ETIOLOGY AND BIOLOGICAL CONTROL OF SHEATH ROT DISEASE OF RICE

BY

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DEDICATED TO
AMMA & APPACHAN

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I hereby declare that this thesis entitled **"Etiology and Biological control of sheath rot disease of rice"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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INTRODUCTION

1. INTRODUCTION

Sheath rot disease of rice caused by Sarocladium oryzae (Sawada) Gams and Hawksworth. has gained much importance in all the rice growing tracts of Kerala during the last decade. Yield losses due to this disease in severe proportion have been reported often from certain pockets of rice growing areas of the state.

In India this disease was first reported by Agnihothrudu in 1973 from Karnataka. The occurrence of this disease in Kerala was first reported by Nair and Sathyarajan (1975). Since then a lot of work has been done on the etiology and chemical control of the disease.

The concept of S.oryzae as the sole incitant of sheath rot of rice is still a matter of controversy. Recent reports show that various types of sheath rot exist, which are incited by different pathogens. More over, the application of fungicides was often found to be ineffective in controlling the disease.

Under the above circumstances the present study was taken up with a view to study in detail the different types of disease development and to derive more details about the pathogens involved, their mode of survival and also to find out the possibility of controlling the disease with some biological agents.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Sheath rot disease of rice is reported to cause serious damage to various promising rice varieties of different rice growing tracts of the world.

Sheath rot of rice was first reported by Sawada (1922) from Formosa and the pathogen was named as Acrocylindrium oryzae Sawada. The pathogen was subsequently reported by Tasugi and Ikeda (1956) from Japan, Chen (1957) from Taiwan, Ou (1963) from Thailand, Jimenez and Panizo (1977) from Peru, Shahjahan et al. (1977) from Peru, Rodriguez and Nass (1990) from USA. Gams and Hawksworth (1975) from Netherlands introduced the new genus Sarocladium for Acrocylindrium and a new combination Sarocladium oryzae Gams and Hawks was made for the organism causing sheath rot of rice.

The disease occurrence in India was first reported by Agnihothrudu in 1973 from Karnataka. Prabakaran et al. (1974) reported a yield loss of about 85 per cent from Tamilnadu. This was followed by Amin et al. (1974) from West Bengal,Nair and Sathyarajan(1975) from Kerala,Ghufran et al.

(1980) from Bihar, Raina and Singh (1980) from Punjab, Upadhay and Diwaker (1984) from Madhya Pradesh, Singh and Roy (1987) from Manipur and Singh (1988) from UttarPradesh.

Chen (1957) observed 20 per cent damage of rice crop due to this disease. Ou (1972) stated that it could cause up to 85 per cent loss in rice. Chakrabarti and Biswas (1978) evaluated the yield loss due to sheath rot in India and reported that the average reduction in grain weight in diseased panicles was 79 per cent.

Estrada et al. (1979) reported a reduction of 28 per cent and 75 per cent in panicle production and grain yield respectively. Later Mohan and Subramanian (1979), Srinivasan (1980) and Vidyasekaran et al. (1984) reported the yield reduction due to this disease from Tamil Nadu. Ou (1984) reported that the pathogen caused damage to 10-20 per cent of tillers in all the rice growing areas. Surin et al. (1988) observed a positive relationship between the disease incidence or disease severity and yield difference. Reddy (1991) reported the effect of infection of Sarocladium oryzae on panicle and grain weight percentage of diseased grains, sterile spikelets, panicle length and seed germination.

2.1 Symptomatology

Tasugi and Ikeda (1956) reported that the fungus chiefly attacked the uppermost leaf sheath and caused rotting. The greyish brown lesions coalesce and form irregular blotches. Ou (1972) observed that the rot occurred on the uppermost leaf sheath enclosing the panicle. The lesions started as oblong spots with brown margins and grey centre or greyish brown throughout. The young panicles remained within the sheath or only partially emerged. White powdery growth can be seen abundantly inside the panicle affected.

Amin et al. (1974) found that the initial lesions were about one centimeter long and about 0.5 cm to 1 cm wide, oval, dark chocolate brown in colour surrounded by a diffused light brown halo. Lesions were most conspicuous on the flag leaf sheath. Under severe infection several lesions overlap. Older lesions had dark brown border 0.2-0.3 cm wide and the green colour of the sheath progressively became light green and finally straw coloured. Severely infected panicles did not emerge out and get compressed inside the flag leaf sheath which is referred as choking.

Nair and Sathyarajan (1975) described the symptoms as follows.

Symptoms appear as oblong lesions on the sheath covering the panicle. Young spots appear as greyish brown which turns to whitish grey with a dark brown margin on maturity. Panicles will be half emerged or completely choked. Whitish powdery mass of fungal growth could be detected over the matured lesions inside the affected sheath.

Shajahan et al. (1977) found that the disease occurs primarily on the flag leaf sheath penetrating stomata or sheath injuries and growing intercellularly in the vascular bundles and in the infected sheath mesophyll tissue. Panicles from the affected plants often did not emerge and the glumes of the infected florets were discoloured dark red or purple brown to black and often were not filled.

Surin (1977) described this disease as `rice abortion'. Balakrishnan (1981) reported that symptom initiated on the middle portion of flag leaf sheath as light purplish brown oblong lesions. Young light brown lesions turned dark brown on maturity. Later the lesions coalesce together to cover

the entire sheath. Panicles fully or partly affected. Grains show discolouration.

Manibhushan Rao et al. (1986) described the symptoms of sheath rot and also the disease development. Mukerjee and Yadov (1989) reported the mode of infection of sheath rot and found out the presence of the fungus in seed husks, floral parts, rachis, rachilla, outer leaf sheath, panicle stem and flag leaf sheath.

2.1.1 Isolation of the Pathogen

The fungus S. oryzae (Sawada) Gams and Hawksworth causing sheath rot of rice belongs to the family Moniliaceae of the order Moniliales of the class Deuteromycetes [Ainsworth (1973)].

Other associated organisms with the disease

Fusarium roseum Link ex Fries, and an unidentified species of Hyalostachybotrys. were reported as associated organisms in sheath rot disease (Shajahan et al. 1977). These organisms could produce typical sheath rot symptoms when inoculated artificially along with A.oryzae. Balakrishnan (1981) observed the occurrence of

Fusarium sp. with the disease. Gaumannomyces graminis var tritici Walker was isolated from the sheath rot affected rice seedlings by Sung et al. (1982). They have also observed that this fungus produced sheath rot symptoms from the crown to the leaf sheath above the ground level. Kang and Rattan (1983) isolated Fusarium equiseti (Corda) Sacc. and Fusarium moniliformae sheldon, from sheaths of sheath rot affected rice. Shajahan et al. (1983) isolated a Pyrenochaeta species from the outer leaf sheath of rice plants and proved its pathogenicity. Ngala (1983) isolated Sarocladium attenuatum Gams and Hawksworth from discoloured grains. Olivos and Jimenez (1984) reported the presence of different pathogens causing sheath rot disease in rice. Nair (1986) reported the presence of Alternaria padwickii from sheath rot infected panicles. Lee et al. (1986) have pointed out that Sheath rot and grain discolouration diseases were more severe when more than one pathogen were involved. Shajahan et al. (1987) isolated and studied two different isolates of Sarocladium which causes sheath rot. Singh and Devi (1991) found that a severe sheath rot incidence was caused by Fusarium graminearum throughout Manipur, India. Lakshmanan (1991) reported a new sheath rot disease of rice caused by Curvularia lunata

2.1.2 Morphology of the causal organism

Acrocylindrium oryzae was first described by Sawada in 1922 from Formosa.

Tasugi and Ikeda (1956) described the conidia as rod shaped and hyaline and it measured 2.1 - 8.5 x 0.5 - 1.6µm from the host and 1.8-13 x 1-1.6 µm from culture.

Ou (1972) described the fungus as follows.

White sparsely branched mycelium measured 1.5 - 2.0 µm in diameter. Conidiophore arise from the mycelium, slightly thicker than the vegetative hyphae, branched one or two times with 3-4 branches in a whorl. The conidia formed consequently on the tip. Conidia were hyaline, smooth, single celled, cylindrical and measured 4.0-9.0 x 1.0-2.5µm.

According to Nair and Sathyarajan (1975) mycelium was, septate, purplish white profusely branched and 1.25 to 2.0 micrometer in diameter. The conidiophores were slightly thicker than the ordinary vegetative hyphae, short and were ending in a whorl of 3-6 branches and often 1 or 2 side branches were also noticed from the main conidiophore. The main branch of conidiophore measured 10-15 µm in length and

2.0-2.5 µm in breadth. Conidia were consecutively formed at the tip of the conidiophores. Conidia were single celled, hyaline and measured 3.5-7.0 x 1.0-1.5 µm in size from the host and 4.0-8.0x1.0-1.5µm from the culture.

Shajahan et al. (1977) described the morphological characters of the fungus. Mycelium was colourless and septate, 1.5 to 3.0µm in diameter. Conidiophores were single or branched 15.0 - 25.0µm long or with secondary branches in whorls of 2-5 phialides and 13.0-19.0 µm long. The conidia was found singly at the tip of the conidiophore and were hyaline, smooth, cylindrical, single celled and measured 3.0-17.0 x 1.0-2.0 µm on PDA culture incubated at 32°C.

Balakrishnan (1981) compared different isolates of the pathogen obtained from rice varieties and weed hosts and found that the conidia from the weed host were comparatively smaller than those from rice varieties. Nair (1986) reported the occurrence of conidiophore in a rice isolate which is double the length than other rice varieties.

2.2 Pathogenicity

Tasugi and Ikeda (1956) established the pathogenicity of S.oryzae by artificially inoculating the plants with

conidial suspension. Chen (1957) reported that different isolates of the fungus differed in their pathogenicity. Amin et al. (1974) found that stem tape inoculation method or spraying of conidial suspension were effective to prove the pathogenicity of the organism. Nair and Sathyarajan (1975) proved that inoculation of rice plants at boot leaf stage with conidial suspension of the fungus could produce typical sheath rot symptom. Shajahan et al. (1977) injected hyphal and conidial suspensions of the fungus behind the outer sheath with a hypodermic needle and established the pathogenicity. Estrada et al. (1979) obtained good results by insertion of a single grain between leaf sheath and culm and also by injecting a spore suspension behind the sheath. Balakrishnan (1981) proved that the successful infection of rice plants by the pathogen could be obtained by inserting actively growing culture bit behind the sheath or by injecting the spore suspension of the pathogen inside the sheath with the help of a hypodermic needle. Mukerjee et al. (1981) found that inserting cultured rice grains inside the flag leaf sheath of rice plant was the most effective method. Nair (1986) successfully inoculated by inserting rice grain culture each behind the boot leaf sheath and the lower two sheaths in each of the test plant.

2.2.1 Varietal reaction

Subramanian and Ramakrishnan (1975) reported that the rice variety Annapoorna was comparatively more susceptible to sheath rot disease of rice. Datta and Purkayastha (1978) found out that sheath rot disease was more severe in high yielding dwarf cultivars. Chien (1981) reported that *japonica* rice cultivars were more susceptible than *indica* types to sheath rot. Srinivasan, 1980; Balakrishnan and Rajan, 1981; Singh and Raju, 1981; Chien and Thseng, 1982 reported the variations in varietal reactions towards the disease.

Amin (1976) reported that a disease index of 1 to 9 scale was convenient to evaluate a large number of cultivars. Satyanarayana and Reddy (1979) scored on a 1 to 9 scale based on the coverage of lesions on boot leaf sheath and infection in panicle due to sheath rot disease.

2.3 Survival of *S. oryzae* in infected grains

Shajahan *et al*. (1977) reported that sheath rot of rice was seed borne. Tschen and Wen (1980) have suggested that the sterility of rice grains of sheath rot affected rice plants might be due to the toxic metabolites produced by the

pathogen. Mohan and Subramanian (1981) reported the external and internal seed borne nature of the sheath rot fungus. The effect of toxic metabolites has also been observed by Balakrishnan (1981). Saktivel and Gnanamanickam (1986a) isolated cerulenin as the toxic metabolite which showed sheath rot symptom. Mukerjee and Yadov (1989) reported the presence of fungus on the seed husks, floral parts, rachis, rachilla, outer leaf sheath, panicle, stem and flag leaf sheath. Maite et al. (1991) found that the fungus could survive in infected seeds and weed inflorescence for about seven months under dry storage conditions.

2.3.1 Survival of *S. oryzae* in soil

Hsien et al. (1980) found that the fungus could survive for 75 days in stubbles but it failed to survive beyond 55 days when buried in soil and puddled. Maite et al. (1991) reported that the pathogen could survive in wet rhizosphere soils for more than 6 months after sowing the infected seeds.

2.4 Cultural Characters

Kawamura (1940) did works on *Acrocylindrium oryzae* and found that the fungus grew best at 30°C to 31°C.

Tasugi and Ikeda (1956) suggested that the optimum conditions for growth of the fungus were 20°C to 28°C and pH of 6.4. For conidial germination the optimum conditions were 23°C to 26°C and PH of 5.5 to 6.4.

Chen (1957) reported the isolates of the fungus differed in their response to temperature, pH, carbon and nitrogen sources as well as on their pathogenicity. Shajahan et al. (1977) got a colony diameter of about 3.0 to 3.5 cm in 10 days on PDA incubated at 28°C. He found that the fungus grew best at 32°C on PDA and at 28°C on corn meal agar. The maximum growth and sporulation was obtained at pH of 7.5 in Potato dextrose agar medium and at 6.5 in Potato dextrose broth.

Mohan and Subramanian (1978) reported, potato dextrose agar and oats agar media as best solid media and czapek's media as the best liquid media. Sucrose and starch were found to be the best sources of carbon and ammonium sulphate and ammonium nitrate as best nitrogen sources.

Datta and Purkayastha (1978) observed that the spore germination was inhibited on highly concentrated spore

suspension. They suggested a temperature of 30°C and pH 5.5 to 6.0 as optimum for the germination of spores.

Alagarsamy (1989) found out that addition of N, P and K individually and in combination to a basal czapek's liquid medium interfered with the production of toxic metabolites.

2.5 Effect of different spectra of light on *S. oryzae*

Kumagai and Hsiao (1983) found that *Verticillium agaricum* sporulated abundantly in blue light. Wulf and Schauz (1983) found out that blue light was effective in the spore germination of *Ustilago maydis*.

Effect of plant extracts on the spore germination of *S. oryzae*

Naidu and John (1981) observed that leaf extracts of *Polyalthia longifolia* L., *Parthenium hysterophorus* L., and *Cymbopogon* sp., and rhizome extracts of *Zingiber officinale* L., and *Curcuma longa* L., inhibited the growth of *S.oryzae* in vitro. The radial growth and the mycelial dry weight of *S.oryzae* were reduced by garlic bulb extract. Kanagarajan (1988) reported that Neem leaf extract was effective against

S.oryzae. Komala et al. (1988) found that leaf extracts of Ocimum sanctum L., Curcuma longa L., Datura metal L., and Azadirachta indica L., inhibited the spore germination of S.oryzae .The leaf extracts of O.Sanctum, A.indica and A.leucophloea were effective against sheath rot diseases under pot culture conditions also (Kumar, 1992). The seed extracts (10%) from Tribulus terrestris and leaf extract (10%) from Agave americana effectively inhibited the spore germination, germ tube elongation and radial growth of S.oryzae and the sporulation of the fungus was reduced by the extracts from young stem of Euphorbia tirucalli and matured leaf of Urginea indica (Selvaraj and Narayanasamy, 1993).

2.6 Antagonistic micro organisms

Saktivel and Gnanamanickam (1986b) suggested that sheath rot might be controlled by treatment of rice seeds or plants with strain of Pseudomonas fluorescens. Saktivel and Gnanamanickam (1987) found that Pseudomonas fluorescens treated rice plants showed enhanced grain yield of 3-16 per cent and a 20-40 per cent suppression of sheath rot. Joseph and Philip (1987) reported that Trichoderma viride,

Myrothecium roridum and Chaetomium gracile exerted specific antagonistic effect on the common pathogens associated with rice seeds. Natarajan et al. (1987) found out that Trichoderma viride exerted antagonistic effect against sheath rot pathogen. Viswanathan and Narayanaswamy (1990) observed that Bipolaris zeicola could inhibit the mycelial growth of Sarocladium oryzae.

Narasimmaraj (1991) could effectively control sheath rot disease of rice by spraying suspension of Bacillus sp. ($1x10^4$ cells/ml). Kumar (1992) observed that pre inoculation of P.fluorescens and Bacillus sp. was effective than their post inoculation in reducing sheath rot disease.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Symptomatology

Detailed studies were made on the symptomatology of sheath rot disease of rice by observing the naturally infected plants in the field. Naturally infected rice plants were collected from selected areas namely Karamana, Pattambi, Vellayani, Kalliyoor, Kayamkulam, Kumarakom and Moncompu.

3.1.1 Isolation of the pathogens

Isolations from infected sheath and grains were carried out following standard techniques. The affected portion of the sheath was cut in to small bits, surface sterilized with 0.1% mercuric chloride solution for two minutes and washed with three changes of sterile distilled water. These bits were then placed in sterile petri dishes, previously poured with Potato dextrose agar medium (PDA). The dishes were then incubated at room temperature (28±2°C). Growth of the fungus was visible within 2 to 3 days. Mycelial bits were then transferred aseptically in to PDA slants. Culture was then purified by frequent transferring of hyphal tips and stock culture was maintained.

3.1.2 Comparative morphology of various isolates of *S. oryzae*

Six isolates of *S. oryzae* were subjected to comparative morphological studies.

The details of isolates of *S. oryzae* used for comparative morphological studies are given below

I_1 - from variety Matta with specific sheath rot symptom collected from Pattambi.

I_2 - from variety White Thriveni with mild sheath rot symptom collected from Moncompu.

I_3 - from variety Jyothi with mild sheath rot symptom collected from Moncompu.

I_4 - from variety White Thriveni with severe sheath rot symptom collected from Moncompu.

I_5 - from variety Matta with typical sheath rot symptom collected from Pattambi,

I_6 - from variety White Thriveni with severe sheath rot symptom collected from Moncompu.

Detailed morphological studies of these isolates were done by using slide culture technique (Riddell 1950). Sterile plain agar medium was poured in to a sterilized petri dish to a thickness of 2 mm and after solidification, blocks of 6 mm square were cut out using a sterile needle. One square was placed in the centre of each sterile glass slide and all the four sides of the block were inoculated with culture bits of the required isolates of the fungus. A coverslip was placed on the top square of the agar and the slide was kept in a damp chamber. (Petri dishes with wet filter paper in the bottom on which two glass rods kept as supports for the slide). The dish with the slide was then incubated at room temperature for 2-3 days. After this, the coverslip was lifted off gently, a drop of 95% alcohol was placed in the centre and before drying, the cover slip was mounted using lactophenol cotton blue on another slide. The square of agar was removed from the culture slide and another mount was prepared without any disturbance to the fungal growth on the slide. These slides were observed for the various morphological characters and were recorded.

