workers suggested that the scoop method (scooping up of floating sclerotia in a given area by a net of 15 x 15 cm and 30 cm in depth) was extremely simple and the results were closely related with disease severity. Ui and associates (1976) developed a sieving floatation technique for determining sclerotial population in soil, using 2 per cent aqueous solution of H_2O_2. The advantage of this method was that the sclerotia did not loose their infectivity. Weinhold (1977) described wet sieving procedure to assay soils for R. solani. Elutriation procedures for quantitative assay of soil for viable propagules of R. solani has described by Clark and his associates (1978) which was further characterised by Belmar et al. (1987).

Sclerotia of R. solani causing sheath blight disease have been quantitatively recovered from soils with wet sieving (Lee, 1980) and hydrogen peroxide floatation method (Ui et al., 1976). The above workers have suggested that the sclerotia of this fungus would appear well suited to extraction from soil by floatation sieving because they are large (about 1 to 2 mm), discrete and buoyant. In the present study involving comparison of floatation sieving technique using Fenwick can and glass cylinder, it is seen that both are effective, eventhough sclerotial recovery was slightly more in Fenwick can method. However, glass cylinder technique is simpler, easier and hence seems to be more acceptable. Modification of glass cylinder sieving using common salt indicated that 2 per cent salt concentration is enough to ensure 97 per cent recovery of sclerotia from soil.

As R. solani is a soil inhabitant, selective counting of its propagules is vital for an understanding of its biological behaviour in soil. Several attempts have been made in the past to develop an efficient selective medium for the recovery of its propagules (Davey and Papavizas, 1962; Martinson, 1963; Ko and Hora, 1971). The selective medium for isolation of R. solani propagules from soil or plant tissues devised by Ko and Hora (1971) has been extensively in use. Subsequently some modification of this

medium for better enumeration of R. solani from soil has also been suggested (Flowers, 1976; Ferriss and Mitchel, 1976). The study of comparison of selective media undertaken in the present investigation indicates the superiority of fosetyl-al in amending mineral antibiotic medium compared to metalaxyl in selective isolation of R. solani from soil. Gangopadhayay and Grover (1985) noticed better recovery of propagules when mineral antibiotic medium was amended with fosetyl-al.

A comparison of plating and pelleting of soil done in the present investigation revealed that soil pelleting was better. This is in line with observations made by Van Bruggen and Arneson (1986).

A comparison of baits and effect of sterilization showed that autoclaving increased percentage of colonisation and among autoclaved baits paddy straw bits were found to be superior to stem bits. This may be attributed to the increased saprophytic colonization of stem bits from assaying soil medium. Sneh and associates (1966) observed that the extent of colonization of R. solani was influenced by autoclaving, which was positive or negative under different situations.

A knowledge on the biological condition of the pathogen in soil will decide the inoculum potential in a

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given time as the various saprophytic flora in soil, especially the rhizosphere and rhizoplane decide it. *In vitro* studies during the present investigation revealed that the saprophytic fungi varied in their extent of suppression of *R. solani*.

Among overgrowing fungi *Rhizopus oryzae* had maximum smothering effect. Next in the order of merit was *Trichoderma* spp. This was followed by *Aspergillus niger* and *Penicillium pinophilum*. *A. terreus*, *A. flavus*, *A. aculeatus* and *Talaromyces stipitatus* were slow growing and have overgrown *Rhizoctonia solani* only late, indicating their weak antagonism. *Chaetomium globosum* intermingled freely with *R. solani* showing its lack of antagonism. This result was contradictory to findings of Das (1986) and Gokulapalan (1989). *P. roseopurpurem* and *Mucor* sp. exhibited cessation at the point of contact with *R. solani* indicating their strong antagonistic interaction. The strongest antagonism was exerted by *P. oxalicum* as on the fourth day of inoculation, there was aversion and the inhibition zone was 8 mm in diameter.

Attempts to control rice sheath blight by employing antagonistic micro-organisms were promising even in the sixties, after the studies of Orgura and Akai (1965) and Hashioka and Fukita (1969) utilizing species of *Trichoderma*,

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Gliocladium and Acremonium. In vitro studies by Gokulapalan (1981) revealed that A. niger and T. viride were the most effective antagonistic fungi inhibiting the linear growth of R. solani. Padmakumary (1989) and Gokulapalan (1989) enumerated several fungi antagonistic to R. solani. The above included A. niger, A. aculeatus, C. globosum, M. hemilis, P. oxalicum, Rhizopus stolonifer, T. harzianum and T. viride. Except in C. globosum similar interactions were observed in this study also. However, the performance of the above fungi has to be evaluated in vivo also for conclusive results.

