# STRAIN VARIATION IN *Rhizoctonia solani* Kuhn [*Thanatephorus cucumeris* (Frank) Donk] CAUSING SHEATH BLIGHT OF RICE

By

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THESIS

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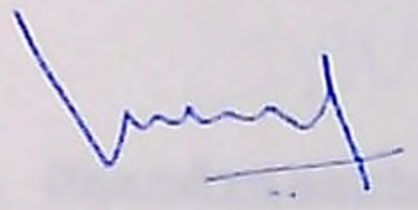
# DECLARATION

I hereby declare that the thesis entitled "Strain variation in Rhizoctonia solani Kühn (Thanatephorus cucumeris (Frank) Donk) causing sheath blight 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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# C O N T E N T S

# LIST OF TABLES

# LIST OF FIGURES

# INTRODUCTION

# INTRODUCTION

Sheath blight of rice is the most serious disease affecting the crop in Kerala. The disease is present endemically in many of the rice growing tracts. Under congenial conditions the disease often attains epiphytotic proportions. This disease was first described from Japan by Miyake (1910) and its occurrence in India was observed as early as in 1918 by Butler.

The causal organism, Rhizoctonia solani Kühn the anamorph of Thanatephorus cucumeris (Frank) Donk is ubiquitous in nature with a wide range of host plants and habitat. The fungus is a facultative saprophyte and it survives in the soil for considerable period of time. The many isolates of R. solani from different sources exhibit differences in pathogenicity, host range, distribution and appearance in culture. Hence, R. solani is often considered as a collective species.

The symposium on Rhizoctonia solani held at Miami, Florida, in 1965, organised by the American Phytopathological Society defined the characteristics of the anamorph and the teleomorph was accepted as Thanatephorus cucumeris (Frank) Donk. Several workers had attempted to group isolates of Rhizoctonia according to various cultural, physiological and pathological criteria. Such groupings were inconsistent due to its diversity in pathogenicity and character. No attempt

has been made so far to study the existence of variants in Rhizoctonia solani, pathogenic to rice, in our State.

The present study is aimed at characterising and grouping the different isolates of Rhizoctonia solani from various host plants and habitat. It is also endeavoured to establish the genetic relationship between these isolates by the Anastomosis group (AG) concept. This scheme was first suggested in Germany by Schultz in 1937 and later developed by Richter and Schneider (1953). According to this scheme hyphal fusion occurs only between isolates of the same anastomosis group indicating that the isolates are related. The significance of AG concept to plant pathologist is that each AG group can be considered an evolutionary unit which is genetically isolated. The inability of anastomosis groups to anastomose each other is a stable character, whereas, morphogenic expression is easily modified by environment. The reason for little progress in breeding for resistance to plant diseases caused by R. solani is attributed to the composite nature of the species which is now being recognised through the AG concept.

Lack of host resistance to Rhizoctonia solani is a limiting factor in the control of sheath blight disease. The genetic diversity of field isolates is responsible for the

differential pathogenic traits. The pathogenic response of common varieties of rice to different isolates of Rhizoctonia solani as an indication of genetic diversity among the isolates is investigated in this study.

The present work was mainly intended to attain an insight into the diverse nature of the disease and the causal organism.

# REVIEW OF LITERATURE

# REVIEW OF LITERATURE

Sheath blight of rice was first described from Japan by Miyake (1910) as a new disease under the name Oriental sheath blight and leaf spot. The causal organism was identified as a new species, Sclerotium irregulare. In India, it was Butler (1918) who mentioned the occurrence of this disease for the first time. Subsequently the pathogen was described as Rhizoctonia solani Kühn from Philippines, Sri Lanka and China (Reinking, 1918; Park & Bertus, 1932; Wei, 1934). The first detailed report on sheath blight disease in India was made by Paracer and Chahal (1963). Since then it has become one of the most destructive diseases in many parts of India including Kerala, Tamil Nadu and Andhra Pradesh.

## The fungus

Sheath blight of rice is caused by Rhizoctonia solani Kühn (Thanatephorus cucumeris (Frank) Donk). Much confusion prevailed regarding the name of the teleomorph of the fungus. It was often referred as Hypochnus sasakii Shirai, Corticium vagum Berk. & Curt., Corticium solani (Prill. & Delacr.) Bourd. & Galz., Pellicularia filamentosa (Pat.) Roger f.sasakii Exner and P. sasakii (Shirai) S. Ito etc., in literature. The fungus is more commonly known by the sclerotial state Rhizoctonia solani Kuhn, since sclerotia are abundantly produced on the affected sheath and lamina of rice leaf and also

in the soil. Rhizoctonia solani was first observed on potato tubers and was first described, named and reported by Julius Kuhn in 1858. The symposium on Rhizoctonia solani held at Miami, Florida in 1965 defined the characteristics of the anamorph (Parmeter & Whitney, 1969) and the teleomorph accepted as Thanatephorus cucumeris (Frank) Donk. They suggested that Rhizoctonia solani is characterised by (1) multinucleate cells in young vegetative hyphae (ii) branching near the distal septum in young vegetative hyphae (iii) a prominent septal pore apparatus (iv) constriction of the branch base (v) formation of a septum in the branch near the point of origin and (vi) some shade of brown pigmentation. Duggar (1915) observed that young hyphal branches were inclined in the direction of growth and constricted at the point of union with the main hyphae. Branching of the young hyphae at right angles but later bending towards the direction of growth was observed by Palo (1926). Frederiksen et al. (1938) measured the sclerotia and they recorded the size as ranging from one mm to several mm in diameter.

Morphological groups

Several workers have attempted to group isolates of Rhizoctonia solani based on morphological, cultural and physiological characters. Elmer (1932) distinguished two groups of Rhizoctonia (Corticium) solani based on the nature

of symptoms produced on potato. Isolates of the first group produced necrotic lesions on potato stem, whereas the second group produced superficial fleck-like lesions on the stem. On artificial media the strains of the second group produced numerous small, white mycelial aggregates of a mealy appearance. Other strains outside these groups were also identified.

Person (1944) differentiated four clear cut groups of Rhizoctonia solani on the basis of pathogenicity to bean in greenhouse. Group 1 comprising of isolates from sugarcane and from sclerotia on potato tubers did not infect beans. Group 2 from peas produced very slight degree of damping-off. Group 3, the rice strain entirely prevented emergence but produced less severe stem lesions than pea isolate. Group 4 isolates from bean, tomato and egg plant were responsible for some reduction in stand with heavy damage to stem.

Sethofer and Jermoljev (1950) identified eight biological forms of Rhizoctonia solani from potato tubers which differed morphologically in culture. Different forms of R. solani attacked different varieties with varying intensity. Kernkamp et al. (1952) concluded that Corticium solani comprises an indefinite number of races differing culturally and pathogenically. Certain races appear to be more specific to certain crops.

Exner (1953) suggested four forms of Pellicularia filamentosa, viz., f.sp. solani causing basal stem and root rots, f.sp. microsclerotia causing Web blight of snap bean, f.sp. sasakii causing sheath spot of grasses and leaf spot of various dicotyledons and f.sp. trinsii causing leaf blight of fig.

Kohli (1966) distinguished three morphological groups of Rhizoctonia solani from rice on Richards' agar. Sadowski (1970) distinguished many strains of Rhizoctonia solani differing in cultural characterisation, ecological requirements and pathogenicity during field and laboratory experiments. In India Raj et al. (1974) grouped isolates of Rhizoctonia solani on potato, which differed in the rate of growth and colour of substrate and density, type and formation of sclerotia. The isolates were grouped into five main and four sub-types based on the pattern of sclerotial formation in culture plates. Based on growth characteristic and pathogenicity Griesbach (1975) assigned isolates of Rhizoctonia solani from 10 differential weeds and potato to a single group whereas isolates from wheat and cowpea were grouped separately.

During an investigation on the cause of poor ratooning in sugarcane in Taiwan, Watanabe and Shiyomi (1975) isolated 121 isolates of Rhizoctonia solani from underground plant parts

of sugarcane plants. The isolates were separated into four different groups based on the frequency of hyphal types.

Ui and Saito (1968) could not group Rhizoctonia solani isolates based on the number of nuclei per cell which ranged from 4 - 8 nuclei per cell.

Anastomosis groups

According to Gregory (1984) hyphal fusion was first recorded by Tulasne and Tulasne during 1861-65 . Ward (1888) was the first to watch the progress of vegetative fusion under the microscope. Major contributions to the understanding of hyphal fusion were made in the works of Buller (1931, 1933), who distinguished three types of fusions, viz. vegetative fusion, sexual fusion and parasitic fusion.

Capacity for hyphal fusion provides an indication of relationship between isolates of Rhizoctonia solani. Matsumoto (1921) tested 15 isolates of Rhizoctonia solani from potato, bean, lettuce, dahlia and egg plant for hyphal fusion. Fusion was found to occur between hyphae arising from different mycelia of the same strain and between hyphae of certain similar strains. Fusion of hyphae occurred more readily between those strains isolated from the same species of host than between isolates from different hosts (Matsumoto, 1923). Capacity for hyphal anastomosis was also observed

by Muller (1924) while studying the homothallic nature of Rhizoctonia solani.

Schultz (1937) made a detailed study on the ecology, morphology and taxonomic relations of a number of strains of the so called propagative fungus (usually referred to as Moniliopsis alderholdi). Both the propagative fungus and Corticium solani were found to be equally pathogenic on tomato and potato so that attempts to maintain the name Moniliopsis alderholdi as distinct from Corticium solani cannot be upheld. Most of the strains under investigation fell under five groups according to the mode of forming hyphal anastomosis. In general members of the same fusion group also agree in respect to morphological characters and temperature requirement. It was concluded that Moniliopsis alderholdi is merely a variety of Corticium solani and as such is not an independent entity. In the case of two of the five groups the teleomorph (Hypochnus) (Corticium) of the fungus developed. The five groups identified based on these results were

I. Rhizoctonia solani K. var. hortensis n.var.

II. Hypochnus solani P.& D. var. brassicae n.var.

III. Hypochnus solani P.& D. var. typica n.var (potato group)

IV. Rhizoctonia solani K. var. cichorii endiviae Thoma

V. Rhizoctonia solani K. var. fuchsiae. n. var.

Hyphal fusion did not occur between Corticium sasakii from rice, C. stevensii from pear twigs, C. koleroga from coffee. Each of these three species could infect Japanese pear and coffee but rice was infected only by rice isolate (Matsumoto & Yamamoto, 1935).

The first systematic grouping of Rhizoctonia solani Kühn based on anastomosis was done by Richter and Schneider (1953). One hundred and seventy six strains from 45 host were grouped into six morphological groups viz., A, B, C, D, E & F based on their capacity of hyphal fusion. The characters which appeared common to a particular group were the width of the hyphae, colour, abundance and texture of the aerial mycelium and production of pseudosclerotium. The 21 isolates from crucifer and 30 from potato fell into two groups (D and F respectively) which anastomosed almost exclusively with members of their own group. They differed markedly from the other four groups in their temperature reaction.

Tu (1968) studied the physiological variations of seven strains of Pellicularia (Corticium) sasakii from rice plants. All these isolates were closely related since hyphal fusion occurred although there was differences in pathogenicity.

Parmeter et al. (1969) concreted the concept of anastomosis grouping of Rhizoctonia solani by elaborating on

the works of Schultz (1937) and Richter and Schneider (1953). Anastomosis among 138 isolates of Thanatephorus cucumeris indicated that most of the isolates fall into one of the four Anastomosis Groups viz., AG 1, AG 2, AG 3 and AG 4 which are genetically isolated and incapable of nuclear exchange due to non-fusion between isolates of the different groups. Anastomosis between field isolates within a group resulted in the death of fused cells and adjacent cells suggesting that cell killing might limit nuclear exchange even among isolates capable of fusion. Variation in host range, infection and pathogenic habit between the groups cautions extension of research data from one group to another. The possibility of existence of additional anastomosis groups and occurrence of genetic recombination within a group leading to new clones capable of anastomosis with members of other groups is discussed by the above workers. It was established that these AG 1, AG 2, AG 3 and AG 4 groups correspond with the groups I, II, III and IV of Schultz (1937) and with Richter and Schneider's (1953) Groups A, D, F and C. It was this work of Parmeter et al. (1969) that initiated world-wide interest in anastomosis grouping of Rhizoctonia solani.

Sherwood (1969) studied the morphology of the various anastomosis groups of Parmeter et al. (1969) and stressed

the verity of using anastomosis grouping rather than using the concept of morphologic - physiologic sub-species of Rhizoctonia solani. The inability of groups to anastomose with one another seems to be a stable character. On the other hand morphologic expression is easily modified by environment. Most isolates of R. solani in AG 1 and AG 4 produced a radial growth of more than 18 mm per day and were moderately to highly virulent. On Potato-dextrose agar medium, AG 1 isolates had prominent subspheroid sclerotia and whitish or reddish brown mycelium; but AG 4 isolates usually had inconspicuous crusty sclerotia and light grey brown mycelium. Isolates of AG 2 and AG 3 groups grew less than 18 mm per day. The two groups could not be distinguished from each other by morphology. The author concludes that AG 1 Type 1 is the same as Pellicularia filamentosa (Pat) Rogers f. sp. microsclerotia (Matz) Exner. AG 1 Type 2 agrees in appearance and growth rate with the fungi described by Matsumoto et al. (1932) and by Exner (1953) as Pellicularia filamentosa f.sp.sasakii (Shirai) Exner.

In Japan, Ogoshi (1972) grouped 214 isolates of Rhizoctonia solani into six groups. Anastomosis between hyphae of the same isolate was perfect fusion while that between isolates of the same group was imperfect fusion. Most isolates in Group AG 1 were from rice, sugarbeet and

soil; those in AG 2 were from soil, flax and Cruciferae; all from AG 3 were from Solanaceae (mainly potato). Most isolates in AG 4 were from Leguminaceae and sugarbeet; in AG 5 the isolates were from soil and AG 6 included isolates from sugarbeet and soils.

Ogoshi (1975) postulated that the AG 5 strain of Japan corresponded with B group of Richter and Schneider (1953).

Ogoshi (1976) in his review on anastomosis groups of Rhizoctonia solani concludes that the anastomosis groups from Japan (Ogoshi, 1972), Germany (Schultz, 1937), (Richter and Schneider, 1953) and USA (Parmeter et al., 1969) are similar. Moreover, AG 1, AG 2 Type 1, AG 2 Type 2, AG 3 and AG 4 corresponded with sasakii web blight type, winter crop type, rush and root rot type, potato type and praticola type respectively of Watanabe and Matsuda (1966). Each anastomosis group except AG 1 developed their teleomorph in the soil.

Tu and Chang (1978) assigned 251 isolates of Rhizoctonia solani from Taiwan into one of five groups (TRAG 1 - TRAG 5) corresponding to groups previously described by Ogoshi (1972) in Japan, Parmeter et al. (1969) in USA and Richter and Schneider (1953) in Europe. The types of fusion observed in each group were perfect fusion and imperfect fusion or contact fusion. No hyphal fusion was observed between isolates of

different groups. Each anastomosis group seems to have specificity in host range, but this specificity was not rigid. TRAG 5 corresponded with AG 5 of Ogoshi (1972) and B group of Richter and Schneider (1953).

Kuninaga et al. (1978) established new anastomosis groups AG 6 and AG-B1. AG 6 was the predominent group in non-cultivated soils in Hokkaido, Japan. From the morphological characters of the teleomorph, isolates of groups AG 6 and AG-B1 were classified as Thanatephorus cucumeris (Kuninaga et al., 1979).

Bolkan (1976) failed to bridge field isolates from each of the four anastomosis groups and their F1, F2 and F3 progenies by pairing in all possible combinations. Hyphal anastomosis occurred only between isolates of the same group. There was no bridging through recombination; but the recent discovery of a group of bridging isolates AG-B1 shows that they anastomose not only with members of their group but also with isolates from AG 2, AG 3 and AG 6 (Kuninaga & Yokosawa, 1985a).

Adams and Butler (1979) compared serologically sixty four isolates of Rhizoctonia solani with the use of antisera developed against one tester strain in each of the six anastomosis groups (AG). Gel diffusion test showed that all

anastomosis groups had several antigens in common. Serological groups corresponded to the anastomosis groups, with the exception of AG 2 Type 1 and AG 2 Type 2 which were serologically indistinguishable.

The base composition of 30 isolates representing eight anastomosis groups compared by Kuninaga and Yokosowa (1980) indicated that each must be regarded as a genetically independent unit.

A collection of 48 isolates of Rhizoctonia solani selected from various anastomosis groups showed atleast nine distinct Zymograms (Matsuyama et al., 1978). Isolates from rice (sasakii type) or its relatives had Zymogram (Zym 1) but other isolates of the same group (AG 1) from other host (Web blight type) showed several other different Zymograms. The Zymogram roughly agreed with anastomosis group but were sometimes more closely related with ecological types.

One hundred and seven isolates of Rhizoctonia solani comprising a single anastomosis group were isolated from seedlings of Japanese radish by Homma et al. (1983). This group did not anastomose with any of the AG 1 to AG 6 and AG-B1 groups. They formed brown or dark brown colonies with aerial hyphae and sclerotia but without clear zonation.

Morphological characteristics of the teleomorph identified the new AG as Thanatephorus cucumeris and was designated as AG 7 of Rhizoctonia solani.

Polyacrylamide gel electrophoresis of soluble proteins extracted from isolates of five anastomosis groups (AG) revealed distinctive protein profile for each AG (Reynolds et al., 1983). It is suggested that this method can be used to help distinguish anastomosis groups of Rhizoctonia solani.

Kuninaga and Yokosawa (1985b) recorded consistently low values (0 - 33.7%) of DNA base sequence homology among isolates of Rhizoctonia solani of different anastomosis groups (AG 1, AG 2 and AG 7), suggesting that each of these anastomosis groups is a genetically isolated group.

Ogoshi and Ui (1983) investigated the presence of clones of Rhizoctonia solani in the field of white potato, sugarbeets, and rice plants. Most of the isolates from potato tuber, soils from sugarbeet fields and rice culms were AG 3, AG 2-2 and AG 1 respectively. Reaction of anastomosed hyphae among the isolates of each group were observed on water agar. The isolates which fused perfectly without death of fused cells (S reaction) were determined to be the same clone and the isolates which showed death of fused cells (K reaction) were

determined to be of distinct clones. In rice plants most AG 1 isolates from several lesions on one culm showed S reaction. K reaction increased between the isolates from neighbouring culms. Fortyone clones were identified by them in a paddy field of 11 acres. The results revealed that there are many clones of the pathogen in one field and that the distribution of one clone is limited within only small areas in the field.

Papavizas et al. (1975) observed that 11 isolates of Rhizoctonia solani from soil were of the AG 4 (Praticola) type while two other soil isolates belonged to AG 3. The isolates ranged from non-pathogenic to highly pathogenic on bean, cotton, lettuce, raddish and sugarbeet.

Isolates of Rhizoctonia solani in AG 2, AG 3 and AG 4 were tested for their ability to cause seedling blight of flax (Anderson, 1977). All isolates caused seed decay but only the AG 4 isolates infected flax hypocotyls. Isolates from spinach were assigned to five anastomosis groups and the most frequent and most pathogenic were AG 4, followed by AG 2 Type 2 (Naiki and Kanoh, 1978). Cedeno (1978) divided isolates from leaves of groundnut and sorghum into AG 1 and AG 4 respectively.

Abe and Tsuboki (1978) assigned 273 isolates from black scurf potato to four anastomosis groups (AG 1, AG 2 Type 2, AG 3 and AG 5) accounting for 0.7, 0.4, 96 and 3 per cent respectively. AG 3 isolates were always obtained from lesions and sclerotia on various plant parts, as well as basidiospores on the base of the stem. AG 5 isolates were obtained from lesion of the stems in the last stage of growth.

Sterne and Jones (1978) tested 18 isolates of Rhizoctonia solani from wheat stem, roots and soil. Fifteen isolates were in AG 4, two in AG 1 and one could not be characterised. Rhizoctonia solani isolates from soybean anastomosed with tester isolate of AG 4 (Cardosa et al., 1978). Herr and Roberts (1980) observed that most of the isolates of Rhizoctonia solani from the soil were of two anastomosis groups AG 2 and AG 4.

A sorghum leaf and sheath blight isolate of Rhizoctonia solani was found to be in AG 1 (O'Neill & Rush, 1982); 15 papaya root isolates were in AG 4 (Yamamoto & Aragaki, 1982); all budding plant isolates were Rhizoctonia solani AG 4 (Stephen et al., 1982); barley stunt strain of Rhizoctonia solani was identified as Thanatephorus cucumeris, AG 3 (Murray, 1981).

Yu (1983) confirmed for the first time a new disease, Brown Sheath Blight of rice in Taiwan caused by Rhizoctonia solani belonging to AG 2, type 2 III B, rush type.

Wasfy et al. (1984) classified 9 isolates of Rhizoctonia solani from various host into 2 Egyptian anastomosis groups. EAG 1 from cotton, and potato and EAG 2 from Hibiscus esculentus, tomato, Capsicum lannuum, tobacco, beans, sour orange and cotton.

Nine of the 50 isolates of Rhizoctonia solani obtained from potato field soils in Maine were found to be members of AG 5 (Bandy et al., 1984).

Abawi and Martin (1985) observed that all isolates of Rhizoctonia solani obtained from cabbage head with foliar blight symptoms belonged to AG 1.

One hundred and thirty isolates of Rhizoctonia solani obtained from different states in Brazil were assigned to the four anastomosis groups of AG 1 (11 isolates), AG 2 (42 isolates) AG 3 (10 isolates) and AG 4 (51 isolates). The groups lacked host specificity although some tendencies were evident (Bolkan & Ribeiro, 1985).

Cultural and Morphological studies

Newton (1931) observed the lethal temperature period for culture of Rhizoctonia (Corticium) solani to be one hour at 50°C. Shorter periods at this temperature caused a lag in the growth rate. No permanent attenuation of vigor occurred

as a consequence of maintaining the culture at temperature near the lethal point.

Microscopic study of 568 cells of stained mycelium of Rhizoctonia solani by Sanford and Skoropad (1955) revealed 3 types of cells. In hyphal tip cells there were 2 to 15 nuclei, in Y type cells 4 to 25 and in unbranched cells 3 to 19 nuclei. Burpee et al. (1978) derived a rapid reliable method of determining nuclear and septal pore character using 0.5% Aniline blue or Trypan blue in lactophenol. HCl Geimsa staining procedure was used by Herr (1979) for staining nuclei of multinucleate and binucleate isolates cultured on thin layer of Difco PDA.

Wide variation was seen in growth rate and colony morphology among Rhizoctonia solani isolates from various grasses and legumes with little specificity in pathogenicity (Luttrell, 1962).

Parmeter et al. (1967) differentiated Rhizoctonia like organism which were binucleate with Ceratobasidium teleomorph from multinucleated Rhizoctonia solani which produced Thanatephorus cucuneris.

Larpent (1965) observed a relationship between growth rate and branching of young mycelium of Rhizoctonia solani. High degree of branching was seen in slow growing cultures.

Growth of the fungus in liquid medium increased to a maximum at 4 to 5 days and then decreased until growth ceased at 8 to 9 days. When the fungus was grown under constant flow condition to replenish nutrients and remove exotoxin, it was found that growth limitation was a function of the entire thallus and was not due to permanent impairment of the peripheral hyphae (Gottlieb, 1971).

Hashiba et al. (1974) observed that Rhizoctonia solani isolates from high temperature regions grew well on PDA at 35°C but tended to grow poorly at 12°C. Isolates from low temperature grew poorly at 35°C and well at 12°C.

Sclerotial formation by Rhizoctonia solani was enhanced in the presence of light than in the absence of light. A sudden fall in temperature also accelerated sclerotial formation (Hemmi and Endo, 1931).

Of the seven culture media used Kohli (1967) observed Richards' medium to be the best for growth which was maximum at 30°C and pH 6.

Ogoshi and Ui (1979) studied the influence of vitamins on the growth of anastomosis groups of Rhizoctonia solani. It was inferred that the need for vitamin is a characteristic of the isolate and not of the group.

Nuclear migration through septa in the direction of growth was observed *in vivo* by Sanford and Skoropad (1955), which is considered as one of the contributory factors to variability of *Corticium solani*. Flentje *et al*. (1967) observed mutation in the crucifer-attacking isolates of *Thanatephorus cucumeris* to occur spontaneously and by UV irradiation.

## Host range of *Rhizoctonia solani*

*Rhizoctonia solani* Kuhn was first reported and described by Kuhn (1853) on potato. Apart from rice the fungus has a wide host range infecting plants belonging to 32 families and 188 genera (Gangopadhyay & Chakrabarti, 1982).

Su (1936) observed a disease caused by *Corticium* sp. in beds of edible straw mushroom (*Volvaria diplasia*) which hampered mushroom production.

Ramakrishnan and Ramakrishnan (1948) observed banded leaf blight of arrowroot caused by *Rhizoctonia solani* in North Malabar.

Strouble (1954) observed *Rhizoctonia* (*Corticium*) *sasakii* (or *Pellicularia filamentosa* f. (sp.) *sasakii*) to occur naturally on soybean and rice. Thread blight was observed on the leaf blades of ginger plants at Pattambi, Malabar by

Sundaram (1953). Foot rot and leaf spot of pan (Piper betle) caused by Rhizoctonia (Corticium) solani was reported from U.P., India (Anon., 1954). Stem rot of Stylosanthes guianensis was observed to be caused by Rhizoctonia (Corticium) solani (Anon., 1967). Wilt of sesamum by the fungus was observed by Palino (1967). White leaf blotch disease of Bermuda grass (Cynodon dactylon) incited by Rhizoctonia solani was recorded by Singh and Seth (1971). Collar rot and web blight of cowpea by Rhizoctonia solani was reported for the first time in Kerala, India by Lakshmanan et al. (1979). Tamietti and Matta (1981) isolated Rhizoctonia solani from decaying carrot in the soil. Chauhan and Jyoti (1982) recorded Rhizoctonia leaf blight of Lemongrass (Cymbopogon flexuosus).

Kannaiyan and Prasad (1979) listed the following weed host from India. Commelina diffusa, Chionachne koenigii, Cyperus rotundus, Digitaria longiflora, Meremia emarginata, Panicum repens, Paspalum scrobiculatum, Digitaria adscendeus, Chloris barbata, Paspalidium flavidum, Brachiaria mutica, Fimbristylis ovata, Desmodium triflorum, Imperata cylindrica, Urochloa panicoides, Alysicarpus monilifer, Dicanthium caricosum, Eriochloa procera, Andropogon asper, Cynodon dactylon and Ischaemum indicum.

Pathogenicity

Malaguti (1951) described the lesions formed by Rhizoctonia solani on rice sheath as pointed, elongated up to 4.3 cm in length, half as width, cream or grey to yellowish in colour with well defined chestnut coloured margin. The necrotic tissues contain very slender, chestnut coloured mycelium, giving rise to sclerotia which gradually turn from white to chestnut coloured and readily fall to the ground. Inoculation experiments with the fungus on the leaf sheaths of rice seedlings resulted in the formation of typical spots. On white bean (Phaseolus vulgaris) seedlings, the fungus produced collar rot.

Luttrell (1962) did not observe host specificity among isolates of Rhizoctonia solani from grasses and legumes that differed widely in growth rate and colony morphology.

Tu (1968) observed physiological variation in seven strains of Pellicularia (Corticium) sasakii which differed in pathogenicity to rice though hyphal fusion occurred between the isolates.

Wu (1976) observed the rice sheath blight pathogen Rhizoctonia solani to be pathogenic to potato tuber, but no anastomosis occurred between the rice pathogen and all isolates of Rhizoctonia solani obtained from a potato field.

Yuno et al. (1978) did not find any difference in pathogenicity of Rhizoctonia solani on rice leaf sheath held in dark or light; but lesions developed much faster when leaf was inoculated on the inner than on the outer surface of the sheath.

Nayak et al. (1979) reported that Rhizoctonia solani infected and survived on several weeds in rice fields viz., Echinochloa crusgallis, E. colona, Cynodon dactylon and Cyperus radiatus and symptoms produced were similar to those on rice plants. None of the wild species of rice was proved to be resistant to the fungus.

Summer (1985) observed high virulence of Rhizoctonia solani belonging to AG 4 and AG 2 type 2 on cultivars of snap bean, pole bean and cowpea. Nandi and Chakrabarti (1984) were able to distinguish four pathotypes of Corticium sasakii by observing the variation in virulence of seven isolates and disease development on ten rice cultivars.

Reaction of Rhizoctonia solani to fungicides

Grover and Chopra (1971) observed that Rhizoctonia solani adapted to grow in lethal concentrations of oxathiin compounds differed morphologically from the parent strains. Four to five transfers to fungicide free medium were sufficient to

restore them to the original type. Isolates adapted to Vitavax showed reduced virulence even after reversion to the parent type by repeated transfers. Altered morphology of adapted isolates of R. solani to Benomyl, Chloroneb and PCNB was reported by Kataria and Grover (1974). Bruggen and Arneson (1984) observed that three of the nine isolates of Rhizoctonia solani were adapted to grow on Potato-dextrose agar amended with Rizotex (tolclofos-methyl) at a concentration 500 times that which initially almost inhibited growth. Pathogenicity of resistant isolates to dry bean (Phaseolus vulgaris) was not reduced, but their growth rates on Potato-dextrose agar medium were significantly lower than the original isolates.

Jhooty and Bains (1973) observed that isolates of Rhizoctonia solani were more sensitive to systemic than to non-synthetic fungicides. No correlation was noted between the dosage required for complete inhibition of Rhizoctonia solani in culture and disease control potential. El-Sawah et al. (1975) reported complete inhibition of growth of Rhizoctonia solani by Vitavax 100, Vitavax 200, Vitavax 300, Demosan, Tecto 60 and Benlate and most of the concentrations tested. Growth of Rhizoctonia solani was inhibited even at 10 ppm of Vitavax and Benlate in in vitro studies by Datta and Sharma (1979).

Bains and Jhooty (1983) noted that isolates of Rhizoctonia solani least pathogenic to different crops were the least sensitive to the fungicides. In vitro studies by Dash and Panda (1984) revealed complete inhibition of mycelial growth of the rice sheath blight pathogen by Carbendazim, Thiram, Edifenphos and Kitazin at 100 ppm each, MEMC at 150 ppm and Captofol and Carboxin at 200 ppm each.

Martin et al. (1984) observed variation in response of anastomosis group tester isolates to the fungicides Carboxin, Quintozene, Chlorothalonil and Triadimefon. They stressed the need for further studies for investigating variation within AG groups.