3.1.3 Comparative morphology of other fungi associated with sheath rot disease

Sheath rot affected rice plants were collected and all the fungi which were associated with the disease were isolated and brought in to pure culture following the standard techniques. Detailed morphological characters of four isolates of the associated fungi collected were also studied following the slide culture technique.

3.2 Pathogenicity test

Isolates of *S. oryzae* and associated fungi obtained were subjected to pathogenicity test on different common varieties of rice viz., Jaya (V_1), Bharathi (V_2), Kanchana (V_3), Aiswarya (V_4), White Mashoori (V_5), Jayathi (V_6), Aathira (V_7) and Sabari (V_8). Inoculation studies were carried out in active tillering and boot leaf stages. Necessary inoculum of all the isolates was cultured in PDA. Ten day old cultures were used for inoculation studies. Effect of individual action of *S. oryzae* and associated fungi were tested in 8 varieties listed above. Effect of combined infection by *S. oryzae* and

associated fungi was tested using the variety Jaya since it was found more susceptible to the disease.

Plants were raised in 1m x 1m cement troughs and artificially inoculated by placing the mycelial bit behind the sheath in active tillering stage and behind the boot leaf sheath in boot leaf stage. The inoculated plants were given sufficient humidity by providing loose polythene wrappers. Control plants were inoculated with sterile distilled water.

Inoculated plants were observed for the development of symptoms and observations were recorded from 5th day onwards. The nature of infection, colour and size of lesion were observed and recorded. The severity of the infection was arbitarily graded in to mild, moderate and severe forms based on the incubation period taken by various isolates of the pathogen, for the initial symptom expression and the differences noticed in the further development of the diseases.

3.2.1 Varietal reaction to various isolates

Varieties used

V_1	-	Jaya	V_5	-	White Mashoori
V_2	-	Bharathi	V_6	-	Jayathi
V_3	-	Kanchana	V_7	-	Aathira
V_4	-	Aiswarya	V_8	-	Sabari

Plants were raised in 1m x 1m cement troughs and inoculated. Inoculation was done by using isolates of *S. oryzae* and the associated fungi singly, in 8 varieties listed above and in combination using the variety Jaya. Inoculations were done in two stages - Active tillering stage and Boot leaf stage. Inoculations were done by placing the mycelial bits behind the leaf sheath in active tillering stage and behind the boot leaf sheath in the boot leaf stage.

Inoculated plants were observed for the development of symptom and observations were recorded from the 5th day onwards. Disease scoring was done to find out the individual effect of *S. oryzae* and associated fungi in the varieties used.

The disease severity was worked out using a score chart as developed by Amin (1976).

* Score values	Description
1.	No visible symptoms on sheath of any leaf, panicles are fully emerged and grains are free from discolouration.
3.	Two to three small lesions of 0.5 to 1.0cm long and 0.2 to 0.5 cm wide developed on flag leaf sheath which are oval, dark, chocolate brown and surrounded by diffused light brown halo, while the colour of the healthy sheaths around the lesions remain green. Grains are not discoloured.
5.	Large lesions of 2 to 3 cm long and 1cm wide are most conspicous on flag leaf sheath, but occur on all the leaf sheaths. Lesions overlap and form irregular large chocolate brown blotches on flag leaf sheath.

Emergence of panicle is affected and it is half way from flag leaf sheath. Grains inside are partially chaffy and covered with pink mycelium and spore masses. Affected panicles range up to an estimated 25 per cent.

7. Flag leaf sheath is completely chocolate brown in colour due to many overlapping lesions. Flag leaves of affected sheath gradually become yellow to straw coloured. Affected panicles are fully compressed by flag leaf sheath and are dark brown, chaffy, and covered with white to pink mycelium and spore masses. This stage is commonly known as "choking". Affected panicles range upto 50 per cent.

9. The entire flag leaf sheath has dark chocolate brown colour. Subsequently become yellow to straw in colour. Flag

leaves are straw in colour. Grains are dark brown and chaffy. Severe choking of panicles. Affected panicles range upto 100 per cent.

Ten tillers were observed from each variety and the intensity of disease was expressed as mean score.

3.3 Survival of *S. oryzae* in infected grains

Infected paddy grains were collected from the field and dried in the usual manner as for storage of the produce and kept in gunny bag and in polythene bag. Samples were taken at monthly intervals, surface sterilized with 0.1% mercuric chloride solution for 2 minutes and washed with 3 changes of sterile distilled water. The surface sterilized grains and grains which were not surface sterilized were then plated on PDA and observed for the growth of organisms.

3.3.1 Survival of *S. oryzae* in soil

Rice culms were chopped and mixed with sand in the ratio 1:3 and sterilized in an autoclave under 1.02 kg/Cm^2 pressure at 121°C temperature. Mycelial bits, were inoculated in to the sterilized media. After 10-15 days of

growth the material was placed in soil at 2 cm and 6 cm depth in wet as well as in dry soil conditions. Samples were taken at monthly intervals from these and the viability of the fungus was tested using Dilution end point technique. Barron (1971).

Ten g soil was taken in a flask containing 90 ml of sterile water and shaken in a mechanical shaker for 20 minutes. While the suspension was in motion, 10ml of the sample were withdrawn and added to 90 ml of sterile water to get 10^{-1} dilution. The process was repeated to get 10^{-4} dilution. The solution was taken and plated with Rose bengal agar on a petridish. Colonies of the pathogen was observed after 6 days.

3.4 Cultural characters

Cultural characters of the pathogen were studied both in solid and liquid media. Isolate I_5 was taken for the study.

3.4.1 Growth of the fungus on different solid culture media

Solid culture media viz., Czapek's agar, Coon's agar, Oat meal agar, Potato dextrose agar and Carrot agar were used for the study. The sterilized media were poured in to

sterilized petri dishes at the rate of 15 ml per each dish and allowed to solidify. Circular mycelial discs of 5 mm diameter were cut out by using a sterile cork borer from the edge of a 7 day old culture of the fungus and placed centrally on the petri dishes. The plates were then incubated at room temperature (28±2°C). Growth characters were observed for 10 days. Three replications were maintained for each treatment.

3.4.2 Growth of *S. oryzae* in liquid media

Liquid culture media like Paddy leaf extract medium, Potato dextrose medium, Czapek's medium, Coon's medium and Richard's medium were used for this study. Fifty ml each of the media was taken in 250 ml conical flasks and sterilized. The flasks were inoculated with mycelial discs of 5 mm diameter, cut out from an actively growing 7 day old culture of the fungus and incubated at room temperature (28±2°C). After 12 days of incubation the culture was filtered through previously weighed Whatman No:1 filter paper and the dry weight of biomass was determined. Three replications of each unit were maintained.

3.4.3 Effect of different Nitrogen Sources on growth of *S. oryzae*

Czapek's medium was taken for the study. Sodium nitrate, the nitrogen source in Czapek's medium was substituted with Ammonium nitrate, Potassium nitrate, Leucine and Tyrosine. 50 ml of each medium was taken in 250ml conical flask and sterilized. The flasks were inoculated with mycelial discs of 5 mm diameter, cut out from an actively growing 7 day old culture of the fungus and incubated at room temperature (28±2°C). After 12 days of incubation, the culture was filtered through previously weighed Whatman No:1 filter paper and the dry weight of the biomass was determined. Three replications were maintained for each case.

3.4.4. Effect of different carbon sources on growth of *S. oryzae*

Czapek's medium was taken for the study. Sucrose, the carbon source in Czapek's medium was substituted by Mannitol, Lactose, glucose, and starch. 50 ml of each medium was taken in 250 ml conical flasks and sterilized. The flasks were inoculated with mycelial disc of 5 mm diameter, cut out from an actively growing 7 day old culture of the

fungus and incubated at room temeprature (28±2°C). After 12 days of incubation, the culture was filtered through previously weighed Whatman No:1 filter paper and the dry weight of biomass was determined. Three replications of each unit were maintained.

3.4.5. Effect of different spectra of light on growth and sporulation of *S. oryzae*

Red, blue and green coloured lights were used for this study. Isolate I_5 was taken for the study. Circular discs of 5 mm diameter were cut out from 7 day old culture of *S. oryzae* and placed centrally on the petri dishes previously poured with sterile potato dextrose media. The petri dishes were then kept under red, blue and green coloured lights after five days of growth under ordinary light conditions. Petri dishes kept under ordinary light condition served as the control. Three replications of the control and treatments were maintained. Observations on the growth of the fungus were recorded for 10 days.

3.4.5.1. Assessment of sporulation

Sporulation studies were done by cutting culture disc of 5 mm diameter from five places and each disc was

transferred to 10 ml of sterile distilled water, macerated well and then strained through a thin cloth. Each filterate was then diluted ten times with sterile distilled water and five samples from each filtrate were taken for spore count. Average spore count of 5 microscopic field were then observed for each sample and the results were recorded using the following scale.

Average spore count per microscopic field	Grade
Less than 50	+
Between 50 and 100	+ +
>100	+ + +

3.5 Effect of Plant extracts on the spore germination of *S. oryzae*

Isolate I[5] was taken for the study. Extracts of *Azadirachta indica* L., *Ocimum sanctum* L., *Allium sativum* L. and *Phyllanthus niruri* L. were taken. Concentrations of 1% and 10% of the extracts were prepared in sterile distilled water. The plant materials collected were thoroughly washed with tap water, then with alcohol and finally with changes

of distilled water. These were ground by using a pestle and mortar by adding sterile water at the rate of one ml/g of leaf tissue. The extract was strained through two layers of muslin cloth and subsequently filtered through two Whatman No:1 filter paper. This formed the standard plant extracts solution (100 percent). The extract was further diluted to 10 per cent and 1 per cent concentrations with sterile distilled water. Spores of S. oryzae and the extract was placed in a cavity slide and observed for 24 hours. A control using sterile plain water was also observed.

3.6 Isolation of microflora from phylloplane of rice plants

Leaf samples were taken from standing crop during Virippu and Mundakan seasons. Samples were taken during active tillering stage and boot leaf stage of the crop growth. The Leaf washing and dilution technique (Waksman, 1922) was used to study the qualitative aspects of the microflora on the leaf surface. The leaf samples were collected using sterile scissors and brought to the laboratory in fresh polythene bags. The samples were taken from Vellayani. Ten leaves were taken and transferred

aseptically in to 250 ml flask containing 100 ml of sterile water and shaken for 20 minutes in a mechanical shaker to detach the propagules from the leaf surface. Five ml of the leaf washing was pipetted out using a sterile pipette and poured in to a sterilized petri dish. In to the same petri dish 20 ml of Rose bengal streptomycin agar medium was poured, swirled and incubated under room temperature. The representative phylloplane microflora obtained were purified and maintained in PDA. Various cultures were subjected to morphological studies and the organisms were identified up to their generic level.

3.6.1 Antagonism of Phylloplane microflora against sheath rot pathogen

The fungal cultures obtained from the phylloplane was tested for their antagonism against *S. oryzae* Isolate I[5] was taken for the study. Methods outlined by Purkayastha and Bhattacharya (1982) were followed for studying the antagonistic action of the microbes against *S. oryzae*. Seven day old cultures of both the pathogen and test fungus were taken and agar discs of 5mm were cut and placed 3.5 cm apart on PDA in a 9 cm petridish and incubated at 28±2°C for 20 days. The paired cultures

were examined at regular intervals throughout the incubation period and the nature of reaction noted. Colony development was observed and the mode of interaction between organisms was assessed. When the growth pattern became stable, the interaction types were grouped in to 4 categories following Purkayastha and Bhattacharya (1982).

Group	Nature of interaction
A. Homogenous	Free intermingling of hyphae
B. Overgrowth	*S. oryzae* overgrown by the test organism
C. Cessation	Cessation of growth at the line of contact
D. Aversion	Development of clear zone of inhibition

3.6.2. Evaluation of the efficiency of the biological agents in the control of sheath rot under field conditions

The biological control agents were obtained from rice plants grown in Vellayani. In addition to this two isolates of *Pseudomonas fluorescens* namely *P. fluorescens* -87 and *P. fluorescens* -2 obtained from Tamil Nadu Agricultural University were also utilised. Rice plants were raised in cement troughs (1mx1m) and managed as per the

Package of Practices Recommendations of KAU. The pathogen incubated at boot leaf stage of the crop using mycelial bits of *S. oryzae* alone served as the control. The following combination of the pathogen and biological control agents were also tested.

1. *Sarocladium* + *P. fluorescens* - 87
2. *Sarocladium* + *P. fluorescens* - 2
3. *Sarocladium* + *chaetomium* sp.
4. *Sarocladium* + *Pestalotia* sp.

After inoculation the plants were covered with polythene wrappers to give sufficient humidity. The plants were then observed for the development of the disease.

RESULTS

4. RESULTS

4.1 Symptomatology

The observations made on the naturally infected rice plants showed that the variety Pavizhachempavu collected from Kalliyoor area of Trivandrum district was that of the typical sheath rot symptoms. Brown oblong lesions were seen on the flag leaf sheath, which were surrounded by light yellow halo initially. On maturity, these lesions turned dark brown with grey centres. Slight brownish discolouration was also seen on the second and third leaf sheaths. Panicles were found fully or partially choked. Grains were also discoloured (Plate 1).

Variations from the above symptoms were noticed in varieties Bhagya, White Mashoori, Matta, Jyothy, Annapoorna, Red Mashoori, White Thriveni, Jaya and Basumathi collected from Karamana, Kalliyoor, Vellayani, Kayamkulam, Kumarakom, Moncompu and Pattambi, during the cropping seasons namely, Virippu, Mundakan and Puncha. A comparative account on the varying symptoms in the naturally infected crops of different varieties at various locality have been presented in Table.1.

TABLE 1

Variations in symptoms of sheath rot on various rice varieties collected from different localities (Natural Infection)

SYMPTOMS NOTICED	* LOCALITY	** VARIETY
1. Sheath rot symptom with boot leaf turning straw colour with pinkish growth of the fungus. Panicles partially emerged and lower side of the stalk showed brown discolouration. Grains also had a pinkish tinge.	B	3
2. Panicles were completely affected. The sheath which covered the panicle was yellow in colour with slight brown discolouration and whitish growth of the fungus was seen externally. Grains were also affected.	B	3
3. Black discolouration seen on the boot leaf sheath. Panicle partially emerged with brown and pinkish coloured grains.	B,F	5,8
4. No discolouration seen on the boot leaf. Panicles completely emerged out. But brown discolouration seen on the stalk region of the panicle.	A,B,C, F,G,D,E	1,2,7 8,9,10
5. Brownish and yellowish discolouration on the boot leaf sheath. Grains discoloured to brown. Panicles either partly emerged or completely choked.	A,B	1,4,3,6

* LOCALITY

Kayamkulam	-	A
Pattambi	-	B
Kalliyoor	-	C
Vellayani	-	D
Kumarakom	-	E
Mancompu	-	F
Karamana	-	G

** VARIETY

Bhagya	-	1
White Mashoori	-	2
Matta	-	3
Jyothi	-	4
Annapoorna	-	5
Red Mashoori	-	6
Pavizha chempavu	-	7
White Thriveni	-	8
Jaya	-	9
Basumathi	-	10

Plate - 1 Natural Infection.

4.1.1 Isolation of the pathogens

Tissue isolation of infected rice plants collected from various localities frequently yielded *S. oryzae* Gams and Hawks. which was brought in to pure culture. Out of the different isolates obtained, six isolates of *S. oryzae* viz.,) I_1, I_2, I_3, I_4, I_5, I_6 were selected based on the symptomatological variations for further study.

I_1 - from variety Matta with specific sheath rot symptom collected from Pattambi.

I_2 - from variety White Thriveni with mild sheath rot symptom collected from Moncompu.

I_3 - from variety Jyothi with mild sheath rot symptom collected from Moncompu.

I_4 - from variety White Thriveni with severe sheath rot symptom collected from Moncompu.

I_5 - from variety Matta with typical sheath rot symptom collected from Pattambi,

I_6 - from variety White Thriveni with severe sheath rot symptom collected from Moncompu.

Along with this fungal pathogen, a few other fungi were also found to be associated in the development of the disease and such isolates were also brought in to pure culture. Out of the different isolates obtained three isolates of Fusarium sp. (A_1, A_2, A_3) and one isolate of Alternaria padwickii (A_4) were selected for the furhter study.

A_1 - From variety Red Mashoori with severe sheath rot symptom collected from Pattambi.

A_2 - From variety Bhagya with moderate sheath rot symptom collected from Kayamkulam.

A_3 - From variety Jyothi with severe sheath rot symptom collected from Kalliyoor.

A_4 - From variety Jyothi with mild sheath rot symptom collected from Karamana.

4.1.2 Comparative morpohology of various isolates of S. oryzae

Comparative studies on various morphological characters were done for six isolates of S. oryzae and the observations made have been summarised in Table 2. It was seen that all the six isolates varied slightly in their

morphological characters. The variation with respect to colony character, size and nature of branching of conidiophore, size of conidia etc were recorded (Fig 1; Plates 2.1 to 2.6).

4.1.3 Comparative morphology of other fungi associated with sheath rot disease

The fungi other than Sarocladium oryzae which were found associated with the disease were also isolated, brought in to pure culture and subjected to morphological studies. The results have been summarized in Table 3. (Fig 2;Plate 2.7 to 2.10). The pathogens isolated and identified were as follows.