The biological control of R. solani using various microorganisms has been reported by many workers from all over the world (Weindling, 1932; 1934; Harder et al., 1979; Chet and Elad, 1982; Mew and Rosales, 1984; Lewis and Papavizas, 1987). Pot trials in the present investigation is in support of the earlier finding that T. harzianum and T. koningii are quite effective to be directly used as biocontrol agents against R. solani. Effectiveness of T. harzianum in biological control of R. solani was emphasised by many workers (Elad et al., 1980; Venkatasubbaiah et al., 1984; Strashnove et al., 1985; Alagarsamy et al., 1987).

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Murugesan (1990) observed that immediately after the contact of hyphae of Trichoderma sp. with Rhizoctonia, the cells separated and the cell wall started to disintegrate. The mycoparasite overgrew on the host mycelium and produced thick mat of mycelium and patches of green conidia especially on the host hyphae and finally it digested the entire host mycelium. Rajan and Alexander (1990) identified T. viride as a successful biocontrol agent in rice sheath blight control and recommended the application of T. viride to suppress the population of R. solani in soil either in combination or in sequence with systemic fungicide Carbendazim for better cost : benefit ratio. Mariappan and Mohan Dass(1990) suggested that among several mycoparasites, Trichoderma spp. are extremely useful in controlling soil-borne pathogen including R. solani. Panicker and Jeyarajan (1990) observed increased population of T. viride and reduced black gram root rot due to R. solani by amending soil with neem leaf @ 2 per cent.

Field experiment conducted in the present investigation clearly demonstrated the efficacy of Trichoderma spp. especially in combination with organic and inorganic soil amendments as well as with the systemic fungicide Carbendazim. Roy (1977) observed that when cultures of T. viride were incorporated into sterilized soil along with R. solani,

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sheath blight infection was reduced slightly but when T. viride spores were sprayed on aerial parts of the plant before inoculation with R. solani, the disease was not checked.

In the field experiment of the present investigation, effect of soil amendments was quite pronounced in early crop stages viz., tillering and stem elongation as evidenced by substantial reduction in disease incidence and intensity. It is persumable that the amendments prevented infection of the pathogen by reducing its survival ability. Rajan and Menon (1975) tried several industrial and agricultural waste materials successfully against the sheath blight pathogen. They suggested that the materials, in general, reduced the intensity of disease by influencing the survival ability of the pathogen. Rajan (1980) opined that non-edible cakes, sawdust and rice husk are equally effective in suppressing sheath blight disease. Dath (1979) suggested that survival period and viability of sclerotia were reduced by incorporation of green manures especially Sesbania aculeata. Kannaiyan and Prasad (1981b) noticed reduction in seedling infection by rice chaff, neem cake, sawdust or cattle manure. George and associates (1984) obtained excellent field control of sheath blight with amendment such as rice husk or neem cake. Padmakumary and Balakrishnan (1987)

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observed reduced saprophytic activity of R. solani in soil amended with punna cake, neem cake, rice husk, saw dust, fish waste or groundnut shell. The superiority of neem cake as a soil amendment in successful biocontrol of R. solani has been emphasized by Rajan and Alexander (1988a) and Kannaiyan (1990).

Amending soil with lime has given the most economic management of sheath blight in the present investigation. Lime has also been found to be cohesive with biocontrol agent T. harzianum as well as systemic fungicide Carbendazim. Balasubramanian and Shanmugam (1986) established the inverse relationship between tissue calcium content of black gram, prior to inoculation and leaf blight intensity due to R. solani indicating the basis for reduced disease incidence, through the application of lime or gypsum. The reduction in disease is possibly due to the formation of calcium pectate, a structure resistant to invasion by the pathogen. Williamson and Dyce (1989) observed reduced inoculum density of Plasmodiophora brassicae, the causal organism of club root of crucifers by the application of calcium cyanamide. Alexander (1987) and Rajan and Alexander (1988b) noticed significant reduction in sheath blight disease of rice by soil application of lime or gypsum.

The present investigation revealed the efficacy of systemic fungicide Carbendazim in combination with lime or neem cake as soil amendments as well as with a biocontrol agent T. harzianum, in sheath blight management. Field experiments conducted by Bhaktavatsalam and associates (1977) revealed that Bavistin was the most effective chemical against the disease. Jaganathan and Kannaiyan (1978) opined that three sprays at ten day interval during maximum tillering stage with Bavistin provided good protection against sheath blight. Reddy and associates (1981) observed significant reduction in disease and increased yield due to application of systemic fungicides. Viswanathan and Mariappan (1980) observed that Carbendazim as a prophylactic or therapeutic spray effectively controlled the disease.