# MATERIALS AND METHODS

# MATERIAL AND METHODS

## I. THE DISEASE

The incidence and development of sheath blight disease of rice were observed from naturally infected rice fields attached to the Instructional Farm, College of Agriculture, Vellayani. Observations on initiation and development of the symptoms were critically studied and recorded.

## II. ISOLATION OF THE CAUSAL ORGANISM

The causal organism, Rhizoctonia solani was isolated from specimens showing typical sheath blight symptoms. Potato-dextrose agar medium was used and isolation done following the procedure described by Booth (1971).

The infected tissues were cut into small bits, surface sterilized with 1 : 1000 ($10^3$) mercuric chloride solution for 60 to 120 seconds and washed repeatedly in three changes of sterile water. The bits were then placed in sterile petri dishes previously poured with Potato-dextrose agar medium. The dishes were incubated at room temperature ($29 \pm 2^{o}C$). After two to three days, when the growth of the fungus was visible, bits of mycelium were transferred to Potato-dextrose agar test tube slants by means of sterile inoculation needle. The fungal growth of these cultures were stained following the procedure of Horr (1979) and morphological details recorded.

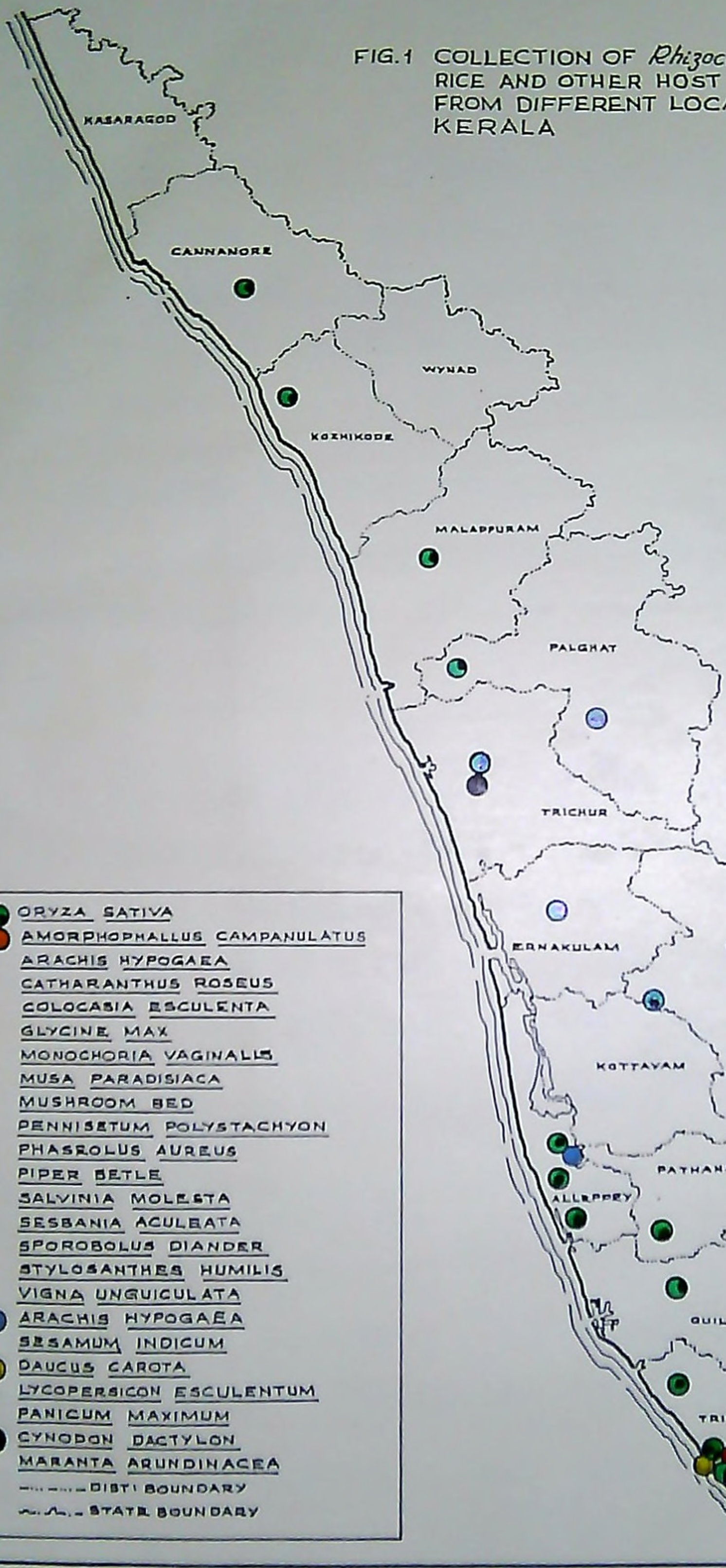
## III. COLLECTION OF RHIZOCTONIA SOLANI FROM RICE AND OTHER HOST PLANTS/HABITAT FROM DIFFERENT LOCALITIES

### A. From rice

Rice plants naturally infected and showing typical sheath blight symptoms were collected from different localities of Kerala. The collections were mainly done from the Research Centres of Kerala Agricultural University and State Seed Farms of the Department of Agriculture (Fig. 1). The variations in symptoms were recorded.

| Sl. No. | Locality | Centre | District |
|---|---|---|---|
| 1. | Adoor | State Seed Farm | Pathanamthitta |
| 2. | Alathur | State Seed Farm | Palghat |
| 3. | Anakayam | State Seed Farm | Malappuram |
| 4. | Chirayinkil | State Seed Farm | Trivandrum |
| 5. | Karamana | Cropping System Research Centre | Trivandrum |
| 6. | Karimanoor | State Seed Farm | Idukki |
| 7. | Kayamkulam | Rice Research Station | Alleppey |
| 8. | Kottarakkara | State Seed Farm | Quilon |
| 9. | Mannuthy | State Seed Farm | Trichur |
| 10. | Moncompu | Rice Research Station | Alleppey |
| 11. | Okkal | State Seed Farm | Ernakulam |
| 12. | Pattambi | Regional Agricultural Research Station | Palghat |

FIG.1 COLLECTION OF *Rhizoctonia solani* FROM RICE AND OTHER HOST PLANTS/ HABITAT FROM DIFFERENT LOCALITIES OF KERALA



| Sl. No. | Locality | Centre | District |
|---|---|---|---|
| 13. | Perambra | State Seed Farm | Kozhikode |
| 14. | Veeyapuram | State Seed Farm | Alleppey |
| 15. | Vellayani | College of Agriculture | Trivandrum |
| 16. | Vengad | State Seed Farm | Cannanore |

B. From other host plants/habitat

Rhizoctonia solani was isolated from the following host plants/habitat as detailed below.

| Sl. No. | Host/habitat | Source | Locality |
|---|---|---|---|
| 1. | Amorphophallus campanulatus Blume | leaf | Vellayani |
| 2. | Arachis hypogaea Linn. | collar region | Vellayani |
| 3. | Arachis hypogaea Linn. | leaf | Kayamkulam |
| 4. | Catharanthus roseus Don. | collar region | Vellayani |
| 5. | Colocasia esculenta Schott (Arac.) | petiole | Vellayani |
| 6. | Cymbopogon flexuosus (Steud.) Wats. | sheath | Odakkali |
| 7. | Cynodon dactylon (L.C.Rich) Pers. | leaf | Trichur |
| 8. | Daucus carota Linn. | tuber | Trivandrum |
| 9. | Glycine max Merr. | collar region | Vellayani |
| 10. | Lycopersicon esculentum Mill. | fruit | Trivandrum |
| 11. | Maranta arundinacea Linn. | tuber | Trichur |

| Sl. No. | Host/habitat | Source | Locality |
|---|---|---|---|
| 12. | Monochoria vaginalis Presl. | leaf | Vellayani |
| 13. | Musa paradisiaca Linn. | rhizome | Vellayani |
| 14. | Mushroom bed | paddy straw | Vellayani |
| 15. | Panicum maximum Jacq. | sheath | Trivandrum |
| 16. | Pennisetum polystachyon (L.) Schults | sheath | Vellayani |
| 17. | Phaseolus aureus Roxb. | collar region | Vellayani |
| 18. | Piper betle Linn. | leaf | Vellayani |
| 19. | Salvinia molesta D.S.Mitchell. | leaf | Vellayani |
| 20. | Sesamum indicum Linn. | collar region | Kayamkulam |
| 21. | Sesbania aculeata Pers. | leaf | Vellayani |
| 22. | Sporobolus diander Beauv. | leaf | Vellayani |
| 23. | Stylosanthes humilis H.B.Orinoco | collar region | Vellayani |
| 24. | Vigna unguiculata Endl. | collar region | Vellayani |

The symptoms and variation in disease expression were recorded. Isolations were made on Potato-dextrose agar medium and pathogenicity of isolates tested on their respective host plants.

## IV. PURIFICATION AND MAINTENANCE OF THE ISOLATES

All the isolates were repeatedly subcultured by the hyphal tip method and maintained at room temperature (29 $\pm$ 2°C)

in Potato-dextrose agar medium slants in test tubes. Stock cultures were incubated at 4°C in a B.O.D. incubator.

V. IDENTIFICATION OF ISOLATES

Detailed studies on the morphological characters of all the isolates were carried out on the nature of hyphal branching, presence of constriction at the point of branching and the presence of septal apparatus. The nuclear status of the isolates was observed by HCl Giemsa staining and rapid staining in plates as described by Herr (1979).

i) HCl Giemsa staining

Culture discs were fixed in a 5 : 1 mixture of 95 per cent ethanol and glacial acetic acid for 10 min, transferred to (i) 95 per cent ethanol for 15 min (ii) acetone for 20 min and finally to 95 per cent ethanol. After storage during overnight in 95 per cent ethanol the discs were transferred to petri dishes containing (i) 70 per cent ethanol for 15 min (ii) 50 per cent ethanol for 15 min and finally tap water for 15 min. The discs were then hydrolysed in 1 N HCl for 8 min at 60°C. The discs were washed in three changes of distilled water, 5 min per change, placed in 1 : 1 buffer-distilled water for 5 min and then in full strength pH 6.8 buffer for 10 min (buffer consisted of a mixture of equal parts of 2.4 g of $KH_2PO_4$ in 1000 ml of distilled water). Discs were stained

for 15 - 18 h (over night) in Giemsa stain. The stain was prepared with three drops of commercial Giemsa stain solution per ml of buffer. Culture discs were transferred to slides and free liquid buffer was absorbed with blotting paper and observed under the microscope.

ii) Rapid staining in plates

Six to eight drops of wetting solution (1000 ml of distilled water, 1 ml of Teepol / Labolene acidified with 1 ml of 85 per cent lactic acid) were distributed on the culture plates avoiding areas of abundant aerial mycelium. These areas were rubbed lightly to wet the hyphae and were stained with a drop of 0.5 per cent aniline blue in lacto-phenol. Cover slips were placed over the treated areas and a small portion of unstained mycelium. After 5 - 30 min, the areas were examined microscopically using a dry 40 X objective.

## VI. MORPHOLOGICAL AND CULTURAL STUDIES

The morphological and cultural characters of the various isolates were recorded by observing the growth of the fungus on Potato-dextrose agar medium in 9 cm petri dishes incubated at room temperature (29 $\pm$ 2$^{o}$C). Type, colour and nature of growth of mycelium, sclerotial colour, shape, density and distribution in culture were recorded. Hyphal and sclerotial dimensions were measured by micrometry. Width of hyphae was measured by

preparing slides using cultures grown for 2 to 3 days on 2 per cent water agar, under a 10 X eyepiece and 40 X objective. Measurement of sclerotial size was done with a 5 X eyepiece and 10 X objective. Twenty measurements were made for each isolate and the average values commuted.

The number of days for completion of growth, for appearance of sclerotial initials and maturation of sclerotia were noted.

Based on the morphological characters of the cultures on Potato-dextrose agar medium in petri dishes, the rate of growth, sclerotial initiation and maturation the isolates were grouped into the various morphological groups.

## VII. PATHOGENICITY OF RICE ISOLATE (ISOLATE 1) ON THE OTHER HOST PLANTS

The pathogenicity of Rhizoctonia solani (Isolate 1) isolated from rice was tested on all the other host plants from which R. solani was isolated as listed out under III B. The inoculation was done on the parts of the respective plants which yielded the fungus viz., leaf, petiole, fruit, tuber, etc., as the case may be.

## VIII. PATHOGENICITY OF ISOLATES FROM OTHER HOST/HABITAT ON RICE

The rice variety Jyothi was raised in pots. All the isolates of Rhizoctonia solani from other host plants/habitat

were inoculated at the maximum tillering phase. Inoculation was done on the outer leaf sheath. The plants were observed for infection and extent of damage.

## IX. ANASTOMOSIS STUDIES

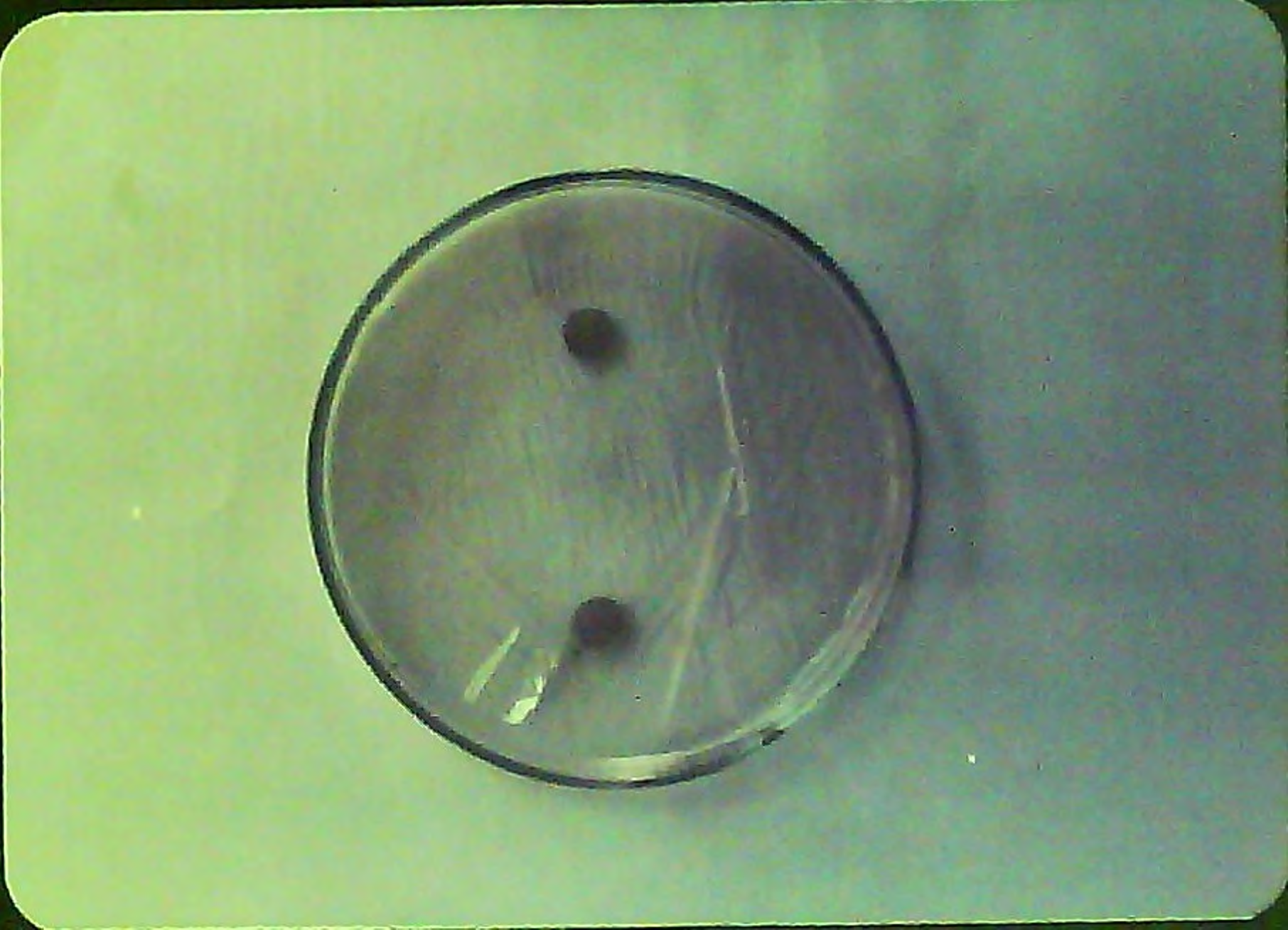
### a. Testing for anastomosis (fusion) between rice isolates of Rhizoctonia solani

The different isolates of Rhizoctonia solani obtained from rice (17 isolates) were tested for their ability to anastomose each other.

Hyphal fusion between isolates were tested by the method adopted by Parmeter et al. (1969). Opposing isolates were plated on cellophane film resting on 2 per cent water agar in 9 cm petri dishes. One pair of isolates was tested per dish. Mycelial transfers from the margin of actively growing young cultures on Potato-dextrose agar medium were placed 2 to 4 cm apart in each dish. The dishes were incubated at room temperature (29 $\pm$ 2°C) until the advancing hyphae made contact usually after 24 to 30 h (Fig. 2). Such portions of the cellophane with the contacting hyphae were removed, mounted on a microscope slide, stained with 0.1 per cent cotton blue-lactophenol and examined under the microscope for fusion. The point of contact/fusion of the opposing hyphae was located by

Fig. 2. Testing for anastomosis between isolates of *Rhizoctonia solani*.

Mycelial disc of two opposing isolates of *Rhizoctonia solani* plated on cellophane resting upon 2 per cent water agar.



tracing the hypha along the direction of growth, branching being inclined towards the direction of growth.

b. Testing for anastomosis (hyphal fusion) between rice isolate (Isolate I) and isolates of Rhizoctonia solani from other host/habitat.

Isolate 1 of Rhizoctonia solani from rice was paired with isolates from all other host plants/habitat, in all possible combinations and observed for hyphal fusion.

c. Anastomosis grouping of the rice group of Rhizoctonia solani

Isolates of Rhizoctonia solani obtained from rice and those isolates anastomosing with rice isolates were tested for their ability to anastomose with the tester isolates of AG 1, AG 2, AG 3 and AG 4.

The anastomosis group tester isolates were obtained from Dr. Edward Butler, Professor, Department of Plant Pathology, University of California, Davis, USA for testing and anastomosis grouping of isolates of Rhizoctonia solani causing sheath blight of rice.

| Isolate | AG | Host | Geographical origin |
|---|---|---|---|
| 465 | 1 | Oryza sativa | Japan |
| 229 | 2 | Raphanus sativus | California |
| 141 | 3 | Phaseolus L. | -- |
| 282 | 4 | Conifer | California |

The cultural and morphogenic characters of these isolates were recorded. The isolates were inoculated on rice plants to test for pathogenicity.

d. Cytology of hyphal fusion in Rhizoctonia solani

Isolates of Rhizoctonia solani were paired on cellophane film on 2 per cent water agar (Parmeter et al., 1969) and closely examined under the microscope to distinguish the different types of fusion. Camera lucida drawings were made of hyphal fusion.

## X. DETERMINATION OF THE PRESENCE OF CLONES OF RHIZOCTONIA SOLANI CAUSING SHEATH BLIGHT OF RICE

The modified method of Ogoshi and Ui (1983) was followed to determine the presence in the field of clones of Rhizoctonia solani. Specimens of sheath blight disease were collected from different locations of a severely infected field at the College of Agriculture, Vellayani. The specimens were examined for lesion variation and isolations made on Potato-dextrose agar medium.

Selection of isolates of Rhizoctonia solani from the field for studies on the presence of clones.

| Row | | 1st | 2nd | 3rd |
|---|---|---|---|---|
| Isolate | $R_5$ | $R_1R_2$ | $R_3$ | $R_4$ |
| Location | IV | I | II | III |

$R_1R_2$ = isolates from the same plant at location I

$R_3$ = isolate from adjacent plant at location II

$R_4$ = isolate from 3rd row at location III

$R_5$ = isolate from location IV at the third row in the opposite direction from location I.

The isolates were maintained in Potato-dextrose agar medium test tube slants and observed for distinguishable variation in morphologic and cultural characters.

The isolates were paired in all possible combinations (Parmeter et al., 1969) and observed for nature of fusion.

## XI. COMPARISON OF GROWTH OF MG 1 ISOLATES OF RHIZOCTONIA SOLANI AND REPRESENTATIVE ISOLATES OF MG 2, MG 3 AND MG 4 IN DIFFERENT CULTURE MEDIA

### a. Liquid medium

Growth of the following isolates of Rhizoctonia solani of MG 1 group were compared with the representative isolates of MG 2 (Mushroom bed), MG 3 (Sesamum indicum) and MG 4 (Vigna unguiculata).

1. Arachis hypogaea
2. Colocasia esculenta
3. Cymbopogen flexuosus
4. Cynodon dactylon
5. Monochoria vaginalis
6. Oryza sativa
7. Panicum maximum
8. Pennisetum polystachyon
9. Sesbania aculeata
10. Sporobolus diander

Potato-dextrose broth and Czapek's broth were used for studying the growth of the isolates. Conical flask (250 ml) containing 100 ml each of the respective media were inoculated after sterilisation with a 5 mm mycelial disc from actively growing culture of the isolates. The flasks were then incubated at room temperature (29 $\pm$ 2°C) for 14 days under static condition. A similar set of inoculated flasks were subjected to continuous shaking in a rotatory shaker for 14 days at room temperature (29 $\pm$ 2°C). Three replications were maintained for each isolate, under both static and rotatory condition. The mycelial mats were harvested on the 14th day and dried in a hot air oven (60°C) to determine the dry weight.

b. Solid medium

The growth of representative isolates of the 4 morphological groups MG 1 (Oryza sativa), MG 2 (Mushroom bed), MG 3 (Sesamum indicum) and MG 4 (Vigna unguiculata) were compared on different solid media. The media used were Potato-dextrose agar medium, Coon's medium, Czapek's medium and Richards' medium (Appendix I).

The media were prepared and poured into sterile petri dishes at the rate of 15 ml in each and allowed to solidify. Circular discs of 5 mm diameter were cut out from the outer edge of actively growing petri dish cultures of the isolates and placed in the centre of the solidified medium at the rate of one in each petri dish. Three replications were run for each isolate in each medium. The dishes were incubated at room temperature (29 $\pm$ 2°C) and the number of days taken for completion of growth, appearance of sclerotial initials and maturation of sclerotia were recorded.

XII. STUDIES ON THE SCLEROTIAL CHARACTERS

Buoyancy of sclerotia

The sclerotia produced on cultures in Potato-dextrose agar medium were tested for their ability to remain floating in water at varying intervals of time. All isolates of MG 1 and representative isolates of MG 2, MG 3 and MG 4 were used.

One hundred sclerotia of each isolate were immersed in 150 ml of water contained in 250 ml beakers. Observation on the percentage of sclerotia remained floating on water were taken at intervals of 1 h, 24 h, 3rd day, 7th day and 14th day.

## XIII. REACTION OF MG 1 ISOLATES OF RHIZOCTONIA SOLANI TO DIFFERENT VARIETIES OF RICE

An experiment was carried out to study the pathogenic variability among MG 1 isolates of Rhizoctonia solani in their reaction to 10 varieties of rice. The ten varieties were arbitrarily selected to include tolerant and susceptible ones. The varieties used were:-

| Variety | Duration | |
|---|---|---|
| Jaya | 133 | |
| MO-5 | 126 | |
| Culture-169 | 133 | High yielding |
| Culture-1537-2 | 123 | |
| IR-36 | 121 | |
| Culture-1954 | 115 | |
| Jyothi | 117 | High yielding |
| Culture-1907 | 113 | |
| Culture-52-3-6 | 108 | |
| Culture-26-1-1 | 105 | |

One month old seedlings were transplanted in 60 x 60 cm cement trays filled with clay soil from rice fields. Ten rows

were planted in each tray at a spacing of 6 x 10 cm, each row comprising of a single variety. Field condition was simulated as far as possible by providing recommended quantity of fertilizers (90 : 45 : 45), basal application of cow dung (@ 5 ton/ha), irrigation and management practices. Inoculation was done at the active tillering phase with the test isolate by pin prick method on the outer leaf sheath. The outer most hill on either sides of each row was inoculated and sufficient moisture provided.

Disease severity and vertical development of the disease were scored and measured on the 7th and 14th day of inoculation. Disease severity was scored with grades ranging from 0 - 9, following the Standard evaluation system for rice diseases (Anon., 1976). Vertical development of disease was expressed as percentage ratio 'X' (Hashiba, 1984).

$$X = \frac{\text{height of upper most lesion}}{\text{plant height}} \times 100$$

XIV. LABORATORY EVALUATION OF FUNGICIDES

a. Reaction of Rhizoctonia solani to increasing concentrations of carboxin

The growth of Rhizoctonia solani isolated from Oryza sativa, on Potato-dextrose agar medium incorporated with increasing concentrations of carboxin was studied by repeated

transfer of culture from the lower most concentration to higher concentrations step by step.

Cultures were transferred from 2.5 ppm to increasing concentration of 5 ppm and then to 10 ppm of carboxin. The 'poisoned food technique' described by Zentmyer (1955) was employed. The required quantity of fungicide was added to 50 ml of sterilized, molten Potato-dextrose agar medium, mixed well and poured into sterilized petri dishes at the rate of 15 ml in each. One 5 mm mycelial disc cut out from an actively growing petri dish culture of the fungus was placed in the centre of each petri dish containing the poisoned medium. Controls consisting of Potato-dextrose agar medium inoculated with the fungus was also maintained. The petri dishes were incubated at room temperature (29 $\pm$ 2°C).

The radial growth and cultural characters of the adapted isolate of the fungus were recorded at each concentration and compared to normal isolate grown on Potato-dextrose agar medium and on Potato-dextrose agar medium incorporated with the same concentration of fungicide.

The tolerant isolate of Rhizoctonia solani adapted to grow at the maximum concentration tested was then cultured on Potato-dextrose agar medium and compared with the normal culture of the isolate.

b. Pathogenicity of tolerant isolate of Rhizoctonia solani

The carboxin tolerant isolate of Rhizoctonia solani grown on Potato-dextrose agar medium was inoculated on rice plants to study its pathogenicity and virulence. Plants were inoculated separately with sclerotia and mycelial disc. Sclerotia were placed inbetween the leaf sheath and covered with moist cotton wool. Mycelial discs of 10 mm diameter were fixed on previously punctured outer leaf sheath with cellophane tape and covered with moist cotton wool (Amin, 1975). Controls with normal isolates were maintained.

c. Reisolation of tolerant isolate and testing for stability of anastomosis character.

The carboxin tolerant isolate of Rhizoctonia solani was reisolated from the infected tissues and tested for anastomosis reaction with normal rice isolate (Isolate 1) by the method of Parmeter et al. (1969).

d. Growth inhibition of Rhizoctonia solani by different fungicides

The following fungicides were used to study the inhibition of growth of Rhizoctonia solani (Isolate I from rice) at different concentrations.

| Fungicide | Active ingredient | Concentration used |
|---|---|---|
| Carbendazim | 2-(Methoxy-carbamoyl)-benzimidazole | 500 ppm<br>1000 ppm<br>1500 ppm |
| Carboxin | 5,6-dihydro-2methyl-1-4 oxathiin-3-carboxanilide | 500 ppm<br>1000 ppm<br>1500 ppm |
| Tridemorph | 2,6,-dimethyl-4-tridecyl morpholine | 500 ppm<br>1000 ppm<br>1500 ppm |

The required quantity of fungicides was incorporated into Potato-dextrose agar medium and growth inhibition tested by the 'poisoned food technique' described by Zentmyer (1955).

# RESULTS

# RESULTS

The typical symptoms of sheath blight disease were studied by observing the development of the symptoms in naturally infected rice fields attached to the Instructional Farm of the College of Agriculture, Vellayani.

## I. THE DISEASE

Symptoms usually developed towards the tillering stage of the crop. Discolouration started at or above the water level which developed into light greenish grey ellipsoidal lesions with dark brown margin. The length of the lesions varied from 1.5 to 2.5 cm. The lesions later coalesced and became off white to light greenish brown with a brown margin. Such coalesced lesions were oblong to irregular and in severe conditions encircled the culm, causing the rotting of the sheath. The lesions then spread to the leaves resulting in the death of the plants. During the vertical development of the disease ovoid greenish grey spots of 5 to 10 cm in length appeared on the upper leaf sheaths and leaf blades (Fig. 3).

Under humid condition, sclerotia of the fungus were seen on severely diseased leaf sheaths and leaves. They were initially white, later becoming brown. Individual sclerotia varied in size up to 5 mm diameter. Such individual sclerotia

Fig. 3. Typical symptom of sheath blight disease of rice.

united to form aggregates. The sclerotia were produced loosely on the surface of the leaf sheath and were spherical to flattened in shape. The sclerotia found in between the leaf sheath were flattened and irregular in shape. Sclerotia were very easily detached from the plant surface. Under very high percentage of relative humidity silvery threads of mycelium appeared on the leaf surface.

Seedlings sown in infested soil developed the disease in three to four weeks after sowing. The base of the plants rot and death of seedlings were observed in patches.