Isolate Number	Name of the isolate
A 1	Fusarium sp.
A 2	Fusarium sp.
A 3	Fusarium sp.
A 4	Alternaria padwickii

TABLE 7

Morphological characters of isolates of Sarocladium oryzae

Characters	Name of Isolates					
	I 1	I 2	I 3	I 4	I 5	I 6
A.Mycelium	Septate	Septate	Septate	Septate	Septate	Septate
Colour of colony	Deep violet	Pink	Violet	Pinkish white	Light pink	Pinkish white
* Hyphal thickness	3.33 - 4.1	3.3 - 4.1	4.1 - 4.9	3.33	3.3 - 4.1	3.33
B.Conidiophore Branching	Branched in 1 or 2 whorls	Branched in 1 or 2 whorls	Branched in 2 or 3 whorls	Branched in 2 or 3 whorls	Branched in 2 or 3 whorls	Branched in 1 or 2 whorls
* Length (Main axis)	29.7 - 49.5	29.7 - 34.32	36.33 - 34.32	33 - 49.5	49.5 - 81.5	33 - 34.32
* Breadth	1 66 - 3.33	2.62 - 3.33	3.33 - 4.1	1.66 - 3.33	1.66 - 3.33	1.66 - 3.33
C.Conidium						
Colour, shape and septation	Hyaline,cylindrical and single celled	Hyaline,cylindrical and single celled	Hyaline,cylindrical and single celled	Hyaline,cylindrical and single celled	Hyaline,cylindrical and single celled	Hyaline,cylindrical and single celled
Attachment to conidiophore	Singly at the tip of branches	Singly at the tip of branches	Singly at the tip of branches	Singly at the tip of branches	Singly at the tip of branches	Singly at the tip rarely two conidia were also noticed in succession
* Length	6.6	4.95 - 6.6	8.91 - 9.9	6.6 - 9.9	8.25 - 8.91	4.9 - 9.9
* Breadth	1.66 - 3.33	1.66 - 3.33	2.6 - 4.1	1.66 - 2.6	3.33 - 4.1	3.3 - 4.1

* All measurements are in microns (µm)

Fig. 1

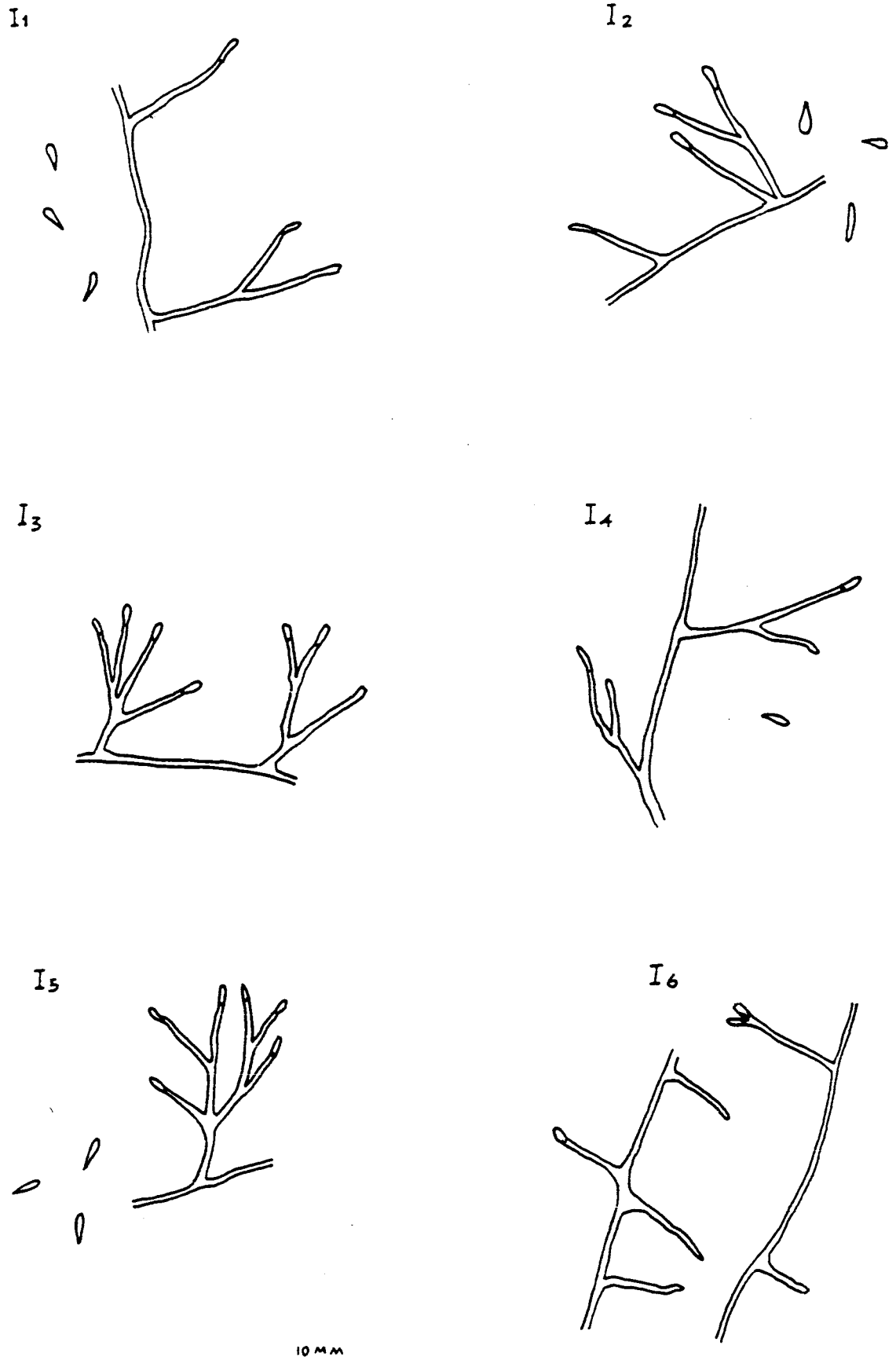


Plate - 2.1 Morphological characters - Mycelium, condiophore and conidia I_1 (x 400).

Plate - 2.2 Morphological characters - Mycelium, conidiophore and conidia I_2 (x 400).

Plate - 2.3. Morphological characters - Mycelium, conidiophore and conidia I (x 400).

3

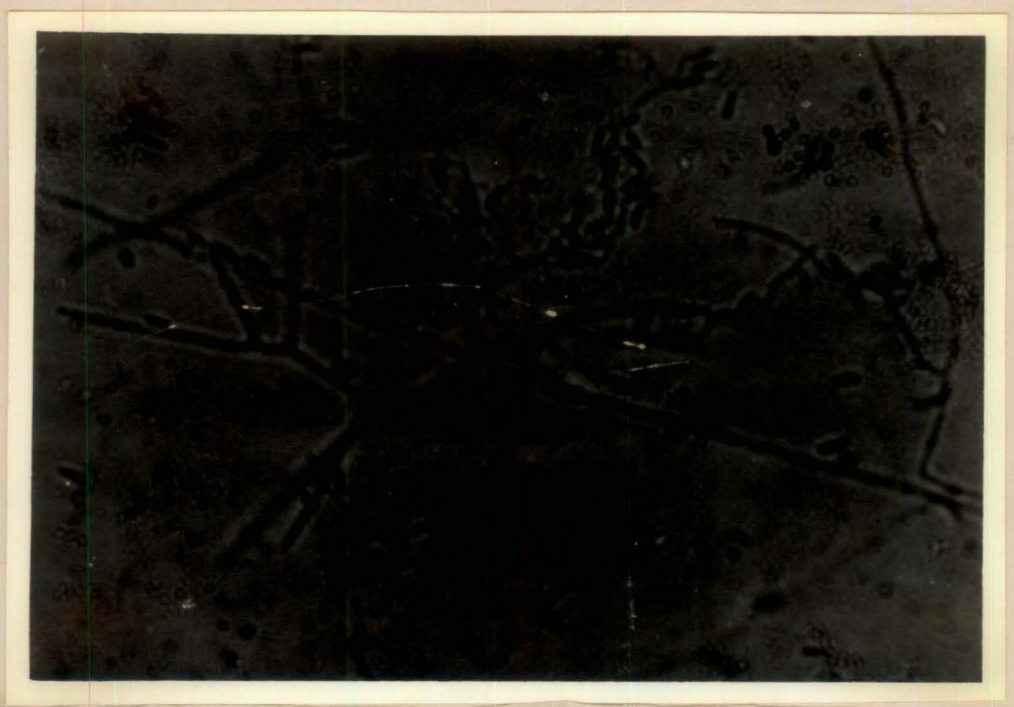


Plate - 2.4 Morphological characters - Mycelium, conidiophore and conidia I (x 400).

4

Plate - 2.5 Morphological characters - Mycelium, conidiophore and conidia I (x 600).

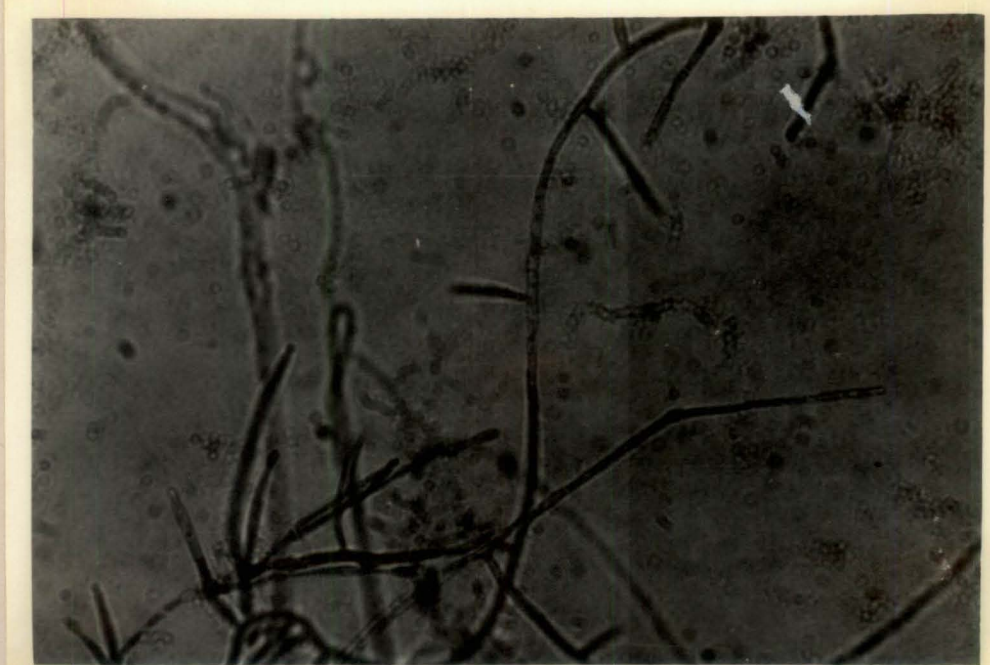


Plate - 2.6 Morphological characters - Mycelium, conidiophore and conidia I (x 600).

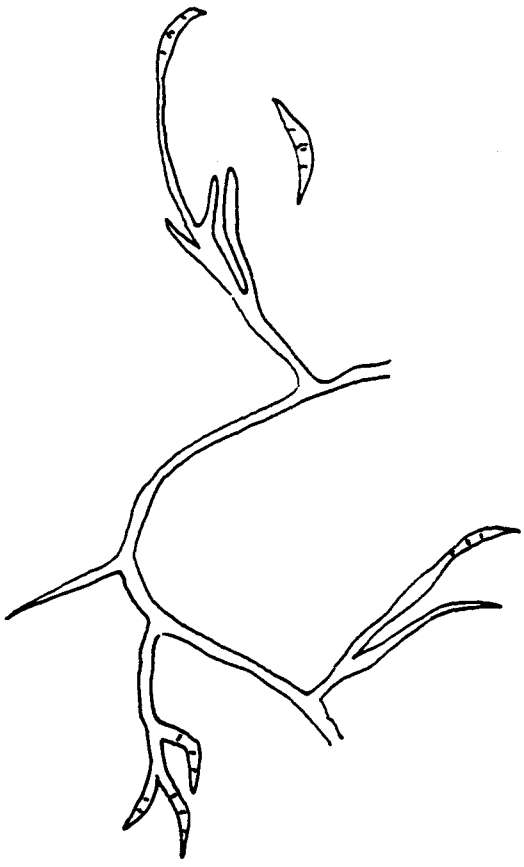
TABLE 3

Morphological characters of other fungi associated with sheath rot

Characters	A 1	A 2	A 3	A 4
Mycelium, septation colour and nature of growth	Septate, deep brown in colour in old cultures	Septate,light brown older cultures	Septate, light brown in older culture	Septate,brownish black colour in older cultures
* Hyphal Thickness	2.99 - 6.66	2.66 - 3.33	3.3 - 6.6	2.66 - 3.33
Conidiophore	Branched	Branched	Branched	Condiophore were not seen demarcated in the mycelium
* length and breadth	39.96 - 49.95 x 3.33	23.1 - 26.4x2.66- 3.33	19.98 - 23.1 x 3.33	
Conidia				
* Colour, shape, size Septation and attachment of conidia	Hyaline, fusiform and slightly curved macro conidia	Macro conidia hyaline, septate	Macroconidia hyaline	Conidia light brown slightly curved 4 to 7 septate with a long beak
	19.98 x 3.33 - 6.66	16.65-29.97x3.33-6.66	16.65-23.1x3.33x6.66	82.5 - 233.1 x 6.6 - 9.94

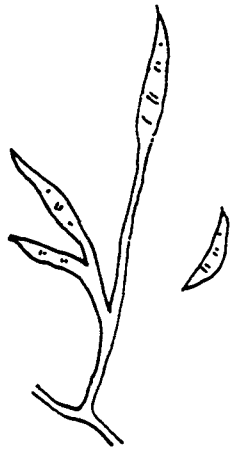
* All measurements are in microns (µm)

Fig. 4

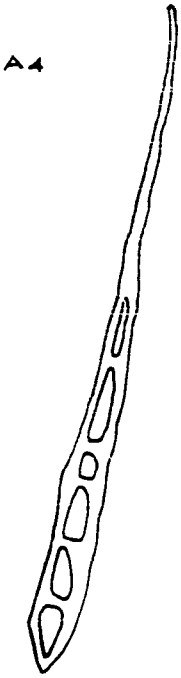


A2

A3



A4



10 MM

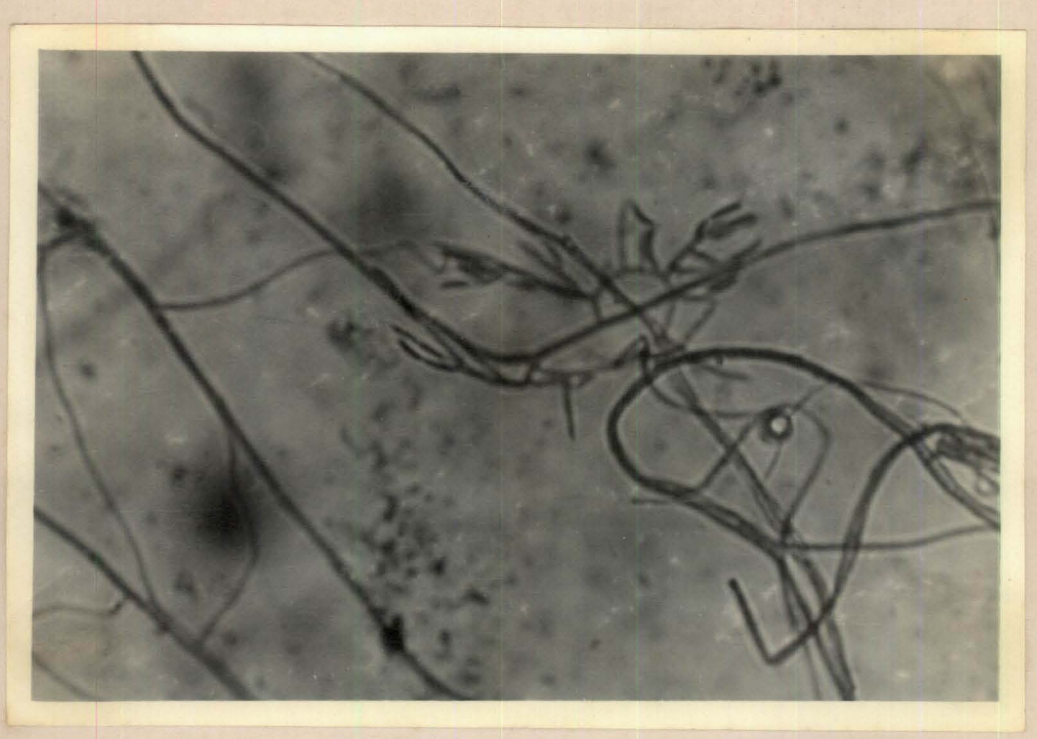


Plate - 2.7 Morphological characters - Mycelium, conidiophore and conidia A_4 (x 400).

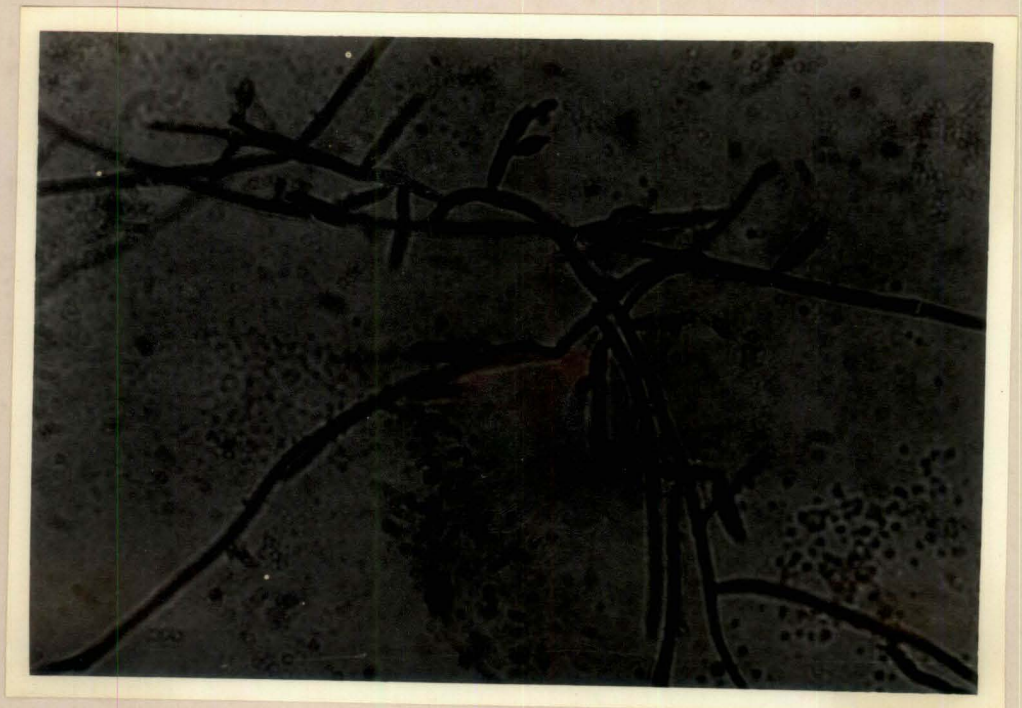


Plate - 2.8 Morphological characters - Mycelium, conidiophore and conidia A_2 (x 400).

Plate - 2.9 Morphological characters - Mycelium, conidiophore and conidia A_3 (x 600).