Plant pathologists have now realised that management is much more desirable than control of any plant disease as it is well known that absolute removal of entire propagules of a pathogen from an environment is neither feasible nor appreciable. In disease management system, the excessive multiplication of a pathogen, causing considerable economic loss, has been prevented. The various methods of plant disease management includes cultural, genetical, chemical and biological. Each of the above has its own advantages

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and disadvantages. Hence, recent approach adopted is integrating the various techniques and the same is designated as integrated disease management.

Several authors in the past have indicated the possible success of integrated disease management in sheath blight disease of rice (Kannaiyan, 1980; Lee and Rush, 1983; Belmar et al., 1987). Rajan and Alexander (1988b) successfully controlled the disease by combination of soil amendment with glyricidia leaves/neem cake with a biocontrol agent T. viride or with a systemic fungicide Carbendazim. In the present investigation amendments, neem cake as well as lime in combination with biocontrol agent T. harzianum and systemic fungicide Carbendazim effectively reduced both intensity as well as incidence of sheath blight disease. Eventhough neem cake combinations gave maximum disease suppression, lime alone as an amendment was the most economic treatment.

In the present investigation, the disease incidence was, in general quite low initially as the same was about 8 per cent in tillering, 13 per cent during stem elongation and 33 per cent at booting. The disease intensity was also quite low as the score was less than three even during flowering. In later stages also, the score reached only about four. The low incidence and intensity of the disease

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might have been the reason for the observation that lime treatment gave the most economic management. It is seen that neem cake in combination with T. harzianum and Carbendazim had least disease incidence and pathogen population. Hence, in situations favouring severe disease development the above treatment combination is likely to offer economically higher yields of grain and straw. Arunyanart and his associates (1984) suggested that the yield losses due to infection at 3, 5, 7 disease scores in 0-9 scale of Standard Evaluation System (IRRI, 1976) were 15, 22, 28 and 40 per cent. Certain pockets in rice fields of Kuttanad, Onattukara and Kole lands of Trissur, Kerala State, wherein sheath blight occurs continuously there is excessive build up of the inoculum in soil. In these "hot spots" integrated management involving cultural, biological and chemical methods have been found to be economic (Rajan, 1983).

Population of sclerotia remained without change among different treatments in the earlier stages. This may be due to the fact that sclerotia formed in the earlier rice crop of the field had remained in soil and were recovered in floatation technique. The causal fungus is able to survive in soil and over winter as sclerotia (Endo, 1931; Nakata and Kawamura, 1939) and these soil-borne sclerotia left in the field from the previous crop

is the main source of survival of the pathogen (Kozaka, 1970; Hori and Anraku, 1971; Yamaguchi et al., 1971). In the later stages of booting and mature grain, sclerotia formed on the host crop varied with treatments as in neem cake/lime combinations with Carbendazim or biocontrol agent, the number of sclerotia was rather low.

Sclerotia of R. solani had poor saprophytic ability in the presence of other soil-inhabiting microorganisms. Sclerotial activity was also influenced by the number of microorganisms present in their surrounding or adhering on their surface (Roy, 1985). Arati Swain (1981) opined that the number of antagonistic microorganisms in soil coupled with the physio-chemical properties of the soil seemed to influence the viability of sclerotia in different soil types.

Some fungi, particularly Trichoderma lignorum (Kohli, 1967), T. viride (Roy, 1977, 1980; Gokulapalan and Nair, 1984), as many as 13 species of Trichoderma (Nagmani and Mew, 1987), Aspergillus niger (Gokulapalan and Nair, 1984), A. terreus (Roy, 1984), Penicillium ehrlichii, Fusarium solani and Pseudeurotium multisporum (Roy, 1985) were found to have antagonistic effect against the growth and sclerotial formation of R. solani in vitro. Mycoparasitic activity of T. brachiatum, T. polysporum

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(Hashioka and Fukita, 1969) and T. harzianum (Roy and Sayre, 1984) on R. solani has also been recorded.