## II. ISOLATION OF THE CAUSAL ORGANISM

Isolations from the sheath blight affected tissues on Potato-dextrose agar medium constantly yielded Rhizoctonia solani Kühn. (Thanatephorus cucumeris (Frank) Donk) (Fig. 4). HCl Giemsa staining revealed multinucleated cells with prominent septal pore apparatus. Branching was near the distal septum with constriction of the branch base (Fig. 5). Young hyphae were colourless and measured 8 to 12 /um in diameter. The culture showed brown pigmentation with age. Sclerotial initials were minute, white in colour, fluffy and reaching a maximum size after 30 h as pigmentation started. Mature sclerotia were subglobose, rough surfaced but regular and size varied from 1.8 mm to 3 mm. Browning of sclerotia

Fig. 4. Culture of *Rhizoctonia solani* Kühn (*Thanatephorus cucumeris* (Frank) Donk) isolated from rice affected with sheath blight disease. (College of Agriculture, Vellayani - Isolate 1)

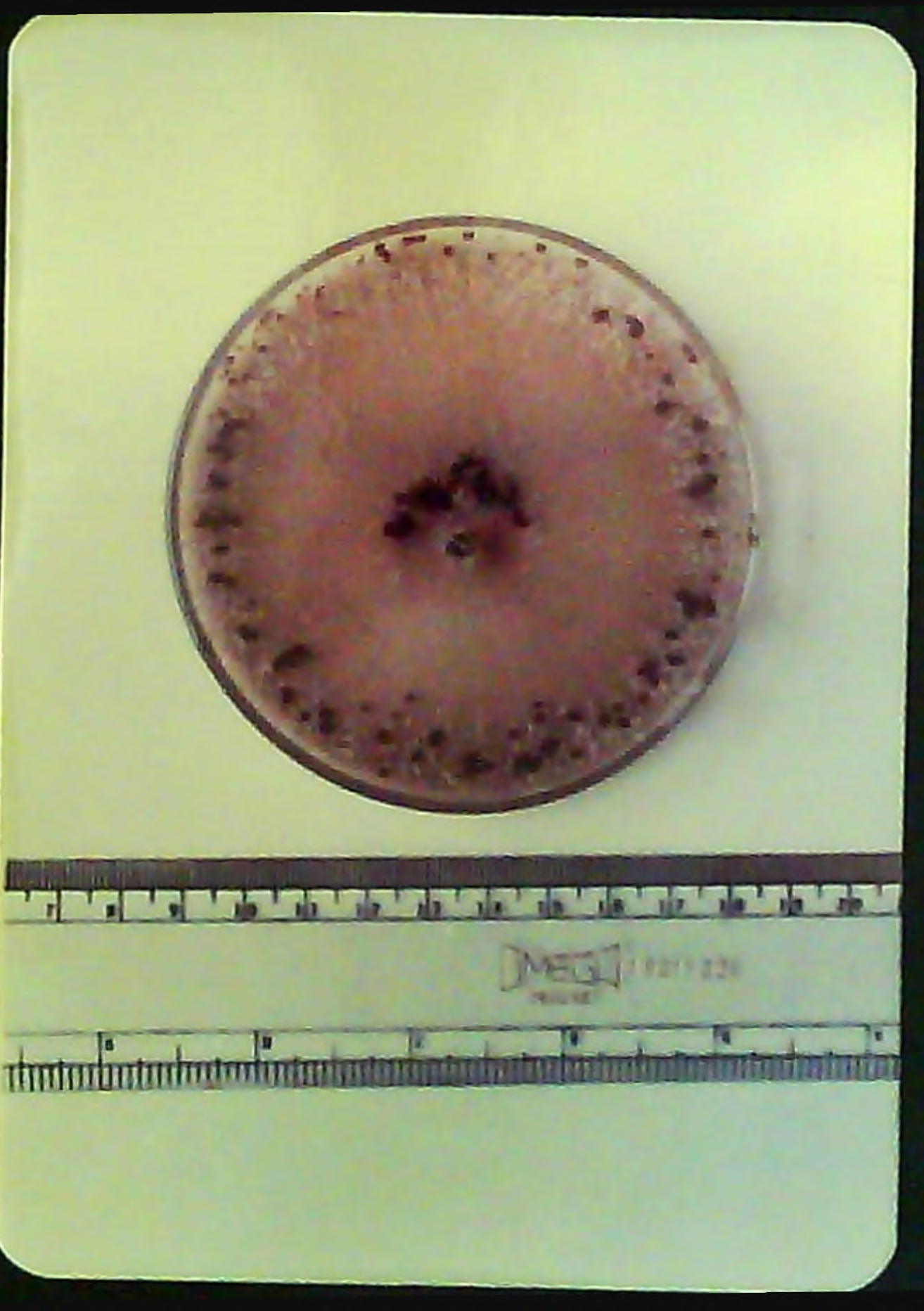
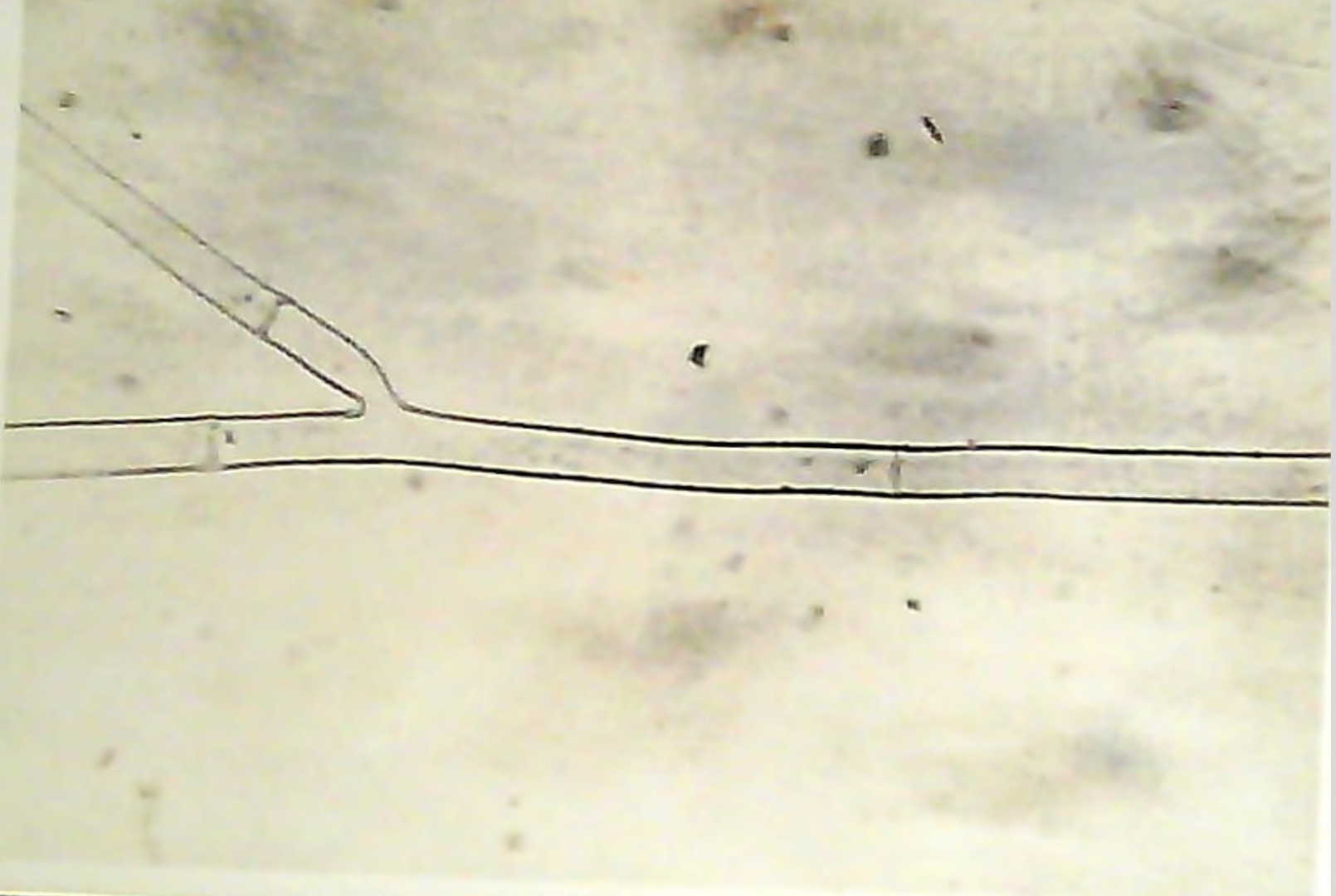


Fig. 5. Hyphal branching of *Rhizoctonia solani*.

Branching near the distal septum with constriction at the branch base.



was completed at about 40 h after formation. The sclerotia were mainly concentrated towards the periphery of the culture plates. Monilioid cells were observed in the ageing cultures.

The cultures were maintained in Potato-dextrose agar medium on slants in test tubes as stock culture (Isolate 1) for comparison with different isolates of Rhizoctonia solani obtained from rice and other host plants/habitat.

## III. COLLECTION OF RHIZOCTONIA SOLANI FROM RICE AND OTHER HOST PLANTS/HABITAT FROM DIFFERENT LOCALITIES

### A. From rice

Specimens of sheath blight affected rice plants were obtained from the following localities in Kerala and the variation in symptoms recorded. The causal fungus was isolated and pathogenicity established.

a. Adoor — Lesions not coalescing with thick brown margin and buff coloured centre (Isolate 2)

b. Alathur — Lesions encircling the culm and greyish green in colour (Isolate 3)

c. Anakayan — Lesion encircling the culm and extending towards the leaves. Dirty grey in colour with indistinct margin (Isolate 4)

d. Chirayinkil — Lesions irregular and elongating. Margin very thin and pale green in colour (Isolate 5)

e. Karamana — Lesions ellipsoidal and regular in outline. Greenish grey and light brown margin (Isolate 6)

f. Karimanoor — Few lesions of below average size with thick brown margin and greyish white centre (Isolate 7)

g. Kayamkulam — Lesions encircling the culm, greenish grey in colour, irregular dark brown margin, extending towards the leaves with sclerotial formation at the culm (Isolate 8)

h. Kottarakkara — Small oval lesions irregularly distributed along with discolouration of the sheath. Lesions greyish in colour with irregular, thin, brown margin (Isolate 9)

i. Mannuthy — Brown discolouration of sheath with irregular lesions coalescing and encircling the culm (Isolate 10)

j. Moncompu — Large lesions uniformly pale green in colour with regular outline of brown colour (Isolate 11)

k. Okkal — Lesions, dirty green with whitish centre and oval to oblong in shape (Isolate 12)

l. Pattambi — Lesions large in size and encircling the sheath. Greyish white in colour having dark brown margin with sclerotial formation (Isolate 13).

m. Perambra — Small oval greenish grey lesions with thick light brown margin (Isolate 14)

n. Veeyapuram — Lesions irregular in outline, elongated and narrow. Greenish grey with white centre and thin brown margin (Isolate 15)

o. Vellayani — (leaf specimen) Lesions varying from oval to elongated on the leaves. Margin irregular, thin and dark brown. Lesions pale green and water soaked with straw coloured centre (Isolate 16)

p. Vengad — Lesions elongated, greenish grey in colour with regular thin margin and broad apex. Lesions tending to coalesce (Isolate 17).

B. From other host plants/habitat

Diseased specimens of other host plants/habitat suspected to be infected by Rhizoctonia solani were collected from

different localities. The symptoms were studied and the causal fungus isolated as detailed under material and methods.

a. Amorphophalus campanulatus (Vellayani) Buff brown coloured, large circular to irregular lesions appeared on the leaves. White striations were present on the lesions. (Isolate 18)

b. Arachis hypogaea (Vellayani) Damping off of young seedlings and collar rot of mature plants were observed. Water soaked lesions appeared on the hypocotyl which turned brown and became dry and sunken. Such seedlings collapsed. On mature plants severe cankers and distortion of tap root and crown occurred. These resulted in the girdling of the collar region and death of the plants. (Isolate 19)

c. Arachis hypogaea (Kayamkulam) Symptoms on the leaves appeared as circular to irregular large water soaked lesions. Dark brown in colour with sign of rotting. (Isolate 20)

d. Catharanthus roseus (Vellayani) Damping off of the infected seedlings occurred. Sunken dark brown lesions were observed on the collar region of the affected seedlings. (Isolate 21)

e. Colocasia esculenta (Vellayani) Symptoms initiated as a water soaked lesion on petiole which enlarged and became

dark brown with irregular outline. Whitish transverse bands occurred along the region where the lesions coalesced. Rotting occurred as disease progressed. (Isolate 22)

f. Cymbopogon flexuosus (Odakkali) Symptoms appeared on the outer leaf sheath as oval to oblong lesions with light brown and thin margins. Spots buff brown in colour and restricted mainly on the sheath. (Isolate 23)

g. Cynodon dactylon (Trichur) Circular to irregular whitish lesions appeared on the leaf tip. The lesions coalesced and extended downwards along the leaf lamina to cover the whole leaf. Straw coloured bands transversed in between the affected area. (Isolate 24)

h. Daucus carota (Trivandrum) Small whitish growth of the fungus appeared on the tubers during storage, causing softening of the infected tissues. Pits appeared which enlarged into sunken craters, covered with whitish mycelial mat. (Isolate 25)

i. Glycine max (Vellayani) Dark brown lesions at the collar region, girdling the stem, causing collar rot. Mycelial strands and sclerotia were present over the affected region under humid condition. (Isolate 26)

j. Lycopersicon esculentum (Trivandrum) Soft rot of the fruits during storage with juicy exudation. Sclerotial formation was observed under humid conditions. (Isolate 27)

k. Maranta arundinacea (Trichur) Storage rot of the tubers occurred with formation of dark brown sunken lesions on the surface of the tubers. (Isolate 28)

l. Monochoria vaginalis (Vellayani) Symptoms appeared on the leaf and leaf stalk. Infected regions showed mingling of dark and light brown areas with whitish streaks. Rotting of leaf stalk occurred during severe infection. (Isolate 29)

m. Musa paradisiaca (Vellayani) Abundant sclerotial formation occurred on the exposed region of the rhizome. Affected portions showed dry rot with disintegration of the infected tissues. (Isolate 30)

n. Mushroom bed (Vellayani) The paddy straw used as substrate for growing Volvariella volvacea showed abundant mycelial growth with copious production of sclerotia in chains. Production of mushroom buttons at the infected region was hindered. (Isolate 31)

o. Panicum maximum (Trivandrum) Oval to oblong greenish grey lesions with white centre and very thin brown margin occurred on the sheath. (Isolate 32)

p. Pennisetum polystachyon (Vellayani) Elongated greyish lesions with buff white centre and thin brown margin were seen on the leaf sheath. (Isolate 33)

q. Phaseolus aureus (Vellayani) Dark brown lesions appeared on the collar region resulting in the girdling of the stem. Mycelial strands and sclerotia were seen leading to collar rot. (Isolate 34)

r. Piper betle (Vellayani) Large water soaked black lesions with irregular outline on the leaves. Mainly originated from the tip and margin of leaves. Sclerotial formation observed under humid condition. (Isolate 35)

s. Salvinia molesta (Vellayani) Dark brown water soaked lesions were noticed on the leaves leading to leaf rot and finally blighting of the leaves. (Isolate 36)

t. Sesamum indicum (Kayamkulam) Wilting of the young seedlings occurred. Affected collar region showed sunken dark brown lesions girdling the stem. Mycelial strands were present over the affected area. (Isolate 37)

u. Sesbania aculeata (Vellayani) Yellowing and curling of the leaflets. The leaflets became brown with irregular lesions, followed by defoliation. (Isolate 38)

v. Sporobolus diander (Vellayani) The lesions produced on the leaves were irregular greenish grey in colour with thin frail brown margin. On the sheath the lesions appeared greyish white in colour. (Isolate 39)

w. Stylosanthes humilis (Vellayani) Drying up of the affected plants. Infected collar region carried abundant sclerotia of the fungus. Dark brown lesions were seen at and above the collar region. (Isolate 40)

x. Vigna unguiculata (Vellayani) Brown lesions on the stem at the collar region caused girdling of the basal portion to produce collar rot symptoms. Mycelial strands and sclerotia were produced during humid condition. (Isolate 41).

The pathogenicity of the isolates were established on the respective host plants.

## IV. PURIFICATION AND MAINTENANCE OF THE ISOLATES

All the isolates of Rhizoctonia solani were repeatedly subcultured by the hyphal tip method and maintained at room temperature (29 $\pm$ 2°C) on Potato-dextrose agar medium slants in test tubes. Stock cultures incubated at 4°C in a B.O.D. incubator required less frequent subculturing at an interval of 6 months.

## V. IDENTIFICATION OF ISOLATES

Rhizoctonia solani was identified mainly based on characteristic features of the fungal mycelium.

All the isolates examined showed young hyphal branches inclined in the direction of growth and constricted at the point of origin from the main hyphae. Branching was always from the distal septum. Prominent septal pore was present in all isolates.

HCl Giemsa staining schedule employed for nuclear staining revealed that all isolates were multinucleated, thus assigning them to the Rhizoctonia complex. The nuclei of rice isolates were larger in size and prominent. The rapid staining method also showed all isolates to be multinucleated, but the nuclei retained the stain only for a shorter period of time.

## VI. MORPHOLOGICAL AND CULTURAL STUDIES

Observation on the morphological and cultural characters of the isolates of Rhizoctonia solani grown on Potato-dextrose agar medium in 90 mm petri dishes were recorded.

Isolate 1 (Oryza sativa) Mycelium subaerial nonfluffy and appressed to substrate. Sclerotial formation concentrated densely and regularly towards the periphery. Sclerotia regular, subglobose, rough with honey dew formation.

Isolate 2 (Oryza sativa) Mycelium subaerial nonfluffy and appressed to substrate. Sclerotia distributed almost uniformly but concentrated more towards the periphery. Sclerotia regular, subglobose and rough.

Isolate 3 (Oryza sativa) Mycelium subaerial nonfluffy and appressed to substrate. Sclerotia concentrated towards the periphery and tending to form clusters. Sclerotia regular, subglobose and rough with honey dew formation.

Isolate 4 (Oryza sativa) Mycelium subaerial, nonfluffy and appressed to substrate. Sclerotia scattered irregularly throughout the culture tending to concentrate towards the periphery. Sclerotia regular, subglobose and rough with honey dew formation.

Isolate 5 (Oryza sativa) Mycelium subaerial nonfluffy and appressed to substrate. Sclerotia concentrated towards the periphery. Sclerotial initials absent towards the centre forming a free zone. Sclerotia regular, subglobose and rough with honey dew formation.

Isolate 6 (Oryza sativa) Mycelium subaerial nonfluffy and appressed to substrate. Abundant sclerotial formation distributed throughout but concentrated towards the periphery as a band. Sclerotia subglobose, regular and rough.

Isolate 7 (Oryza sativa) Mycelium subaerial, nonfluffy and appressed to substrate. Sclerotia concentrated towards the periphery. Sclerotia subglobose, regular and rough.

Isolate 8 (Oryza sativa) Mycelium subaerial, nonfluffy and appressed to substrate. Sclerotia concentrated towards the periphery. Sclerotia subglobose regular and rough. Honey dew formation.

Isolate 9 (Oryza sativa) Mycelium subaerial, nonfluffy and appressed to substrate. Sclerotia scanty, scattered towards the periphery. Sclerotia subglobose, regular and rough. Abundant honey dew formation.

Isolate 10 (Oryza sativa) Mycelium subaerial, nonfluffy and appressed to substrate. Sclerotia distributed irregularly throughout the culture concentrating in clumps towards the periphery. Sclerotia subglobose, surface regular and rough.

Isolate 11 (Oryza sativa) Mycelium subaerial, nonfluffy and appressed to substrate. Sclerotia scanty and concentrated regularly towards the periphery. Sclerotial initials scattered throughout. Sclerotia subglobose, surface rough and regular.

Isolate 12 (Oryza sativa) Mycelium subaerial, nonfluffy and appressed to substrate. Sclerotia distributed irregularly

throughout the culture concentrating in clumps towards the periphery. Sclerotia subglobose, surface rough and regular.

Isolate 13 (Oryza sativa) Mycelium subaerial nonfluffy and appressed to substrate. Sclerotia distributed irregularly throughout the culture but tending to concentrate towards the periphery. Sclerotia subglobose, surface rough and regular. Honey dew formation.

Isolate 14 (Oryza sativa) Mycelium subaerial nonfluffy and appressed to substrate. Sclerotia scanty and distributed irregularly throughout the culture. Sclerotia subglobose, surface rough and regular. Abundant honey dew formation.

Isolate 15. (Oryza sativa) Mycelium subaerial, nonfluffy and appressed to substrate. Sclerotia scanty and distributed irregularly but tending to concentrate towards the periphery. Sclerotia subglobose, surface rough and regular.

Isolate 16 (Oryza sativa) Mycelium subaerial, nonfluffy and appressed to substrate. Sclerotia scattered densely and regularly towards the periphery. Sclerotia subglobose, surface regular and rough.

Isolate 17 (Oryza sativa) Mycelium subaerial, nonfluffy and appressed to substrate. Sclerotia concentrated towards

the periphery. Sclerotia subglobose, surface regular and rough. Honey dew formation.

Isolate 18 (Amorphophallus campanulatus) Mycelium fluffy cottony growth. Sclerotia scanty and distributed in clumps on the margin of the culture. Sclerotia globose and tending to elongate. Surface smooth and irregular.

Isolate 19 (Arachis hypogaea) Mycelium radiating, subaerial and scattered individually. Sclerotia irregularly distributed towards the periphery. Honey dew formation present. Sclerotia globose to irregular, surface smooth and irregular.

Isolate 20 (Arachis hypogaea) Mycelium subaerial, nonfluffy and appressed to the substrate. Sclerotia concentrated densely and regularly towards the periphery of the culture to form a band. Sclerotia flat, tending to elongate with rough irregular surface. Scanty honey dew formation.

Isolate 21 (Catharanthus roseus) Mycelium radiating aerial and fluffy towards the margin of the culture. Sclerotia abundant, uniformly distributed throughout the culture. Sclerotia globose to slightly irregular. Surface smooth and regular.

Isolate 22 (Colocasia esculenta) Mycelium subaerial, nonfluffy and appressed to substrate. Sclerotia distributed throughout. Sclerotia subglobose with regular rough surface.

Isolate 23 (Cymbopogon flexuosus) Mycelium subaerial, nonfluffy and appressed to substrate. Sclerotia irregularly distributed in clumps towards the periphery. Sclerotia subglobose, regular with rough surface.

Isolate 24 (Cynodon dactylon) Mycelium subaerial nonfluffy and appressed to substrate. Sclerotia distributed irregularly. Sclerotia subglobose, regular with rough surface. Abundant honey dew formation.

Isolate 25 (Daucus carota) Mycelium radiating, subaerial, scattered individually. Sclerotia abundant distributed regularly along the sides of the plates. Sclerotia globose, regular and smooth.

Isolate 26 (Glycine max) Fluffy cottony growth with advancing mycelial strands. Sclerotia distributed in clumps on the side and top of plates. Sclerotia globose to oblong, surface smooth and regular.

Isolate 27 (Lycopersicon esculentum) Mycelium radiating, subaerial and scattered individually. Scanty sclerotial formation and present in clumps towards the periphery. Sclerotia globose with smooth and regular surface. Honey dew formation.

Isolate 28 (Maranta arundinacea) Mycelium subaerial, radiating and scattered individually. Sclerotia distributed irregularly tending to concentrate in clumps towards the periphery. Sclerotia globose, smooth walled and regular.

Isolate 29 (Monochoria vaginalis) Mycelium subaerial, nonfluffy and appressed to substrate. Sclerotia concentrated densely and regularly towards the periphery. Sclerotia subglobose, regular, rough surfaced with abundant honey dew formation.

Isolate 30 (Musa paradisiaca) Mycelium radiating, subaerial and scattered individually. Abundant sclerotial formation on the sides of the plates. Evenly distributed and mostly present individually adjacent to each other. Sclerotia globose, smooth walled and regular.

Isolate 31 (Mushroom bed) Mycelium radiating, subaerial and scattered individually. Abundant sclerotial formation on the sides of petri dishes and periphery of culture in a regular manner. Sclerotia globose, smooth walled and regular.

Isolate 32 (Panicum maximum) Mycelium subaerial, nonfluffy and appressed to substrate. Sclerotia scanty and distributed irregularly throughout the culture. Sclerotia subglobose, surface rough and regular.

Isolate 33 (Pennisetum polystachyon) Mycelium subaerial, nonfluffy and appressed to substrate. Sclerotia scanty and distributed irregularly throughout the culture. Sclerotia subglobose, surface regular and rough.

Isolate 34 ( Phaseolus aureus) Mycelium radiating, aerial and fluffy towards the margin. Sclerotia distributed almost uniformly throughout the plate individually. Sclerotia globose to irregular. Surface smooth and regular.

Isolate 35 (Piper betle) Fluffy cottony growth of mycelium. Sclerotial formation scanty and distributed in clumps. Abundant honey dew formation. Sclerotia globose tending to elongate, surface smooth and irregular.

Isolate 36 (Salvinia molesta) Mycelium radiating and aerial. Hyphae in thick aggregates towards the margin. Scanty sclerotial formation occurring in clumps. Irregularly distributed throughout the culture. Sclerotia globose, surface smooth and regular.

Isolate 37 (Sesamum indicum) Mycelium radiating and aerial. Hyphae in thick aggregates. Sclerotia distributed almost uniformly. Present individually rather than in aggregates. Sclerotia globose, surface smooth and regular with honey dew formation.

Isolate 38 (Sesbania aculeata) Mycelium subaerial nonfluffy and appressed to substrate. Sclerotia abundant distributed throughout, more towards the periphery to form a band. Sclerotia subglobose, regular and rough surfaced.

Isolate 39 (Sporobolus diander) Mycelium subaerial, non-fluffy and appressed to substrate. Sclerotia concentrated densely and regularly towards the periphery. Sclerotia subglobose, tending to elongate with rough regular surface.

Isolate 40 (Stylosanthes humilis) Mycelium radiating, aerial and fluffy towards the margin. Sclerotia distributed in clumps more towards the periphery. Sclerotia not abundant. Sclerotia globose to slightly irregular, surface smooth and regular.

Isolate 41 (Vigna unguiculata) Mycelium fluffy and cottony in growth. Sclerotial formation in clumps and distributed irregularly. Sclerotia globose, surface smooth and irregular.

The minimum, maximum and average dimensions of hyphae and sclerotia of the different isolates are presented in Table 1.

The growth rate of the isolates on Potato-dextrose agar medium and time required for sclerotial initiation and maturation were recorded (Table 2). Variations in time for

Table 1. Hyphal and sclerotial dimensions of different isolates of *Rhizoctonia solani*

| Isolate No. | Host/habitat | Mycelial dimension (/um) | | | Sclerotial dimension (mm) | | |
|---|---|---|---|---|---|---|---|
| | | Minimum | Maximum | Average | Minimum | Maximum | Average |
| 1 | *Oryza sativa* (Vellayani) | 10.75 | 17.90 | 11.65 | 1.80 | 3.00 | 2.60 |
| 2 | *Oryza sativa* (Adoor) | 8.00 | 11.00 | 10.50 | 2.00 | 2.70 | 2.50 |
| 3 | *Oryza sativa* (Alathur) | 7.00 | 10.00 | 8.00 | 1.80 | 2.80 | 2.60 |
| 4 | *Oryza sativa* (Anakayam) | 6.25 | 9.50 | 8.25 | 1.90 | 2.50 | 2.30 |
| 5 | *Oryza sativa* (Chirayinkil) | 7.25 | 10.00 | 9.25 | 1.60 | 2.90 | 2.50 |
| 6 | *Oryza sativa* (Karamana) | 7.15 | 14.35 | 11.50 | 1.60 | 3.00 | 2.50 |
| 7 | *Oryza sativa* (Karimanoor) | 9.00 | 15.25 | 13.50 | 1.80 | 3.00 | 2.70 |
| 8 | *Oryza sativa* (Kayankulam) | 8.25 | 12.50 | 10.25 | 1.70 | 2.90 | 2.40 |
| 9 | *Oryza sativa* (Kottarakkara) | 7.50 | 13.25 | 11.15 | 2.00 | 3.00 | 2.50 |
| 10 | *Oryza sativa* (Mannuthy) | 3.60 | 4.50 | 4.75 | 1.50 | 2.60 | 2.40 |
| 11 | *Oryza sativa* (Moncompu) | 7.15 | 10.75 | 8.40 | 2.00 | 2.80 | 2.50 |
| 12 | *Oryza sativa* (Okkal) | 6.50 | 10.00 | 9.50 | 1.90 | 3.10 | 2.70 |
| 13 | *Oryza sativa* (Pattambi) | 4.75 | 9.00 | 7.50 | 1.60 | 2.70 | 2.40 |
| 14 | *Oryza sativa* (Perambra) | 8.00 | 12.00 | 9.50 | 1.70 | 2.80 | 2.40 |
| 15 | *Oryza sativa* (Veeyapuram) | 10.00 | 15.25 | 12.50 | 1.80 | 2.60 | 2.50 |
| 16 | *Oryza sativa* (Vellayani - leaf specimen) | 10.50 | 15.00 | 12.00 | 3.20 | 1.70 | 2.40 |
| 17 | *Oryza sativa* (Vengad) | 8.00 | 14.00 | 11.75 | 1.70 | 2.10 | 2.30 |
| 18 | *Amorphophallus campanulatus* | 2.70 | 5.40 | 4.00 | 1.60 | 2.00 | 1.90 |
| 19 | *Arachis hypogaea* (collar) | 3.70 | 8.00 | 7.50 | 1.30 | 1.90 | 1.80 |
| 20 | *Arachis hypogaea* (leaf) | 7.15 | 9.80 | 9.00 | 1.30 | 3.00 | 2.30 |

Table 1. Contd.

| Isolate No. | Host/habitat | Mycelial dimension (/um) | | | Sclerotial dimension (mm) | | |
|---|---|---|---|---|---|---|---|
| | | Minimum | Maximum | Average | Minimum | Maximum | Average |
| 21 | Catharanthus roseus | 2.70 | 5.40 | 4.00 | 1.60 | 1.90 | 1.80 |
| 22 | Colocasia esculenta | 7.15 | 14.30 | 9.50 | 1.50 | 2.60 | 2.00 |
| 23 | Cymbopogon flexuosus | 4.00 | 6.25 | 5.50 | 1.80 | 3.20 | 2.50 |
| 24 | Cynodon dactylon | 4.50 | 6.25 | 6.70 | 1.20 | 2.90 | 2.20 |
| 25 | Daucus carota | 4.50 | 7.15 | 5.00 | 2.20 | 1.30 | 1.90 |
| 26 | Glycine max | 3.60 | 7.15 | 6.25 | 1.50 | 2.00 | 1.80 |
| 27 | Lycopersicon esculentum | 3.75 | 7.00 | 6.25 | 1.50 | 2.00 | 1.90 |
| 28 | Maranta arundinacea | 4.00 | 7.50 | 6.00 | 1.50 | 2.60 | 2.00 |
| 29 | Monochoria vaginalis | 4.50 | 8.85 | 6.25 | 1.30 | 3.30 | 2.50 |
| 30 | Musa paradisiaca | 3.60 | 7.15 | 5.00 | 1.30 | 1.80 | 1.50 |
| 31 | Mushroom bed | 2.70 | 4.50 | 3.40 | 1.30 | 1.80 | 1.50 |
| 32 | Panicum maximum | 3.58 | 6.25 | 5.00 | 1.70 | 3.30 | 2.30 |
| 33 | Pennisetum polystachyon | 5.40 | 8.95 | 7.20 | 1.50 | 2.50 | 2.30 |
| 34 | Phaseolus aureus | 4.50 | 9.50 | 7.50 | 1.40 | 2.00 | 1.70 |
| 35 | Piper betle | 2.70 | 6.25 | 4.70 | 1.60 | 2.50 | 2.10 |
| 36 | Salvinia molesta | 3.75 | 8.00 | 7.15 | 2.90 | 1.30 | 2.00 |
| 37 | Sesamum indicum | 7.00 | 12.50 | 10.25 | 3.10 | 1.30 | 2.00 |
| 38 | Sesbania aculeata | 7.15 | 9.50 | 9.00 | 1.50 | 2.60 | 2.00 |
| 39 | Sporobolus diander | 7.15 | 4.50 | 6.25 | 1.70 | 3.40 | 2.40 |
| 40 | Stylosanthes humilis | 3.60 | 8.00 | 7.15 | 1.40 | 2.20 | 1.70 |
| 41 | Vigna unguiculata | 3.60 | 6.25 | 4.70 | 1.20 | 2.30 | 1.80 |