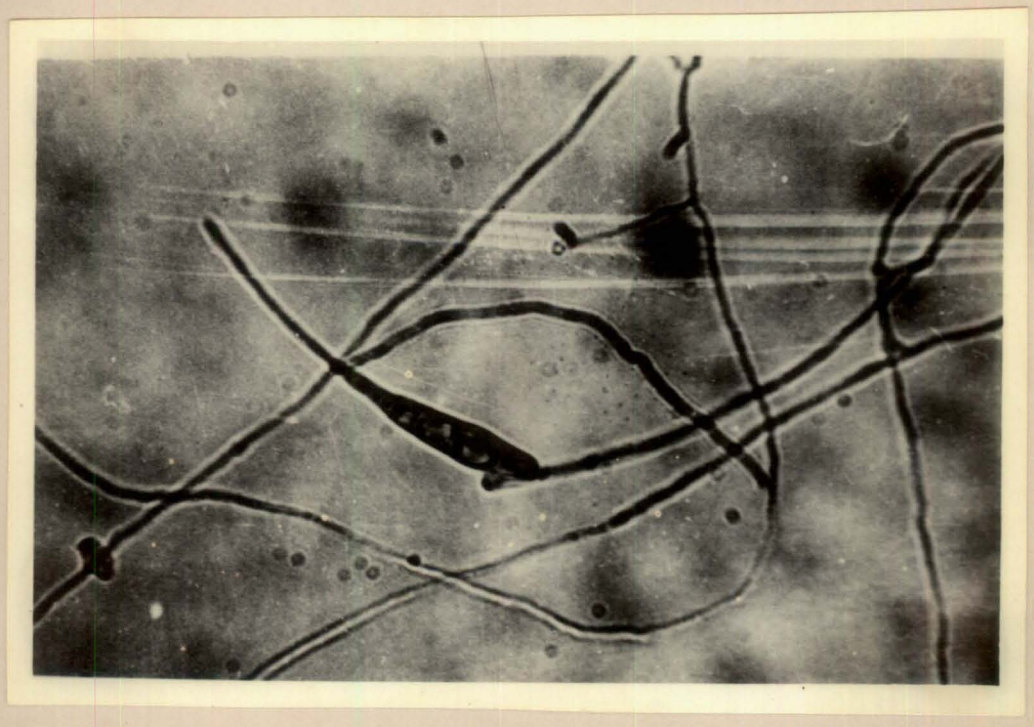


Plate - 2.10 Morphological characters - Mycelium, conidiophore and conidia A_4 (x 600).

4.2 Pathogenicity

It was found that all the isolates of Sarocladium oryzae (I_1 to I_6) and the associated fungi (A_1, A_2, A_3, A_4) individually and in combination could produce disease on the varieties tested. The nature of symptoms varied with virulence of the pathogen and rice varieties. The observations on varying symptoms were made as explained under (3.2) of materials and methods and for convenience in the comparison of severity of the disease the symptoms were classified in to following three groups.

Mild - Infection initiated after 9 days of inoculation. Symptoms first appeared as light brownish discolouration which later spread on the lower portion of the stalk and also on the boot leaf. Panicles were fully emerged without any grain discolouration.

Moderate - Infection initiated after six days of inoculation. Dark brown discolouration developed on the inoculated area which later extended and covered the boot leaf and also the lower portion of the stalk. Panicles were partially emerged with pinkish growth of the pathogen.

Severe - Symptoms initiated on 5th day of inoculation. Dark brown lesions seen on the inoculated site, some times with profuse sporulation of the fungus. Complete choking or partial choking of the panicle were observed. Boot leaf was either brown, pinkish or straw coloured which was found often covered with mycelial growth of the fungus. Grains were either partially filled or chaffy and seem to be brown or pinkish in colour. Inoculation of rice plants with S. oryzae alone at boot leaf stage produced typical sheath rot symptoms more often than thier inoculation done at active tillering stage. This was the same in the case of inoculation done with the other associated fungi.

Isolate I_5 showed the maximum virulence. both at active tillering and boot leaf stages. S. oryzae and the associated fungi in combination gave the typical choking symptom more often than their individual infection. I_5 + A combination and I_5 + A_1 combination was found to give typical choking symptom. The degree of virulence of the pathogen isolates based on the symptoms grouped as above are presented in table 4 to 6 (Plate 3.1 to 5.3).

Table 4
Virulence of various isolates on symptom expression in different varieties

Isolate Number	Types of Symptoms on rice varieties under inoculation															
	V 1		V 2		V 3		V 4		V 5		V 6		V 7		V 8	
	ATS	BLS	ATS	BLS	ATS	BLS	ATS	BLS	ATS	BLS	ATS	BLS	ATS	BLS	ATS	BLS
I 1	Severe	Severe	Mild (Plate-3.2)	Mild	Mild	Moderate	Mild	Severe (Plate-4.6)	Moderare	No Symptom	Mild	Mild	Moderate	Moderate	Severe	Severe
I 2	Mild	Mild	Mild	Mild	Mild	Moderate (Plate-4.1)	Severe	Severe (Plate-4.5)	Mild	Mild	Mild	Severe	Severe (Plate-3.3)	Severe (Plate-4.4)	Mild	Severe
I 3	Mild	Severe (Plate-4.2)	Mild	Mild	Mild	Mild	Mild	Mild	Moderate	Severe	Mild	Moderate	Moderate	Moderate	Mild	Severe
I 4	Mild	Severe (Plate-4.2)	Moderate (Plate-3.5)	Mild	Mild	Severe	Moderate (Plate-3.1)	Severe (Plate-4.6)	Severe	Severe	Severe	Severe	Severe	Mild	Mild (Plate-3.4)	Severe
I 5	Severe	Severe	Moderate	Severe	Moderate	Moderate	Severe (Plate-3.1)	Severe (Plate-4.3)	Mild	Mild	Severe	Severe	Mild	Severe	Mild (Plate-3.4)	Severe
I 6	Severe	Moderate (Plate-4.2)	Mild	Mild	Mild	Severe (Plate-4.1)	Mild	Mild (Plate-4.5)	Mild	Mild	Mild	Severe	Severe	Severe (Plate-4.4)	Mild (Plate-3.4)	Moderate

V - Varieties
I - Isolates
ATS - Inoculation at Active tillering stage
BLS - Inoculation at Boot leaf stage
I to I - Symptoms noticed under natural infection
1 6

I - Typical sheath rot symptom. Affected panicles were straw coloured with pinkish growth of the fungus. Grains of affected panicles showed pinkish colour
1

I - Brownish discolouration seen on the leaf sheath. Continuous brown discolouration was seen on the second leaf sheath. Pinkish orange discolouration seen on the grains, Panicles
2 partially emerged.

I - Panicles completely emerged out. Brownish discolouration seen on the stalk region.
3

I - Brown discolouration seen on the boot leaf. Panicle partially emerged.
4

I - Typical sheath rot symptom. Complete boot leaf was affected. The grains were affected and have a pinkish tinge.
5

I - Partially emerged panicle with discoloured grains. Mycelial growth seen on the grains. Brown lesions seen on the leaf sheath.
6

Table 5

Virluence of various associated fungi on symptom expression in different varieties

Isolate Number	Types of symptoms on rice varieties under inoculation															
	V 1		V 2		V 3		V 4		V 5		V 6		V 7		V 8	
	ATS	BLS	ATS	BLS	ATS	BLS	ATS	BLS	ATS	BLS	ATS	BLS	ATS	BLS	ATS	BLS
A 1	Mild	Mild	Mild	Mild	Mild	Severe	Mild	Severe (Plate-4.6)	Mild	Mild	Mild	Mild	Moderate	Mild	Moderate	Severe
A 2	Mild	Mild	Moderate	Mild	Mild	Mild	Moderate	Moderate (Plate-4.6 and 4.3)	Mild	Severe	Mild	Severe	Mild	Severe	Mild	Severe
A 3	Moderate (Plate-3.7)	Mild	Mild	No Symptom	Mild (Plate-3.7)	Moderate	Mild (Plate-3.7)	Moderate	Mild (Plate-3.6)	Mild (Plate-3.6)	Mild (P-3.6)	No Symptom	Moderate (Plate-3.3 & 3.6)	Moderate (Plate-4.4)	Mold (Plate-3.6)	Mild
A 4	Mild	Moderate	Mild	Moderate	Mild	Mild	Mild	Mild	Mild	Moderate	Mild	Mild	Mild	Mild	Moderate	Moderate

V - Varities
A - Associated Fungi
ATS - Inoculation at Active tillering stage
BLS - Inoculation at Boot leaf stage

A to A - Symptoms under natural infection
1 4

A - Partially emerged panicle, Brown lesions on the boot leaf sheath, brown discolouration was not continuous.
1

A - Brown small lesion surrounded by yellow colour, Grains discoloured, partially emerged panicle.
2

A - Brown oval spots seen on the boot leaf sheath. Grains discoloured partially emerged panicle.
3

A - Brownish discolouration seen on the lower portion of the stalks. Panicles completely emerged.
4

Table 6

Effect of combined inoculation of isolates of Sarocladium oryzae and associated fungi on disease development using variety Jaya

Isolates of Sarocladium oryzae	Associated Fungi							
	A 1		A 2		A 3		A 4	
	ATS	BLS	ATS	BLS	ATS	BLS	ATS	BLS
I 1	Severe	Severe	Mild	Mild	Severe	Severe	Mild	Severe
I 2	Mild	Mild	Severe (Plate-5.1)	Severe	Severe (Plate-5.2)	Severe	Severe	Mild
I 3	Moderate	Moderate	Mild	Mild	Severe (Plate 5.1)	Severe	Moderate	Moderate
I 4	Severe (Plate 5.3)	Moderate	Severe	Moderate	Severe (Plate-5.2)	Mild	Severe	Mild
I 5	Severe (Plate 5.1)	Severe	Severe (Plate 5.2)	Mild	Severe	Mild	Severe	Severe
I 6	Severe (Plate 5.3)	Moderate	Mild	Mild	Mild	Mild	Mild	Mild

I - Isolates of Sarocladium oryzae

A - Isolates of associated fungi

ATS - Inoculation at active tillering stage

BLS - Inoculation at boot leaf stage

Plate - 3.1 Pathogenicity test on Aiswarya (V_4) done at Active tillering stage .

6 - I_5 3 - I_4

Plate - 3.2 Pathogenicity test on Bharathi (V_4) done at Active tillering stage .

7 - I_1

Plate - 3.3 Pathogenicity test on Aathira (V_7) done at Active tillering stage .

10, 12 - I_2 , 22 - A_3

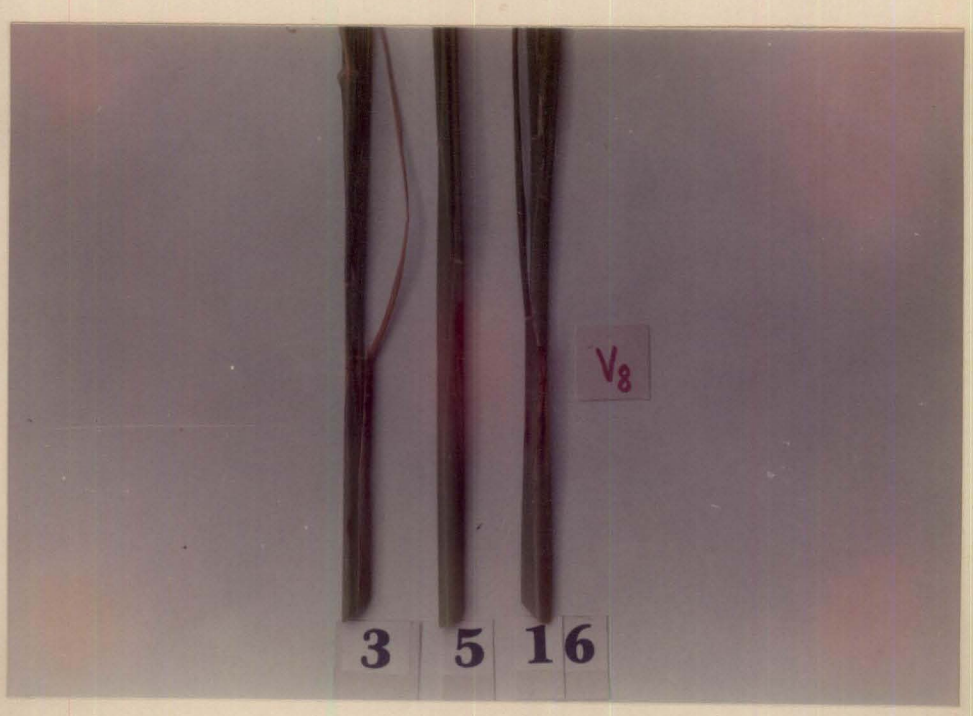


Plate - 3.4 Pathogenicity test on Sabari (V_8) done at Active tillering stage .

3 - I_4 , 5 - I_5 , 16 - I_6

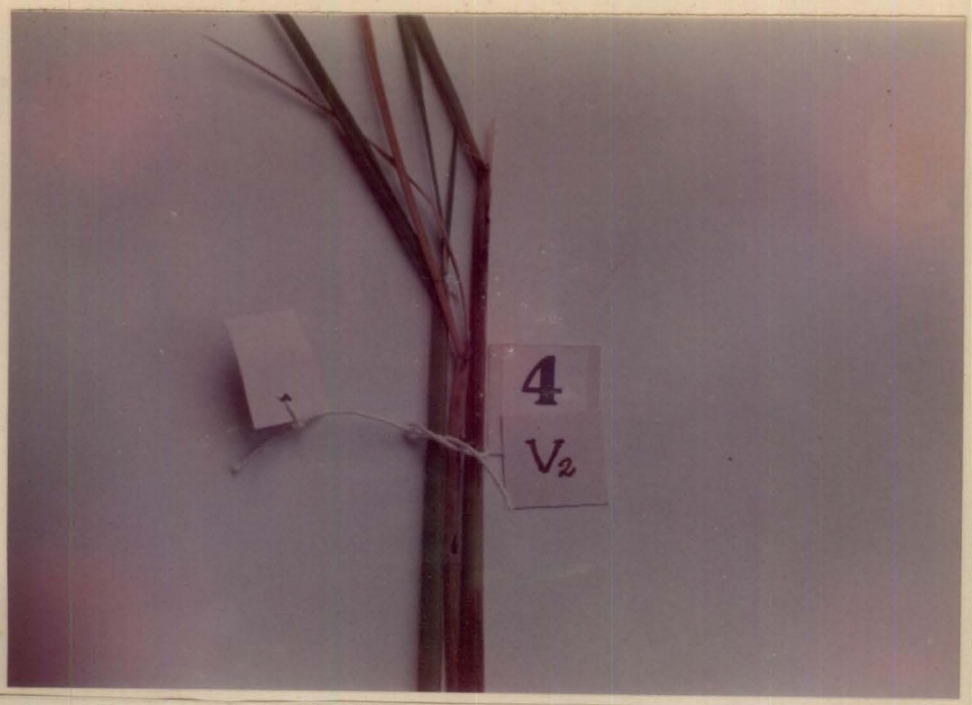


Plate - 3.5 Pathogenicity test on Bharathi (V_2) done at Active tillering stage .

4 - I_4

Plate - 3.6 Pathogenicity test on different varieties done at Active tillering stage.

21 - A_3

V_5 - White Mashoori V_7 - Aathira

V_6 - Jayathi V_8 - Sabari

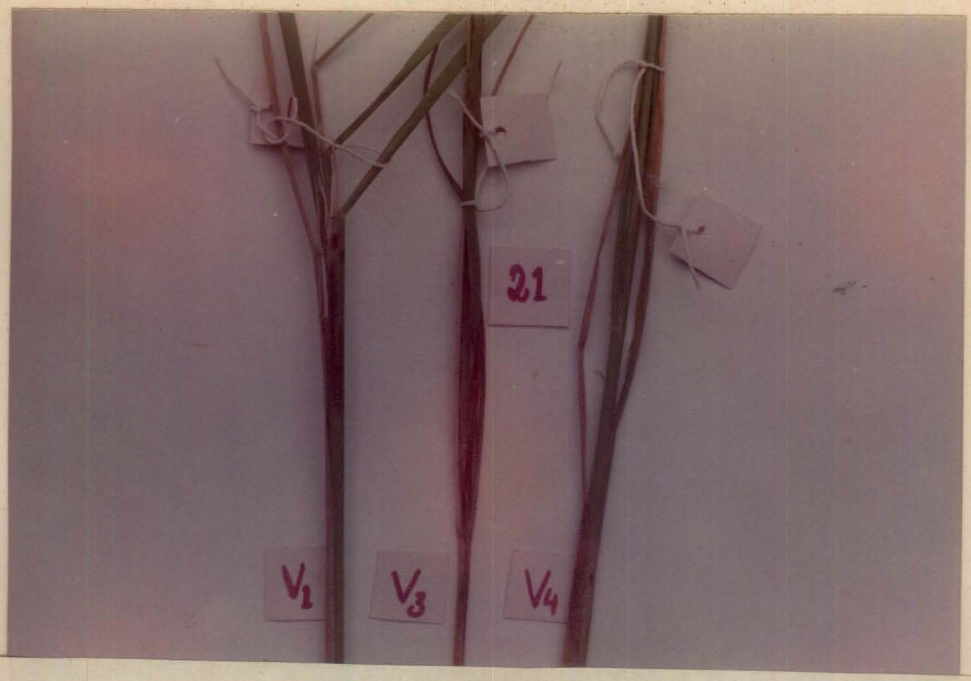


Plate - 3.7 Pathogenicity test on different varieties done at Active tillering stage .

21 - A_3

V_1 - Jaya

V_3 - Kanchana

V_4 - Aiswarya

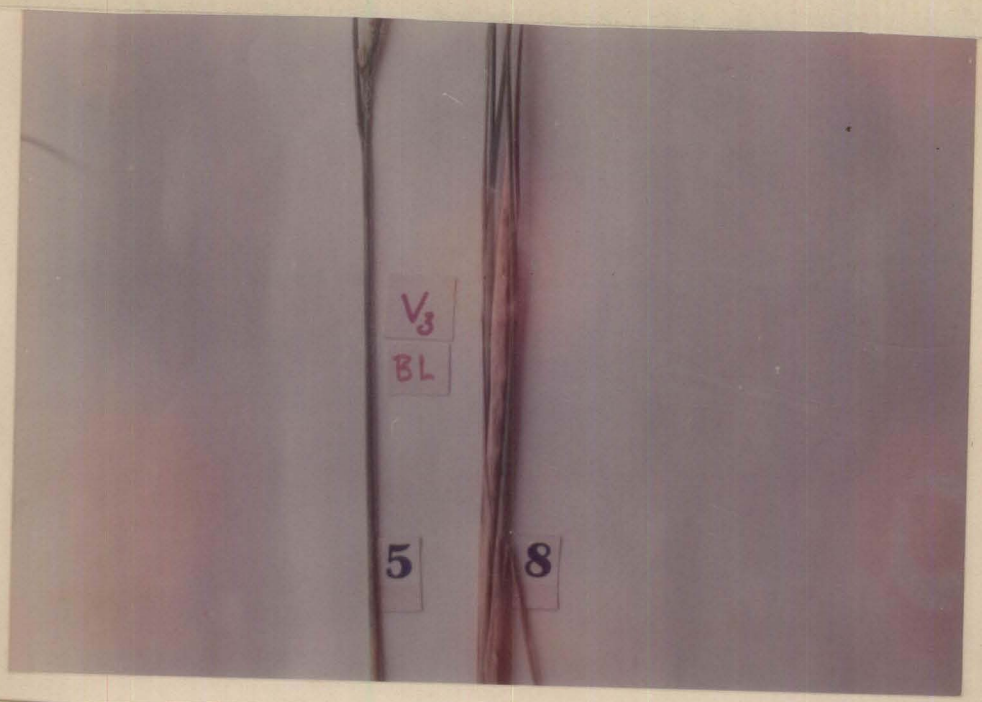


Plate - 4.1 Pathogenicity test on Kanjana (V_3) done at Boot leaf stage.