Population of total fungi at planting stage was higher in lime amended plots compared to neem cake amended plots, though neem cake amended plots had higher number than untreated control. The observed difference is possibly due to changes in soil reaction favouring the growth and development of fungi. Similar results were observed by Alexander (1987). In stem elongation stage both neem cake and lime amended plots maintained higher fungal population. Increased population of soil fungi corresponding to organic amendment application has been observed by several workers in the past (George, 1981; Alexander, 1987; Padmakumary, 1989). The results indicate that systemic fungicide Carbendazim can be successfully used in the management without the fungal population being detrimentally influenced. The fungicide does not possess a broad spectrum of bio-activity sparing many of the antagonistic microorganisms. However, this aspect requires a closer probe and the position would become clear by plating species of Trichoderma, Aspergillus, Penicillium and other antagonists in selective media for each of them. Krishnamohan and Kandasamy (1986), Padmakumari and Balakrishnan (1987) and Rajan and Alexander (1988b) have observed increased population of bacteria and

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actinomycetes in rice plots amended with organic materials. Reduction in sheath blight intensity has also been observed in amended plots. Among them, species of Bacillus and Streptomyces were predominant. However, no remarkable progress in the population of actinomycetes was observed in the present investigation. Hence, the biological activity of the soil may be attributed to the various fungal species identified and bacterial colonies developed in plates. However, no qualitative study of the bacterial colonies appearing in plates has been taken up. This is perhaps an important aspect of study which deserves immediate attention of rice pathologists.

R. solani is an ubequitous pathogen responsible for causing various maladies in plants. It has a remarkable capacity for saprophytic survival in soil or in crop residues in the absence of host plants (Roy, 1989). The teeming microbes present in soil, each occupying its own niche, co-exist in a dynamic equilibrium. The crux of biological control is to learn how biological equilibrium can be upset to the detriment of the pathogen. The above disturbance can be brought about by different techniques. Gangopadhyay and Chakrabarti (1982) suggested that reports of chemical control of sheath blight are so contradictory that at present no blanket recommendation can be made.

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They have emphasised the necessity to understand the biology of the sclerotia in soil and in hosts so that by manipulation of cultural and agronomic practices, the sclerotial population in the soil can be kept within an acceptable fungistatic condition, if not completely inactivated or destroyed. It is well known that development of resistant cultivars is the best solution to control such diseases but for sheath blight resistant doners are yet to be identified.

It is well known that the biological equilibrium can be modified by addition of organic matter which creates new niches for which both the pathogen and the antagonists compete. Moreover, the addition of already decomposed amendment can introduce new biota into the pathosystem. Finally, the decomposition of organic matter can result in toxic transformation products. In some system, the addition of amendment could, by stimulating the saprophytic microbial population, diminish the availability of root exudates to pathogen propagules and exclude the suppression of fungistasis. The pathogen would then be submitted to the process of starvation lysis (Papavizas et al., 1970). Henis and his associates (1967) observed a correlation between antagonists active in vitro against R. solani and the protection effects of amendments. Sneh et al. (1972) concluded that the suppression effect was due to either antibiosis or competition or both. The results of the

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present investigation endorsed the above findings. Significant negative correlation coefficient between population of total fungi and R. solani in the stem elongation and booting stages and those of bacteria with the pathogen in the stem elongation stage suggests that stimulated fungal and bacterial populations caused the reduction of pathogen in early and late crop stages, respectiyely. Broadbent and Baker (1974) observed that number of bacterial and actinomycetes propagules were higher in a "suppressive" than conducive soils. This correlation suggests the role of antagonists in biological control and advocates their use to eliminate pathogen in soil.

The use of selected antagonists is a direct method based on the theory that these organisms when introduced into soil can act directly on the behaviour of the pathogen. Baker and Cook (1974) considered that soil bacteria are extremely important in biological control because bacteria constitute the most numerous group of organisms of the soil. Among bacterial genera, Bacillus sp., Pseudomonas sp. and Azotobactor sp. are important as antagonists. Encouragement of soil bacterial flora, especially in the late crop stages, has been observed in the present study. The bacterial population was found to be responsible for the reduction in pathogen population, at least in part, as

evidenced by significant negative correlation between the two. Broadbent and his associates (1971) and Merriman et al., (1974) suggested that actinomycetes like Streptomyces sp. are very effective in controlling pathogens such as R. solani, despite their slow growth and low competitiveness. Their efficiency in biological control is probably based on the ability to produce antibiosis. However, actinomycetes did not show much prominence in the present investigation. Encouraged saprophytic fungal and bacterial populations have caused a substantial reduction in the population density of the pathogen in plots amended with neem cake/lime along with T. harzianum and/or Carbendazim. This suggests that the disease suppression observed in plots of encouraged saprophytic microbial population was through direct/indirect action of the saprophytes on the pathogen. Studies on the correlation between populations of pathogen with different saprophytes indicated that significant negative correlations existed between total fungi and pathogen density as well as between bacteria and pathogen density. Efforts were made in the past to explain the mechanism of biological control by assaying the inoculum density as a function of time. Rajan and Singh (1972) suggested that organic amendments reduced soft rot of ginger caused by Pythium aphanidermatum through direct action of total fungi in the early period followed