Table 2. Growth rate and time required for sclerotial initiation and maturation of different isolates of Rhizoctonia solani on Potato-dextro agar medium

| Isolate No. | Host/habitat | Days for completion of growth | Days for appearance of sclerotial initials | Days for sclerotial maturation |
|---|---|---|---|---|
| 1 | Oryza sativa (Vellayani) | 2 | 3 | 4 |
| 2 | Oryza sativa (Adoor) | 2 | 4 | 5 |
| 3 | Oryza sativa (Alathur) | 3 | 4 | 5 |
| 4 | Oryza sativa (Anakayam) | 2 | 3 | 4 |
| 5 | Oryza sativa (Chirayinkil) | 3 | 3 | 4 |
| 6 | Oryza sativa (Karamana) | 3 | 3 | 4 |
| 7 | Oryza sativa (Karimanoor) | 3 | 4 | 5 |
| 8 | Oryza sativa (Kayamkulam) | 2 | 3 | 4 |
| 9 | Oryza sativa (Kottarakkara) | 2 | 3 | 4 |
| 10 | Oryza sativa (Manmuthy) | 3 | 3 | 4 |
| 11 | Oryza sativa (Moncompu) | 2 | 3 | 4 |
| 12 | Oryza sativa (Okkal) | 2 | 3 | 4 |
| 13 | Oryza sativa (Pattambi) | 2 | 3 | 4 |
| 14 | Oryza sativa (Perambra) | 2 | 3 | 4 |
| 15 | Oryza sativa (Veeyapuram) | 3 | 3 | 4 |
| 16 | Oryza sativa (Vellayani - leaf specimen) | 3 | 4 | 5 |
| 17 | Oryza sativa (Vengad) | 2 | 3 | 4 |
| 18 | Amorphophallus campanulatus | 8 | 12 | 14 |
| 19 | Arachis hypogaea (collar) | 5 | 7 | 11 |
| 20 | Arachis hypogaea (leaf) | 3 | 3 | 4 |

Table 2. Contd.

| Isolate No. | Host/habitat | Days for completion of growth | Days for appearance of sclerotial initials | Days for sclerotial maturation |
|---|---|---|---|---|
| 21 | Catharanthus roseus | 4 | 7 | 11 |
| 22 | Colocasia esculenta | 3 | 3 | 4 |
| 23 | Cymbopogon flexuosus | 3 | 3 | 4 |
| 24 | Cynodon dactylon | 3 | 3 | 4 |
| 25 | Daucus carota | 4 | 6 | 8 |
| 26 | Glycine max | 5 | 7 | 11 |
| 27 | Lycopersicon esculentum | 5 | 9 | 11 |
| 28 | Maranta arundinacea | 4 | 8 | 12 |
| 29 | Monochoria vaginalis | 2 | 3 | 4 |
| 30 | Musa paradisiaca | 4 | 8 | 10 |
| 31 | Mushroom bed | 4 | 5 | 8 |
| 32 | Panicum maximum | 2 | 3 | 5 |
| 33 | Pennisetum polystachyon | 3 | 4 | 5 |
| 34 | Phaseolus aureus | 4 | 7 | 11 |
| 35 | Piper betle | 5 | 7 | 11 |
| 36 | Salvinia molesta | 4 | 8 | 11 |
| 37 | Sesamum indicum | 4 | 7 | 11 |
| 38 | Sesbania aculeata | 2 | 3 | 4 |
| 39 | Sporobolus diander | 2 | 3 | 4 |
| 40 | Stylosanthes humilis | 4 | 8 | 11 |
| 41 | Vigna unguiculata | 4 | 6 | 11 |

completion of growth ranged from 0 to 24 h between rice isolates and isolates 20, 22, 23, 24, 29, 32, 33, 38 and 39. Similar variation in time required for sclerotial initiation and maturation of these isolates were observed. All other isolates required longer time for completion of growth, sclerotial initiation and maturation.

Based on the similarity of morphological and cultural characters the different isolates of Rhizoctonia solani were grouped into 4 morphological groups, MG 1, MG 2, MG 3 and MG 4 (Table 3, Fig. 6). The general characters of each group are as follows:-

MG 1. Mycelium subaerial, nonfluffy and appressed to substrate. Colour ranging from light brown to dark brown. Hyphal width ranging from 4.75 /um to 13.5 /um. Sclerotia scattered irregularly but tending to concentrate towards the periphery to form clusters and bands. Sclerotium subglobose, surface regular and rough with and without honey dew formation. Colour dark brown and size ranging from 1.8 mm to 2.7 mm (Fig. 7a).

MG 2. Mycelium radiating, subaerial and scattered individually. White coloured and hyphal width ranging from 3.4 /um to 7.5 /um. Abundant sclerotial formation concentrating towards the

Table 3. Morphological grouping of forty one isolates of Rhizoctonia solani

| Morphological group | Host/habitat | Source | Mycelial character on maturity | Sclerotial character on maturity |
|---|---|---|---|---|
| MG 1 | Arachis hypogaea | Leaf | Mycelium subaerial non-fluffy and appressed to substrate. Colour ranging from light brown to dark brown. Hyphal width ranging from 4.5/um to 13.5/um. | Sclerotia scattered irregularly but tending to concentrate towards the periphery to form clusters and bands. Sclerotium subglobose, surface regular and rough with and without honey dew formation. Colour dark brown and size ranging from 1.8 mm to 2.7 mm. |
| | Colocasia esculenta | Petiole | | |
| | Cymbopogon flexuosus | Sheath | | |
| | Cynodon dactylon | Leaf | | |
| | Monochoria vaginalis | Leaf | | |
| | Oryza sativa (17 isolates) | Sheath & leaf | | |
| | Panicum maximum | Sheath | | |
| | Pennisetum polystachyon | Sheath | | |
| | Sesbania aculeata | Leaf | | |
| | Sporobolus diander | Leaf | | |
| MG 2 | Daucus carota | Tuber | Mycelium radiating subaerial and scattered individually. White coloured and hyphal width ranging from 3.4/um to 7.5/um. | Abundant sclerotial formation concentrating towards the periphery of the culture and sides of plate. Sclerotium globose, surface regular and smooth. Size ranging from 1.5 mm to 1.9 mm and reddish brown to dark brown in colour. |
| | Lycopersicon esculentum | Fruit | | |
| | Maranta arundinacea | Tuber | | |
| | Musa paradisiaca | Rhizome | | |
| | Mushroom bed | Paddy straw | | |

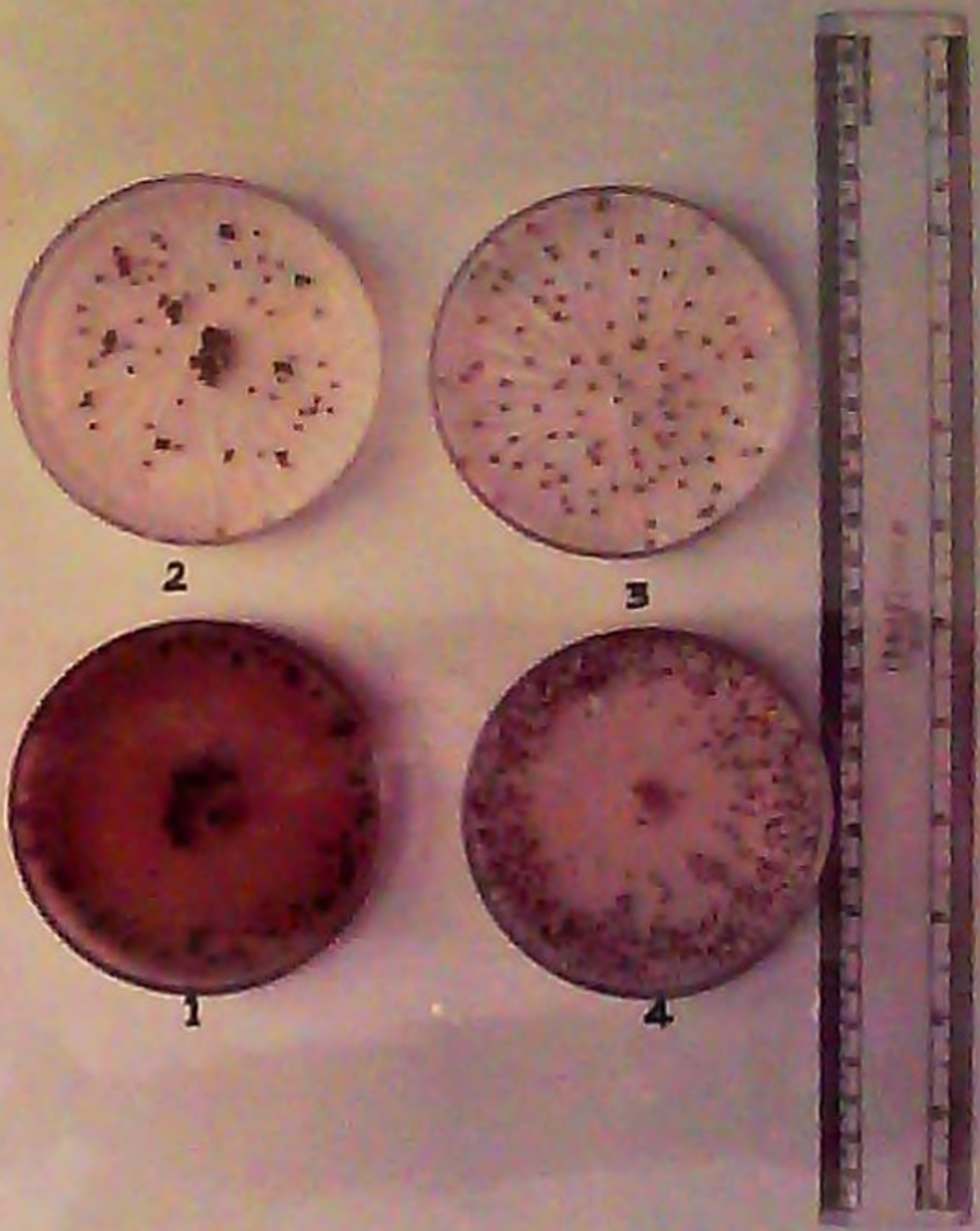
Table 3. Contd.

| Morpho-logical group | Host/habitat | Source | Mycelial character on maturity | Sclerotial character on maturity |
|---|---|---|---|---|
| MG 3 | Catharanthus roseus | Collar region | Mycelium radiating aerial, hyphae scattered and fluffy to thick aggregates towards the margin. White in colour and hyphal width ranging from 6 /um to 10.2 /um. | Sclerotia distributed almost uniformly in plate. Sclerotium globose to irregular. Surface regular and smooth. Colour ranging from dark orange to brown. |
| | Phaseolus aureus | Collar region | | |
| | Salvinia molesta | Leaf | | |
| | Sesamum indicum | Collar region | | |
| | Stylosanthes humilis | Collar region | | |
| MG 4 | Amorphophallus campanulatus | Leaf | Mycelium fluffy, cottony growth and white in colour. Hyphal width ranging from 4 /um to 6.5 /um. | Sclerotial formation not abundant and distributed irregularly in clumps. Sclerotium globose to oblong, surface irregular and smooth. Colour yellowish brown to brown. Size ranging from 1.9 mm to 2.0 mm. |
| | Arachis hypogaea | Collar region | | |
| | Glycine max | Collar region | | |
| | Piper betle | Leaf | | |
| | Vigna unguiculata | Collar region | | |

Fig. 6. Morphological groups (MG) of *Rhizoctonia solani*

Representative isolates of morphological groups

1. From *Oryza sativa* (MG 1)
2. From *Vigna unguiculata* (MG 4)
3. From *Sesamum indicum* (MG 3)
4. From Mushroom bed (MG 2)


2
3
1
4

periphery of the culture and sides of dish. Sclerotium globose, surface regular and smooth. Size ranging from 1.5 mm to 1.9 mm and reddish brown to dark brown in colour (Fig. 7b).

MG 3. Mycelium radiating, aerial, hyphae scattered and fluffy to thick aggregates towards the margin. White in colour and hyphal width ranging from 6 /um to 10.2 /um. Sclerotia distributed almost uniformly in dish. Sclerotium globose to irregular. Surface regular and smooth. Colour ranging from dark orange to brown (Fig. 7c).

MG 4. Mycelium fluffy, cottony growth and white in colour. Hyphal width ranging from 4 /um to 6.5 /um. Sclerotial formation not abundant and distributed irregularly in clumps. Sclerotium globose to oblong. Surface irregular and smooth. Colour yellowish brown to brown. Size ranging from 1.9 mm to 2.0 mm (Fig. 7d).

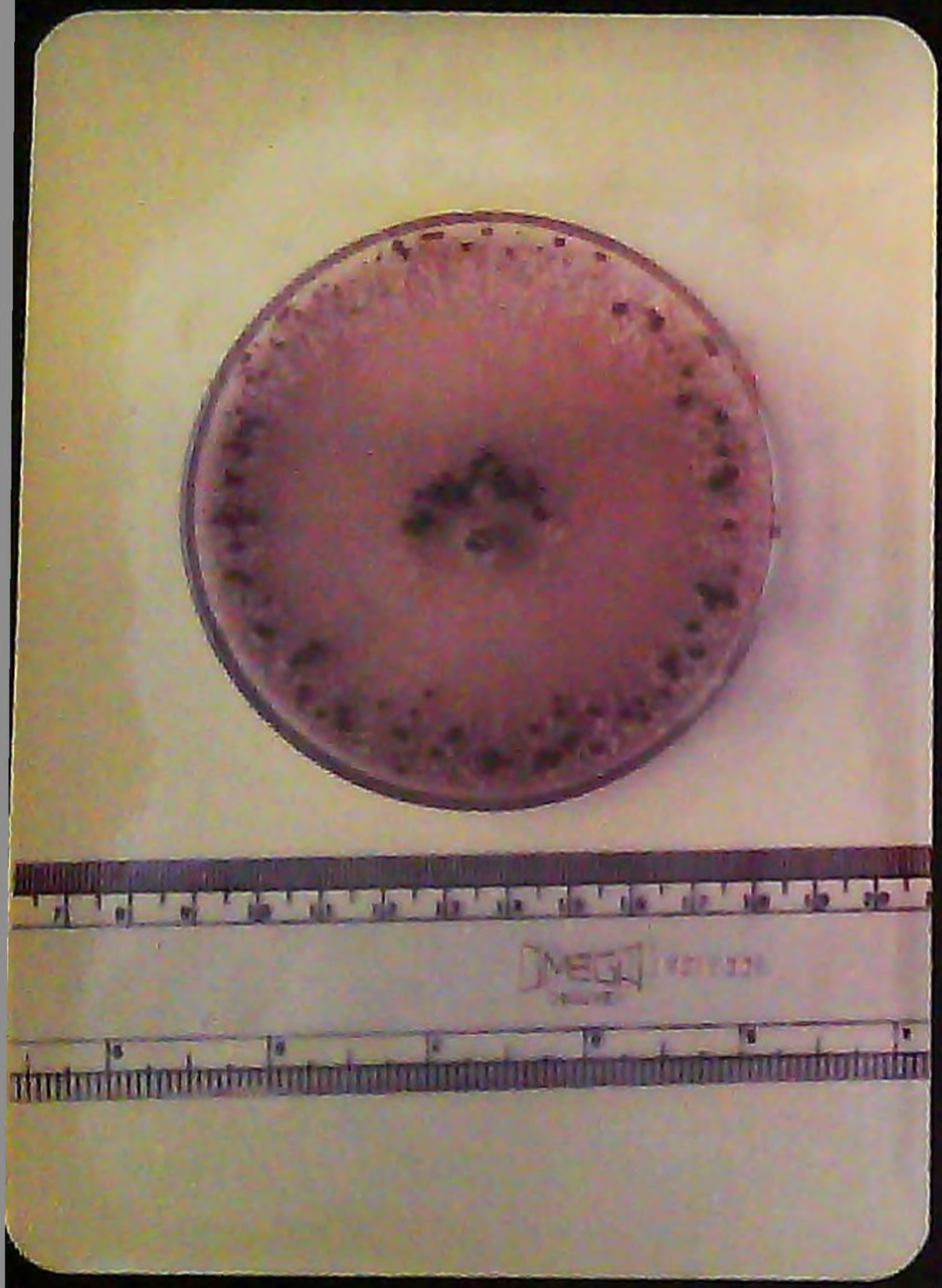
## VII. PATHOGENICITY OF RICE ISOLATE (ISOLATE 1 ) ON THE OTHER HOST PLANTS

Rhizoctonia solani from rice (Isolate 1) when inoculated on the other 22 host plants took infection with symptom development on the following host plants, fruits and tubers.

Fig. 7. Growth habit of representative isolates of *Rhizoctonia solani* from each morphological group

a. MG 1 - Isolate from *Oryza sativa*

b. MG 2 - Isolate from Mushroom bed



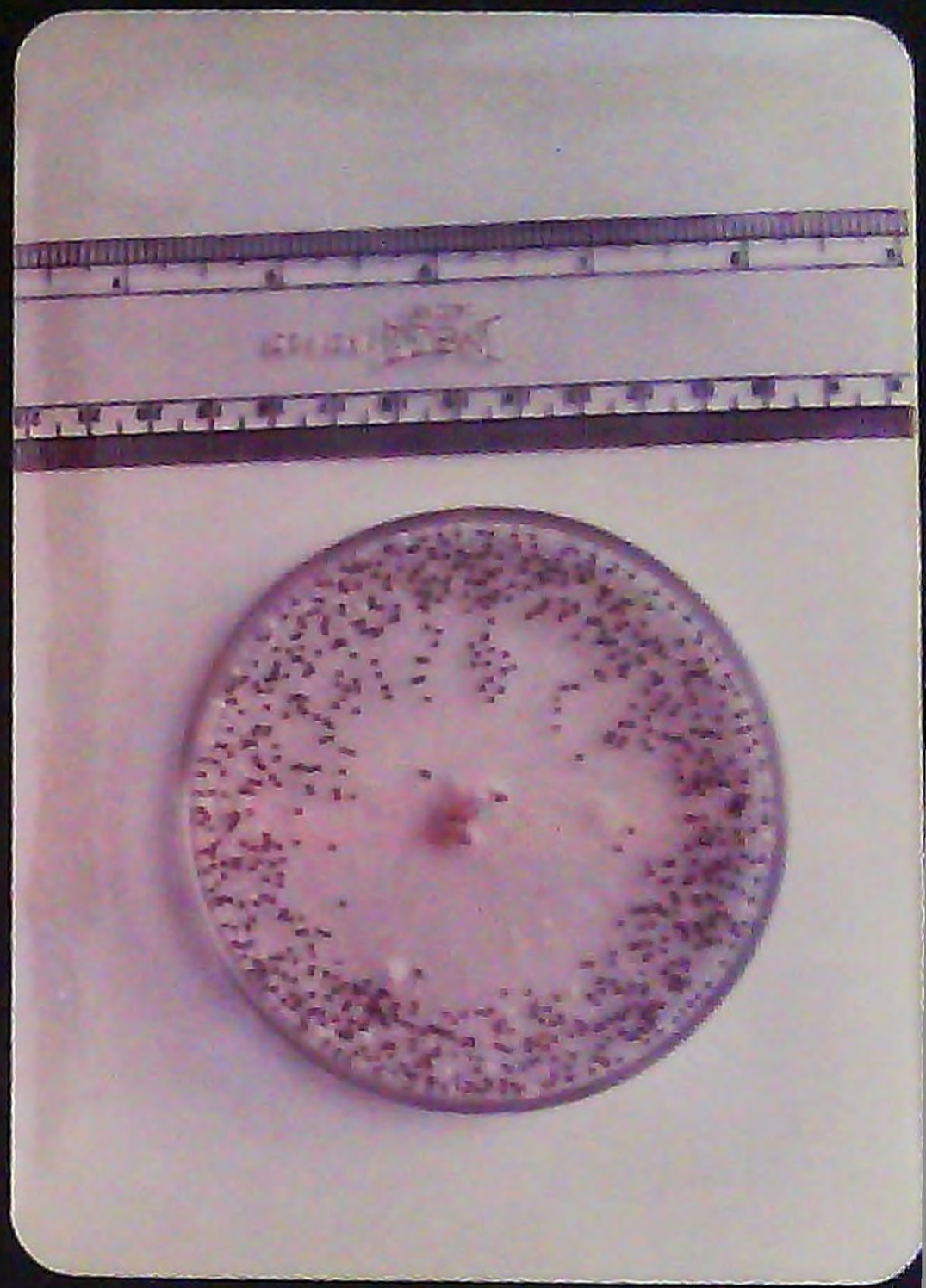
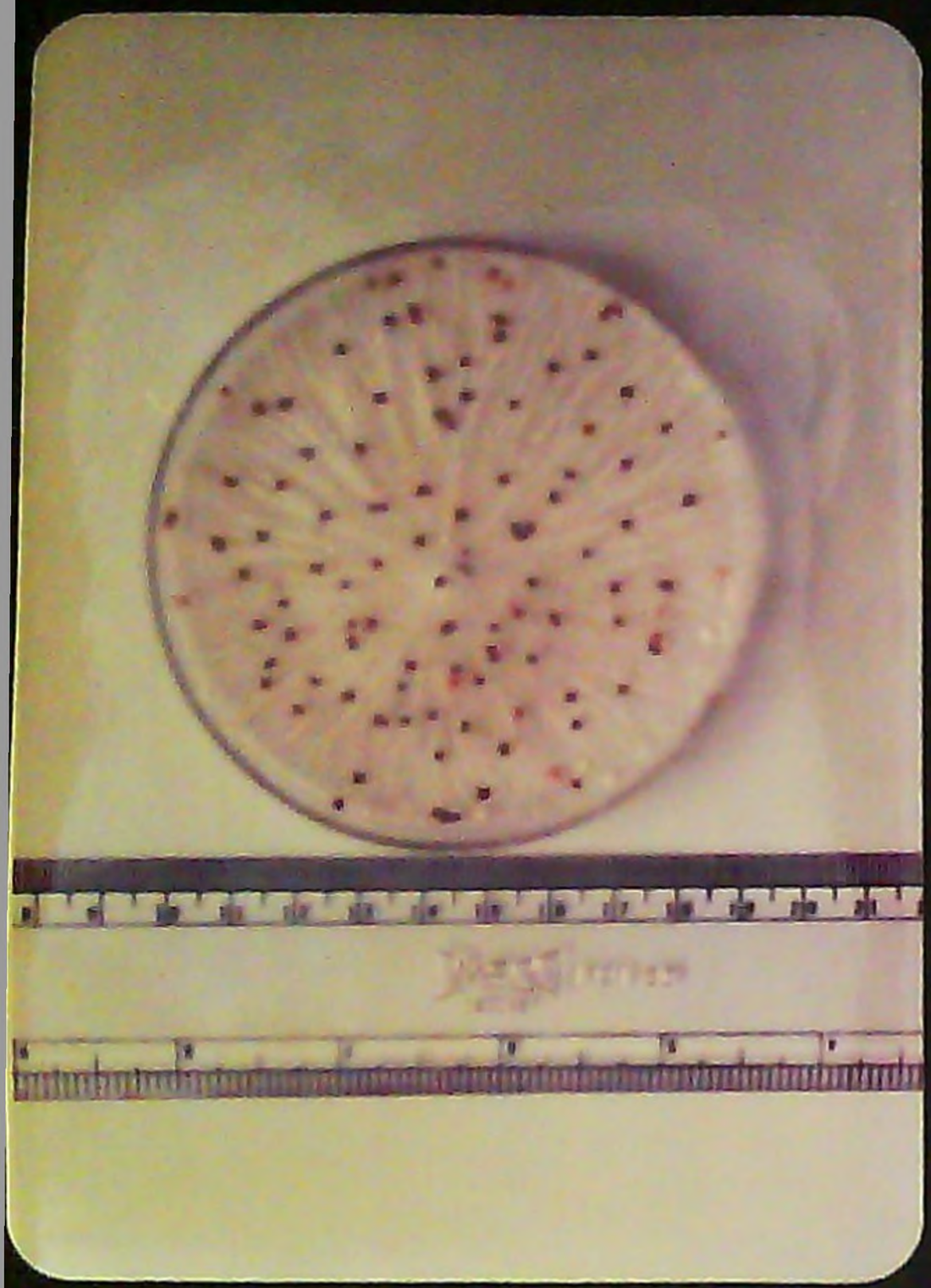


Fig. 7 Contind.

c. MG 3 - Isolate from Sesamum indicum

d. MG 4 - Isolate from Vigna unguiculata



1. Amorphophallus campanulata Brown water soaked lesions appeared resulting in leaf rot.

2. Arachis hypogaea Collar region did not take infection. Web blight symptoms were produced on the leaves.

3. Colocasia esculenta The leaves were infected leading to leaf rot.

4. Cymbopogon flexuosus The inoculated sheath produce lesion of oblong shape and light brownish grey colour. Lesion failed to spread to the leaves.

5. Cynodon dactylon Greyish white irregular lesions were formed on the inoculated leaves.

6. Daucus carota Rotting of the tubers with white mycelial growth of the fungus.

7. Glycine max Collar rot symptoms did not occur. Leaves were infected causing leaf blight.

8. Lycopersicon esculentum Rotting of fruits with exudation.

9. Monochoria vaginalis Severe rotting of the leaves and stem occurred. Sclerotial formation seen on the infected parts.

10. Panicum maximum Greyish green lesions formed on the inoculated leaf sheath

11. Pennisetum polystachyon Dull whitish brown lesions restricted to the inoculated leaf sheath.

12. Phaseolus aureus The leaves were infected causing web blight symptoms. Collar rot symptom did not develop.

13. Piper betle Inoculated leaves produced leaf spots resulting in the rotting of the leaves.

14. Salvinia molesta Water soaked lesions appeared resulting in leaf rot.

15. Sesamum indicum Leaf spot symptoms appeared killing the young plants. But no collar rot symptom was produced.

16. Sesbania aculeata Browning of the infected leaves and defoliation occurred.

17. Sporobolus diander Inoculated leaf sheath produced irregular lesions spreading to the leaf blight.

18. Stylosanthes humilis Leaf spot symptoms followed by leaf blighting of inoculated plants were seen.

19. Vigna unguiculata Collar rot symptoms failed to occur on mature plants. But leaves were infected causing leaf

blight. Seedlings of up to 3 weeks were readily infected at the collar region to produce typical collar rot symptoms.

Monochoria vaginalis and Sporobolus diander were found to be very susceptible to infection and symptom development occurred very rapidly (Figs. 8 & 9). On Arachis hypogaea, Glycine max and Vigna unguiculata typical web blight symptoms were produced, the collar region remaining free of infection. Cowpea seedlings were susceptible to infection up to 3 weeks of age when inoculated at the collar region, resulting in severe collar rot (Fig. 10). Infection did not occur on the following host plants.

1. Catharanthus roseus
2. Maranta arundinacea
3. Musa paradisiaca

## VIII. PATHOGENICITY OF ISOLATES FROM OTHER HOST/HABITAT ON RICE

The isolates of Rhizoctonia solani from the following host plants could infect rice of the variety Jyothi on artificial inoculation.

1. Arachis hypogaea
2. Colocasia esculenta
3. Cymbopogon flexuosus

Fig. 8. Symptoms produced on leaf of Monochoria vaginalis on inoculation with rice isolate of Rhizoctonia solani.

Fig. 9. Symptoms produced on leaf sheath and leaves of Sporobolus diander on inoculation with rice isolate of Rhizoctonia solani

Fig. 10. Collar rot of cowpea seedling on inoculation with rice isolate of *Rhizoctonia solani*

4. Cynodon dactylon
5. Monochoria vaginalis
6. Pennisetum polystachyon
7. Panicum maximum
8. Sesbania aculeata
9. Sporobolus diander

The extent of infection by these isolates on rice is given in Table 4. Maximum infection is produced by the isolate from Sporobolus diander (Isolate 39) followed by Monochoria vaginalis isolate (Isolate 29). The morphological and cultural characters of these isolates are presented in Table 5 (Fig. 11).

## IX. ANASTOMOSIS STUDIES

### a. Testing for anastomosis (hyphal fusion) between rice isolates of Rhizoctonia solani

The different isolates of Rhizoctonia solani obtained from rice (17 isolates) when tested for anastomosis showed hyphal fusion between the isolates in all possible combinations.

### b. Testing for anastomosis (hyphal fusion) between rice isolate (Isolate 1) and isolates of Rhizoctonia solani from other host/habitat

Rice isolate (Isolate 1) when paired with isolates of the 23 hosts/habitat showed hyphal fusion with the following isolates (Table 6a).