5 - I_2 8 - I_6

Plate - 4.2 Pathogenicity test on Jaya (V_1) done at Boot leaf stage.

1 - I_3 8 - I_6 2 - I_4

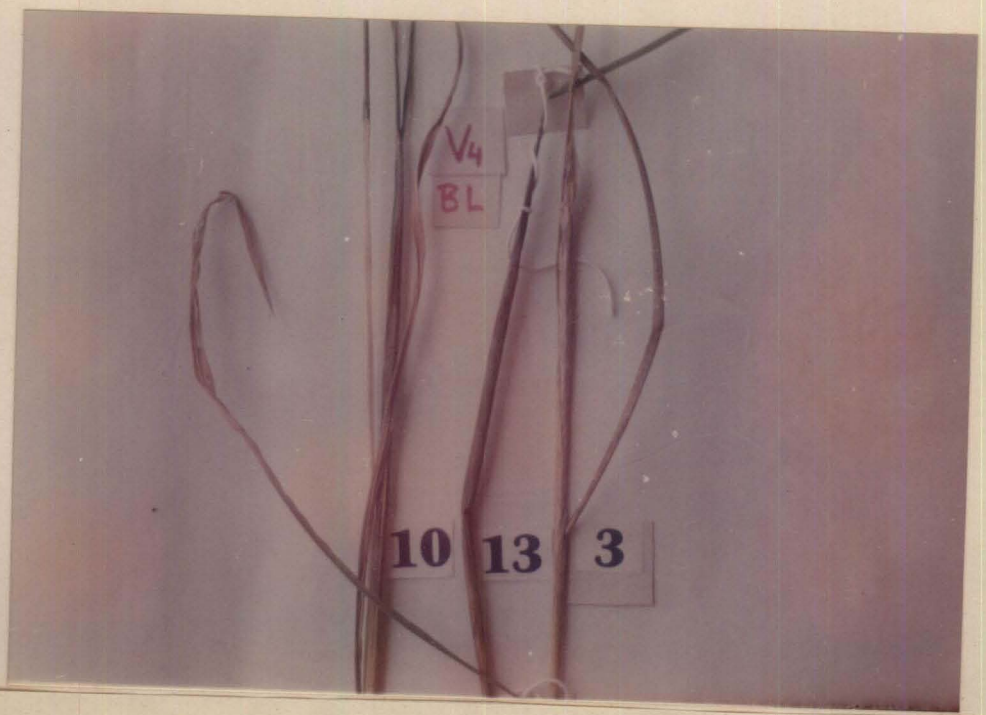


Plate - 4.3 Pathogenicity test on Aiswarya (V_4) done at Boot leaf stage.

10 - A 13 - A 3 - I

Plate - 4.4 Pathogenicity test on Aathira (V_7) done at Boot leaf stage.

6 - I_2 , 7 - I_6 , 13 - A_3 8 - I_6

Plate - 4.5 Pathogenicity test on Aiswarya (V_4) done at Boot leaf stage.

11 - A_2 , 6 - I_2 , 7 - I_6

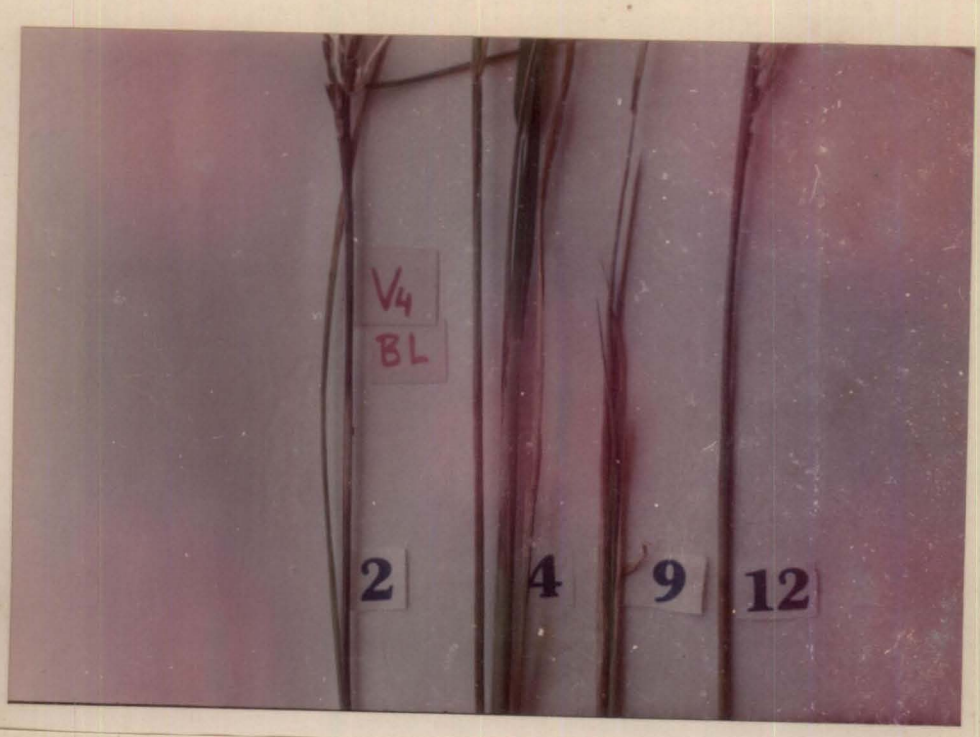


Plate - 4.6 Pathogenicity test on Aiswarya (V_4) done at Boot leaf stage.

2 - I_4, 4 - I_1, 9 - A_1, 12 - A_2

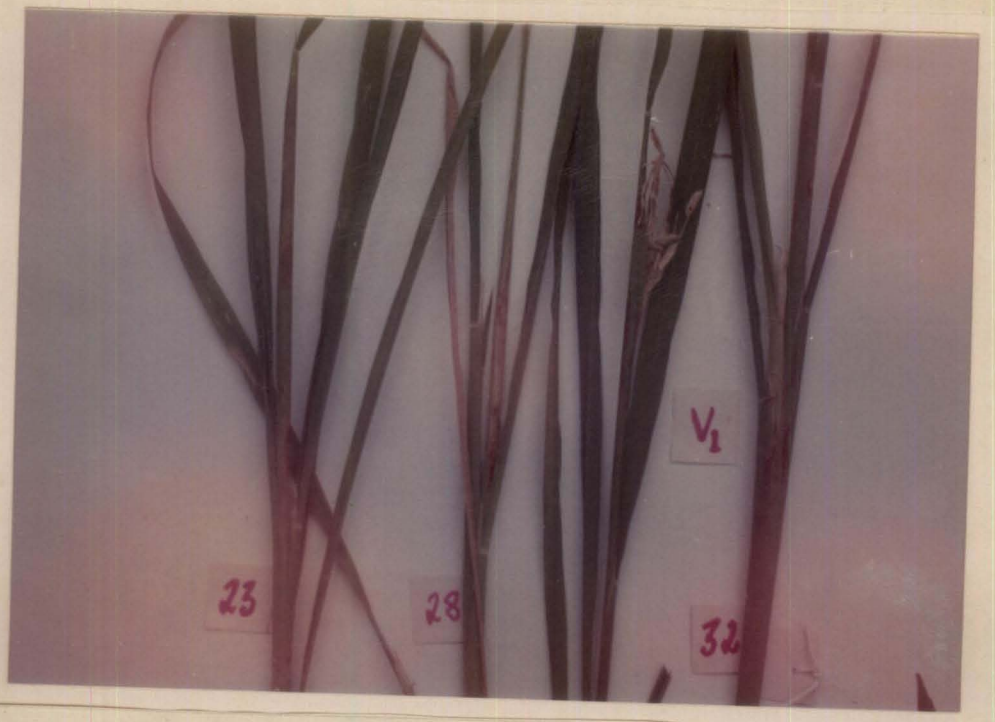


Plate - 5.1 Effect of combined inoculation on Jaya (V_1) done at Active tillering stage.

23 - I_2 + A_2, 28 - I + A, 32 - I + A

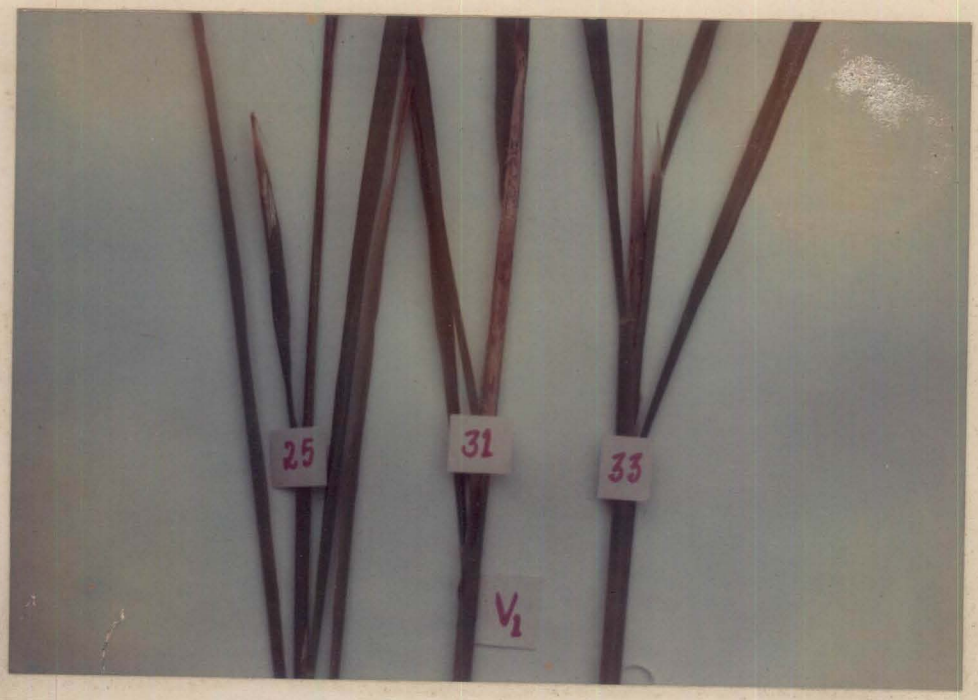


Plate - 5.2 Effect of combined inoculation on Jaya (V_1) done at Active tillering stage.

25 - $I_2 + A_3$, 31 - $I_4 + A_3$, 33 - $I_5 + A_2$

Plate - 5.3 Effect of combined inoculation on Jaya (V_1) done at Active tillering stage.

35 - $I + A$, 29 - $I + A$,

4.2.1. Varietal reaction to different isolates

All the varieties showed susceptibility to sheath rot pathogen. Disease intensity of various rice varieties were measured using a score chart as described under (3.2.1) materials and methods.

Variety Jaya showed the highest susceptibility and was similar to Jayathi. Susceptible nature of Bharathi, Kanchana, Aiswarya, White Mushoori, Aathira and Sabari was on par (Table 7) Fig 3.

Table 7

Disease Intensity

Serial Number	Variety	Mean score
1	Jaya	4.94
2	Bharathi	3.61
3	Kanchana	2.96
4	Aiswarya	3.38
5	White Mashoori	3.82
6	Jayathi	4.58
7	Aathira	3.16
8	Sabari	3.74

CD - 1.098

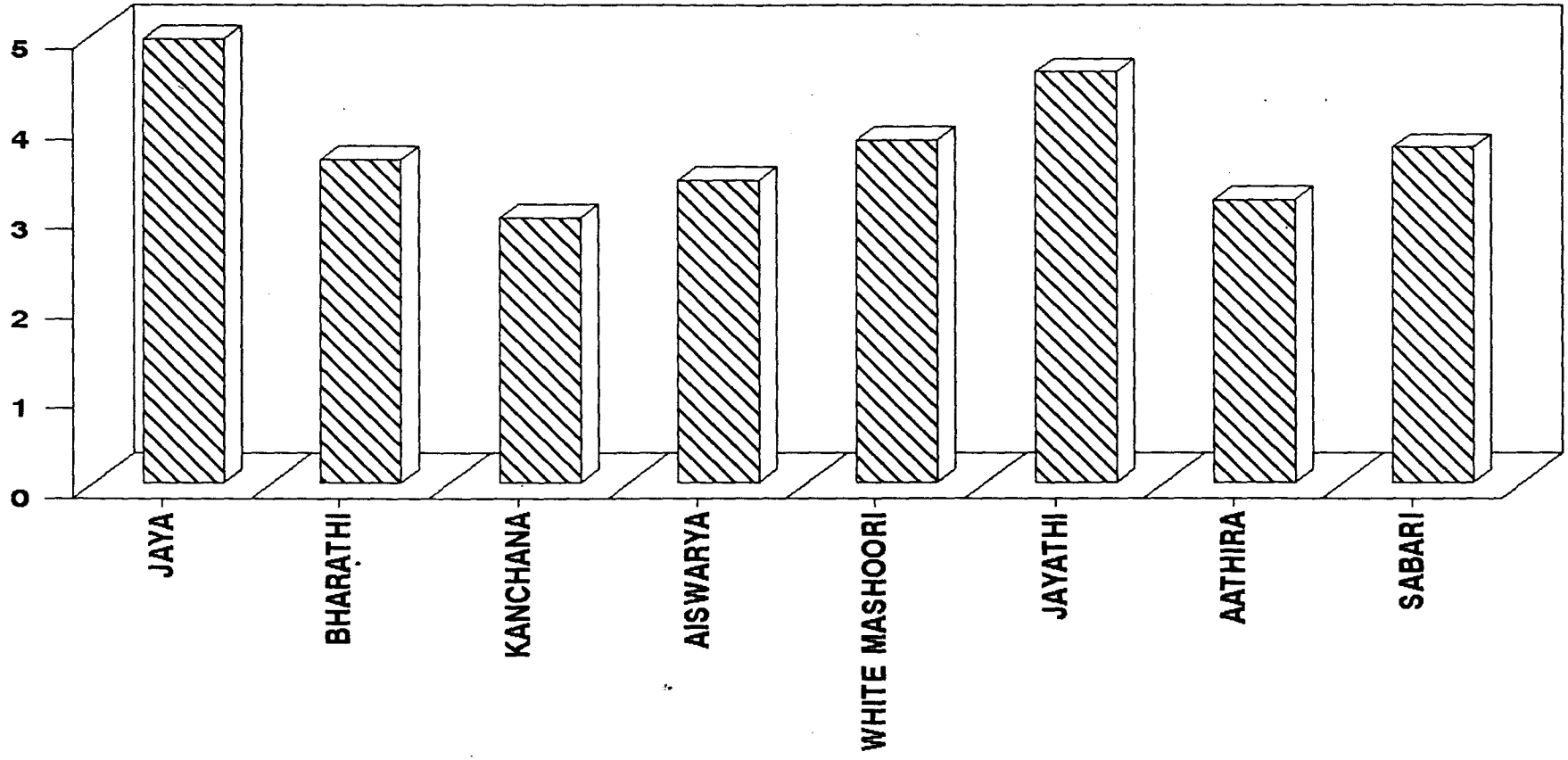


Fig. 3. Disease Intensity

4.3 Survival of *Sarocladium oryzae* in infected grains

Viability of the pathogen in infected grains kept under ordinary storage conditions were studied. The samples were tested at 30 days interval for a period of six months. The results revealed that the pathogen could remain viable for six months in paddy grains, both in the surface sterilized and non surface sterilized grains. But from the third month onwards, the frequency of occurrence of the pathogen was found to be reduced (Table 8).

Table 8

Viability of *S. oryzae* in infected grains

Sample No		Frequency of occurrence of *S.oryzae* Period (months) of storage					
		1	2	3	4	5	6
1	NSS	+	+	+	+	+	+
	SS	+	+	+	-	+	+
2	NSS	+	+	+	+	+	-
	SS	+	+	+	+	-	-
3	NSS	+	+	-	-	-	-
	SS	+	+	-	-	-	-
4	NSS	+		-		-	
	SS	+	-	+	-	+	-
5	NSS	+			-	+	-
	SS		-	+	-	-	-
6	NSS		+	-	-	-	-
	SS	-	-	-	-	-	-

\+ ----> Pathogen present
\- ----> Pathogen not present
NSS ----> Not surface sterlized
SS ----> Surface sterlized.

4.3.1 Survival of *S. oryzae* in soil

Viability of the pathogen in soil was tested at monthly intervals. The results showed that the pathogen was viable steadily for 3 months both under wet and dry conditions of soil conditions at a depth of 2 cm and 6 cm (Table 9)

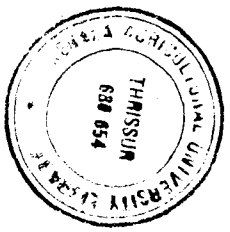


Table 9

Viability of Sarocladium oryzae in soil

Days after soil inoculation	Soil Condition	Frequency of occurence of Sarocladium oryzae											
		Depth of Soil (in cm)											
		2	6	2	6	2	6	2	6	2	6	2	6
30	Wet	+	-	+	-	+	+	+	+	+	+	-	+
	Dry	+	+	+	+	-	+	-	+	+	+	+	+
60	Wet	-	+	-	+	+	+	+	+	-	+	-	+
	Dry	+	+	-	+	+	-	+	-	+	+	+	+
90	Wet	+	-	+	+	+	+	-	+	+	+	+	+
	Dry	+	+	-	+	+	+	-	+	+	-	+	-
120	Wet	+	-	-	-	+	-	-	-	+	-	-	-
	Dry	-	+	-	-	-	-	-	+	-	-	-	-
150	Wet	-	-	-	-	+	-	-	-	-	-	-	-
	Dry	-	-	-	-	-	-	-	-	-	-	-	-
180	Wet	-	-	-	-	-	-	-	-	-	-	-	-
	Dry	-	-	-	-	-	-	-	-	-	-	-	-

+ —> Pathogen present

- —> Pathogen not present

4.4 Cultural characters

Cultural characters of the fungus were studied by growing the fungus in different solid and liquid media.

4.4.1 Growth of the fungus on different solid media

The effect of different solid media on the growth of the fungus was studied. The media tested include Potato dextrose agar, Czapek's agar, Oat meal agar, Coon's agar and Carrot agar. The mean radial growth and the growth characters of the organism in different culture media was observed for ten days. It was found that carrot media gave the maximum radial growth for isolate I_6 followed by I_5 and I_4. Different isolates showed varying growth pattern under different media condition. Coon's medium was found to be least effective since it gave the least mycelial growth for I_2, I_3, I_5 and I_6. Czapek's agar, Oatmeal agar and Carrot agar supported maximum growth for most of the isolates. Potato dextrose agar gave the least growth for I_2. The results are presented in Tables 10 and 11. (Plates - 6.1 to 6.6; Fig 4).