by action of bacteria in the later period. Jayaprakash (1977) and Rajan and Jayaprakash (1978) observed that combination of soil amendments with systemic bactericide resulted in increased saprophytic fungal population with a consequent reduction in the population of the pathogen, Pseudomonas solanacearum. Rajan and Alexander (1988b) obtained significant negative correlation between saprophytic fungal and bacterial populations with the population of R. solani, the rice sheath blight pathogen.

Gilbert and his associates (1968) suggested that during early decomposition of organic materials, the breakdown of fungistasis results in increase in the number of propagules of the pathogen in soil together with increase in the saprophytic flora. However, soon the activity of the enhanced microflora reduces the population of pathogen through various antagonistic and/or competition effects.

In the field trial, different treatments had no influence on height of the plant or on tiller production suggesting that increased yields of grain and straw are the result of disease suppression. However, increased plant height in certain floricultural crops has been reported by application of T. harzianum (Chang et al., 1986). Windham et al. (1986) observing enhanced plant growth in plots of T. harzianum suggested increased seed

germination and dry weight of shoots of stem due to production of growth promoting substance by the pathogen.

The present trial revealed that significant increase in yield and growth attributes has occurred in plots treated with neem cake/lime with T. harzianum and Carbendazim, eventhough plant height and tiller count did not vary. There was significant increase in grain per panicle and yield of grain and straw in plots of combination of amendment, biocontrol agent and fungicide.

An economic evaluation of different treatments revealed that eventhough neem cake in combination of T. harzianum and Carbendazim was the best treatment for encouraging microbial population, reduced pathogen population and incidence and intensity of sheath blight as well as highest yields of grain and straw, the same was not economically viable. The benefit-cost ratio was far less satisfactory than plots receiving lime alone. The obvious reason for the above might be due to the general poor disease pressure. Even in untreated control, the disease pressure was quite low till flowering stage. Beyond flowering stage also development of the disease was only to a medium level. As the disease pressure was low, the loss in yields of grain and straw due to sheath blight was not compensated the costly input due to neem cake

and systemic fungicide Carbendazim. Nevertheless, the observed result revealed that combination of neem cake with T. harzianum and Carbendazim reduced the incidence and intensity of sheath blight substantially through encouragement of saprophytic flora and consequent reduction in the inoculum potential of the pathogen which will be extremely useful in sheath blight endemic areas.

SUMMARY

SUMMARY

Sheath blight disease caused by Rhizoctonia solani Kuhn is one of the important diseases of rice causing considerable economic loss to rice cultivators. Chemical control alone will not be an adequate answer to the problem. The present investigation has been aimed to evolve an economically feasible management strategy integrating biological and chemical aspects of control measures to eradicate this disease. In biological control aspect, potential biocontrol agent suited to field conditions was identified by isolating various antagonistic fungi from the experimental site and screening them against sheath blight pathogen R. solani in vivo and pot culture conditions. Another important aspect of the study is the development of an easy and cheap method to assay the population dynamics of R. solani in paddy soil.

Methods for assaying the R. solani population were floatation technique, selective medium and baiting technique. In the floatation technique both Fenwick can and glass cylinder floatation sieving were effective. But, modified glass cylinder method using two per cent salt solution was found to be cheap, simple and easily accessible. Among the selective media, mineral antibiotic medium amended with fosetyl-al was superior to MA media amended with

metalaxyl. A comparison of baits and effect of sterilization showed that autoclaving increased percentage colonisation and among autoclaved baits paddy straw bits were found to be superior than stem bits.

Several soil fungi isolated from the paddy soil were found to exhibit in vitro antagonism towards R. solani. These included Aspergillus aculeatus, A. flavus, A. niger, A. terreus, Chaetomium globosum, Mucor sp. Penicillium roseopurpureum, P. oxalicum, P. pinophilum, Rhizopus oryzae, Talaromyces stipitatus, Trichoderma harzianum and T. koningii. Among overgrowing fungi Rhizopus oryzae, Trichoderma spp. A. niger and P. pinophilum were superior. P. roseopurpureum and Mucor sp. exhibited cessation of growth at the point of contact and P. oxalicum showed aversion.