Table 4. Extent of infection of isolates of *Rhizoctonia solani* from other host plants inoculated on rice variety Jyothi

| Isolate No. | Host/habitat | Length of infection on sheath (mm) | Infection on leaf |
|---|---|---|---|
| 20 | *Arachis hypogaea* | 65 | + |
| 22 | *Colocasia esculenta* | 35 | |
| 23 | *Cymbopogon flexuosus* | 30 | |
| 24 | *Cynodon dactylon* | 40 | |
| 29 | *Monochoria vaginalis* | 70 | + |
| 32 | *Panicum maximum* | 30 | |
| 33 | *Pennisetum polystachyon* | 30 | |
| 38 | *Sesbania aculeata* | 30 | |
| 39 | *Sporobolus diander* | 80 | + |

\+ Positive reaction

Table 5. Morphological and cultural characters of 10 isolates of AG 1 group of *Rhizoctonia solani* infecting rice

| Isolate No. | Host | Nature of mycelial growth | Shape of sclerotia | Density and distribution |
|---|---|---|---|---|
| 20 | *Arachis hypogaea* | Mycelium subaerial, nonfluffy and appressed to substrate. | Sclerotia flat, tending to elongate with rough irregular surface. | Sclerotia concentrated densely and regularly towards the periphery. Sclerotial initials scattered throughout. |
| 22 | *Colocasia esculenta* | Mycelium subaerial, nonfluffy and appressed to substrate. | Sclerotia subglobose, regular with rough surface. | Sclerotia distributed throughout. |
| 23 | *Cymbopogon flexuosus* | Subaerial, nonfluffy and appressed to substrate. | Sclerotia subglobose, regular with rough surface. | Sclerotia irregularly distributed in clumps towards the periphery. |
| 24 | *Cynodon dactylon* | Mycelium subaerial nonfluffy and appressed to substrate. | Sclerotia subglobose, regular with rough surface. | Sclerotia distributed irregularly. |
| 29 | *Monochoria vaginalis* | Mycelium subaerial nonfluffy and appressed to substrate. | Sclerotia subglobose, regular with rough surface. | Sclerotia concentrated densely and regularly towards the periphery. |

Table 5. Contd.

| Isolate No. | Host | Nature of mycelial growth | Shape of sclerotia | Density and distribution |
|---|---|---|---|---|
| 1 | Oryza sativa | Mycelium subaerial nonfluffy and appressed to subatrate. | Sclerotia subglobose, regular with rough surface | Sclerotia concentrated densely and regularly towards the periphery. |
| 32 | Panicum maximum | Mycelium subaerial nonfluffy and appressed to substrate. | Sclerotia subglobose, surface rough and regular. | Sclerotia scanty and distributed irregularly throughout the culture. |
| 33 | Pennisetum polystachyon | Mycelium subaerial, nonfluffy and appressed to substrate. | Sclerotia subglobose, surface rough and regular. | Sclerotia distributed irregularly throughout the culture and scanty. |
| 38 | Sesbania aculeata | Mycelium subaerial nonfluffy and appressed to substrate | Sclerotia subglobose, regular and surface rough. | Sclerotia abundantly distributed throughout more towards periphery. |
| 39. | Sporobolus diander | Mycelium subaerial, nonfluffy and appressed to substrate. | Sclerotia subglobose tending to elongate with rough regular surface. | Sclerotia concentrated densely and regularly towards the periphery. |

Table 5. Contd.

| Isolate No. | Host | Nature of mycelial growth | Shape of sclerotia | Density and distribution |
|---|---|---|---|---|
| 1 | Oryza sativa | Mycelium subaerial nonfluffy and appressed to subatrate. | Sclerotia subglobose, regular with rough surface | Sclerotia concentrated densely and regularly towards the periphery. |
| 32 | Panicum maximum | Mycelium subaerial nonfluffy and appressed to substrate. | Sclerotia subglobose, surface rough and regular. | Sclerotia scanty and distributed irregularly throughout the culture. |
| 33 | Pennisetum polystachyon | Mycelium subaerial, nonfluffy and appressed to substrate. | Sclerotia subglobose, surface rough and regular. | Sclerotia distributed irregularly throughout the culture and scanty. |
| 38 | Sesbania aculeata | Mycelium subaerial nonfluffy and appressed to substrate | Sclerotia subglobose, regular and surface rough. | Sclerotia abundantly distributed throughout more towards periphery. |
| 39. | Sporobolus diander | Mycelium subaerial, nonfluffy and appressed to substrate. | Sclerotia subglobose tending to elongate with rough regular surface. | Sclerotia concentrated densely and regularly towards the periphery. |

Fig. 11. Growth habit of ten isolates of Rhizoctonia solani of morphological group 1.

Isolates of Rhizoctonia solani from -

1. Arachis hypogaea
2. Colocassia esculenta
3. Cymbopogon flexuosus
4. Cynodon doctylon
5. Monochoria vaginalis
6. Oryza sativa
7. Panicum maximum
8. Pennisetum polystachyon
9. Sesbania aculeata
10. Sporobolus diander


1
2
3
4
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8
9
10

Table 6a. Anastomosis between rice isolate of Rhizoctonia solani (Isolate 1) and other isolates of MG 1, MG 2, MG 3 and MG 4.

| Isolate No. | Host/habitat | Rice isolate (Isolate 1) |
|---|---|---|
| 18 | Amorphophallus campanulatus | - |
| 19 | Arachis hypogaea (collar) | - |
| 20 | Arachis hypogaea (leaf) | + |
| 21 | Catharanthus roseus | - |
| 22 | Colocasia esculenta | + |
| 23 | Cymbopogon flexuosus | + |
| 24 | Cynodon dactylon | + |
| 25 | Daucus carota | - |
| 26 | Glycine max | - |
| 27 | Lycopersicon esculentum | - |
| 28 | Maranta arundinacea | - |
| 29 | Monochoria vaginalis | + |
| 30 | Musa paradisiaca | - |
| 31 | Mushroom bed | - |
| 32 | Panicum maximum | + |
| 33 | Pennisetum polystachyon | + |
| 34 | Phaseolus aureus | - |
| 35 | Piper betle | - |
| 36 | Salvinia molesta | - |
| 37 | Sesamum indicum | - |
| 38 | Sesbania aculeata | + |
| 39 | Sporobolus diander | + |
| 40 | Stylosanthes humilis | - |
| 41 | Vigna unguiculata | - |

\+ Hyphal fusion.

| Isolate No. | Host |
|---|---|
| 20 | Arachis hypogaea |
| 22 | Colocasia esculenta |
| 23 | Cymbopogon flexuosus |
| 24 | Cynodon dactylon |
| 29 | Monochoria vaginalis |
| 32 | Panicum maximum |
| 33 | Pennisetum polystachyon |
| 38 | Sesbania aculeata |
| 39 | Sporobolus diander |

Isolates from the above host plants showed hyphal fusion in all possible combinations (Table 6b).

None of the isolates of the other host/habitat could anastomose with the rice isolate.

c. Anastomosis grouping of rice group of Rhizoctonia solani

The four AG tester isolates obtained from Dr. E. Butler were maintained in Potato-dextrose agar medium test tube slants for testing for fusion with the Rhizoctonia solani isolates of the rice group. The morphological and cultural characters of each of the AG tester isolate recorded were as follows:-

AG 1. Mycelium dark reddish brown, sparsely aerial. Sclerotia moderately abundant with large sclerotia on agar

Table 6b. Anastomosis between different combinations of *Rhizoctonia solani* of the MG 1 group

| Sl. No. | Combination of isolates | Hyphal fusion |
|---|---|---|
| 1 | *Arachis hypogaea* (leaf) x *Colocasia esculenta* | + |
| 2 | *Arachis hypogaea* (leaf) x *Cymbopogon flexuosus* | + |
| 3 | *Arachis hypogaea* (leaf) x *Cynodon dactylon* | + |
| 4 | *Arachis hypogaea* (leaf) x *Monochoria vaginalis* | + |
| 5 | *Arachis hypogaea* (leaf) x *Panicum maximum* | + |
| 6 | *Arachis hypogaea* (leaf) x *Pennisetum polystachyon* | + |
| 7 | *Arachis hypogaea* (leaf) x *Sesbania aculeata* | + |
| 8 | *Arachis hypogaea* (leaf) x *Sporobolus diander* | + |
| 9 | *Colocasia esculenta* x *Cymbopogon flexuosus* | + |
| 10 | *Colocasia esculenta* x *Cynodon dactylon* | + |
| 11 | *Colocasia esculenta* x *Monochoria vaginalis* | + |
| 12 | *Colocasia esculenta* x *Panicum maximum* | + |
| 13 | *Colocasia esculenta* x *Pennisetum polystachyon* | + |
| 14 | *Colocasia esculenta* x *Sesbania aculeata* | + |
| 15 | *Colocasia esculenta* x *Sporobolus diander* | + |
| 16 | *Cymbopogon flexuosus* x *Cynodon dactylon* | + |
| 17 | *Cymbopogon flexuosus* x *Monochoria vaginalis* | + |
| 18 | *Cymbopogon flexuosus* x *Panicum maximum* | + |
| 19 | *Cymbopogon flexuosus* x *Pennisetum polystachyon* | + |
| 20 | *Cymbopogon flexuosus* x *Sesbania aculeata* | + |
| 21 | *Cymbopogon flexuosus* x *Sporobolus diander* | + |
| 22 | *Cynodon dactylon* x *Monochoria vaginalis* | + |
| 23 | *Cynodon dactylon* x *Panicum maximum* | + |
| 24 | *Cynodon dactylon* x *Pennisetum polystachyon* | + |
| 25 | *Cynodon dactylon* x *Sesbania aculeata* | + |
| 26 | *Cynodon dactylon* x *Sporobolus diander* | + |
| 27 | *Monochoria vaginalis* x *Panicum maximum* | + |
| 28 | *Monochoria vaginalis* x *Pennisetum polystachyon* | + |
| 29 | *Monochoria vaginalis* x *Sesbania aculeata* | + |
| 30 | *Monochoria vaginalis* x *Sporobolus diander* | + |
| 31 | *Panicum maximum* x *Pennisetum polystachyon* | + |
| 32 | *Panicum maximum* x *Sesbania aculeata* | + |
| 33 | *Panicum maximum* x *Sporobolus diander* | + |
| 34 | *Pennisetum polystachyon* x *Sesbania aculeata* | + |
| 35 | *Pennisetum polystachyon* x *Sporobolus diander* | + |
| 36 | *Sesbania aculeata* x *Sporobolus diander* | + |

\+ Positive reaction.

surface. Subglobose with aggregation into compound sclerotia. Surface rough and woolly.

AG 2. Mycelium light to dark brown appressed to moderately aerial. Uniform colour over the colony except for occasional concentric zonation. Sclerotia moderate on agar surface. Sclerotia round, surface woolly and colour similar to mycelial colour.

AG 3. Mycelium dark chocolate brown in colour. Slightly aerial sclerotia, round in shape and embedded in agar. Surface woolly, loose and colour similar to mycelial colour.

AG 4. Mycelium whitish to light brown, compact with conspicuous brown runner hyphae radiating from the inoculated disc. Sclerotia few in number, small and flat. Surface crusty, grey to nearly black.

AG 1, AG 2, AG 3 and AG 4 tester isolates when paired in all possible combinations with the rice group of Rhizoctonia solani isolates revealed that all isolates could anastomose with AG 1 but not with AG 2, AG 3 and AG 4 (Table 6c).

Inoculation of the tester isolates on rice plants of the variety Jyothi showed that only AG 1 was pathogenic, causing typical sheath blight disease. Reisolation yielded the fungus

Table 6c. Anastomosis between rice isolate of *Rhizoctonia solani* and the different AG tester isolates

| Sl. No. | Rice isolates | AG 1 | AG 2 | AG 3 | AG 4 |
|---|---|---|---|---|---|
| 1 | Adoor | + | - | - | - |
| 2 | Alathur | + | - | - | - |
| 3 | Anakayam | + | - | - | - |
| 4 | Chirayinkil | + | - | - | - |
| 5 | Karamana | + | - | - | - |
| 6 | Karimanoor | + | - | - | - |
| 7 | Kayamkulam | + | - | - | - |
| 8 | Kottarakkara | + | - | - | - |
| 9 | Mannuthy | + | - | - | - |
| 10 | Moncompu | + | - | - | - |
| 11 | Okkal | + | - | - | - |
| 12 | Pattambi | + | - | - | - |
| 13 | Perambra | + | - | - | - |
| 14 | Veeyapuram | + | - | - | - |
| 15 | Vellayani | + | - | - | - |
| 16 | Vellayani (leaf) | + | - | - | - |
| 17 | Vengad | + | - | - | - |

+ Hyphal fusion.

Fig. 12. Perfect fusion between hyphae in <u>Rhizoctonia solani</u>

a. Between two isolates

b. Within the same isolate
(Self-anastomosis)

which showed similar anastomosis reaction with the MG 1 isolates of Rhizoctonia solani.

d. Cytology of hyphal fusion in Rhizoctonia solani

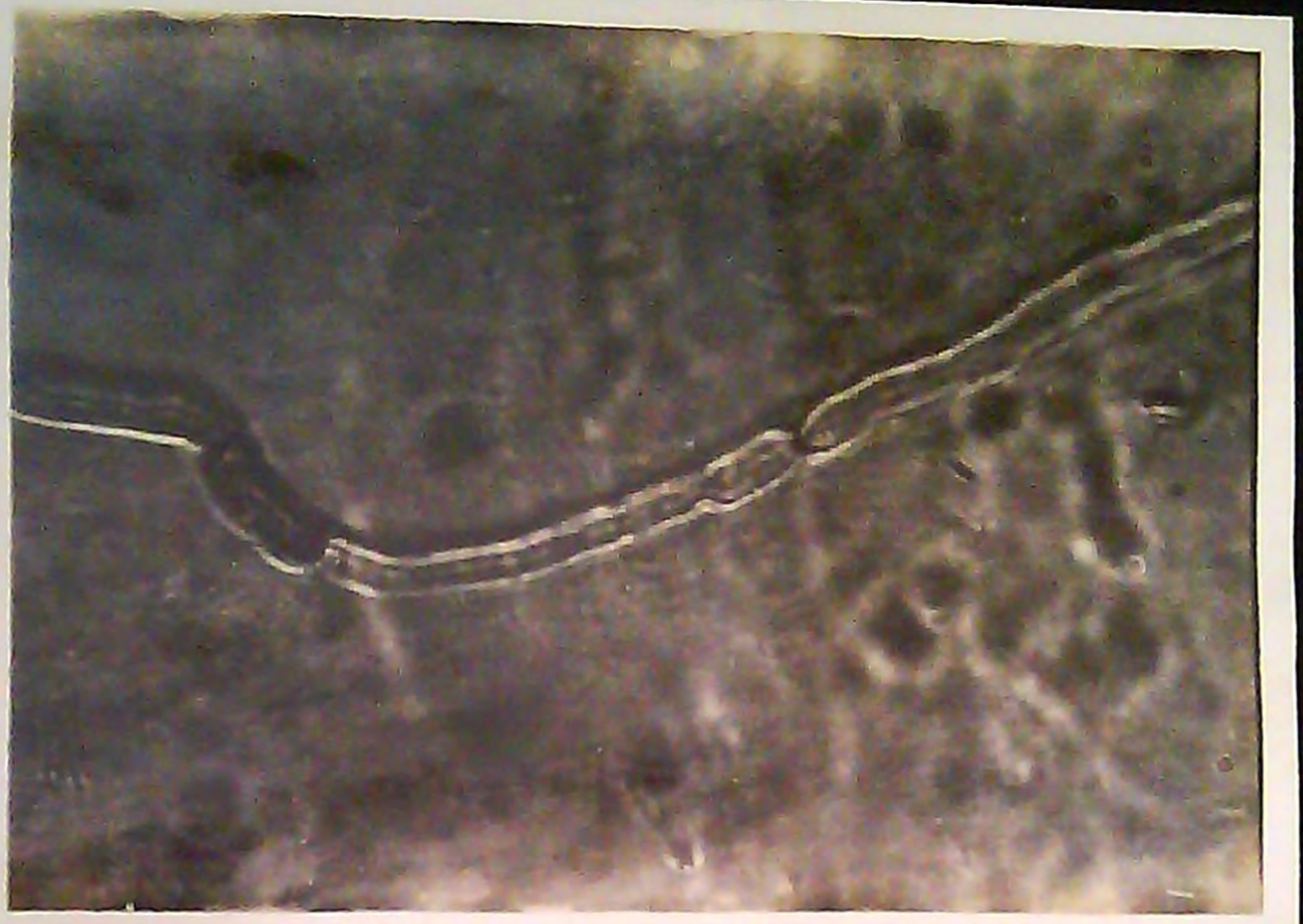
Two main kinds of hyphal fusion were observed viz., perfect fusion and imperfect fusion. In perfect fusion, immediately after hyphal contact cell wall fusion occurred. This was followed by cytoplasmic fusion leading to a successful anastomosis; cytoplasmic exchange freely occurred after a perfect fusion (Fig. 12 a,b). Perfect fusion was invariably seen in self-anastomosis within an isolate and occurred sparingly between isolates of the same anastomosis group.

Imperfect fusion occurred between two isolates of the same anastomosis group. Immediately after cell wall fusion, killing reaction occurred. This reaction was characterised by disintegration of the cytoplasm of the fused cells leading to its death along with a few adjacent cells. The affected cells showed vacuolation typifying the killing reaction (Fig. 13). Here cytoplasmic fusion did not occur.

A third kind of fusion observed during anastomosis of isolates of the same anastomosis group (rice group) was contact fusion. The contact fusion showed only hyphal contact without further hyphal growth, cell wall fusion and cytoplasmic fusion which is followed by killing reaction (Fig. 14).

Fig. 13. Imperfect fusion between hyphae of two isolates of Rhizoctonia solani showing killing reaction. (Vacuolation of dead cells)

Fig. 14. Contact fusion between hyphae of two isolates of Rhizoctonia solani followed by vacuolation of cells.



The different types of hyphal fusion predominantly observed during anastomosis between two isolates of Rhizoctonia solani were:-

i. Tip to tip fusion

The tips of the approaching hyphae of the opposing isolates while reaching each other at a proximity of 7 - 15 $\mu$m, inclined towards each other and quickly made contact. This was followed by the flattening of the hyphal tips leading to anastomosis (Fig. 15a).

ii. Tip to side wall fusion

The tip of the hypha of the opposing isolate coming into contact with the side wall of the opposite isolate flattened leading to anastomosis (Fig. 15b).

iii. Peg to side wall fusion

The hyphae of two opposing isolates when laid adjacent to each other at very close proximity of about 7 - 15 $\mu$m, a peg like branch without a separating septum projected from one of the hyphae to contact the other. Flattening of the peg at the point of contact occurred leading to anastomosis (Fig. 15c).

FIG. 15 DIFFERENT TYPES OF HYPHAL FUSION DURING ANASTOMOSIS

a. TIP TO TIP FUSION

b. TIP TO SIDE WALL FUSION

C. PEG TO SIDE WALL FUSION

80 μ

d. SIDE WALL TO SIDE WALL FUSION

iv. Side wall to side wall fusion

The side wall of two hyphae when laid in contact to each other resulted in the dissolution of the side wall at the point of contact leading to anastomosis (Fig. 15d).

## X. DETERMINATION OF THE PRESENCE OF CLONES OF RHIZOCTONIA SOLANI CAUSING SHEATH BLIGHT OF RICE

The specimens of sheath blight disease obtained from the different locations were examined for variation in symptoms.

Location 1 Severe infection on sheath. Lesion oblong to circular with dark narrow margin and greyish centre. Lesions coalescing.

Location 2 Lesion elongated with thin light brown margin and greenish grey centre. Lesions not coalescing.

Location 3 Lesions elongating. Margin indistinct, dirty brown in colour with light centre.

Location 4 Young lesions, circular to elongated. Lesion dark green and water soaked with thin margin and greenish centre.

Isolates of Rhizoctonia solani obtained from the various locations did not show distinguishable variation in morphogenic and cultural descriptions. Isolates $R_1$ and $R_2$ were obtained from the same plant at Location 1. Isolates $R_3$, $R_4$ and $R_5$ were obtained from specimen of Location 2, Location 3 and Location 4 respectively.

Pairing of these isolates were made in all possible combinations to determine the presence of imperfect fusion. It was observed that only isolates $R_1$ and $R_2$ could anastomose perfectly without killing reaction. Fusion in all other combinations were imperfect resulting in killing reaction of fused cell. The cytoplasm of the fused cells was disintegrated resulting in vacuolation (Table 7).

## XI. COMPARISON OF GROWTH OF MG 1 ISOLATES OF RHIZOCTONIA SOLANI AND REPRESENTATIVE ISOLATES OF MG 2, MG 3 AND MG 4 IN DIFFERENT CULTURE MEDIA

### a. Liquid medium

#### 1. Potato-dextrose broth

Among the MG 1 isolates, Oryza sativa isolate grew best in Potato-dextrose broth with a mycelial dry weight of 680 mg when the cultures were subjected to rotatory shaking. This was followed by Cymbopogon flexuosus isolate (590 mg) and

Table 7. Anastomosis pairing of the different isolates, $R_1$, $R_2$, $R_3$, $R_4$ and $R_5$ of Rhizoctonia solani from rice to determine kind of fusion

| Sl. No. | Combination (pairs) | Perfect fusion | Imperfect fusion | No fusion |
|---|---|---|---|---|
| 1 | $R_1$ x $R_2$ | + | - | - |
| 2 | $R_1$ x $R_3$ | - | + | - |
| 3 | $R_1$ x $R_4$ | - | + | - |
| 4 | $R_1$ x $R_5$ | - | + | - |
| 5 | $R_2$ x $R_3$ | - | + | - |
| 6 | $R_2$ x $R_4$ | - | + | - |
| 7 | $R_2$ x $R_5$ | - | + | - |
| 8 | $R_3$ x $R_4$ | - | + | - |
| 9 | $R_3$ x $R_5$ | - | + | - |
| 10 | $R_4$ x $R_5$ | - | + | - |

+ Positive reaction

Cynodon dactylon isolate (480 mg). Minimum growth was shown by Colocasia esculenta isolate (120 mg) preceeded by Sesbania aculeata (160 mg) and Panicum maximum (300 mg). Among the MG 2, MG 3 and MG 4 isolates, Vigna unguiculata of MG 4 showed maximum growth (460 mg) followed by Sesamum indicum isolate of MG 3 (380 mg) and Mushroom bed isolate of MG 2 (370 mg).

Under static condition, Vigna unguiculata showed maximum growth (490 mg) followed by Mushroom bed isolate (450 mg) and Sesamum indicum (400 mg), the isolates representing MG 4, MG 2 and MG 3 respectively. Among the MG 1 isolates maximum growth was by Oryza sativa isolate (560 mg) followed by Cymbopogon flexuosus isolate (400 mg) and Arachis hypogaea isolate (400 mg). Minimum growth was by Panicum maximum (170 mg) preceeded by Cynodon dactylon (200 mg) (Table 8).

ii. Czapek's broth

When the cultures in Czapek's broth were subjected to rotatory shaking Sesbania aculeata isolate and Sporobolus diander isolate showed maximum growth (280 mg) among the MG 1 isolates. This was followed by Colocasia esculenta isolate (270 mg). Minimum growth was by Pennisetum polystachyon isolate (150 mg) preceeded by Cynodon dactylon (170 mg). Among the other MG groups, mushroom bed isolate

Table 8. Growth of different isolates of Rhizoctonia solani of MG 1 and representative isolates of MG 2, MG 3 and MG 4 in shake and non-shake broth of Potato-dextrose medium

| MG group | Isolate No. | Host/habitat | Non-shake* (mg) | Shake* (mg) |
|---|---|---|---|---|
| MG 1 | 1 | Oryza sativa | 560 | 680 |
| | 20 | Arachis hypogaea (leaf) | 400 | 340 |
| | 22 | Colocasia esculenta | 280 | 120 |
| | 23 | Cymbopogon flexuosus | 400 | 590 |
| | 24 | Cynodon dactylon | 200 | 480 |
| | 29 | Monochoria vaginalis | 350 | 450 |
| | 32 | Panicum maximun | 170 | 300 |
| | 33 | Pennisetum polystachyon | 340 | 320 |
| | 38 | Sesbania aculeata | 320 | 160 |
| | 39 | Sporobolus diander | 230 | 350 |
| MG 2 | 31 | Mushroom bed | 450 | 370 |
| MG 3 | 37 | Sesamun indicum | 400 | 380 |
| MG 4 | 41 | Vigna unguiculata | 490 | 460 |

* dry weight of mycelium
(average of three replications)

of MG 2 showed the maximum growth of 280 mg followed by Sesamum indicum isolate (125 mg). No growth was shown by Vigna unguiculata of MG 4.

With respect to the MG 1 isolates cultured under static condition, Sesbania aculeata isolate grew best (400 mg) succeeded by Colocasia esculenta isolate (300 mg) and Arachis hypogaea (250 mg). Minimum growth was by Cynodon dactylon and Sporobolus diander isolates (80 mg) preceeded by Oryza sativa isolate (100 mg). Mushroom bed isolate of MG 2 and Sesamum indicum isolate of MG 3 grew to 270 mg and 155 mg respectively whereas Vigna unguiculata isolate of MG 4 failed to grow (Table 9).

b. Solid medium

Growth of the representative isolates of MG 1, MG 2, MG 3 and MG 4 in different solid media, viz., Potato-dextrose agar medium, Coon's medium, Czapek's medium and Richards' medium showed that Oryza sativa isolate of MG 1 had a greater rate of growth and quicker sclerotial initiation and maturation in all the media; maximum being in Potato-dextrose agar medium and Richards' medium. The rate of growth of the other isolates were more or less uniform in all the media, the fastest being Mushroom bed isolate of MG 2 in Czapek's medium (Table 10).

Table 9. Growth of different isolates of Rhizoctonia solani of MG 1 and representative isolates of MG 2, MG 3 and MG 4 in shake and non-shake broth of Czapek's medium

| MG Group | Isolate No. | Host/habitat | Non-shake* (mg) | Shake* (mg) |
|---|---|---|---|---|
| MG 1 | 1 | Oryza sativa | 100 | 230 |
| | 20 | Arachis hypogaea (leaf) | 250 | 200 |
| | 22 | Colocasia esculenta | 300 | 270 |
| | 23 | Cymbopogon flexuosus | 110 | 250 |
| | 24 | Cynodon dactylon | 80 | 170 |
| | 29 | Monochoria vaginalis | 175 | 250 |
| | 32 | Panicum maximum | 170 | 200 |
| | 33 | Pennisetum polystachyon | 220 | 150 |
| | 38 | Sesbania aculeata | 400 | 280 |
| | 39 | Sporobolus diander | 80 | 280 |
| MG 2 | 31 | Mushroom bed | 270 | 280 |
| MG 3 | 37 | Sesamum indicum | 155 | 125 |
| MG 4 | 41 | Vigna unguiculata | N.G | N.G |

* Dry weight of mycelium
(Average of three replications)

N.G No growth.

Table 10. Growth characters of isolates of Rhizoctonia solani from Oryza sativa (MG 1), Mushroom bed (MG 2), Sesamum indicum (MG 3) and Vigna unguiculata (MG 4)

| Culture media | Isolate No. | Host/habitat | No. of days required for | | |
|---|---|---|---|---|---|
| | | | Completion of growth | Appearance of sclerotial initials | Maturation of sclerotia |
| Potato-dextrose agar medium | 1 | Oryza sativa | 2 | 3 | 4 |
| | 31 | Mushroom bed | 4 | 9 | 10 |
| | 37 | Sesamum indicum | 4 | 7 | 11 |
| | 41 | Vigna unguiculata | 5 | 7 | 11 |
| Coon's medium | 1 | Oryza sativa | 3 | 4 | 6 |
| | 31 | Mushroom bed | 5 | 9 | 12 |
| | 37 | Sesamum indicum | 5 | 8 | 11 |
| | 41 | Vigna unguiculata | 5 | 8 | 12 |
| Czapek's medium | 1 | Oryza sativa | 2 | 4 | 5 |
| | 31 | Mushroom bed | 3 | 6 | 10 |
| | 37 | Sesamum indicum | 4 | 7 | 12 |
| | 41 | Vigna unguiculata | 4 | 6 | 10 |
| Richards' medium | 1 | Oryza sativa | 2 | 3 | 4 |
| | 31 | Mushroom bed | 5 | 7 | 10 |
| | 37 | Sesamum indicum | 6 | 9 | 12 |
| | 41 | Vigna unguiculata | 6 | 8 | 12 |

## XII. STUDIES ON THE SCLEROTIAL CHARACTERS

### Buoyancy of sclerotia

All Rhizoctonia solani isolates of MG 1 showed cent per cent floatation of sclerotia when observations were taken at 1 h and 6 h after immersion of the sclerotia in water. The percentage declined at the 24th h for Panicum maximum isolate (90%), on the third day for Pennisetum polystachyon (90%) and Colocasia esculenta isolates (80%), and on the seventh day for Cymbopogon flexuosus (90%) and Cynodon dactylon isolates (90%). The sclerotia of isolates from Arachis hypogaea, Monochoria vaginalis, Oryza sativa, Sesbania aculeata and Sporobolus diander remained floating on the 14th day of observation.

None of the sclerotia of Mushroom bed (MG 2), Sesamum indicum (MG 3) and Vigna unguiculata (MG 4) isolates could float even at the first hour of observation (Table 11).

## XIII. REACTION OF MG 1 ISOLATES OF RHIZOCTONIA SOLANI TO DIFFERENT VARIETIES OF RICE

The reaction of the various varieties of rice to the 10 isolates of Rhizoctonia solani of MG 1 showed variation in virulence of the isolates. Variation was observed in disease development and severity between the varieties.