Table 10

Growth of Sarocladium oryzae on different solid media

Name of isolates	Mycelial growth (in cm) (8th day)				
	Czepek's agar	Oat meal agar	Coon's agar	Carrot agar	PDA
I 1	9 (3)	9 (3)	9 (3)	9 (3)	8.94 (2.99)
I 2	9 (3)	9 (3)	8.10 (2.84)	9 (3)	6.74 (2.59)
I 3	9 (3)	8.04 (2.83)	8.09 (2.84)	8.19 (2.86)	9 (3)
I 4	9 (3)	9 (3)	9 (3)	9 (3)	9 (3)
I 5	8.75 (2.95)	9 (3)	8.99 (2.98)	9 (3)	9 (3)
I 6	9 (3)	9 (3)	8.99 (2.99)	9 (3)	9 (3)

CD - 0.021
Figures in parantheses are the transformed values

Table 11

Growth characters of isolates of Sarocladium oryzae in different solid media

Isolates	Carrot agar	Czapek's agar	Coon's agar	Oat meal agar	PDA
I 1	Feeble growth with light pink colour	Dirty cream colour	Dirty cream colour	Deep pink colour without fluffy growth	Deep pink colour with fluffy growth
I 2	Feeble growth with light pink colour	White fluffy growth later turned to pinkish violet colour	White fluffy growth later turned to pink	Pinkish violet colour	Pinkish violet colour
I 3	Colourless mycelial growth	White fluffy growth later turned to light pinkish violet colour	White fluffy growth which remained white	Dull coloured growth which later turned pinkish violet	Violet colour growth
I 4	Feeble growth	Fluffy growth which later turned light pink colour	Fluffy growth turning dull cream colour	Pinkish violet colour	Pink colour
I 5	Feeble colourless growth	Light pink colour	Fluffy white growth which turned dirty white	Fluffy growth turned violet later	Fluffy growth which is light pink
I 6	Feeble growth	Fluffy growth with pink colour	White fluffy growth	Deep violet colour	Slight pinkish white colour

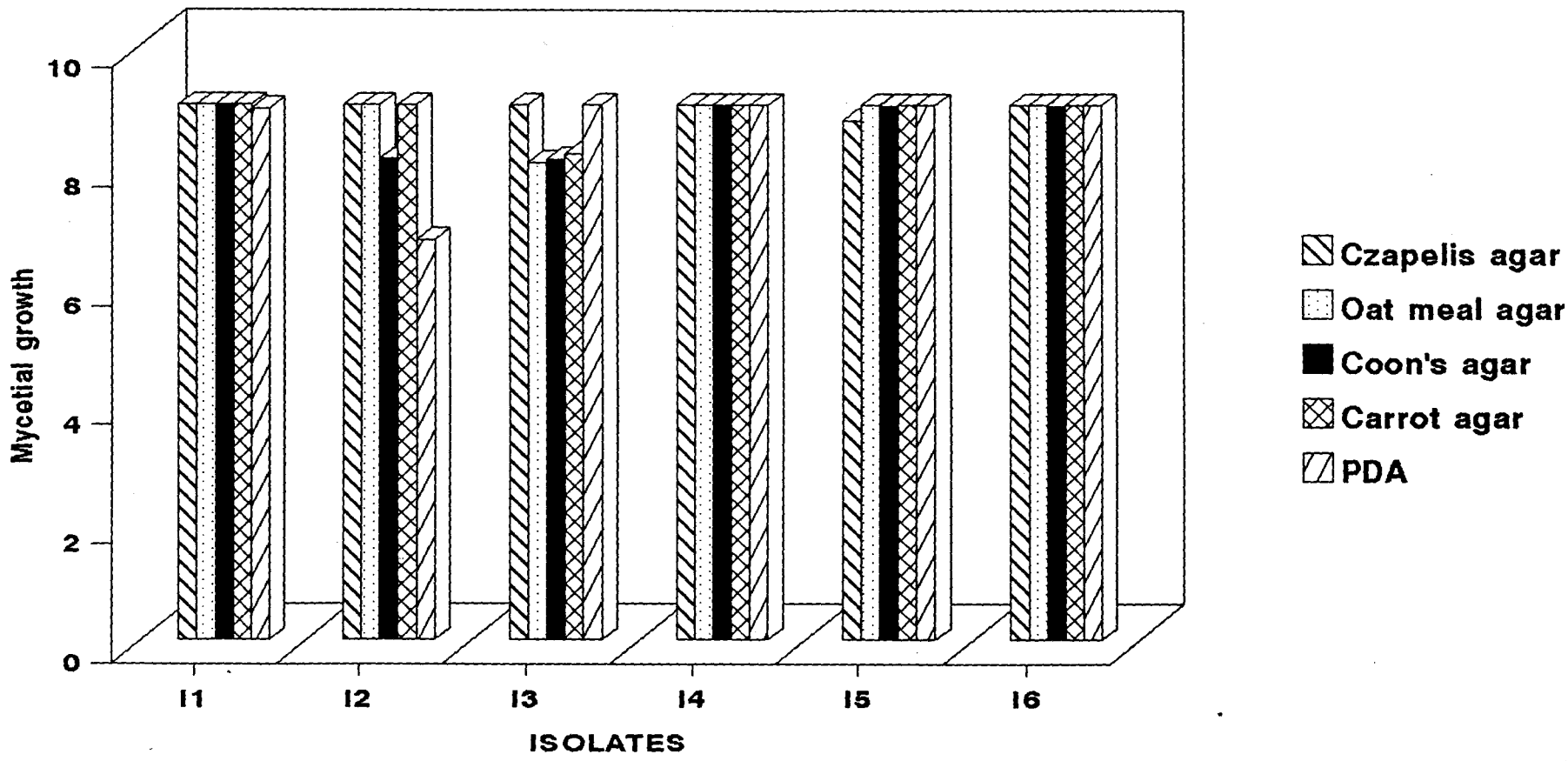


Fig. 4. Growth o Sarocladium oryzae on different solid media

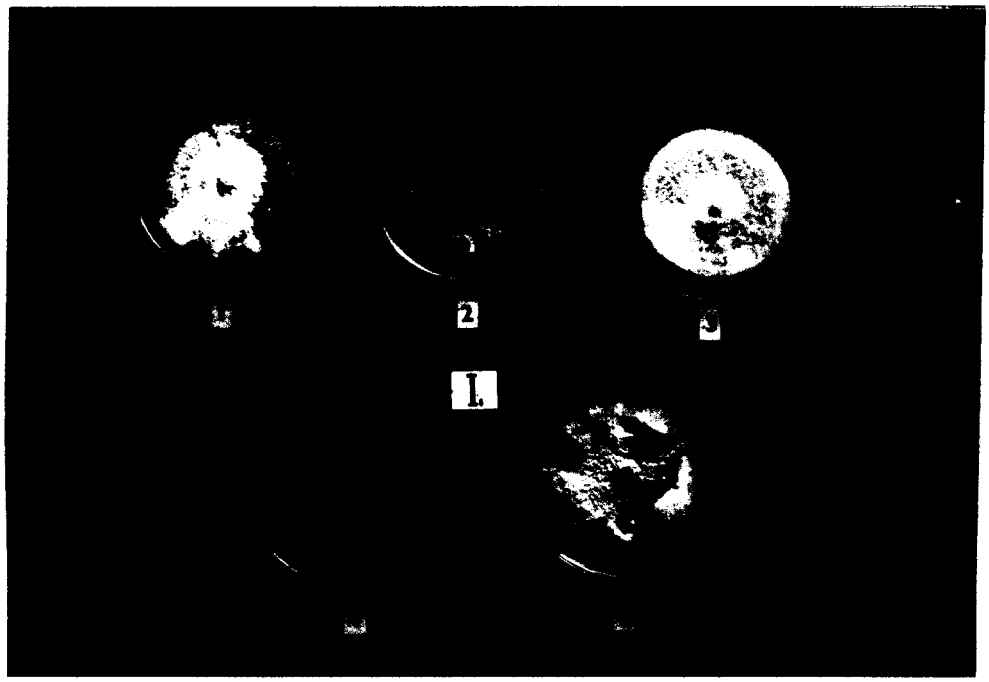


Plate - 6.1 Growth characters of I_1 on different solid culture media.

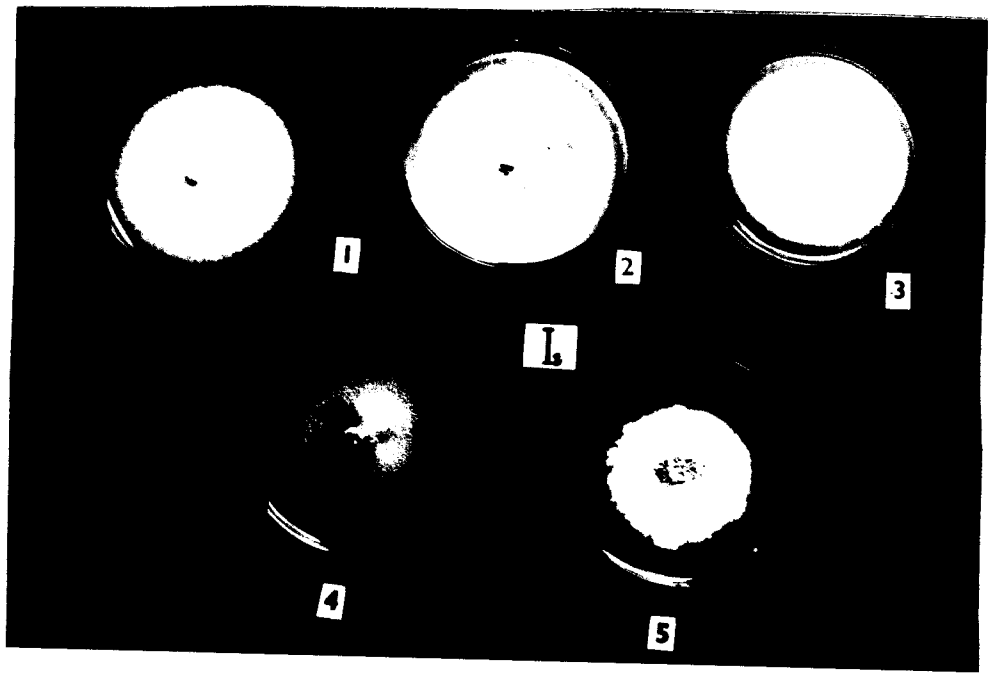


Plate - 6.2 Growth characters of I_2 on different solid culture media

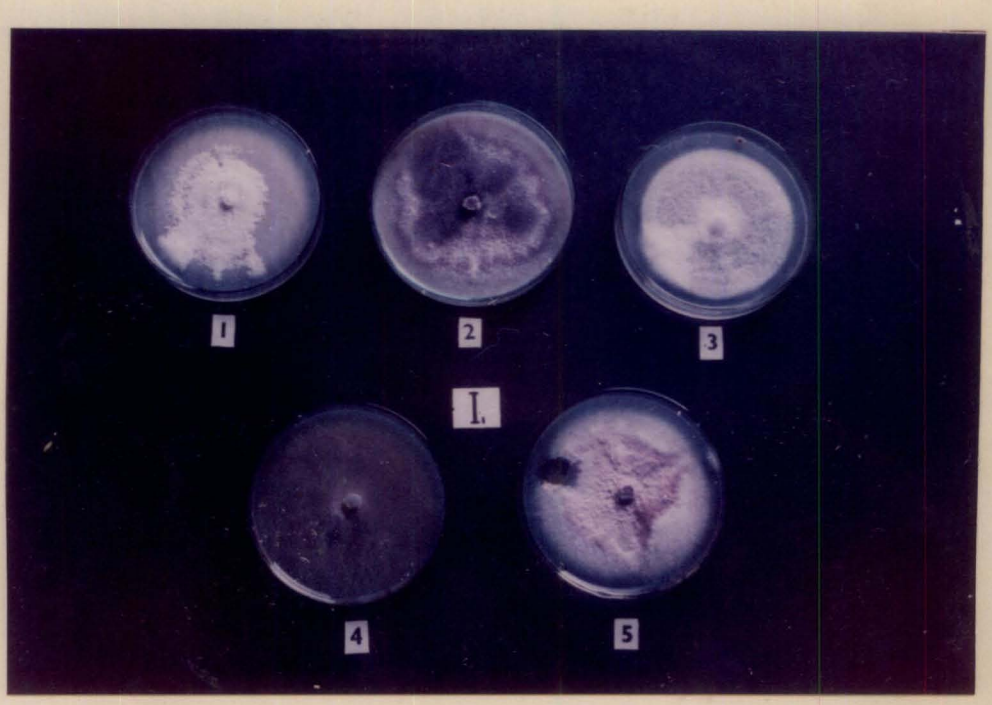


Plate - 6.1 Growth characters of I_1 on different solid culture media.

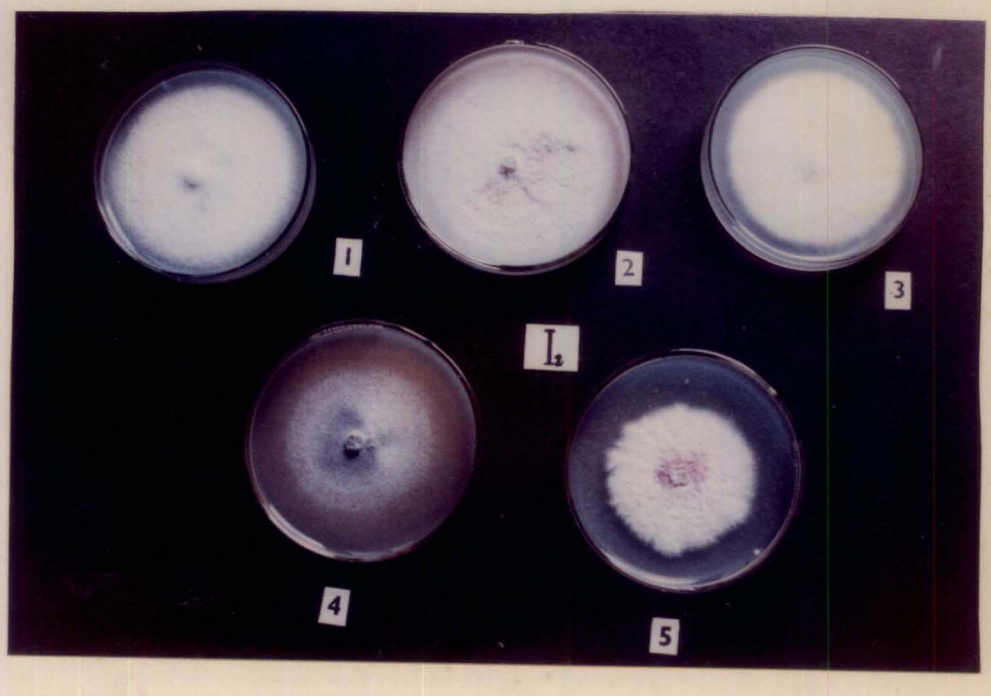


Plate - 6.2 Growth characters of I_2 on different solid culture media

Plate - 6.3 Growth characters of I_3 on different solid culture media

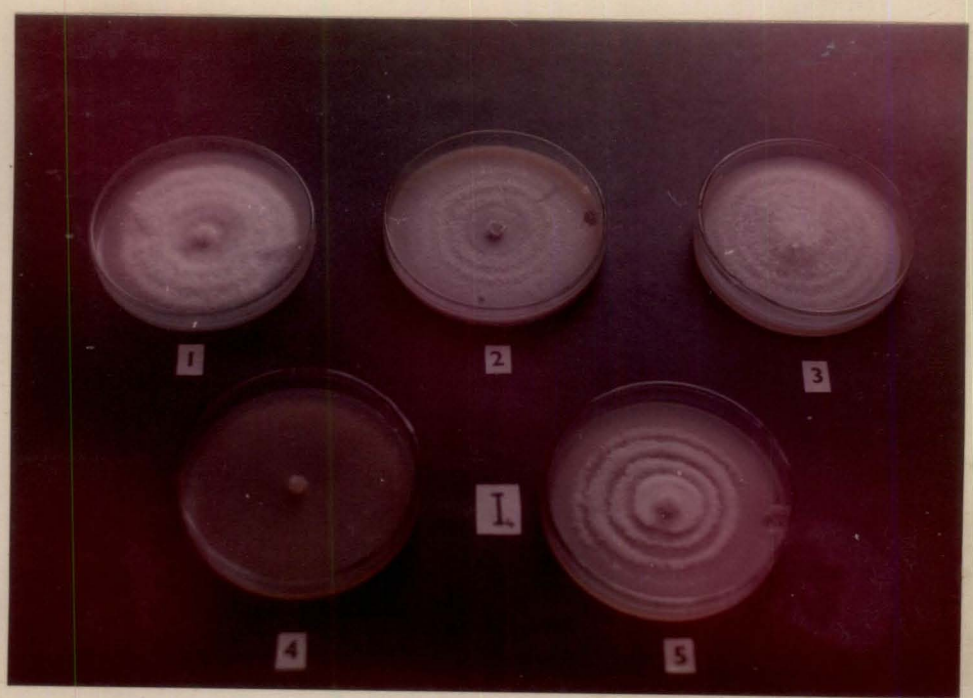


Plate - 6.4 Growth characters of I_4 on different solid culture media.

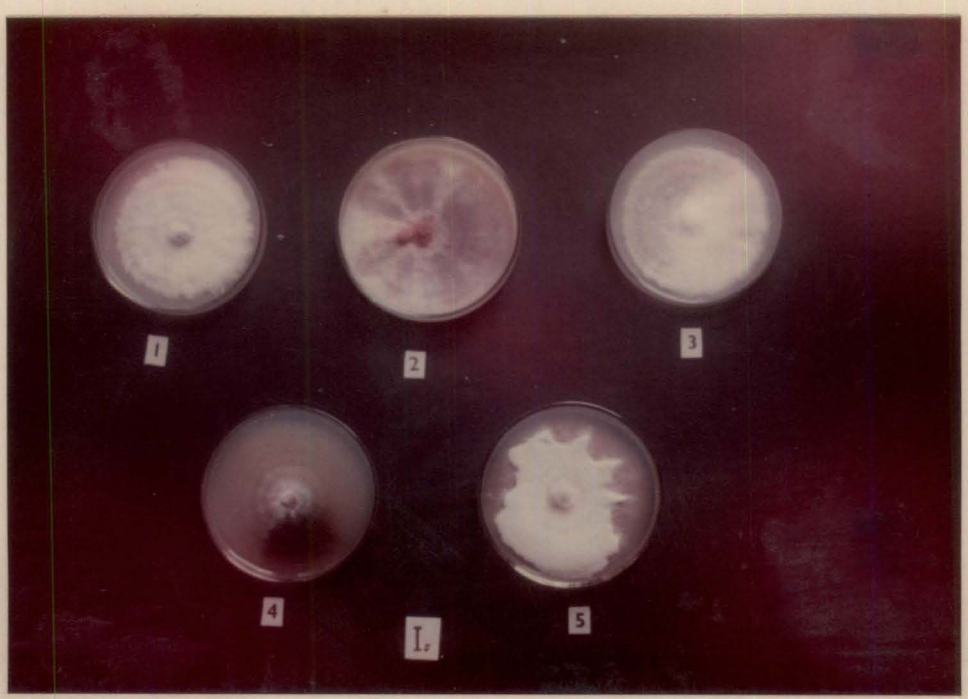


Plate - 6.5 Growth characters of I_5 on different solid culture media.