When the efficacy of these soil antagonists were screened against sheath blight disease in pot culture studies, T. harzianum showed least disease intensity followed by T. koningii. In another pot culture study, when addition of antagonists was done in both conditions viz., one week before the addition of R. solani as well as one week after the addition of R. solani also, T. harzianum proved its significant disease controlling ability compared to other fungi.

When field trials were conducted incorporating various soil amendments (neem cake and lime), systemic fungicide (Carbendazim) and biocontrol agent (T. harzianum), a combination of amendments with Carbendazim and T. harzianum reduced disease severity and enhanced the yields of grain and straw. Neem cake was better than lime for both disease suppression and yield increase.

When population of soil saprophytes and R. solani were estimated at different periods of crop growth, the population varied according to treatments and stage of the crop. Population of R. solani was estimated at different stages by soil pelleting on selective media (MA medium amended with fenaminosulf). During initial stages amendments had suppressing effect on R. solani, but in later stages treatments with biocontrol agent had least population. Population of sclerotia of R. solani was also estimated by glass cylinder floatation method. Eventhough treatment effect on population of sclerotia was insignificant in earlier stages, amendments with all combinations of Carbendazim and biocontrol agent were the best treatments in later stages.

Population of soil saprophytes viz., fungi, bacteria and actinomycetes were estimated by dilution plating in respective selective media. Higher fungal and bacterial populations were observed in amended plots in initial stages.

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In later stages amendments in combination with Carbendazim and biocontrol agent had higher populations of fungi and bacteria. Population of actinomycetes did not show any variation among treatments. Another important aspect of this study was the significance of negative correlation between soil microorganisms and R. solani population in soil, especially between total fungi/bacteria and R. solani at stem elongation stage.

Eventhough neem cake in combination with T. harzianum and Carbendazim was the best treatment, the same was less superior than lime along treatment from the economic point of view. This may be due to poor disease pressure. However, it is presumable that in situations favouring severe disease incidence/intensity, combination of systemic fungicide Carbendazim and biocontrol agent T. harzianum with soil amendment neem cake is likely to offer economically higher yield of grain and straw.

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*Originals not seen

APPENDICES

APPENDICES

Appendix I. Composition of media

Potato-dextrose agar (Riker and Riker, 1936)

Potato	-	200.0 g
Dextrose	-	20.0 g
Agar	-	20.0 g
Distilled water	-	1000.0 ml
pH	-	6.8 - 7.0

Potatoes were cleaned and sliced (not peeled). Weighed out 200 g and boiled until it was soft. Extract was taken, agar was added and boiled. Dextrose was also added and made up the volume to one litre.

Peptone-dextrose-rosebengal agar (Martin, 1950)

Dextrose	-	10.0 g
Peptone	-	5.0 g
$KH_2\ Po_4$	-	1.0 g
$Mg\ So_4\ 7H_2O$	-	0.5 g
Rosebengal (10%)	-	3.3 ml
Streptomycin	-	80.0 mg
Agar	-	20.0 g
Distilled water	-	1000.0 ml

Streptomycin was added after autoclaving when it was cooled to 44°C.

Soil extract agar (Allen, 1957)

Glucose	-	1.0 g
K_2HPo_4	-	0.5 g

Soil extract	-	100.0 ml
Agar	-	15.0 g
Water	-	900.0 ml
pH	-	6.8 - 7.0

Soil extract was prepared by autoclaving 1 kg of soil with one litre of water, cooled and filtered.

Kenknights and Munaier's medium

Dextrose	-	1.0 g
$KH_2 Po_4$	-	0.1 g
$Na No_3$	-	0.1 g
KCl	-	0.1 g
$MgSo_4 7H_2o$	-	0.1 g
Agar	-	15.0 g
Distilled water	-	1000.0 ml

Mineral-antibiotic medium amended with Fosetyl-al (Gangopadhyay and Grover, 1985)

$K_2H Po_4$	-	1 g	
$Mg So_4 \cdot 7H_2O$	-	0.5 g	
KCl	-	0.5 g	
$FeSo_4$	-	10.0 mg	
Fosetyl-al	-	250.0 g/ml	Added after autoclaving
Chloramphinicol	-	50.0 mg	
Streptomycin sulphate	-	50.0 mg	
Gallic acid	-	0.4 g	
Agar	-	20.0 g	
Distilled water	-	1000.0 ml	