Table 11. Buoyancy of sclerotia of different isolates of *Rhizoctonia solani* of MG 1 and representative isolates of MG 2, MG 3 and MG 4

| MG No. | Isolate No. | Host/habitat | Percentage of Sclerotia buoyant | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1 h | 6 h | 24 h | 3 days | 7 days | 14 days |
| MG 1 | 1 | *Oryza sativa* | 100 | 100 | 100 | 100 | 100 | 100 |
| | 20 | *Arachis hypogaea* (leaf) | 100 | 100 | 100 | 100 | 100 | 100 |
| | 22 | *Colocasia esculenta* | 100 | 100 | 100 | 80 | 80 | 80 |
| | 23 | *Cymbopogon flexuosus* | 100 | 100 | 100 | 100 | 90 | 90 |
| | 24 | *Cynodon dactylon* | 100 | 100 | 100 | 100 | 90 | 90 |
| | 29 | *Monochoria vaginalis* | 100 | 100 | 100 | 100 | 100 | 100 |
| | 32 | *Panicum maximum* | 100 | 100 | 90 | 80 | 70 | 70 |
| | 33 | *Pennisetum polystachyon* | 100 | 100 | 100 | 90 | 80 | 70 |
| | 38 | *Sesbania aculeata* | 100 | 100 | 100 | 100 | 100 | 100 |
| | 39 | *Sporobolus diander* | 100 | 100 | 100 | 100 | 100 | 100 |
| MG 2 | 31 | Mushroom bed | 0 | 0 | 0 | 0 | 0 | 0 |
| MG 3 | 37 | *Sesamum indicum* | 0 | 0 | 0 | 0 | 0 | 0 |
| MG 4 | 41 | *Vigna unguiculata* | 0 | 0 | 0 | 0 | 0 | 0 |

a. On the seventh day after inoculation

Arachis hypogaea isolate caused maximum infection on Jyothi (grade 1-5/X = 15.4) followed by MO-5 (1-3/13.35). No infection was noticed on Cul-1537-2 and IR-36. Cul-1954 showed a score of 0 - 1 while the other varieties recorded a score of 1. Colocasia esculenta isolate produced uniform disease severity on all varieties (Grade 1) except Cul-1954, on which infection failed to develop. Maximum X value of 19.6 was seen in Cul-169. Disease severity by Cymbopogon flexuosus isolate ranged from 0 - 1 in Cul-169, Cul-1537-2, IR-8 and Cul-1907, and 1 in the remaining varieties except Cul-1954 which was not infected. X value did not exceed 15.22 observed in Cul-26-1-1. In the case of Cynodon dactylon isolate grades ranged from 0 - 1 in Jaya, MO-5, Cul-169 and Cul-1954 while the remaining varieties showed disease severity of grade 1. The maximum X value was in variety Jyothi (12.2). Monochoria vaginalis isolate did not produce infection on Cul-52-3-6. Grades of 1 were observed in MO-5, Cul-169 and Cul-26-1-1, while grades of the remaining varieties ranged from 0 - 1. Maximun X value was in variety MO-5 (16.2). Cul-1954 was not infected by Oryza sativa isolate on the 7th day of inoculation. Grades of 0 - 1 were observed in Jaya, Cul-1537-2 and Cul-26-1-1 while the remaining varieties had a value of 1. X ratio was maximum in variety

MO-5 and Cul-169 (15.5). Panicum maximum isolate produced grade of 1 in all varieties except Cul 1954 which ranged from 0 - 1. X value was maximum (16.0) in Cul-1907. Pennisetum polystachyon isolate caused disease severity of grade 1 in Cul-169, Cul-1954 and Cul-26-1-1; the score of the remaining varieties being 0 - 1. Sesbania aculeata isolate produced maximum disease on Jyothi (3/20) and minimum disease on Culture-1537-2 (0-1/7.0). Cul-1954 scored a grade of 1 - 3 and all remaining varieties had a score of 1. Sporobolus diander did not cause infection on Cul-52-3-6. Varieties MO-5 and Cul-169 had maximum disease (1-3) with highest X value of 14.75 on MO-5. Grade 1 was observed on the other varieties (Table 12).

b. On the 14th day of inoculation

Variety Jyothi was the most susceptible to isolate of Arachis hypogaea (Grade 3-5/X = 24.7) followed by Cul-169 (1-5/21.6). The least disease was produced on Cul-1954 (1/5.4). Colocasia esculenta isolate did not infect Cul-1954 even on the 14th day of inoculation. Maximum disease was seen on Cul-169 (1-3/25.7). Cymbopogon flexuosus isolate caused maximum disease on Jyothi (3-5/20.35) and IR-36 (3-5/14.1) followed by Cul-169 (3/26.3). Minimum disease was seen on Cul-1537-2 (0-1/5.4). Cynodon dactylon isolate caused

Table 12. Reaction of 10 varieties of rice to 10 isolates of Rhizoctonia solani (MG 1) (7th day of inoculation)

| Isolate No. | Host Variety | Grade/percentage ratio (X) on the 7th day of inoculation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Jaya | MO-5 | Cul-169 | Cul-1537-2 | IR-36 | Cul-1954 | Jyothi | Cul-1907 | Cul-52-3-6 | Cul-26-1-1 |
| 1 | Oryza sativa | 0-1 / 5 | 1 / 15.50 | 1 / 15.50 | 0-1 / 4.90 | 1 / 12 | 0 / 0 | 1 / 8 | 1 / 13.50 | 1 / 10 | 0-1 / 0.50 |
| 20 | Arachis hypogaea | 1 / 9.95 | 1-3 / 13.35 | 1 / 15.10 | 0 / 0 | 0 / 0 | 0-1 / 4.40 | 1-5 / 15.40 | 1 / 8.80 | 1 / 13.25 | 1 / 8.30 |
| 22 | Colocasia esculenta | 1 / 7.25 | 1 / 9.60 | 1 / 19.60 | 1 / 13.7 | 1 / 8.90 | 0 / 0 | 1 / 11.80 | 1 / 9.65 | 1 / 10.90 | 1 / 11.40 |
| 23 | Cymbopogon flexuosus | 1 / 4.35 | 1 / 15.00 | 0-1 / 10.40 | 0-1 / 3.55 | 0-1 / 5.25 | 0 / 0 | 1 / 8.60 | 0-1 / 2.65 | 1 / 12.35 | 1 / 15.22 |
| 24 | Cynodon dactylon | 0-1 / 2.60 | 0-1 / 5 | 0-1 / 3.30 | 1 / 9.15 | 1 / 6.80 | 0-1 / 5.38 | 1 / 12.20 | 1 / 3.15 | 1 / 6.60 | 1 / 4.80 |
| 29 | Monochoria vaginalis | 0-1 / 4.50 | 1 / 16.20 | 1 / 9 | 0-1 / 4.50 | 0-1 / 5.50 | 0-1 / 3.50 | 0-1 / 3.50 | 0-1 / 1.50 | 0 / 0 | 1 / 6.50 |
| 32 | Panicum maximum | 1 / 8.20 | 1 / 11.40 | 1 / 12.50 | 1 / 8.35 | 1 / 9.75 | 0-1 / 5.35 | 1 / 15.85 | 1 / 16.00 | 1 / 8.95 | 1 / 10 |
| 33 | Pennisetum polystachyon | 0-1 / 3.10 | 0-1 / 6.25 | 1 / 8.65 | 0-1 / 4.35 | 0-1 / 5.70 | 1 / 8 | 0-1 / 3.20 | 0-1 / 3.15 | 0-1 / 2.50 | 1 / 12.35 |
| 38 | Sesbania aculeata | 1 / 14.14 | 1 / 6.80 | 1 / 18.15 | 0-1 / 7.00 | 1 / 14.00 | 1-3 / 17.50 | 3 / 20.00 | 1 / 8.95 | 1 / 9.35 | 1 / 8 |
| 39 | Sporobolus diander | 1 / 11.30 | 1-3 / 14.75 | 1-3 / 14.00 | 1 / 8.60 | 1 / 5.65 | 1 / 9.00 | 1 / 7.75 | 1 / 7.50 | 0 / 0 | 1 / 7.25 |

Grade 1 to 9. Percentage ratio (X) = $\frac{\text{height of upper most lesion}}{\text{plant height}} \times 100$

maximum disease on Jyothi (1-3/15.7) and IR-36 (1-3/11.8). Least disease was on Cul-1954 (0-1/7.5). Variety Jyothi (3-5/31), MO-5 (3-5/21.6) and IR-36 (3-5/17) were the most susceptible to Monochoria vaginalis isolate. Cul-1537-2 (1/10.2) was the most tolerant preceeded by Cul-1954 (1/11.8). Oryza sativa isolate could not infect Cul-1954, and produced maximum disease on Jyothi (5-7/38.5) followed by IR-36 (3-5/26) and Cul-169 (1-5/20.3). Panicum maximum isolate caused maximum disease on Cul-169 (3-5/22) followed by Jyothi (3/24.3). Least disease was on Cul-1954 (1/7.35). Pennisetum polystachyon isolate produced disease severity of grade 0 - 1 for Jaya, Cul-169, Cul-1537-2 and IR-36, and 1 for the remaining isolates. Sesbania aculeata infected MO-5 to the greatest extent (3/22.1) followed by Jyothi (1-3/28.9), minimum infection being on Cul-1537-2 (0-1/3.1). Sporobolus diander isolate showed maximum disease on MO-5 (3/20.9) followed by Jyothi (3/19.4) and least infection on Cul-52-3-6 (1/7.25) (Table 13, Fig. 16a to 16j).

## XIV. LABORATORY EVALUATION OF FUNGICIDES

### a. Reaction of Rhizoctonia solani to increasing concentrations of carboxin

Rhizoctonia solani from Oryza sativa completed its radial growth with sclerotial formation on Potato-dextrose

Table 13. Reaction of 10 varieties of rice to 10 isolates of Rhizoctonia solani (MG 1) (14th day of inoculation

| Isolate No. | Host Variety | Grade/percentage ratio (X) on the 14th day of inoculation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Jaya | MO-5 | Cul-169 | Cul-1537-2 | IR-36 | Cul-1954 | Jyothi | Cul-1907 | Cul-52-3-6 | Cul-26-1-1 |
| 1 | Oryza sativa | 1 / 15.60 | 3 / 21.70 | 1-5 / 20.30 | 1 / 18.90 | 3-5 / 26 | 0 / 0 | 5-7 / 38.50 | 3 / 18.80 | 1-3 / 34 | 1-3 / 29 |
| 20 | Arachis hypogaea | 1 / 15.30 | 1-3 / 17.60 | 1-5 / 21.60 | 0-1 / 6.70 | 0-3 / 10.80 | 1 / 5.40 | 3-5 / 24.70 | 1-3 / 15.80 | 3 / 17.50 | 1 / 11.45 |
| 22 | Colocasia esculenta | 1 / 8.80 | 3 / 17.50 | 1-3 / 25.70 | 1-3 / 20.30 | 1 / 11.70 | 0 / 0 | 1 / 20.10 | 1 / 14.80 | 1-3 / 21.10 | 1 / 15.60 |
| 23 | Cymbopogon flexuosus | 1 / 6.70 | 1-3 / 21.30 | 3 / 26.30 | 0-1 / 5.40 | 3-5 / 14.10 | 1 / 7.50 | 3-5 / 20.35 | 1 / 16.95 | 1-3 / 13.85 | 1 / 17.20 |
| 24 | Cynodon dactylon | 1 / 14.10 | 1 / 16 | 1 / 9.20 | 1 / 11.40 | 1-3 / 11.80 | 0-1 / 7.50 | 1-3 / 15.70 | 1 / 8.80 | 1 / 10.30 | 1 / 10.75 |
| 29 | Monochoria vaginalis | 1 / 24 | 3-5 / 21.6 | 1 / 21.60 | 1 / 10.20 | 3-5 / 17 | 1 / 11.80 | 3-5 / 31 | 1-3 / 25.50 | 1-3 / 23.70 | 1 / 19 |
| 32 | Panicum maximum | 1 / 16.90 | 3 / 19.50 | 3-5 / 22 | 1 / 15.70 | 1-3 / 17.90 | 1 / 7.35 | 3 / 24.30 | 1 / 18.00 | 1 / 19.90 | 1 / 11.70 |
| 33 | Pennisetum polystachyon | 0-1 / 13.40 | 1 / 12.10 | 0-1 / 17.40 | 0-1 / 2.90 | 0-1 / 8.50 | 1 / 5.00 | 1 / 12.90 | 1 / 14.50 | 1 / 21.20 | 1 / 12.40 |
| 38 | Sesbania aculeata | 1 / 12.80 | 3 / 22.20 | 1-3 / 19.60 | 0-1 / 3.10 | 1-3 / 21.70 | 1-3 / 18.70 | 1-3 / 28.90 | 1 / 13.40 | 1-3 / 13.70 | 1 / 15.40 |
| 39 | Sporobolus diander | 1 / 12.80 | 3 / 20.90 | 1-3 / 30.00 | 1 / 18.20 | 1-3 / 20.80 | 1 / 14 | 3 / 19.40 | 1 / 9 | 1 / 7.25 | 1 / 12.60 |

Note: Grade 1 to 9. Percentage ratio (X) = $\frac{\text{Height of the uppermost lesion}}{\text{Plant height}} \times 100$

Fig. 16. Varying symptoms of sheath blight produced by the ten isolates of Rhizoctonia solani of MG 1 on the common varieties of rice

a. Isolate from Arachis hypogaea on Jyothi (Grade - 3)

b. Isolate from Colocassia esculenta on MO-5 (Grade - 3)

c. Isolate from Cymbopogon flexuosus on Jyothi. (Grade - 5)

d. Isolate from Cynodon dactylon on Jyothi (Grade - 3)

e. Isolate from Monochoria vaginalis on Jyothi (Grade - 7)

Fig. 16. Varying symptoms of sheath blight produced by the ten isolates of Rhizoctonia solani of MG 1 on the common varieties of rice

a. Isolate from Arachis hypogaea on Jyothi (Grade - 3)

b. Isolate from Colocassia esculenta on MO-5 (Grade - 3)

c. Isolate from Cymbopogon flexuosus on Jyothi. (Grade - 5)

d. Isolate from Cynodon dactylon on Jyothi (Grade - 3)

e. Isolate from Monochoria vaginalis on Jyothi (Grade - 7)

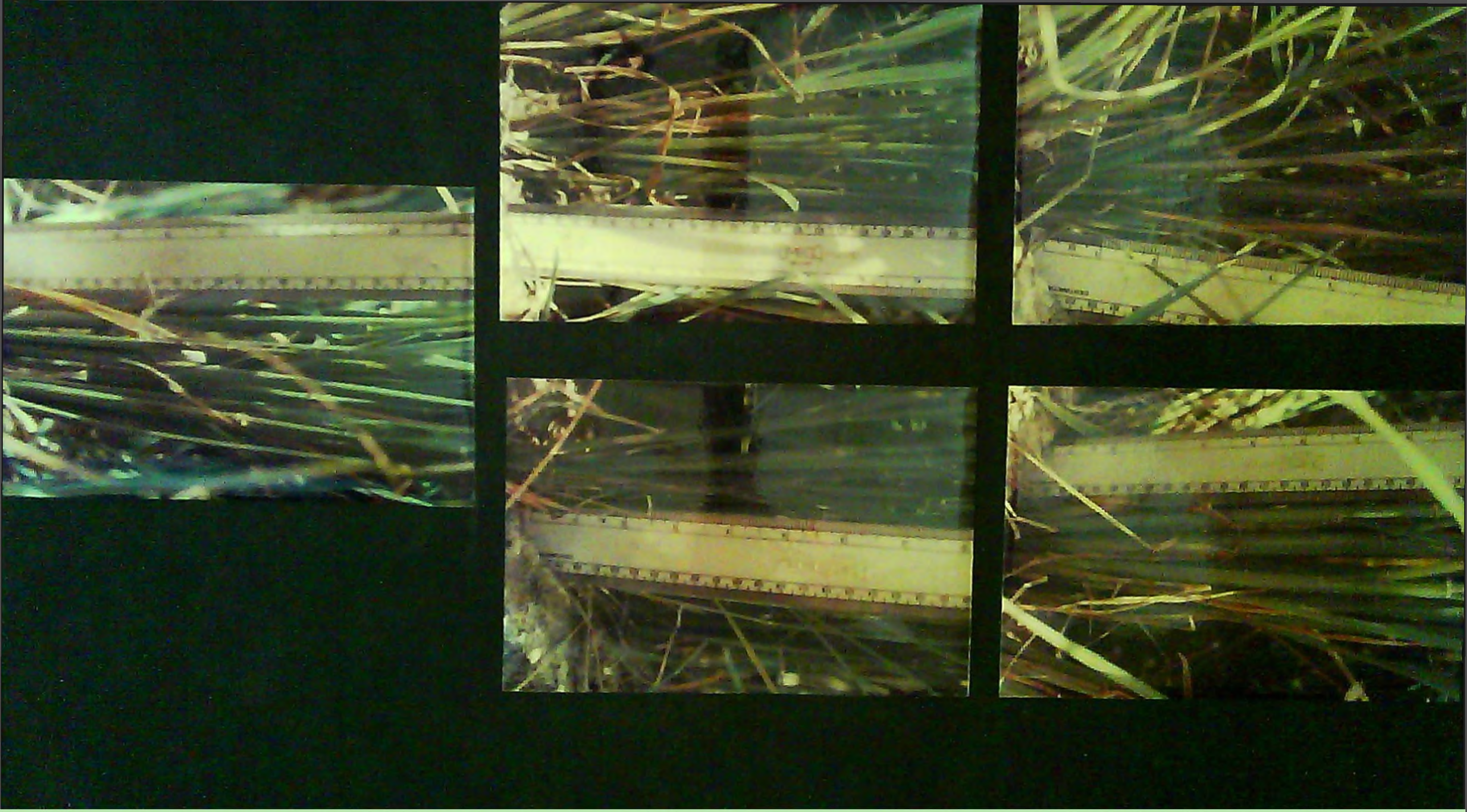


Fig. 16 Contd.

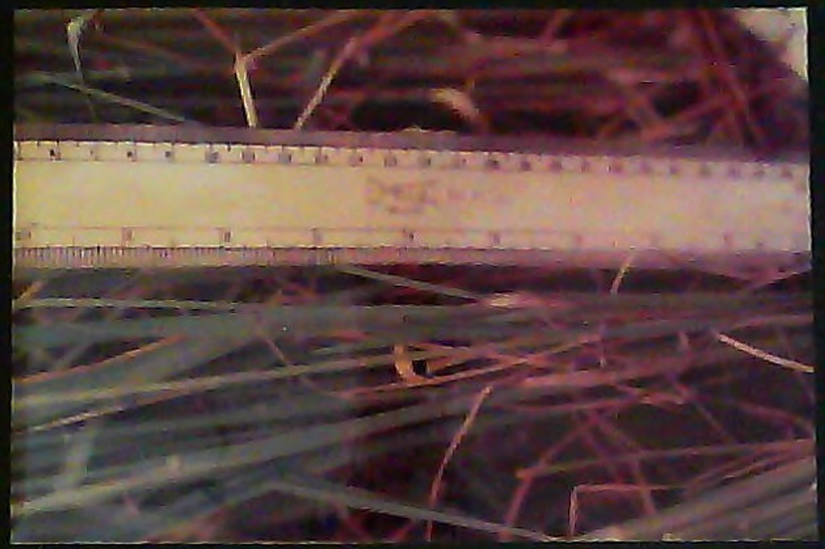
f. Isolate from *Oryza sativa* on Jyothi (Grade - 7)

g. Isolate from Panicum maximum on Cul-169 (Grade - 5)

h. Isolate from *Pennisetum polystachyon* on Jyothi (Grade - 3)

i. Isolate from *Sesbania aculeata* on IR-36 (Grade - 3)

j. Isolate from *Sporobolus diander* on Jyothi (Grade - 3)



agar medium incorporated with 2.5 ppm carboxin. Growth started on the second day of incubation and was completed on the sixth day (Table 14a). Mycelium brownish white and radiating at the periphery at right angles to the lid of petri dish. Thin mycelial strands were seen radiating to the lid from a narrow band about 35 mm from the centre. Sclerotial initials were formed on the seventh day and distributed irregularly towards the periphery. Sclerotia attained maturity on the ninth day and were larger in size than normal with honey dew formation. Sclerotia aggregated into masses (Fig. 17). The Isolate was designated as $V_1$.

Isolate $V_1$ tolerant to carboxin at 2.5 ppm was transferred to 5 ppm carboxin incorporated medium and growth compared to that of normal isolate on 5 ppm carboxin and on normal medium. Growth was arrested for $V_1$ isolate after attaining a growth of 35 mm whereas normal isolate could grow only to 25 mm on the 6th day. In both cases sclerotial formation was arrested, mycelium was fluffy with numerous vertical hyphae (Table 14b). The isolate $V_1$ adapted to grow on 5 ppm carboxin was designated as $V_2$.

Isolate $V_2$ inoculated on Potato-dextrose agar medium containing 10 ppm of carboxin could grow to a diameter of 35 mm (Isolate $V_3$) whereas control of normal isolate grow

Table 14a. Growth of Rhizoctonia solani (rice isolate) on Potato-dextrose agar medium incorporated with 2.5 ppm carboxin

| No. of days | Radial growth (mm) | Mycelial and sclerotial characters on completion of growth |
|---|---|---|
| 1 | No growth | Mycelium brownish white, the periphery radiating as thin strands to the lid at right angles. Similar strands radiating to the lid from a narrow band at 35 mm from the centre. Sclerotial initials appearing on the 7th day and distributed irregularly towards the periphery. Sclerotial maturation on the 9th day with honey dew formation. |
| 2 | 12 | |
| 3 | 17 | |
| 4 | 30 | |
| 5 | 75 | |
| 6 | 90 | |

Table 14b. Growth of $V_1$ (carboxin tolerant isolate - 2.5 ppm) and $C_1$ (normal isolate) on Potato-dextrose agar medium incorporated with 5 ppm carboxin

| No. of days | Radial growth (mm) | | |
|---|---|---|---|
| | $V_1$ | $C_1$ | $C_1$ (unamended PDA) |
| 1 | No growth | No growth | 40 |
| 2 | 15 | Just started | Completed growth |
| 3 | 25 | 20 | Sclerotial formation |
| 4 | 30 | 25 | Sclerotial maturation |
| 5 | 35 | 25 | |
| 6 | Growth stopped | Growth stopped | |

only to 20 mm in such medium. Mycelium in both isolates were fluffy with vertical hyphae and without sclerotial formation (Table 14c).

Isolate $V_3$ was compared with normal isolate of Rhizoctonia solani in growth characteristic when cultured on Potato-dextrose agar medium. Both isolates completed growth on the second day with sclerotial initiation on the fourth day and sclerotial maturation on the fifth day (Table 14d). Isolate $V_3$ produced lesser number of sclerotia, but were larger in size. Mycelial crust distributed throughout the culture and mycelium fluffy towards the margin and sides of the petri dish (Fig. 18).

b. Pathogenicity of the tolerant isolate of Rhizoctonia solani

Rice plants inoculated with sclerotia from the tolerant isolate of Rhizoctonia solani ($V_3$) on Potato-dextrose agar medium produced infection and symptom of reduced intensity than control plants inoculated with normal isolate. Inoculation with mycelial disc on the outer sheath failed to produce infection. Control plants developed normal symptoms of sheath blight disease (Fig. 19).

c. Reisolation of tolerant isolate and testing for stability of anastomosis character

The tolerant isolate reisolated from the diseased specimen of the inoculated plants tended to reverse to the normal isolate

Table 14c. Growth of $V_2$ (carboxin tolerant isolate - 5 ppm) and $C_1$ (normal isolate on Potato-dextrose agar medium incorporated with 10 ppm carboxin)

| No. of days | Radial growth (mm) | | |
|---|---|---|---|
| | $V_1$ | $C_1$ | $C_1$ (unamended) |
| 1 | No growth | No growth | 40 |
| 2 | 15 | Just started | Completed growth |
| 3 | 30 | 10 | Sclerotial formation |
| 4 | 30 | 15 | Sclerotial maturation |
| 5 | 35 | 20 | |
| 6 | Growth stopped | Growth stopped | |

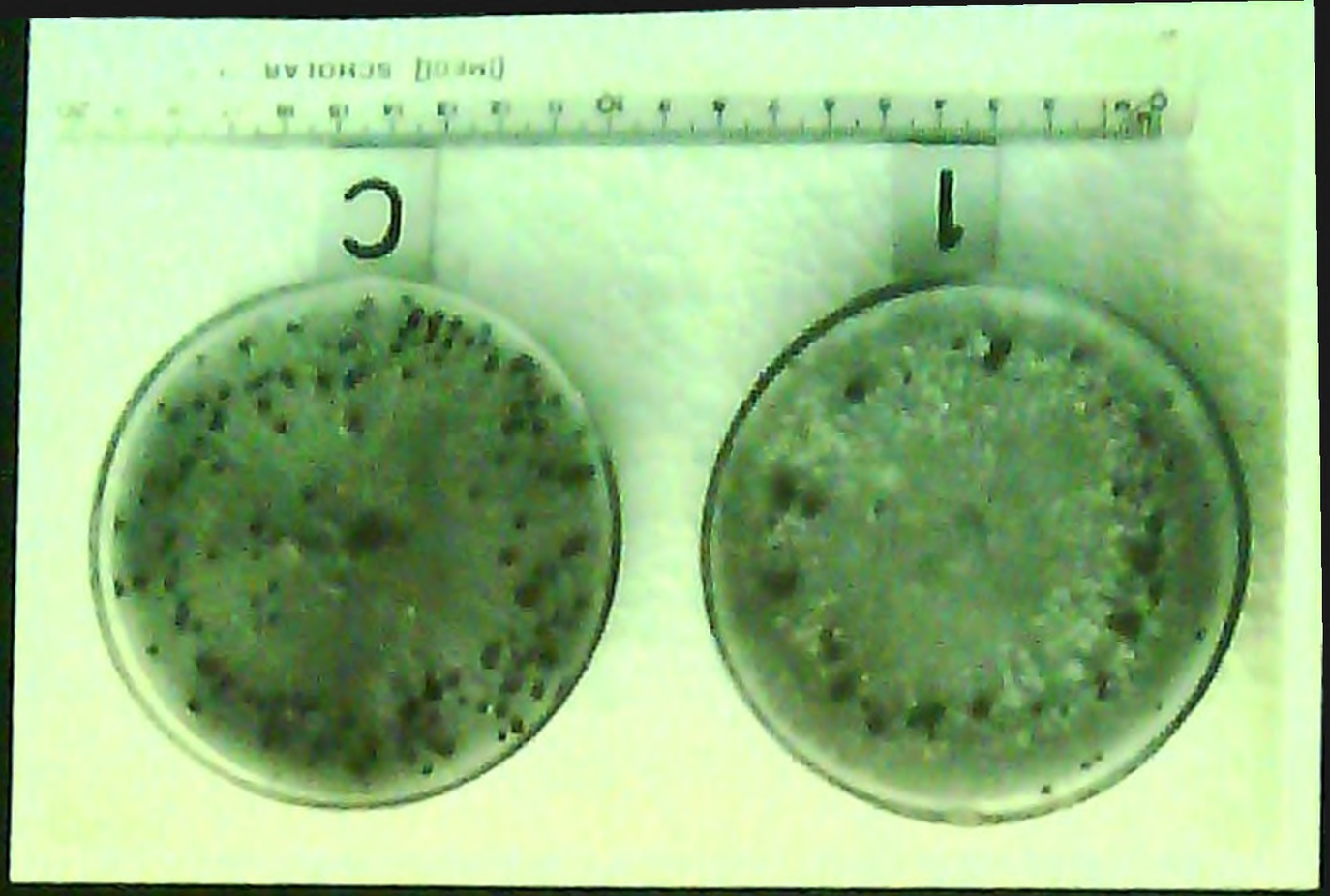
Table 14d. Growth of $V_3$ (carboxin tolerant isolate - 10 ppm) and $C_1$ (normal isolate on unamended Potato-dextrose agar medium)

| No. of days | $V_3$ | $C_1$ |
|---|---|---|
| 1 | 35 mm | 35 mm |
| 2 | Growth completed | Growth completed |
| 3 | Sclerotial initials (smaller in size) | Sclerotial initials |
| 4 | Maturation of sclerotia | Maturation of sclerotia |
| 5 | Lesser number of sclerotia, larger in size with abundant honey dew formation distributed to the periphery. Mycelium crusty and distributed throughout the culture. Mycelium fluffy towards the margin and sides of petri dish. | |

Fig. 17. Altered cultural characters in Rhizoctonia solani isolate grown on Potato-dextrose agar medium incorporated with 2.5 ppm carboxin. (Isolate $V_1$)

Fig. 18. Growth characteristics of Isolate $V_3$ (10 ppm carboxin tolerant isolate) on unamended Potato-dextrose agar.

1. $V_3$
C. Parent isolate


C
1


Blue Star
1

Fig. 19. Inoculation of rice plants with *Rhizoctonia solani* by cellophane technique

1. Isolate $V_3$ - Negative reaction

2. Normal isolate - Positive reaction

1
2
MEGI SCHOLAR 1974

in morphology and culture. Two to three transfers completed the process of reversion. Pairing with normal isolate of Rhizoctonia solani (Isolate 1) showed perfect fusion with cytoplasmic exchange.

d. Growth inhibition of Rhizoctonia solani by different fungicides

Bavistin, calixin and carboxin caused complete inhibition of growth of Rhizoctonia solani (Oryza sativa isolate) in all the three concentrations of 500 ppm, 1000 ppm and 1500 ppm tested.

# DISCUSSION

# DISCUSSION

Rice plants at the Instructional Farm, College of Agriculture, Vellayani, affected with sheath blight showed typical symptoms of the disease as reported in literature. The greenish grey ellipsoidal lesions developed from the original discolourations were the first conspicuous symptom of the disease. The lesions usually originated at or above the water line. The pointed nature of the lesions which were elongated with well-defined brown margin agrees with the description of the disease by Malaguti (1951). The lesions coalesced and the encircling of the culm caused the rotting of the sheath.

Sclerotia formed from the fungal mycelia on the lesions were easily detachable and served as inoculum in the stubble and soil. Valdez (1955) observed the mycelium to be viable for more than a month in stubbles in the soil, and the sclerotia for six months in the soil.

Isolation from sheath blight affected rice tissues yielded Rhizoctonia solani Kuhn. Morphological and cultural characters agreed to the descriptions of Parmeter and Whitney (1969). Multinucleated hyphal cells were confirmed by HCl Giemsa staining as described by Herr (1979). Parmeter et al. (1967) distinguished Rhizoctonia solani with multinucleate

hyphal cells which produced the teleomorph Thanatephorus cucumeris Frank (Donk) (Corticium solani Prill & Declacr.) from the binucleated Rhizoctonia solani like fungi with a Ceratobasidium teleomorph. The hypha had prominent septal pore and branching was near the distal septum with constriction of the branch base. Culture of the organism on Potato-dextrose agar medium showed brown pigmentation. Mature sclerotia were subglobose, rough surfaced and regular. Size varied from 1.8 mm to 3 mm. Characteristic light brown to dark brown aerial mycelium with usually abundant large brown sclerotia were observed in isolates of Rhizoctonia solani by Herr and Roberts (1980).

Specimens of sheath blight affected rice plants from the different localities in Kerala showed variation in the symptoms of the disease. By a critical analysis, the varying type of symptoms as detailed under Results can be grouped into four different categories.

1. Lesions remaining isolated
2. Individual lesions coalescing into larger elongated lesions
3. Lesions encircling the culm
4. Lesions encircling the culm and extending to the leaves.

The shape of the lesions was usually ellipsoidal and varied from oval to irregular and oblong to elongated. The

colour of mature lesions was in the range of pale green, greenish grey, dirty grey, grey, greyish white and buff white.

No uniform pattern of symptoms could be observed either on geographical or on varietal basis.

Symptoms produced by Rhizoctonia solani on other host plants varied depending on the part of the plant affected.

Typical collar rot syndrome was seen on host plants with infection on the stem at the soil region. Infection invariably resulted in the girdling of the collar region of Arachis hypogaea, Glycine max, Phaseolus aureus, Sesamum indicum and Vigna unguiculata. In Stylosanthes humilis, infected collar region supported the development of abundant sclerotia along the dark brown lesions. Damping off of infected seedlings of Catharanthus roseus occurred due to collar infection by Rhizoctonia solani.