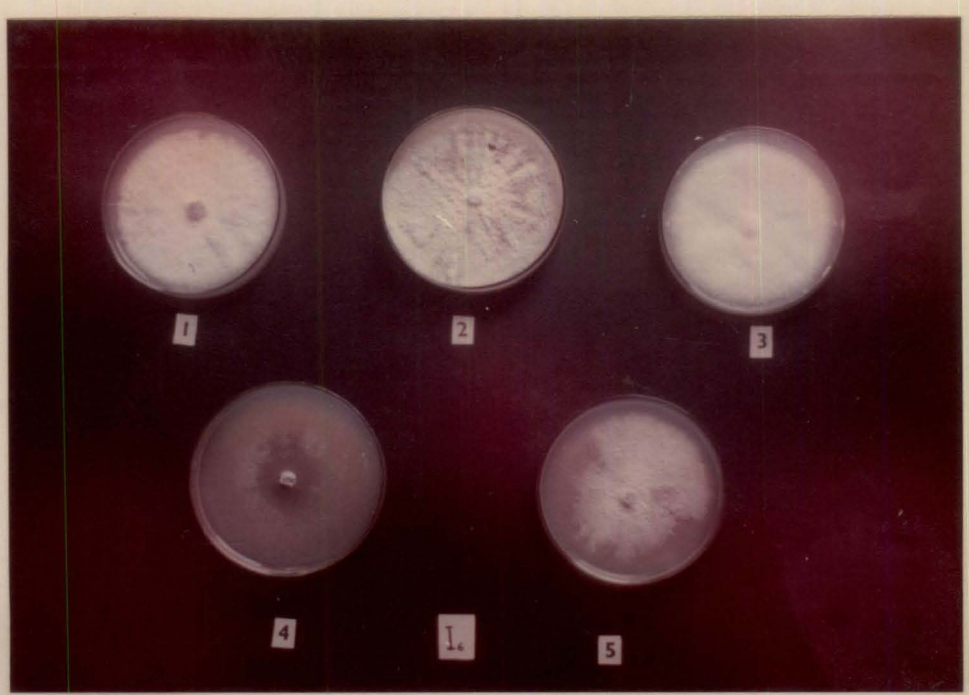


Plate - 6.6 Growth characters of I_6 on different solid culture media.

4.4.2 Growth of *S. oryzae* in liquid media

The most virulent isolate I_5 was used for the study. Among the five different liquid media tested maximum dry weight of biomass was obtained in Czapek's medium followed by Richard's medium and Coon's medium. Potato Dextrose Agar was found to be inferior with respect to the dry weight of biomass. Host leaf extract was the least respondent for biomass production (Table 12; Fig.5).

Table 12

Growth of *S. oryzae* in different liquid media

Sl.No.	Media	Avg.dry wt of biomass (g)	
1	Paddy leaf extract	0.07	(0.2792)
2	Potato Dextrose media	0.159	(0.3991)
3	Czapek's media	0.436	(0.6607)
4	Coon's media	0.236	(0.48581)
5	Richard's media	0.40	(0.6350)

CD - 0.03809

Figures given in parantheses are transformed values

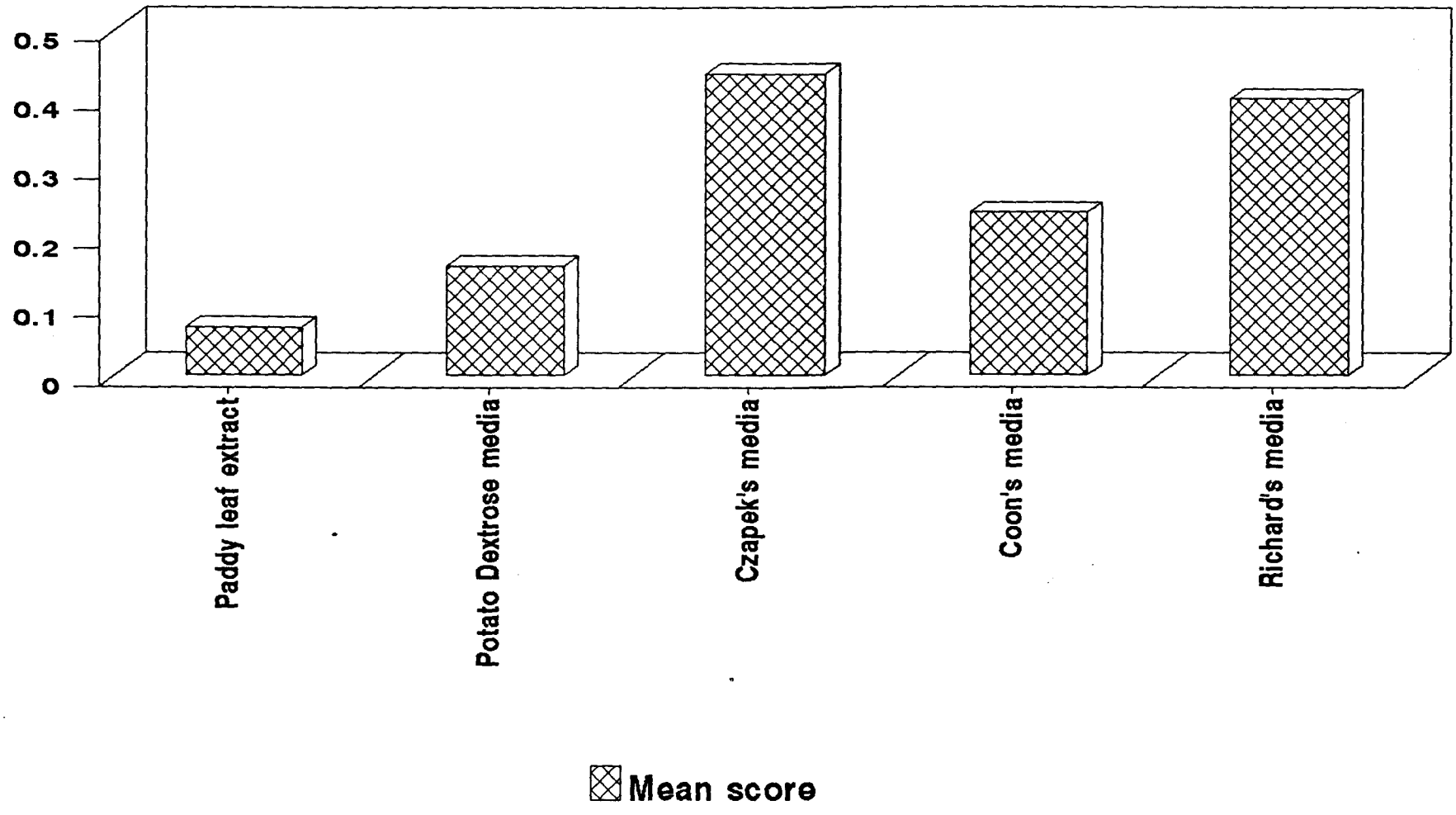


Fig. 5. Growth of Sarocladium oryzae in different liquid media

4.4.3. Effect of different nitrogen sources on growth of *S. oryzae*

Of the different nitrogen sources substituted in Czapek's media, Ammonium nitrate and Sodium nitrate gave the best results with respect to dry weight of biomass. This was followed by Potassium nitrate, Tyrosine and Leucine (Table 13, Fig 6)

Table 13

Effect of different Nitrogen sources on growth of *S. oryzae*

Sl.No.	Nitrogen sources	Avg.dry wt of biomass (g)
1	Tyrosine	0.239 (0.4898)
2	Potassium nitrate	0.278 (0.5281)
3	Sodium nitrate	0.350 (0.5921)
4	Leucine	0.161 (0.4026)
5	Ammonium nitrate	0.360 (0.6002)

CD - 0.00904

Figures given in parantheses are transformed values.

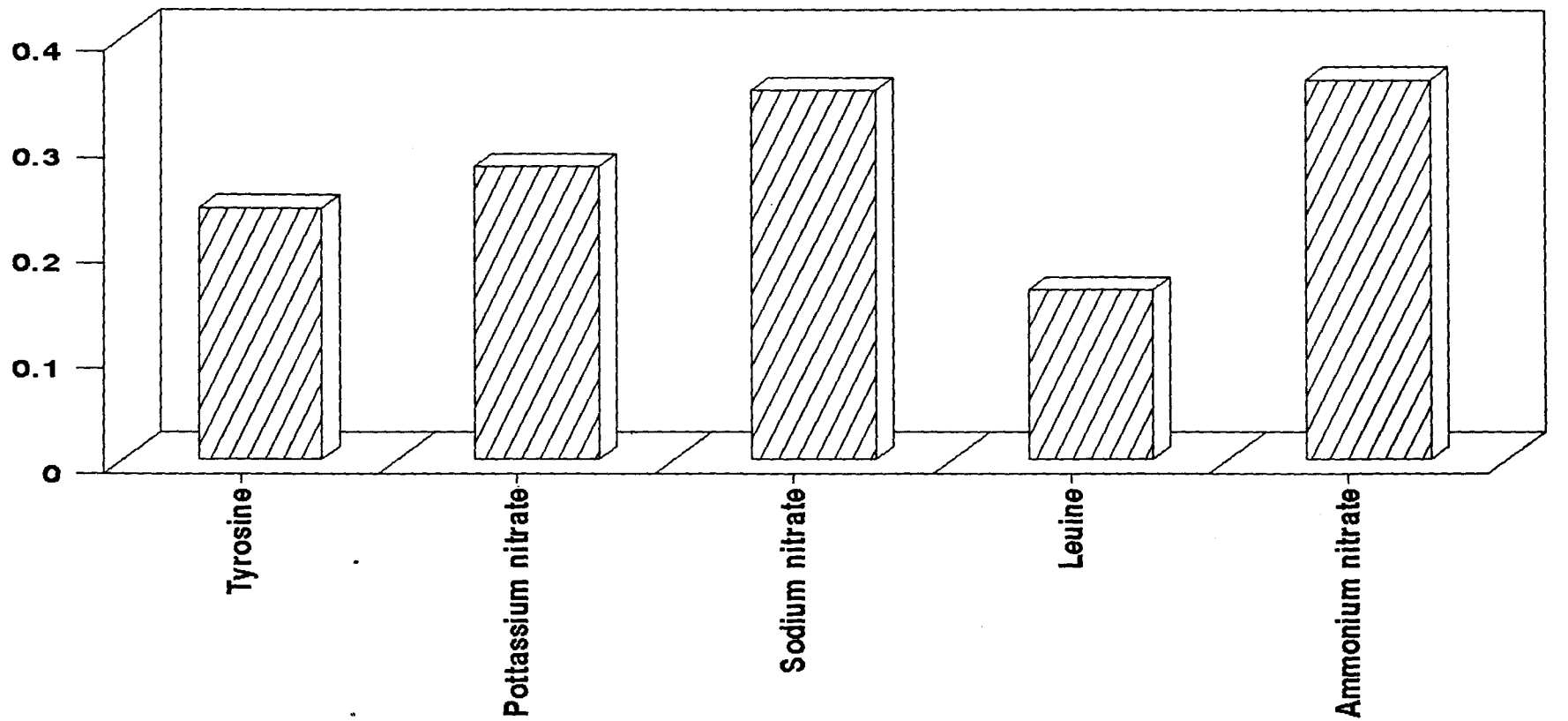


Fig. 6. Effect of different Nitrogen sources on growth of Sarocladium oryzae

4.4.4 Effect of different carbon sources on growth of *S. oryzae*

The Carbon sources were found to be significantly different in their response. When these were substituted in Czapek's media, mannitol supported maximum growth. Glucose was found to be the least effective carbon source (Table 14; Fig.7).

Table 14

Effect of different carbon sources on growth of : *S. oryzae* :

Sl.No.	Carbon sources	Avg.dry wt of biomass(g)	
1	Mannitol	0.642	(0.8012)
2	Lactose	0.280	(0.5292)
3	Glucose	0.250	(0.5006)
4	Starch	0.427	(0.6536)
5	Sucrose	0.460	(0.6789)

CD - 0.0239

Figures given in parantheses are transformed values.

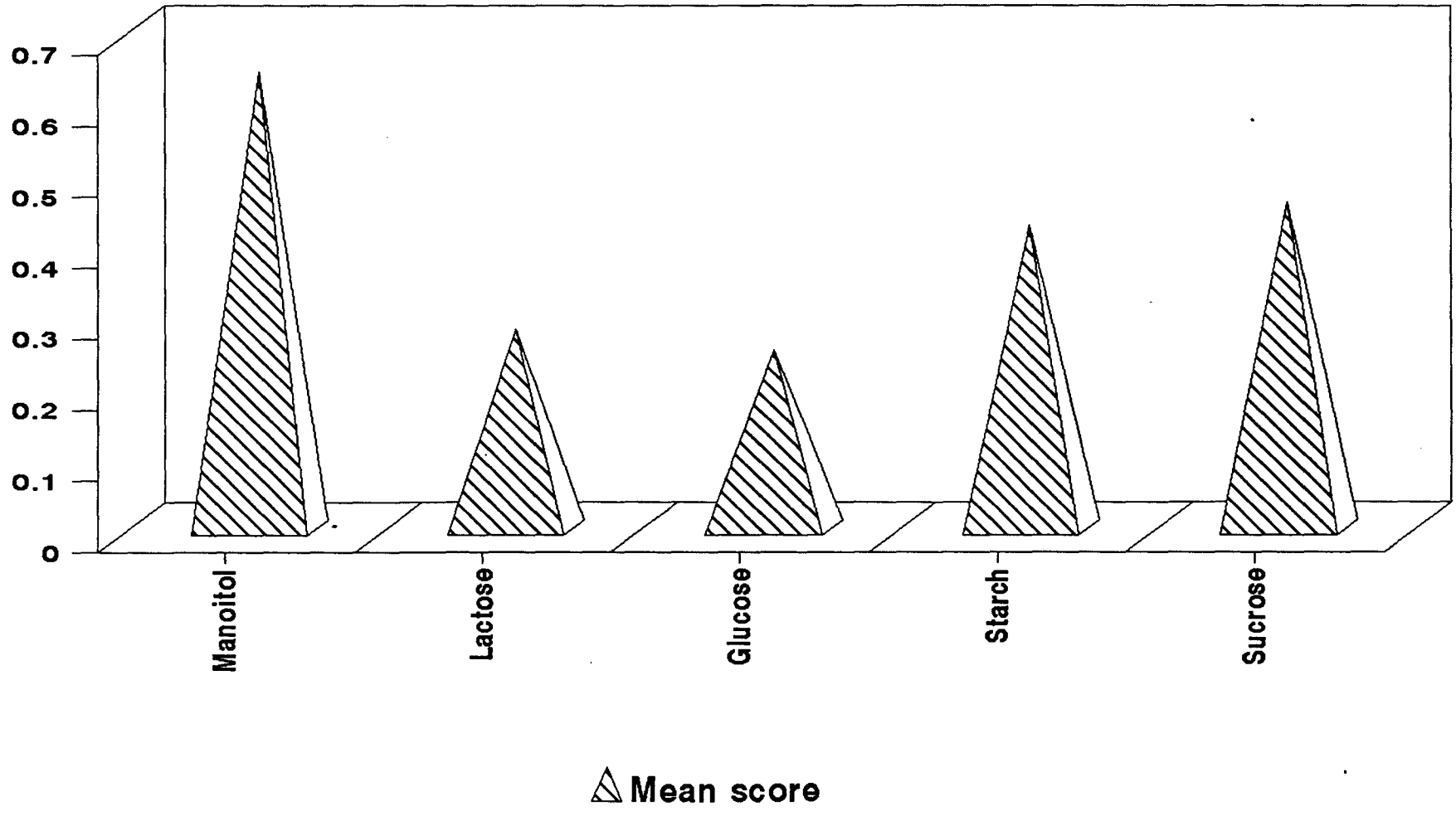


Fig. 7. Effect of different Carbon sources on growth of Sarocladium oryzae

4.4.5 Effect of different spectra of light on growth and sporulation of *S. oryzae*

The various spectra of light were found to influence the growth of *S. oryzae* significantly. All the colours of light viz., red, blue and green showed a reduction in the growth, the rate of reduction on growth being more pronounced from 8th day of incubation (Table 15.1 Fig.8)

Sporulation was maximum when the fungus was grown under blue light and least under red light (Table 15.2).

Table 15.1

Effect of different spectra of light on growth of *S. oryzae*

Sl. No.	Colours of Light	Average radial grwoth (cm)		
		Days 6th	7th	8th
1	Control	6.07 (2.464)	7.33 (2.708)	9 (3)
2	Red	6.03 (2.456)	6.86 (2.620)	7.46 (2.73)
3	Blue	5.96 (2.442)	6.36 (2.523)	6.66 (2.584)
4	Green	6.09 (2.469)	6.93 (2.633)	7.20 (2.683)

CD - 0.014

Figures given in parantheses are transformed values.