Mineral-antibiotic medium amended with Metalaxyl

$K_2H\ Po_4$	-	1 g	
$MgSo_4.7H_2O$	-	0.5 g	
KCl	-	0.5 g	
$FeSo_4$	-	10.0 mg	
$NaNo_3$	-	0.2 g	
Metalaxyl	-	250.0 g/ml	Added after autoclaving
Chloramphinicol	-	50.0 mg	
Streptomycin sulphate	-	50.0 mg	
Gallic acid	-	0.4 g	
Agar	-	20.0 g	
Distilled water	-	1000.0 ml	

Appendix II. Abstract of Anovas (M.S.S. values)

Abstract of Anova for Table 1

Source	df	M.S.S.
Treatments	7	88.551
A	1	69.433
B	3	148.232
AB	3	35.233
Error	24	142.041

Abstract of Anova for Table 2

Source	df	M.S.S.
Treatments	5	1322.953
Error	18	927.754

Abstract of Anova for Table 3 and 4

Source	df	M.S.	
		Table 3	Table 4
Treatments	15	475.082	470.111
A	1	319.375	0.301
B	1	4135.827	1000.623
C	3	161.132	1557.737
AB	1	242.177	288.069
BC	3	58.577	177.247
AC	3	341.922	95.993
ABC	3	247.992	89.941
Error	48	34.423	10.412

Abstract of Anova for Table 6

Source	df	Tiller-ing	Stem elonga-tion	Booting	Filling
Treat-ments	5	0.341	0.213	0.235	0.245
Error	18	0.044	0.037	0.035	0.040

Abstract of Anova for Table 7

Source	df	Intensity			Incidence		
		Tiller-ing	Stem elon-ga-tion	Boot-ing	Tiller-ing	Stem elon-ga-tion	Boot-ing
Treat-ments	4	0.235	0.241	0.201	232.441	86.462	94.892
Error	15	0.034	0.030	0.016	82.943	10.732	12.823

Abstract of Anova for Table 8

Source	df	Intensity			Incidence		
		Tillering	Stem elongation	Booting	Tillering	Stem elongation	Booting
Treatments	4	0.246	0.358	0.320	253.657	108.696	150.925
Error	15	0.036	0.162	0.006	74.435	14.528	12.353

Abstract of Anova for Table 9

Source	df	Intensity of sheath blight disease					
		Tillering	Stem elongation	Booting	Flowering	Filling	Mature grain
Replication	3	0.000	0.004	0.014	0.022	0.020	0.003
Treatments	11	0.018	0.031	0.114	0.158	0.167	0.166
Error	33	0.004	0.009	0.024	0.028	0.032	0.033

Abstract of Anova for Table 10

Source	df	Incidence of sheath blight disease					
		Tillering	Stem elongation	Booting	Flowering	Filling	Mature grain
Replication	3	58.891	0.170	13.974	16.557	13.536	57.937
Treatments	11	134.298	67.596	137.787	201.643	196.028	190.138
Error	33	31.410	16.691	45.813	20.776	26.395	35.436

Abstract of Anova for Table 11

Source	df	Population of *R. solani* propagules				
		Before addition of amendments	Planting	Stem elongation	Booting	Mature grain
Treatments	11	0.178	0.384	0.378	0.393	0.268
Error	24	0.134	0.2036	0.123	0.211	0.108

Abstract of Anova for Table 12

Source	df	Population of Sclerotia of *R. solani*				
		Before addition of amendments	Planting	Stem elongation	Booting	Mature grain
Treatments	11	0.013	0.037	0.022	0.108	0.109
Error	24	0.023	0.023	0.042	0.035	0.043

Abstract of Anova for Table 13

Source	df	Population of total fungi				
		Before addition of amendments	Planting	Stem elongation	Booting	Mature grain
Treatments	11	0.234	0.130	0.284	0.212	0.511
Error	24	0.113	0.058	0.108	0.080	0.079

Abstract of Anova for Table 14

Source	df	Population of bacteria				
		Before addition of amendments	Planting	Stem elongation	Booting	Mature grain
Treatments	11	0.553	0.447	0.495	0.661	0.410
Error	24	0.223	0.039	0.130	0.131	0.119

Abstract of Anova for Table 15

Source	df	Population of actinomycetes				
		Before addition of amendments	Planting	Stem elongation	Booting	Mature grain
Treatments	11	0.073	0.126	0.098	0.064	0.048
Error	24	0.067	0.109	0.057	0.055	0.065