The symptoms observed on the foliar region showed wide difference between host plants. In general, the graminaceous host plants, Cymbopogon flexuosus, Cynodon dactylon, Panicum maximum, Pennisetum polystachyon and Sporobolus diander showed symptoms resembling sheath/leaf blight of rice. Symptoms on Cynodon dactylon had distinct variation in lesion colour. Whitish lesions with straw coloured bands covered the whole leaf lamina.

Rhizoctonia solani infection produced web blight symptoms on Arachis hypogaea. Water soaked lesions were seen in Colocasia esculenta, Monochoria vaginalis, Salvinia molesta and Piper betle.

Rotting of tubers and fruits occurred in Daucus carota, Maranta arundinaceae and Lycopersicon esculentum. The rhizome of Musa paradisiaca showed dry rot with abundant sclerotial formation. Such abundant sclerotial formation was also seen on paddy straw used as substrate for growing the mushroom Volvariella volvacea.

Isolations from the various host plants yielded Rhizoctonia solani, identified based on the descriptions of Parmeter and Whitney (1969) as detailed above.

Detailed morphological and cultural studies conducted on the forty one isolates of Rhizoctonia solani are presented in Table 1, 2 and 3. The isolates were grouped into morphological groups MG 1, MG 2, MG 3 and MG 4 based on the similarity of characters like mycelial and sclerotial colour, sclerotial density and distribution, hyphal and sclerotial dimensions and number of days for completion of growth and for sclerotial initiation and maturation. Raj et al. (1974) grouped Rhizoctonia solani isolates from potato, based on rate of

growth, colour of substrate and, density, type and formation of sclerotia.

Morphological group 1 (MG 1) included all isolates of Rhizoctonia solani from sheath/leaf of rice and isolates infecting the foliage of Arachis hypogaea, Colocasia esculenta, Cymbopogon flexuosus, Cynodon dactylon, Monochoria vaginalis, Panicum maximum, Pennisetum polystachyon, Sesbania aculeata and Sporobolus diander. Mycelium of all these isolates were subaerial, nonfluffy and appressed to the substrate. Colour ranging from light brown to dark brown. Hyphal width ranging from 4.75 /um to 13.5 /um. Sclerotium scattered irregularly but tending to concentrate towards the periphery to form clusters and bands. Sclerotia subglobose, surface regular and rough with and without honey dew formation. Colour dark brown and size ranging from 1.8 mm to 2.7 mm. Sclerotial distribution within the MG 1 isolates was similar for isolates from rice (Isolate 1), Arachis hypogaea, Monochoria vaginalis and Sporobolus diander; the sclerotial formation being concentrated towards the periphery. The distribution of all the other isolates were more or less irregular throughout the culture (Table 5, Fig. 11). Kohli (1966) distinguished three morphological groups of Rhizoctonia (Corticium) solani from rice on Richards' agar. In the present study all the seventeen isolates

from rice showed uniformity in growth rate, mycelial characters and sclerotial distribution which tended to concentrate towards the periphery of the cultures. Sclerotial formation was scanty for isolates 9, 11, 14 and 15.

The mycelium of isolates coming under the group MG 2 was radiating, subaerial and scattered individually, white coloured and hyphal width ranging from 3.4 /um to 7.5 /um. Abundant sclerotial formation occurred concentrating towards the periphery of the cultures and sides of plates. Sclerotium globose, surface regular and smooth. Size ranging from 1.5 mm to 1.9 mm and reddish brown to dark brown in colour. The isolates of MG 2 mainly caused the rotting of tubers of Daucus carota and Maranta arundinaceae and fruits of Lycopersicon esculentum. The isolate infecting rhizomes of Musa paradisiaca produced dry rot symptoms. The isolate on paddy straw of mushroom bed was saprophytic in nature. The role of Isolate 31 as a weed parasite affecting mushroom cultivation was discussed by Bhavani Devi (1982). The isolate inhibited normal growth and production of mushroom buttons at the affected region of the bed.

The MG 3 isolates had radiating aerial mycelium with scattered hyphae forming fluffy to thick aggregates towards the margin. White in colour and hyphal width ranging from

6/um to 10.2/um. Sclerotia were almost uniformly distributed. Sclerotium globose to irregular, surface regular and smooth. Colour ranging from dark orange to brown. All isolates in this group barring that from Salvinia molesta infected the collar region of the host plants viz., Catharanthus roseus, Phaseolus aureus, Sesamum indicum and Stylosanthes humilis. Isolate from Salvinia molesta produced leaf rot symptom.

Majority of the isolates in MG 4 namely those isolated from Arachis hypogaea, Glycine max and Vigna unguiculata produced collar rot of the respective host plants. The mycelium of the isolates was fluffy, cottony in growth and white in colour. Hyphal width ranging from 4/um to 6.5/um. Sclerotial formation was not abundant and distributed irregularly in clumps. Sclerotium globose to oblong, surface irregular and smooth. Colour yellowish brown to brown. Size ranging from 1.9 mm to 2 mm. Isolates of Amorphophallus campanulatus and Piper betle caused leaf rot.

Each morphological group showed a distinctive morphological and cultural norm as discussed above. MG 2, MG 3 and MG 4 isolates had mycelium with whitish shade which differed conspicuously from the MG 1 isolate having typical brown pigmentation of Rhizoctonia solani (Parmeter and Whiteney, 1969). Herr and Roberts (1980) distinguished isolates with

appressed, tan-white to silvery grey brown mycelium from isolates with abundant light brown to dark brown aerial mycelium. Such isolates were assigned to AG 4 of Rhizoctonia solani. MG 1 isolates mainly produced abundant large brown sclerotia having a rough hairy surface. Aggregation or fusion of individual sclerotia into bands or clusters is a common feature. The sclerotia of the other morphological groups were more or less globose, tending to elongate in a few instances and rarely forming aggregates. The average hyphal width of isolates was also different between the groups. MG 1 isolates had greater hyphal width (4.75 /um to 13.5 /um) as compared to MG 2 (3.4 /um to 7.5 /um), MG 3 (6 /um to 10.2 /um) and MG 4 (4 /um to 6.5 /um).

MG 1 isolates had a faster growth rate on Potato-dextrose agar medium. Growth in 90 mm petri dishes was completed in two to three days with appearance of sclerotial initials on the third and fourth day and sclerotial maturation on the fourth or fifth day. The isolates of the other morphological groups, except that from Amorphophallus campanulatus (Isolate 18) of MG 4 could complete growth by the fourth to fifth day. The sclerotial initiation was on the fifth to ninth day and sclerotial maturation on the eighth to eleventh day. Isolate 18 had the slowest growth

rate, completing its growth only on the eighth day with sclerotial initiation and maturation on the twelfth and fourteenth day respectively (Table 2).

The pathogenicity of the different isolates showed some similarity within the groups. MG 1 included isolates infecting the aerial parts of plants, MG 2 those infecting tubers and storage fruits with an active saprophytic phase. MG 3 and MG 4 isolates infected the stolon or collar region of plants.

Rhizoctonia solani (Isolate 1) from rice was used as the representative isolate of MG 1 for studies on pathogenicity to the other host plants. The fungus could infect a wide range of 22 plant species tested. Inoculation on the leguminous plants viz., Phaseolus aureus, Stylosanthes humilis, Glycine max, Arachis hypogaea, Sesbania aculeata and Vigna unguiculata produced typical web/leaf blight symptoms. Collar rot symptoms did not develop in any of the above plants. However, cowpea (Vigna unguiculata) seedlings up to three weeks of age were readily infected at the collar region to produce typical collar rot symptom. Malaguti (1951) observed that Rhizoctonia solani isolated from sheath blight infected rice plants caused collar rot on seedlings of white bean (Phaseolus vulgaris). Symptoms resembling

sheath blight were produced on the graminaceous host plants, Cymbopogon flexuosus, Cynodon dactylon, Panicum maximum, Pennisetum polystachyon and Sporobolus diander. Rotting occurred on the tubers of Daucus carota, fruits of Lycopersicon esculentum, leaves of Salvinia molesta, Sesamum indicum, Amorphophallus campanulatus, Piper betle, Colocasia esculenta and leaf/stem of Monochoria vaginalis.

Pathogenicity trials on rice plants with the isolates of Rhizoctonia solani obtained from other host plants showed that only isolates of MG 1 could infect and produce typical sheath blight symptoms on rice. All the other isolates of MG 2, MG 3 and MG 4 failed to produce infection even when the plants were punctured and relative humidity near saturation point was provided.

The indications are that isolates of Rhizoctonia solani from rice and isolates from other host plants infecting rice belong to the same group both in its pathological reaction and also in its morphological and cultural characters. Even-though none of the MG 2, MG 3 and MG 4 isolates could infect rice plants, rice isolates of MG 1 could infect many of the host plants of the isolates of other morphological groups. This is a clear indication that the pathogenicity of rice group is not confined to the group of host plants of the rice group pathogen.

An interesting observation is that rice isolate caused typical web blight symptoms on pulses, whereas it could not develop collar rot symptoms on mature plants. Seedlings of cowpea could take up infection up to the third week after sowing beyond which the isolate failed to infect and cause collar rot. This points out the potential danger of infection of pulses raised as fallow crop in rice fields of the State where sheath blight is known to be endemic. ONesirosan (1977) observed differences in isolates causing web blight and basal canker in cowpea. The isolates varied in cultural characters on Potato-dextrose agar medium and showed difference in pathogenicity.

The presence of two variant types of Rhizoctonia solani isolates infecting cowpea differing morphologically and in pathological reaction warrants further studies in the field. The extent of infection by the rice isolate to the different plant parts of the pulse crop should be monitored at the different growth stages of the crop.

The different isolates of Rhizoctonia solani obtained from rice (17 isolates) tested for anastomosis showed typical fusion between the isolates in all possible combinations. This suggest that the isolates are related and belong to the same anastomosis group. Capacity for hyphal fusion provides an indication of relationship within groups of isolates

(Schultz, 1937; Richter & Schneider, 1953). Tu (1968) observed 7 strains of Pellicularia (Corticium) sasakii from rice plants to be closely related, since hyphal fusion occurred between the isolates.

Hyphal fusion occurred between rice isolate (Isolate 1) and all the isolates of MG 1, viz., isolates from Arachis hypogaea, Colocasia esculenta, Cymbopogon flexuosus, Cynodon dactylon, Monochoria vaginalis, Panicum maximum, Pennisetum polystachyon, Sesbania aculeata and Sporobolus diander.

None of the isolates of MG 2, MG 3 and MG 4 could anastomose with rice isolate of MG 1. This indicates that isolates of MG 1 are related and genetically isolated from those of MG 2, MG 3 and MG 4. Isolates within each group anastomosed with isolates of the same group but not with isolates from the other groups (Parmeter et al., 1969).

Anastomosis grouping of rice isolates showed that the AG 1 tester isolate anastomosed with rice group of isolates (MG 1 isolates) but not with MG 2, MG 3 and MG 4 isolates. This reveals that MG 1 corresponds to the AG 1 of Parmeter et al. (1969) and also to the Group 1 of Schultz (1937), Group A of Richter and Schneider (1953), the Sasakii type and web blight type of Watanabe and Matsuda (1966) and TRAG 1 of Tu and Chang (1978).

The 60 isolates of Rhizoctonia solani assigned to AG 1 by Ogoshi (1966) included many of the sheath blight fungus (Corticium sasakii) and the web blight fungus (Corticium microsclerotia). Anderson (1977) observed that AG 1 isolates mainly infect the aerial parts of many plant species.

The relationship within the MG 1 isolates can be listed as

1. Similar cultural and morphogenic nature of the isolates. Richter and Schneider (1953) found greater morphogenic and physiologic similarity within anastomosis groups than between groups.
2. Genetic affinity between isolates. Hyphal fusion indicates genetic similarity of the isolates (Schultz, 1937).
3. Pathological similarity among the isolates. All isolates cause aerial infection of their respective host plants. Only these isolates infect rice to cause sheath blight (Ogoshi, 1976; Anderson, 1977; Tu & Chang, 1978).

Of the different AG tester isolates only AG 1 infected and caused sheath blight disease of rice on artificial inoculation. AG 1 tester isolate reisolated from the infected

tissues showed similar positive anastomosis reaction with the MG 1 group of isolates. Repeated transfers of the MG 1 isolates did not change their anastomosis nature. Tu et al. (1969) also observed that anastomosis tendencies remained the same even with change of culture medium, growth temperature and other environmental conditions. This signifies that each anastomosis group is genetically independent.

Among the kinds of fusion within the same group (MG 1) the most common was imperfect fusion or contact fusion, followed by perfect fusion. Imperfect fusion was described by Flentje and Stretton (1964) as a mycelial reaction in which consummation of fusion occurred followed by mycelial rejection with death of groups of cells. Imperfect fusion restricted cytoplasmic flow and migration of nuclei between the fused hyphae (Buller, 1931; 1933). Perfect fusion is followed by protoplasmic streaming which signified the perfect genetic identity between the fused hyphae. Ogoshi (1976) observed anastomosis within the same group to be imperfect or contact fusion and anastomosis within one and the same isolate to be perfect fusion, suggesting that they belong to the same anastomosis group.

Flentje et al. (1969) postulated that among the different isolates, anastomosis relationship proceeded from perfect

fusion with heterokaryon formation to imperfect fusion without genetic exchange and finally to complete failure to anastomose. MG 1 isolates failed to anastomose with isolates outside the group. Anastomosis relationship within the MG 1 group proceeded from perfect fusion between same rice isolates to imperfect fusion between rice isolates and isolates of other host plants infecting rice.

With regard to the frequency of occurrence of the different types of fusion, the tendency was more towards tip to side wall followed by peg to side wall, side wall to side wall and finally tip to tip fusion. This depended mainly on the chance of contact rather than inherent tendencies.

Lesions of sheath blight disease on the specimen obtained from different locations selected for determination of clones of Rhizoctonia solani showed the normal variation in size, shape and colour. The young lesions from location 5 were typically greenish and water soaked. The centre of lesions from location 2 was greenish grey while the coalescing lesions of location 1 was grey in colour. Lesions of location 3 varied from the other specimens in having indistinct margins with light centres.

The isolates of Rhizoctonia solani obtained from the different locations were alike in cultural characters and in their morphology.

Anastomosis relationship between these isolates was imperfect fusion with killing reaction for all combinations except between isolates $R_1$ and $R_2$ obtained from the same location. Isolates $R_1$ and $R_2$ showed perfect fusion indicating that the lesions originated from a single locus of infection or from different loci by the same strain.

Anderson (1982) observed that imperfect fusion is seen only within the same anastomosis group and the killing reaction is a somatic or vegetative incompatibility reaction. This reaction contributed to the stability of the hetero-karyon. Somatic incompatibility in fungi was attributed by Esser (1964) to limit outbreeding and to make the race the evolutionary unit instead of the species.

In the present study, Rhizoctonia solani isolates showing somatic incompatibility can be considered as a distinct variant with genetic potential for variation in pathogenicity and response to antifungal compounds. Ogoshi and Ui (1983) used the term clone for rice isolates showing K (killing) reaction. Isolates from several lesions on the

same culm showed perfect fusion (S reaction). K reaction increased between the isolates from neighbouring culm.

Aerated culture of the organism in Potato-dextrose broth showed that isolate of Rhizoctonia solani from Oryza sativa showed the maximum growth followed by isolates from Cymbopogon flexuosus and Cynodon dactylon. Under static condition too isolate from Oryza sativa showed maximum growth. Of the other MG isolates, isolate from Vigna unguiculata had maximum growth under both conditions. It is obvious from the data that shaking did not have marked influence on the growth of the isolates.

In Czapek's broth, isolate from Sesbania aculeata of MG 1 grew best under both the conditions. Among the other MG isolates, Mushroom bed isolate grew best whereas isolate from Vigna unguiculata failed to grow under both conditions.

Growth in solid media showed the supremacy of MG 1 isolate from Oryza sativa which exceeded the other isolates in growth rate, sclerotial initiation and maturation in all the media tested.

Sclerotia of all the MG 1 isolates were capable of floating for one day. Only a marginal percentage of sclerotia sunk after 24 hours of suspension, as in isolates

from Panicum maximum, Pennisetum polystachyon, Colocasia esculenta, Cymbopogon flexuosus and Cynodon dactylon. All the other isolates including that from Oryza sativa showed cent per cent floatation even after 14 days. Representative isolates of the other groups were not adapted to float in water, all of them sinking at the time of immersion.

Tu et al. (1979) were able to trap sclerotia of Rhizoctonia solani floating on irrigation water in paddy fields one month after transplantation. The ability of MG 1 isolates to float plays an important role in the spread of sheath blight disease of rice.

The reaction of the various varieties of rice to the ten MG 1 isolates of Rhizoctonia solani showed variation in virulence among the isolates. The rice varieties responded differently to the ten isolates in disease severity and development (Table 13). The rice isolate (Isolate 1) was found to be the most virulent with maximum disease severity and development on variety Jyothi (Grade 5 to 7, X = 38.5), followed by variety IR-36 (Grade 3 to 5, X = 26) and Cul-169 (Grade 1 to 5, X = 20.3). Isolate from Monochoria vaginalis produced maximum disease on Jyothi (Grade 3 to 5, X = 31) followed by MO-5 (Grade 3 to 5, X = 21.6) and IR-36 (3 to 5, X = 17). Isolates from Arachis hypogaea, Panicum maximum and

Sporobolus diander produced grades of 3 to 5, 3 and 3 respectively, on variety Jyothi. All the above isolates, except that of Panicum maximum showed some similarity in culture and morphology (Table 5). Isolate from Pennisetum polystachyon showed minimum infection with grades not exceeding 1 in all the varieties tested. Varieties Jaya and Cul-1954 were the most tolerant to all the isolates, with varying degree of infection. Isolates from Colocasia esculenta and Oryza sativa failed to produce infection on Cul-1954.

The variability in pathogenicity of the isolates on the different varieties suggest inherent genetic difference which also accounts for the cytoplasmic incompatibility during hyphal fusion among these isolates. This suggests the presence of strains of the pathogen in the ecosystem of rice fields which is responsible for the breakdown in the resistance offered to the pathogen by the rice plants. Nandi and Chakrabarti (1984) were able to distinguish four pathotypes of Corticium sasakii by observing the variation in virulence of seven isolates and disease development on ten rice cultivars.

Rhizoctonia solani from rice grown on Potato-dextrose agar medium amended with 2.5 ppm carboxin (Isolate $V_1$)

departed from the normal isolate (Isolate 1) in culture and morphology. Kataria and Grover (1974) observed altered morphology of isolate of Rhizoctonia solani adapted to benomyl, chloroneb and PCNB.

Successive transfer of the adapted isolates to increasing concentrations of 5 ppm and 10 ppm carboxin limited the growth to 35 mm. This was only marginally greater than the growth of 25 and 20 mm by the original isolate in 5 ppm and 10 ppm carboxin amended medium.

The adapted isolate $V_3$ grown on unamended Potato-dextrose agar medium produced sheath blight of lesser intensity than the normal isolate, when the sclerotia were used as inoculum. Mycelial disc inoculated by the cellophane method (Amin, 1975) failed to produce infection, whereas the disc of the normal isolate readily infected and produced symptoms. Grover and Chopra (1971) reported reduced virulence of isolates adapted to carboxin even after reversion to the parent type by repeated transfers.

The adapted isolate reisolated from the inoculated plants reverted to the normal morphology and culture after 2 to 3 transfers on Potato-dextrose agar medium (Grover and Chopra, 1971) showing that the alteration is only a temporarily acquired tendency.

Perfect fusion occurred with the reisolated adapted (tolerant) isolate and the parent isolate indicating that capacity to anastomose is a stable character unaltered by environmental condition (Tu et al., 1969).

# SUMMARY

## SUMMARY

Rice plants affected with sheath blight observed at the College of Agriculture, Vellayani, showed the typical symptoms of the disease. Symptoms usually developed towards the tillering stage of the crop. Light greenish grey ellipsoidal lesions with dark brown margin developed from initial discolourations originating at or above the water level. These later coalesced into larger lesion of light greenish brown and brown margin. Such lesions were oblong to irregular and in severe condition encircled the culm to cause rotting of the sheath. The lesions then spread to the leaves and the plants were killed. Under high humid condition, sclerotia and silvery threads of the fungal mycelium were seen on the affected regions.

Isolation from the diseased tissues yielded Rhizoctonia solani Kühn (Thanatephorus cucumeris (Frank) Donk). Hyphae were multinucleate with septal pore apparatus. Branching was near the distal septum with constriction of the branch base. The culture showed brown pigmentation. Mature sclerotia were subglobose with rough regular surface.

Specimens of sheath blight affected rice plants from the different localities showed variation in the symptoms of the disease. The shape of the lesions was usually ellipsoidal and

varied from oval to irregular and oblong to elongated. No uniform pattern of symptoms could be observed either on geographical or on varietal basis.

Symptoms observed on the other host plants varied depending on the part of the plant infected by this organism. Typical collar rot symptoms were seen in Arachis hypogaea, Glycine max, Phaseolus aureus, Sesamum indicum and Vigna unguiculata. Symptoms resembling sheath blight disease developed on the graminaceous host plants. Web blight symptoms were seen on Arachis hypogaea. Rotting of fruits and tubers and leaf rot were observed on other host plants.

Isolation from the various host plants/habitat yielded Rhizoctonia solani and in total forty one isolates were used for the study.

Based on a critical analysis of the morphological and cultural characters, the 41 isolates of Rhizoctonia solani were grouped into four morphological groups, viz., MG 1, MG 2, MG 3 and MG 4 following distinctive morphological and cultural norm.

MG 1 isolates have the typical brown pigmentation of Rhizoctonia solani which differed from the MG 2, MG 3 and MG 4 isolates which have mycelium of whitish shade. MG 1 isolates produced large brown sclerotia having a rough hairy

surface. Aggregation or fusion of individual sclerotia into bands or clusters is a common feature. The sclerotia of the other morphological groups were more or less globose and rarely forming aggregates. MG 1 isolates had greater hyphal width compared to the other groups. Mycelial growth on Potato-dextrose agar medium was faster for MG 1 isolates.

The pathogenicity of the isolates of Rhizoctonia solani showed some specificities within the groups. MG 1 included isolates infecting rice and aerial parts of other host plants. MG 2 isolates infected tubers and stored fruits with an active saprophytic phase. MG 3 and MG 4 isolates infected the stolon and collar region of plants.

Only isolates of MG 1 could infect and produce sheath blight disease on rice. None of the MG 2, MG 3 or MG 4 isolates was pathogenic on rice. On the other hand rice isolates of MG 1 could infect many of the host plants of the other MG isolates. This indicates that the pathogenicity of rice group is not confined to the group of host plants of the rice group pathogen. These observations draw to the conclusion that, MG 1 isolates have similar pathogenic and morphogenic tendencies.

Rice isolate caused web blight symptoms on pulses but collar rot symptom did not develop on mature plants. Seedlings

of cowpea could take up infection up to the third week after sowing, beyond which the isolate failed to infect and cause collar rot.

Hyphal fusions occurred between rice isolates (17 isolates). Rice isolate could anastomose with all isolates of MG 1 group. None of the isolates of the other MG groups could anastomose with MG 1 isolates, showing that the MG 1 isolates are genetically related and belong to the same anastomosis group.

Anastomosis group tester isolate AG 1 anastomosed only with the rice group (MG 1) of isolates. This suggests that MG 1 corresponds with AG 1 of Parmeter et al. (1969), to the Group 1 of Schultz (1937), Group A of Richter and Schneider (1953), the sasakii type and web blight type of Watanabe and Matsuda (1966) and TRAG 1 of Tu and Chang (1978). Similar cultural and morphogenic nature of isolates, genetic affinity between isolates and pathogenic similarity among the isolates signify the relationship within the MG 1 isolates.

Among the kinds of fusion within the same group (MG 1) the most common was imperfect fusion or contact fusion followed by perfect fusion. Imperfect fusion occurs between two isolates of the same group. Immediately after cell wall fusion, killing reaction occurred, characterised by the disintegration of the

cytoplasm of the fused cells. Perfect fusion was invariably seen in anastomosis within the same isolate and occurred sparingly between isolates of the same anastomosis group. In this type, fusion is complete with cytoplasmic exchange. Contact fusion is an imperfect fusion wherein killing reaction occurs upon contact of the hyphal walls without fusion.

In the study for determination of clones of Rhizoctonia solani in rice, only isolates $R_1$ and $R_2$ from the same culm showed perfect fusion, whereas the anastomosis reaction with the other isolates from neighbouring culms was imperfect. Isolates showing somatic incompatibility can be considered as a distinct variant with genetic potential for variation in pathogenicity and response to antifungal compounds.

The reaction of the ten common varieties of rice to ten isolates of Rhizoctonia solani of MG 1 showed difference in virulence of the isolates. This variation suggests inherent genetic differences which also account for the cytoplasmic incompatibility during hyphal fusion among the isolates. The presence of strains of the pathogen in the ecosystem of rice fields is responsible for the break down in the resistance of rice plants to the pathogen.

Imperfect or contact fusion was observed between two isolates of the same group which resulted in killing reaction. Disintegration of cytoplasm occurred immediately after cell wall fusion. During contact fusion killing reaction occurred upon contact of the hyphal wall without fusion. Perfect fusion was observed in anastomosis within the same isolate, and sparingly between isolates of the same group. Perfect fusion was followed by cytoplasmic exchange.

In the anastomosis reaction between rice isolates of Rhizoctonia solani in the same field, only isolates from the same culm showed perfect fusion. Anastomosis of these isolates with isolates of the neighbouring culms was imperfect. Isolates showing cytoplasmic incompatibility can be considered as a distinct variant which vary in pathogenicity and response to fungicides.

The ten isolates of MG 1 varied in their virulence and pathogenicity on ten common varieties of rice. This variation suggests inherent genetic differences which also accounted for their somatic incompatibility during hyphal fusion among these isolates. The presence of strains of the pathogen is responsible for the break down in the resistance of the rice plants to the pathogen.

Fig. 20. Strain variation in Rhizoctonia solani the causal organism of sheath blight of rice - Summary of results.

FIG: 20. STRAIN VARIATION IN RHIZOCTONIA SOLANI THE CAUSAL ORGANISM OF SHEATH BLIGHT OF RICE

41 isolates

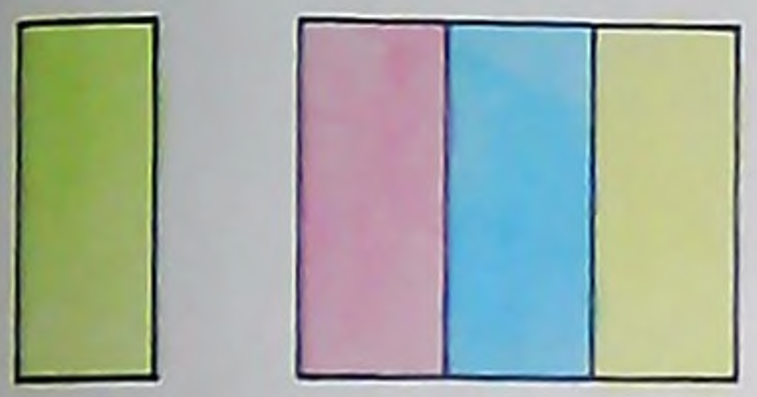
24 host plants / habitat

Grouped into four morphological groups ( MG 1, 2, 3 & 4 )

ANASTOMOSIS STUDIES

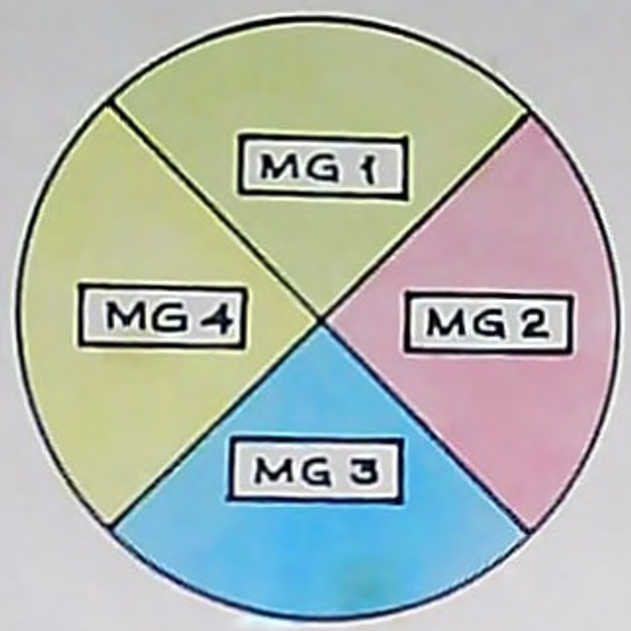
PATHOLOGICAL STUDIES

ANASTOMOSIS REACTION WITH RICE ISOLATE (ISOLATE 1)



POSITIVE REACTION

NEGATIVE REACTION

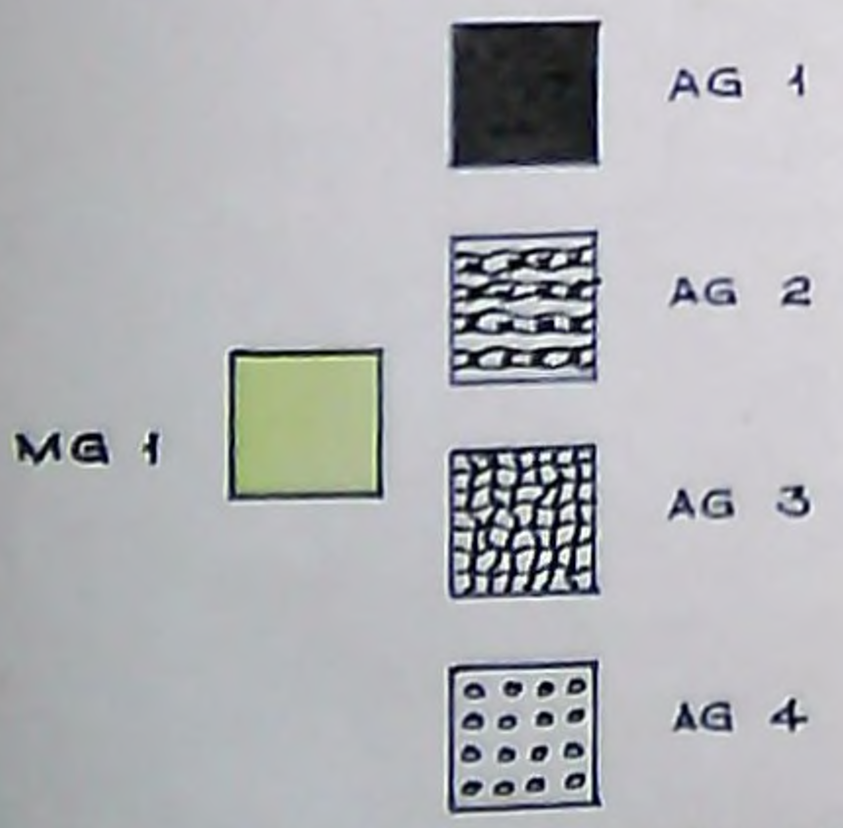



PATHOGENICITY ON RICE

POSITIVE REACTION

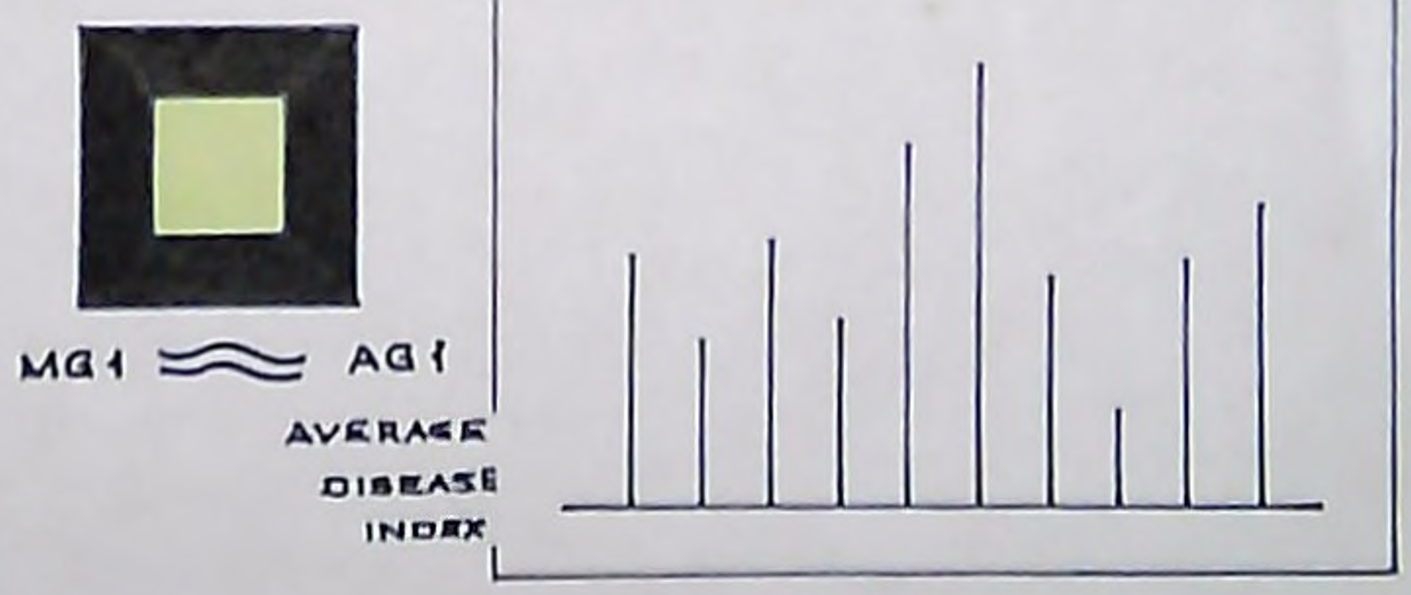
NEGATIVE REACTION

ANASTOMOSIS REACTION WITH AG TESTER ISOLATES




IMPERFECT FUSION WITHIN MG 1

DIFFERENTIAL REACTION OF MG 1 ISOLATES TO VARIOUS RICE VARIETIES




10 ISOLATES

CYTOPLASMIC INCOMPATIBILITY → PRESENCE OF CLONES

DIFFERENTIAL PATHOGENICITY → GENETIC VARIATION

PRESENCE OF STRAINS OF RHIZOCTONIA SOLANI IN RICE FIELDS

# REFERENCES

## REFERENCES


Abawi, G.S. and Martin, S.B. (1985). Rhizoctonia foliar blight of cabbage in New York State. Plant Disease 69 : 158-161.

Abe, H and Tsuboki, K. (1978). Anastomosis groups of isolates of Rhizoctonia solani Kuhn from potatoes. Bull. Pref. Hokkaido agric. Exp. Stn. 40 : 61-71.

Adams, G.C. Jr. and Buller, E.E. (1979). Serological relationships among anastomosis groups of Rhizoctonia solani. Phytopathology 69 : 629-633.

Amin, K.S. (1975). An improved method of evaluating rice sheath blight. Phytopathology 65 : 214-215.

Anderson, N.A. (1977). Evaluation of the Rhizoctonia complex in relation to seedling blight of flax. Pl. Dis. Reptr. 61 : 140-142

Anderson, N.A. (1982). The genetics and pathology of Rhizoctonia solani. Ann. Rev. Phytopathol. 20 : 329-347.

Anonymous (1954). Control of serious diseases of foot rot and leaf spot of pan crop. Agric. Anim. Husb. Uttar Pradesh 5(2) : 8-10.

* Anonymous (1967). Plant Pathology. Rep. Res. Brch. Minist. Agric. Zambia : 95-109.

Anonymous (1976). Standard evaluation system for rice. IRRI. Laguna, Philippines. 64 pp.

Bains, S.S. and Jhooty, J.S. (1983). Sensitivity to fungitoxicants, cultural behaviour and pathogenicity of Rhizoctonia solani isolates naturally occurring in Punjab. Indian J. Ecol. 10 : 274-278.

Bandy, B.P., Zanzinger, D.H. and Tavantzin, S.M. (1984). Isolation of anastomosis group 5 of Rhizoctonia solani from potato field soil in Maine. Phytopathology 74 : 1220-1224.

Bhavani Devi, S. (1982). Studies on the Edible Mushrooms of Kerala with special reference to paddy straw mushroom Volvariella sp. Ph.D. Thesis. Kerala Agricultural University. pp.221.

Bolkan, H.A. (1976). Attempts to bridge anastomosis groups of Rhizoctonia solani. Fitopatol. Bras. 1 : 14-17.

Bolkan, H.A. and Ribeiro, W.R.C. (1985). Anastomosis groups and pathogenicity of Rhizoctonia solani isolates from Brazil. Plant Disease 69 : 599-601.

Booth, C. (1971). Introduction to General Methods. In Methods in Microbiology Vol. 4, ed. C. Booth, pp 1-47. Academic Press, London and N.Y. 795 pp.

Bruggen, A.H.C. Van, and Arneson, P.A. (1984). Resistance in Rhizoctonia solani to tolchofosmethyl. Neth. J. Plant Pathol. 90 : 95-106.

Buller, A.H.R. (1931). Social organisation and sex in the Hymenomycetes. In Researches on Fungi, Vol. IV. pp 139-186. Hafner Publishing Co., New York 1958. 329 pp.

Buller, A.H.R. (1933). The formation of hyphal fusion in the mycelium of the higher fungi. In Researches on Fungi Vol. V. pp 1-74. Hafner Publishing Co., New York, 1958. 416 pp.

Burpee, L.L., Sanders, P.L., Cole, H.J.R. and Kim, S.H. (1978). A staining technique for nuclei of Rhizoctonia solani and related fungi. Mycologia 70 : 1281-1282.

Butler, E.J. (1918). Fungi and Diseases in Plants. M/s. Bishen Singh Mahendra Pal Singh and M/s. Periodical Experts, Delhi. 547 pp.

Cardosa, J.E., Hildebrandt, A.C. and Grau, C.R. (1978). Taxonomic and cultural characterisation of Rhizoctonia solani Kühn isolate from soybean (Glycine max Merril). Fitopatol. Bras. 3 : 241-247.

Cedeno, L. (1978). Cultural characters, condition of the nucleus and anastomosis groups in isolates of Rhizoctonia solani Kühn. Agronnia trop. 28 : 151-161.

Chauhan, L.S. and Jyoti Singh. (1982). Rhizoctonia leaf blight of lemongrass (Cymbopogon flexuosus). Indian J. Mycol. Pl. Path. 11 : 245.

Dash, S.C. and Panda, S. (1984). Chemical control of rice sheath blight disease. Indian Phytopath. 37 : 79-82.

Datta, T.P. and Sharma, B.D. (1979). A preliminary study on the control of soreshin disease of cotton in West Bengal. Pesticides 10 : 28-29.

* Duggar, B.M. (1915). Rhizoctonia crocorum (Pers) DC. and R. solani Kühn (Corticium vagum B & C) with notes on other species. Ann. Mo. bot. Gdn. 2 : 403-458.

Elmer, O.H. (1932). Pathogenic and cultural comparison of strains of Rhizoctonia solani. Phytopathology 22 : 8-9.

* El-Sawah, M.Y., Ziedan, M.I. and Abdelhalim, S.T. (1975). Laboratory and green house evaluation of various systemic fungicides for control of Rhizoctonia damping-off of cotton seedlings. Agri. Res. Rev. 53(2) : 65-77.

Esser, K. (1964). Heterogenic incompatibility. In Incompatibility in Fungi, ed. K. Esser & J.R. Raper, pp 6-13. Springer-Verlag. 124 pp.

Exner, B. (1953). Comparative studies of four Rhizoctonias occurring in Louisiana. Mycologia 45 : 698-719.

Flentje, N.T. and Stretton, H.M. (1964). Mechanism of variation in Thanatephorus cucumeris and Thanatephorus praticola. Aust. J. biol. Sci. 17 : 686-704.

Flentje, N.T., Stretton, H.M. and McKensie, A.R. (1967). Mutation in Thanatephorus cucumeris. Aust. J. biol. Sci. 20 : 1173-1180.

Flentje, N.T., Stretton, H.M. and McKenzie, A.R. (1969). Mechanism of variation in Rhizoctonia solani. In Rhizoctonia solani, Biology and Pathology, ed. Parmeter, pp 52-65. Univ. California Press, Berkeley. 255 pp.

* Frederiksen, T., Jorgensen, C.A. and Neilsen, O. (1938). Undersogelser over Kartoflens Rodfiltsvamper dens Bekaempelse. Tidsskr. Plavl. 42 : 1-64.

Gangopadhyay, S. and Chakrabarti, N.K. (1982). Sheath blight of Rice. Rev. Plant Pathol. 61 : 451-460.

Gottlieb, D. (1971). Limited growth in fungi. Mycologia 63 : 619-629.

Gregory, P.H. (1984). The fungal mycelium : an historical perspective. (First benefactor's lecture). Trans Br. mycol. Soc. 82 : 1-11.

* Grieshbach, E. (1975). The significance of weeds for the transmission of Rhizoctonia solani Kühn. II. On the morphology and physiology of isolates from different source. Zur Morphol. Physiol. Versch. Erreg. Hygiene 130(1) : 64-81.

Grover, R.K. and Chopra, B.C. (1971). Adaptation of Rhizoctonia species to two oxathiin compounds and manifestation of the adapted isolates. Acta Phytopathol. Acad. Sci. Hung. 5 (2-4) : 113-121.

Hashiba, T. (1984). Forecasting model and estimation of yield loss by rice sheath blight disease. JARQ 18(2) : 92-98.

Hashiba, T. and Mogi, S. (1975). Developmental changes in sclerotia of the rice sheath blight fungus. Phytopathology 65 : 159-162.

* Hashiba, T., Mogi, S. and Yagi, S. (1974). The relationship between the mycelial growth of rice sheath blight fungus isolates and the air temperature of the collecting regions. Proc. Ass. Pl. Prot. Hokuriku 22 : 8-14.

* Hemmi, T. and Endo, S. (1931). Studies on the sclerotium disease of rice plants. III. Some experiments on the sclerotial formation and the pathogenicity of certain fungi causing sclerotium disease of rice plants. Forsch. Geb. Pfl. Krankh 1 : 111-125.

Herr, L.J. (1979). Practical nuclear staining procedure for Rhizoctonia-like fungi. Phytopathology 69 : 958-961.

Herr, L.J. and Roberts, D.L. (1980). Characterisation of Rhizoctonia populations obtained from sugar beet fields with differing soil texture. Phytopathology 70 : 476-480.

Homma, Y., Yamashita, Y. and Ishii, M. (1983). A new anastomosis group (AG 7) of Rhizoctonia solani Kuhn from Japanese radish fields. Ann. phytopath. Soc. Japan 49 : 184-190.

Jhooty, J.S. and Bains, S.S. (1973). Studies on Rhizoctonia solani. III. Reaction of various isolates to different fungitoxicants. Indian J. Microbiol. 13 : 27-32.

Kannaiyan, S. and Prasad, N.N. (1979). Sheath blight incidence in weed hosts. IRRN 4(3) : 17.

Kataria, H.R. and Grover, R.K. (1974). Adaptation of Rhizoctonia solani to systemic and non-systemic fungitoxicants. Z. Pflkrankh. Pflschutz. 81 : 472-478.

* Kernkamp, M.F., Dezeeuw, D.J., Chen, S.N., Ortega, B.C., Tsiang, C.T. and Khan, A.M. (1952). Investigations on physiologic specialisation and parasitism of R. (Corticium) solani. Tech. Bull. Minn. agric. Exp. Sta. 200 : 36.

Kohli, C.K. (1966). Pathogenicity and host range studies on the paddy sheath blight pathogen (Rhizoctonia solani Kühn). J. Res. Ludhiana 3 : 37-40.

Kohli, C.K. (1967). Some cultural studies on Rhizoctonia solani the causal organism of sheath blight of paddy. J. Res. Ludhiana 4 : 401-405.

* Kuhn, J.G. (1858). Die Krankheiten der Kulturegewachse ihre ursachen und ihre Verhutung. Gustav Bosselmann, Berlin. pp. 312.

Kuninaga, S. and Yokosawa, R. (1980). A comparison of DNA base composition among anastomosis groups in Rhizoctonia solani Kühn. Ann. phytopath. Soc. Japan 46 : 150-158.

Kuninaga, S. and Yokosawa, R. (1985a). DNA base sequence homology in Rhizoctonia solani Kühn. VII. Genetic relatedness between AGBI and other anastomosis groups. Ann. phytopath. Soc. Japan 51 : 121-132.

Kuninaga, S. and Yokosawa, R. (1985b). DNA base sequence homology in R. solani Kuhn. VI. Genetic relatedness among seven anastomosis groups. Ann. phytopath. Soc. Japan 51 : 121-132

Kuninaga, S., Yokosawa, R. and Ogoshi, A. (1978). Anqstomosis grouping of Rhizoctonia solani Kuhn isolated from noncultivated soils. Ann. phytopath. Soc. Japan 44 : 591-598.

Kuninaga, S., Yokosawa, R. and Ogoshi, A. (1979). Some properties of anastomosis group 6 and BI in Rhizoctonia solani Kuhn. Ann. phytopath. Soc. Japan 45 : 207-214.

Lakshmanan, P., Nair, M.C. and Menon, M.R. (1979). Collar rot and web blight of cowpea caused by Rhizoctonia solani in Kerala, India. Pl. Dis. Reptr. 63 : 410-413.

* Larpent, J.P. (1965). Relationship between growth rate and branching of young mycelium of some fungi. C.r. hebd. Seane. Acad. Sci. 260 : 265-267.

Luttrell, E.S. (1962). Rhizoctonia blight of tall fescue grass. Pl. Dis. Reptr. 46 : 661-664.

Malaguti, G. (1951). Sheath spot disease of rice caused by Rhizoctonia solani. Agronomia trop. Maracay 1 : 71-75.

Martin, S.B., Lucas, L.T. and Campbell, C.L. (1984). Comparative sensitivity of Rhizoctonia solani and Rhizoctonia-like fungi to selected fungicides in vitro. Phytopathology 74 : 778-781.

* Matsumoto, T. (1921). Studies in the physiology of the fungi. XII. Physiological specialisation in Rhizoctonia solani Kuhn. Ann. Mo. bot. Gdn. 7 : 1-62.

* Matsumoto, T. (1923). Further studies on the physiology of Rhizoctonia solani Kuhn. Bull. Imp. Coll. Agric. and Forestry 5 : 63 pp.

* Matsumoto, T. and Yamamoto, W. (1935). Hypochnus sasakii in comparison with Corticium stevensii and Corticium koleroga. Trans. nat. Hist. Soc. Taiwan 25 : 161-175.

Matsumoto, T., Yamamoto, W. and Hirane, S. (1932). Physiology and parasitology of the fungi generally referred to as Hypochnus sasakii Shirai. I. Differentiation of the strains by means of hyphal fusion and culture in differential media. J. Soc. Trop. Agric. 4 : 370-388.

Matsuyama, N., Morozimato, Z., Ogoshi, A. and Wakimoto, S. (1978). Grouping Rhizoctonia solani Kuhn with nonspecific esterase zymogram. Ann. phytopath. Soc. Japan 44 : 652-658.

* Miyake, I. (1910). Studies uber die Pilze der Reispflanzen. J. Coll. Agric. Tokyo 2 : 237-275.

* Muller, K.O. (1924). Investigations on the evolution and biology of Hypochnus solani P.U.D. (Rhizoctonia solani Kühn). Arb. biol. Reichsanst. Land-u Forstw.8 : 198-262.

Murray, D.I.L. (1981). Rhizoctonia solani causing barley stunt disorder. Trans. Br. mycol. Soc. 76 : 383-395.

Naiki, T. and Kanoh, M. (1978). Grouping of Rhizoctonia solani Kühn. causing root disease of spinach in plastic house cropping. Ann. phytopath. Soc. Japan 44 : 554-560.

Nandi, S. and Chakrabarti, N.K. (1984). Differential virulence of isolates of Corticium sasakii, the sheath blight pathogen to some rice cultivars and type of resistance offered by the host rice plants. Z. Pflkrakh. Pflschutz. 91 : 182-188.

Nayak, P., Row, K.V.S.R.K. and Sridhar, R. (1979). Host range of Rhizoctonia solani, the causal organism of sheath blight disease of rice. Indian Phytopath. 32 : 604-605.

Newton, W. (1931). The physiology of Rhizoctonia. Scient. Agric. 7 : 178-182.

Ogoshi, A. (1972). Grouping of Rhizoctonia solani Kühn with hyphal anastomosis. Ann. phytopath. Soc. Japan 38 : 117-122.

Ogoshi, A. (1975). Studies on the grouping of Rhizoctonia solani Kühn. JARQ 9 : 198-203.

Ogoshi, A. (1976). Studies on the grouping of Rhizoctonia solani Kühn with hyphal anastomosis and the perfect stages of the groups. Bull. natn. Inst. agric. Sci. Tokyo. Series C, No. 30, 1-63.

Ogoshi, A. and Ui, T. (1979). Specificity of vitamin requirement among anastomosis groups of Rhizoctonia solani Kuhn. Ann. phytopath. Soc. Japan 45 : 47-53.

Ogoshi, A. and Ui, T. (1983). Diversity of clones within an anastomosis group of Rhizoctonia solani Kühn in a field. Ann. phytopath. Soc. Japan 49 : 239-245.

O'Neill, N.R. and Rush, M.C. (1982). Etiology of sorghum sheath blight and pathogen virulence on rice. Plant Disease 60 : 1115-1118.

ONesirosan, P.T. (1977). Comparison of Rhizoctonia solani isolates from web blight and basal canker of cowpea. Pl. Soil 46 : 135-143.

* Palo, M.A. (1926). Rhizoctonia disease of rice. I. A study of the disease and of the influence of certain conditions upon the viability of the sclerotial bodies of the causal fungus. Philippine Agric. 15 : 361-376.

Papavizas, G.C., Adams, P.B., Lumsden, R.D., Lewis, J.A., Dow, R.L., Ayer, W.A. and Kentzes, J.G. (1975). Ecology and epidemiology of Rhizoctonia solani in field soil. Phytopathology 65 : 871-877.

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

* Park, M. and Bertus, L.S. (1932). Sclerotial disease of rice in Ceylon. II. Sclerotium oryzae Catt. Ceylon J. Sci. 11 : 343-359.

Parmeter, J.R. and Whitney, H.S. (1969). Taxonomy and nomenclature of the imperfect state. In Rhizoctonia solani: Biology and Pathology, ed. J.R. Parmeter, pp 7-49. Univ. Calif. Press, Berkeley. 255 pp.

Parmeter, J.R., Sherwood, R.T. and Platt, W.D. (1969). Anastomosis grouping among isolates of Thanatephorus cucumeris. Phytopathology 59 : 1270-1278.

Parmeter, J.R., Whitney, H.S. and Platt, W.D. (1967). Affinities of some Rhizoctonia species that resemble mycelium of Thanatephorus cucumeris. Phytopathology 57 : 218-223.

* Patino, H.C. (1967). Diseases of oleaginous annuals in Colombia. Agricultura trop. 23 : 532-539.

Person, L.H. (1944). Parasitism of Rhizoctonia solani on beans. Phytopathology 34 : 1056-1068.

Raj, S., Dutt, S.C. and Sahai, D. (1974). Cultural variation in potato isolates of Rhizoctonia solani Kühn. Acta Phytopathol. Acad. Sci. Hung. 9 : 71-79.

Ramakrishnan, K. and Ramakrishnan, T.S. (1948). Banded leaf blight of Arrow root, Maranta arundinaceae. Indian Phytopath. 1 : 129-136.

* Reinking, O.A. (1918). Philippine economic plant diseases. Philippine J. Sci. 13 : 165-274.

Reynolds, M., Weinhold, A.R. and Morris, T.J. (1983). Comparison of anastomosis groups of Rhizoctonia solani by polyacrylamide gel electrophoresis of soluble proteins. Phytopathology 73 : 903-906.

Richter, H. and Schneider, R. (1953). Studies on the morphological and biological differentiation of Rhizoctonia solani. Phytopath. Z. 20 : 167-226.

Sadowski, S. (1970). Properties and ecological requirements of some Mazurian isolates of Rhizoctonia. Zasz. nauk. wyzsz. Szk. roln. Olsztyn ser. Azo (Suppl.2), 3-17.

Sanford, G.B. and Skoropad, W.P. (1955). Distribution of nuclei in hyphal cells of Rhizoctonia solani. Can. J. Microbiol. 1 : 412-415.

* Schultz, H. (1937). Comparative studies on the ecology, morphology and systematic position of the propagation fungus. Arb. biol. Anst (Reichsanst.) Berl. 22 : 1-41.

* Sethofer, V. and Jermoljev, E. (1950). On the resistance of potato varieties of Rhizoctonia (R. solani). Ochr. Rost. 23 : 89-100.

Sherwood, R.T. (1969). Morphology and physiology in four anastomosis groups of Thanatephorus cucumeris. Phytopathology 59 : 1924-1929.

Singh, D.V. and Seth, M.C. (1971). White leaf blotch of Bermuda grass in India. Indian Phytopath. 24 : 207-208.

Stephens, C.T., Herr, L.J., Schmittenner, A.F. and Powell, C.C. (1982). Characterisation of Rhizoctonia isolates associated with damping-off of bedding plants. Plant Disease 66 : 700-703.

Sterne, R.E. and Jones, J.P. (1978). Sharp eye spot of wheat in Arkansas caused by Rhizoctonia solani. Pl. Dis. Reptr. 62 : 56-60.

Stroubles, W.H. (1954). Host range of Rhizoctonia aerial blight fungus in Louisiana. Pl. Dis. Reptr. 38 : 789-790.

Su, M.T. (1936). Report of the Mycologist, Burma, Mandalay for the year ending the 31st March 1936. 5 pp.

Summer, D.R. (1985). Virulence of anastomosis groups of Rhizoctonia and Rhizoctonia-like fungi on selected germplasm of snap bean, lima bean and cowpea. Plant Disease 69 : 25-27.

Sundaram, N.V. (1953). Thread blight of Ginger. Indian Phytopath. 6 : 80-85.

* Tamietti, G. and Matta, A. (1981). Decay of carrots during field storage in winter. Riv. Patol. veg. 17 : 45-54.

Tu, J.C. (1968). Physiological specialisation of strains of Pellicularia sasakii isolated from rice plants. Pl. Dis. Reptr. 52 : 323-326.

Tu, C.C. and Chang, Y.C. (1978). Studies on the anastomosis groups of Rhizoctonia solani Kühn in Taiwan. J. agri. Res. China 27 : 325-343.

Tu, C.C., Chang, Y.C. and Wang, C.W. (1979). Studies on ecology of Rhizoctonia solani, the causal organism of sheath blight of rice. Natn. Sci. Coun. Monthly 7 : 1208-1219.

Tu, C.C., Roberts, D.A. and Kinbrough, J.W. (1969). Hyphal fusion, nuclear condition and perfect stages of three species of Rhizoctonia. Mycologia 61 : 775-783.

* Uĭ, T. and Saito, I. (1968). Notes on the number of nuclei in hyphal cells of Rhizoctonia solani. Mem. Fac. Agric. Hokkaido Univ. 6 : 359-363.

* Valdez, R.B. (1955). Sheath spot of rice. Philippine Agric. 39 : 317-336.

* Ward, H.M. (1888). On a lily disease. Ann. Bot. 2 : 319-382.

Wasfy, E.H., Sheir, H.M. and Darweesh. (1984). Comparative studies on some isolates of Rhizoctonia solani in Egypt. Phytopath. Z. 100 : 346-353.

Watanabe, T. and Shiyomi, M. (1975). Hyphal morphology of Rhizoctonia solani Kuhn and related fungi isolated from sugarcane in Taiwan. Trans. Mycol. Soc. Japan 16 : 253-263.

Watanabe, B. and Matsuda, A. (1966). Studies on the grouping of Rhizoctonia solani Kuhn pathogenic to upland crops. Designated Experiment No. 7, Agriculture, Forestry and Fisheries Research Council and Ibaraki Agr. Exp. Sta.

* Wei, C.T. (1934). Rhizoctonia sheath blight of rice. Bulletin of the College of Agriculture and Forestry, University of Nanking No. 15, 21 pp.

Wu, W.S. (1976). The incidence of pathogen of rice sheath blight on potato. Pl. Prot. Bull. Taiwan 18 : 354-360.

Yamamoto, D.T. and Aragaki, M. (1982). Pathogenicity of Rhizoctonia isolates to papaya in Hawaii. Plant Disease 66 : 1136-1137.

Yu, C.M. (1983). Brown sheath blight disease of rice - a new disease of rice in Taiwan. Pl. Prot. Bull. Taiwan 25 : 53-56.

* Yuno, I., Hashiba, T. and Mogi, S. (1978). Effect of temperature on disease development of rice sheath blight caused by Rhizoctonia solani. Proc. Assoc. Pl. Prot. Hokurika 26 : 4-9.

Zentmyer, C.A. (1955). A laboratory method for testing soil fungicides with Phytophthora cinnamomi as test organism. Phytopathology 45 : 398-404.

* Original not seen.

# APPENDIX

# APPENDIX I

## Composition of the various media used.

### Coon's agar

| | |
|---|---|
| Sucrose | 7.20 g |
| Dextrose | 3.60 g |
| Magnesium sulphate | 1.23 g |
| Potassium dihydrogen phosphate | 2.72 g |
| Potassium nitrate | 2.02 g |
| Agar | 20.00 g |
| Water | 1 litre |

### Czapek's medium

| | |
|---|---|
| Sodium nitrate | 2.00 g |
| Potassium dihydrogen phosphate | 1.00 g |
| Magnesium sulphate | 0.50 g |
| Potassium chloride | 0.50 g |
| Ferrous sulphate | 0.01 g |
| Sucrose | 30.00 g |
| Agar | 20.00 g |
| Water | 1 litre |

### Potato-dextrose agar

| | |
|---|---|
| Potato | 200 g |
| Dextrose | 20 g |
| Agar | 20 g |
| Water | 1 litre |

### Richards' agar

| | |
|---|---|
| Potassium nitrate | 10.00 g |
| Potassium dihydrogen phosphate | 5.00 g |
| Magnesium sulphate | 2.50 g |
| Ferric chloride | 0.02 g |
| Sucrose | 50.00 g |
| Agar | 20.00 g |
| Water | 1 litre |

# STRAIN VARIATION IN *Rhizoctonia solani* Kuhn [*Thanatephorus cucumeris* (Frank) Donk] CAUSING SHEATH BLIGHT OF RICE

By

**M. VIJAYAN**

ABSTRACT OF A THESIS

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## ABSTRACT

Symptoms of sheath blight disease usually develop towards the tillering stage of the rice crop. Discolourations initially appearing on the sheath at or above the water level develop into ellipsoidal lesions. The lesions are light greenish grey in colour with dark brown margin. As the disease progress the lesions coalesce and become oblong to irregular in shape. In severe condition the lesions encircle the culm and cause rotting of the sheath. The disease then spreads to the leaves and such plants are killed. Sclerotia and silvery threads of the fungal mycelium appear on the affected regions under high humid condition.

The causal organism was isolated and identified as Rhizoctonia solani Kuhn. The hyphae branched near the distal septum with a constriction at the branch base. Hyphae were multinucleate and in culture showed brown pigmentation. Mature sclerotia were subglobose with rough regular surface.

Lesions of sheath blight were generally ellipsoidal in shape on a number of diseased specimens collected from different localities. Variation in shape was from oval to irregular and oblong to elongated. The types of symptons seen on other host plants were collar rot, sheath/leaf blight, web blight, leaf rot and rotting of fruits and tubers.

Four morphological groups, viz., MG 1, MG 2, MG 3 and MG 4 were identified from the 41 isolates of Rhizoctonia solani

obtained from the various host plants/habitat. Each morphological group has distinctive morphological and cultural characters.

The isolates of Rhizoctonia solani also exhibited pathogenic specificity within the groups. Isolates of MG 1 infected rice and aerial parts of other host plants. MG 2 isolates infected tubers and stored fruits with an active saprophytic phase. MG 3 and MG 4 isolates infected the stolon and collar region of plants.

Of the 41 isolates only isolates of MG 1 infected rice. Eventhough none of the isolates of the other groups could infect rice, many of their host plants were susceptible to infection by rice isolates. This shows that the pathogenicity of rice group is not confined to the group of host plants of the rice group pathogen.

MG 1 isolates were genetically related and belonged to the same anastomosis group since hyphal fusion occurred between these isolates. None of the isolates of the other MG groups could anastomose with the MG 1 isolates.

The rice group (MG 1) isolates anastomosed with the Anastomosis group tester isolate AG 1 indicating that MG 1 corresponded with AG 1 of United States (Parmeter et al., 1969).