Abstract of Anova for Table 17

Source	df	Nutrient levels							
		pH	N	P_2O_5	K_2O	Calcium	Magnesium	Zinc	Iron
Replication	3	0.374	0.033	0.007	0.212	0.033	0.205	0.053	0.072
Treatments	11	0.394	0.035	0.012	0.238	0.054	0.033	0.076	0.087
Error	33	0.416	0.029	0.005	0.129	0.012	0.212	0.063	0.073

Abstract of Anova for Table 18

Source	df	Plant height	Total number of tillers	Number of productive tillers	Grain/ panicle	Grain yield	Straw yield
Replication	3	71.739	16.854	6.042	16.853	1.100	227.091
Treatments	11	24.869	7.657	2.947	7.657	0.453	94.937
Error	33	35.061	5.187	3.524	5.187	0.235	32.114

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Identification Services

REPORT Nº H566/89/YI2 — 7 August, 1989

YOUR REF Professor K.I. Wilson
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Specimen Number	Herb. IMI Number	IDENTIFICATION
		Report from Dr. P.M. Kirk
15	333481	*Mucor* sp. Close to *Mucor hiemalis* Wehmer sensu lato I have placed this isolate in our culture collection.
6	333472	*Rhizopus oryzae* Went & Prinsen Geerligs A common species throughout the world, particularly in tropical and subtropical countries, where it occurs in soil and on a wide variety of plant parts contaminated with soil. I have discarded this collection.
		Report from Dr. M.A.J. Williams
1	333467	*Trichoderma koningii* Oudem.
8	333474	*Trichoderma harzianum* Rifai
13	333479	*Trichoderma harzianum* Rifai All isolates discarded.
		Report from Dr. Z. Lawrence
4	333470	*Penicillium roseopurpureum* Dierckx
12	333478	*Talaromyces stipitatus* (Thom) C.R. Benjamin Anamorph: *Penicillium emmonsii* Pitt
2	333468	*Aspergillus flavus* Link
5	333471	
7	333473	*Penicillium oxalicum* Currie & Thom
9	333475	
14	333480	*Aspergillus terreus* Thom
10	333476	*Aspergillus aculeatus* Iizuka
11	333477	*Penicillium pinophilum* Hedgcock Cultures discarded.
3	333469	*Chaetomium globosum* Kunze. *C. globosum* is the type species of the genus, and is extremely common throughout temperate and tropical regions, frequently being isolated from soil and plant debris. It is recognizable most easily by its limoniform-ellipsoidal ascospores, which are slightly flattened on one lateral surface, and by its coiled, usually olive green, ascomatal hairs. The best account of the classification of *Chaetomium* is by von Arx, Guarro & Figueras, *Beih. Nova Hedwigia* 84: 162 pp. (1986). Discarded. Report from Dr P.F. Cannon.

INTEGRATED MANAGEMENT OF SHEATH BLIGHT DISEASE OF RICE

BY

GEOGY ZACHARIA

ABSTRACT OF THE THESIS
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DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE
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ABSTRACT

The present investigation was undertaken to evolve an economically feasible management strategy integrating biological and chemical aspects of control measures of sheath blight disease of rice. An attempt has been made to identify a potential biocontrol agent to combat sheath blight disease causing considerable loss to rice cultivation. Another important aspect of the programme was to develop an easy and cheap method of assaying the population of R. solani in paddy soil.

Among different techniques screened, floatation sieving using both Fenwick can and glass cylinder were effective in selective recovery and the latter was further modified using different concentrations of salt. Among selective media mineral antibiotic media amended with Fosetyl-al and in studies using different baits autoclaved straw bits were superior.

Among the microorganisms isolated from rice soils of Thiruvananthapuram, Kerala, thirteen species of fungi were found to be antagonistic to R. solani. Among the antagonists, Trichoderma harzianum was found to be the best biocontrol agent against sheath blight disease. This was followed by T. koningii.

A field study incorporating various soil amendments viz., neem cake and lime, Carbendazim and T. harzianum, a combination of amendments with Carbendazim and T. harzianum reduced disease severity and increased yield of grain and straw. These combinations also showed higher fungal and bacterial population and reduced R. solani population. Negative correlation between total fungi/bacteria and R. solani was observed suggesting inoculum reduction of the pathogen in critical growth stages of the crop. From the economic point of view, lime alone was found to have highest cost-benefit ratio compared to other treatments. The above result is possibly due to poor disease pressure observed during the investigation.