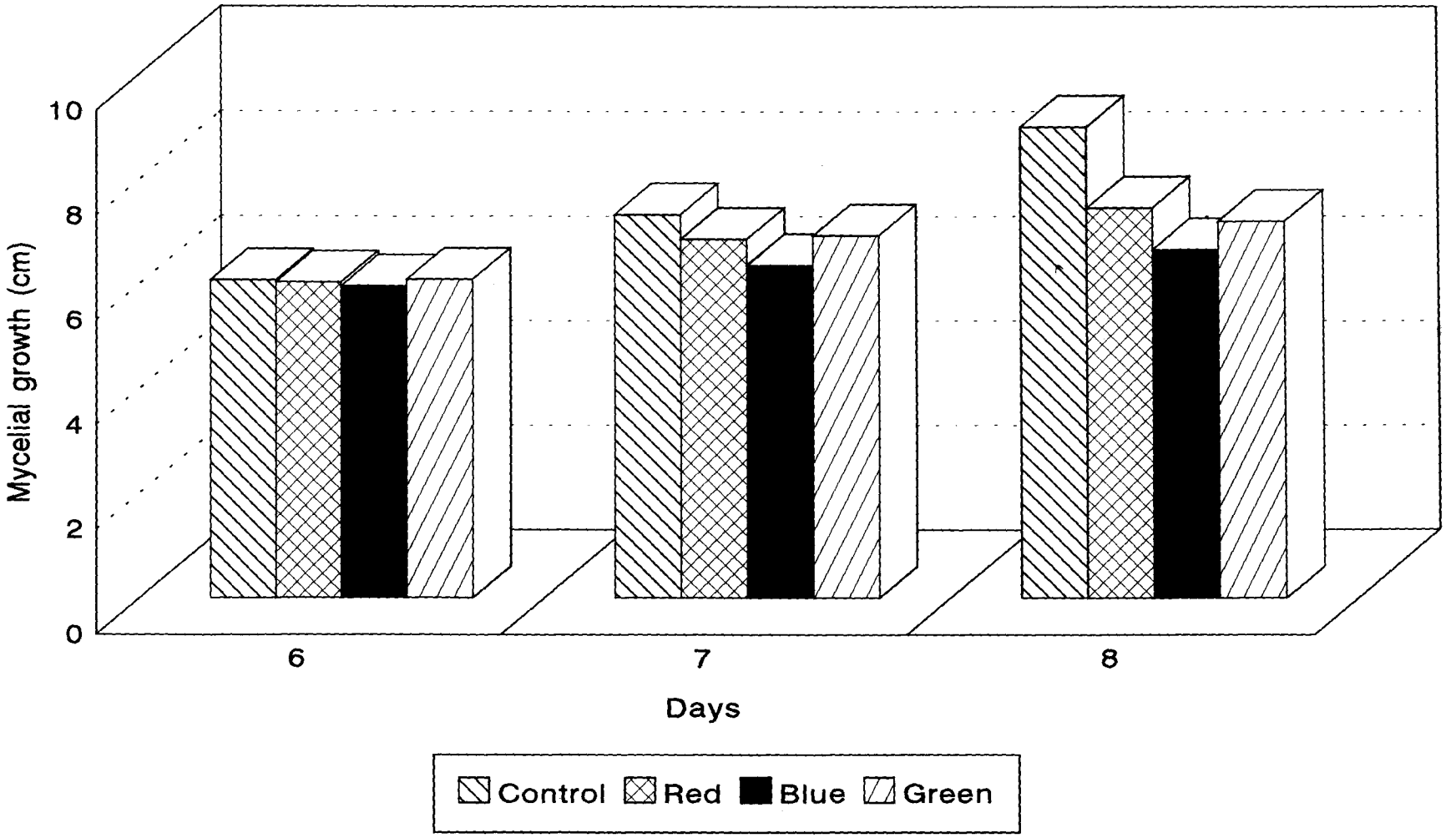


Fig. 8. Effect of different spectra of light on growth of Sarocladium oryzae

Table 15.2

Effect of different spectra of light on sporulation of S. oryzae

Sl. No.	Colours of Light	Days 6th	7th	8th
1	Control	+ +	+ +	+ + +
2	Red	+	+	+
3	Blue	+ + +	+ + +	+ + +
4	Green	+	+ +	+ +

4.5. **Effect of plant extracts on the spore germination of S. oryzae**

Plant extracts were found to produce inhibitory effect on spore germination of S. oryzae. All the plant extracts tested in both the concentrations (1% and 10%) had inhibitory effect on the germination of spores of S. oryzae (Table 16)

Table 16

Effect of Plant extracts on spore germination

Plant extracts	Percentage inhibition	
	1% extract	10% extract
Azadirachta indica	24	50
Allium sativum	20	43
Phyllanthus niruri	10	12
Ocimum sanctum	18	40
Water	0	0

4.6 Isolation of microflora from phylloplane of rice plants

The microflora isolated from phylloplane of rice plants were identified and have been listed out in Table 17.

Table 17

Microflora obtained from phylloplane of rice plants

Phylloplane microbes obtained	Frequency of occurence Virippu		Mundakan	
	ATS	BLS	ATS	BLS
FUNGI				
Chaetomium sp.	+	+	-	+
Aspergillus sp.	+	+	+	+
Botrytis sp.	-	+	-	+
Fusarium sp.	+	+	+	+
Pestalotia sp.	-	-	+	-
Mucor sp.	-	+	+	+
Yeast	+	+	+	+
BACTERIA				
Bacillus sp.	+	+	-	+
Xanthomonas sp.	+	+	-	+

ATS - Active tillering stage

BLS - Boot leaf stage

4.6.1 Antagonism of phylloplane microflora against sheath rot pathogen

Varying reactions were observed when phylloplane microflora and S. oryzae were grown side by side on PDA.

A clear zone of inhibition between the paired cultures was observed in the case of Pestalotia sp. and Chaetomium sp.

Overgrowth by antagonists was observed in the case of fungi like Fusarium sp.

Aspergillus sp. caused cessation of growth of S. oryzae at the point of contact.

Botrytis sp. and Mucor sp. were found to grow freely intermingled with S. oryzae.

The Bacterial isolates namely Bacillus sp. and Xanthomonas sp. freely intermingled with the pathogen where as P. fluorescens-87 and Pseudomonas fluorescens-2 showed a clear zone of inhibiton. The various types of reactions observed has been summarized in Table 18, (Plate - 7.1 to 7.7).

Table 18

Antagonistic action of the phylloplane microbes isolated from rice plants against sheath rot pathogen

Test Fungus	Type of Reaction
FUNGI	
Chaetomium sp.	D
Aspergillus sp.	C
Botrytis sp.	A
Fusarium sp.	B
Pestalotia sp.	D
Mucor sp.	A
Yeast	A
BACTERIA	
Bacillus sp.	A
Xanthomonas sp.	A
Pseudomonas fluorescens - 2	D
Pseudomonas fluorescens - 87	D

* A Homogenous - Free intermingling between pairing organism

B Overgrowth - *Sarocladium oryzae* overgrown by the test organism.

C Cessation - at the line of contact of growth

D Aversion - A clear zone of inhibition between the two organisms.

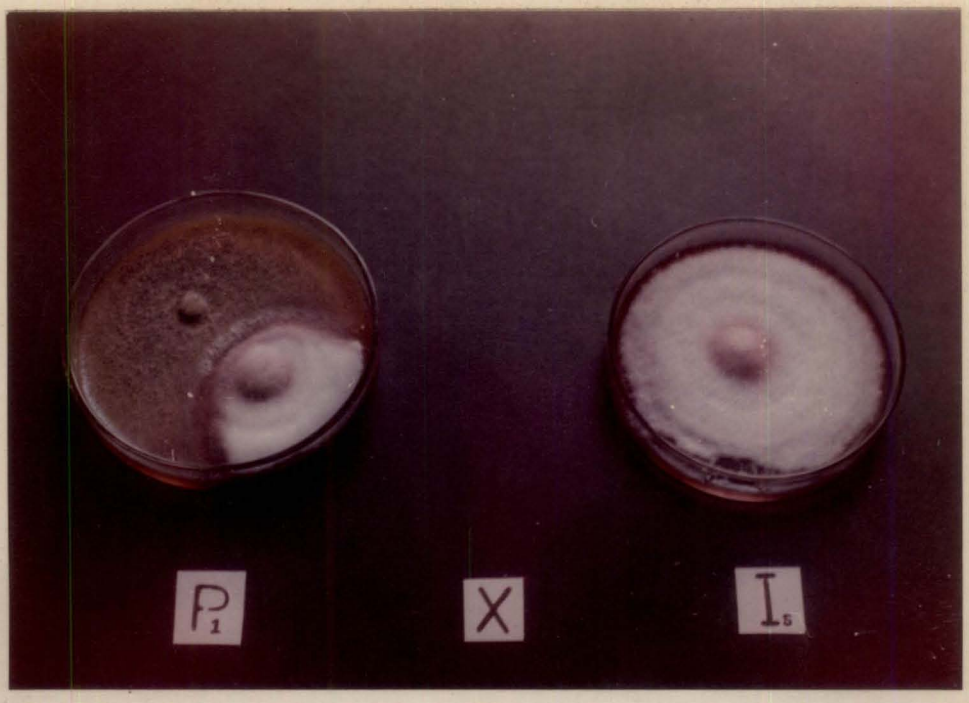


Plate - 7.1 Antagonism between Sarocladium oryzae (I_5) and Chaetomium sp (P_1)

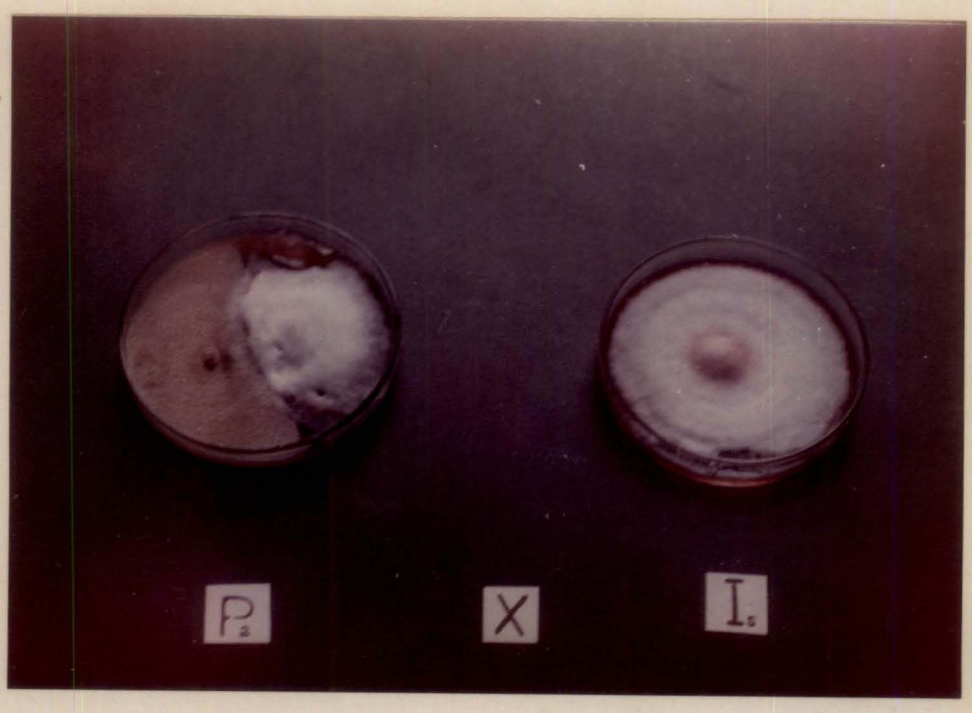


Plate - 7.2 Antagonism between Sarocladium oryzae (I_5) and Botrytis sp (P_2)

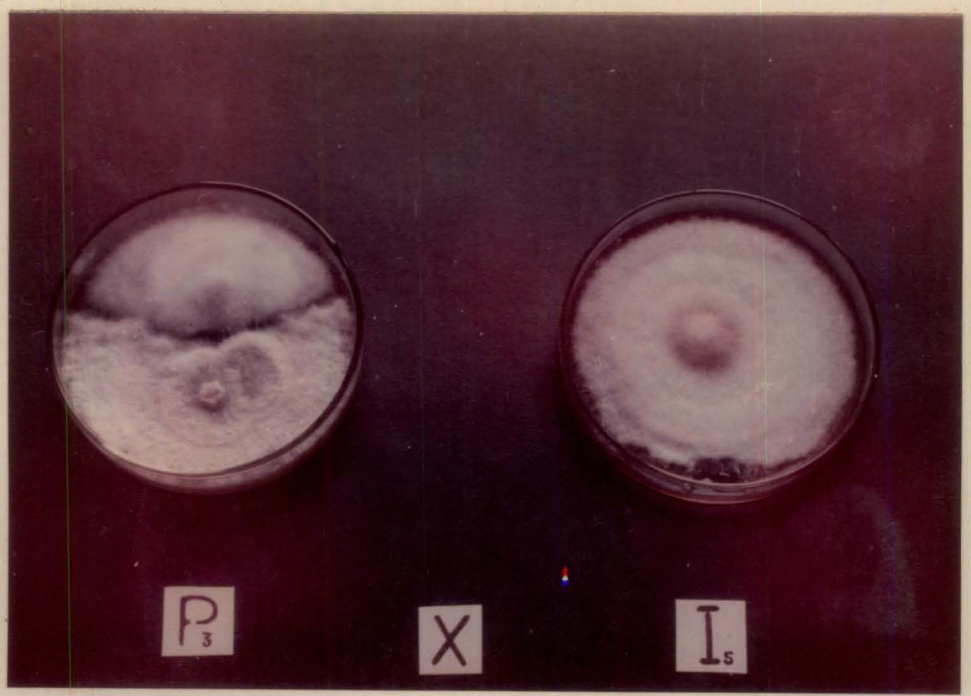


Plate - 7.3 Antagonism between Sarocladium oryzae (I_5) and Pestalotia sp (P_3)

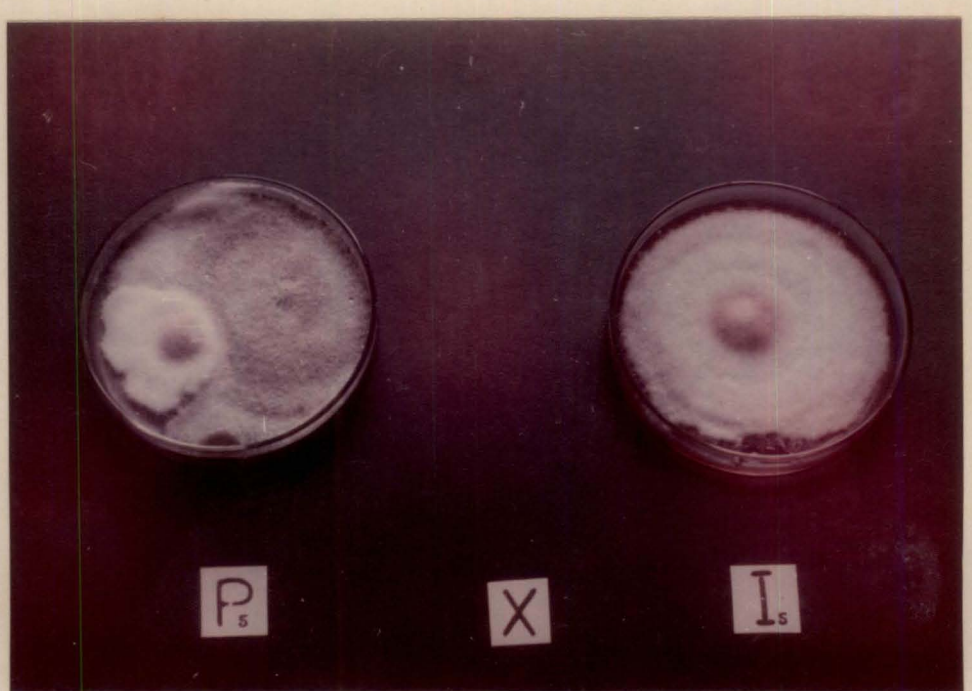


Plate - 7.4 Antagonism between Sarocladium oryzae (I_5) and Fusarium sp (P_5)

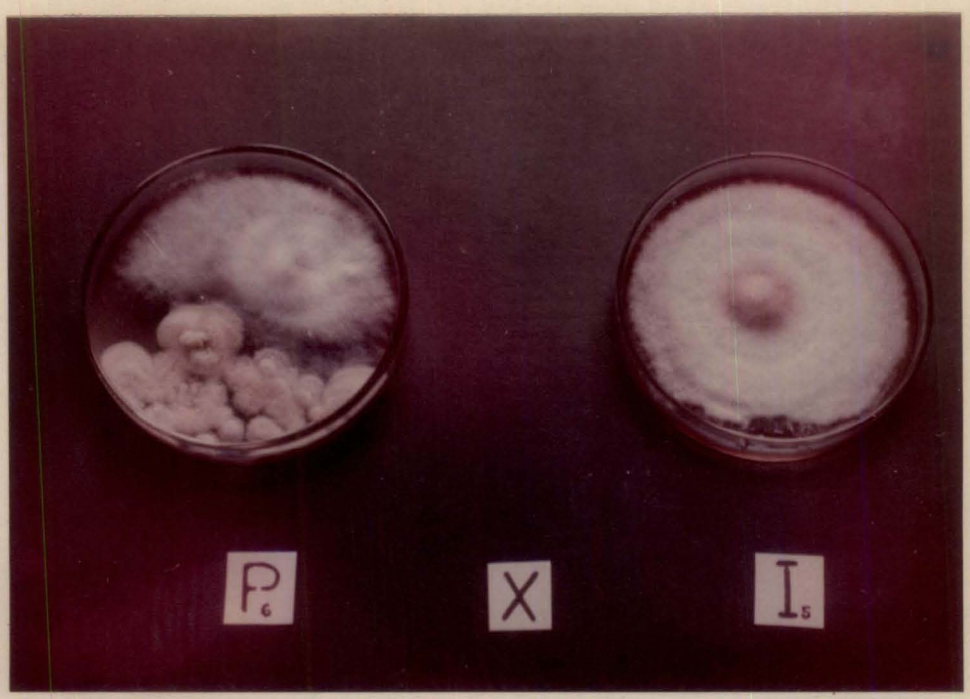


Plate - 7.5 Antagonism between Sarocladium oryzae (I_5) and Aspergillus sp (P_6)

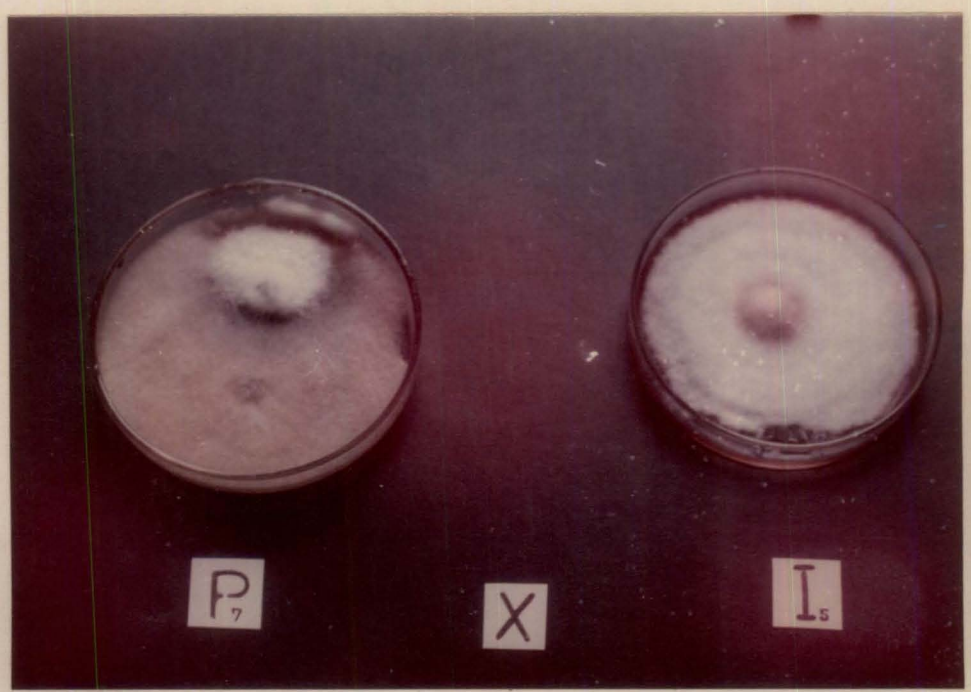


Plate - 7.6 Antagonism between Sarocladium oryzae (I_5) and Mucor sp (P_7)

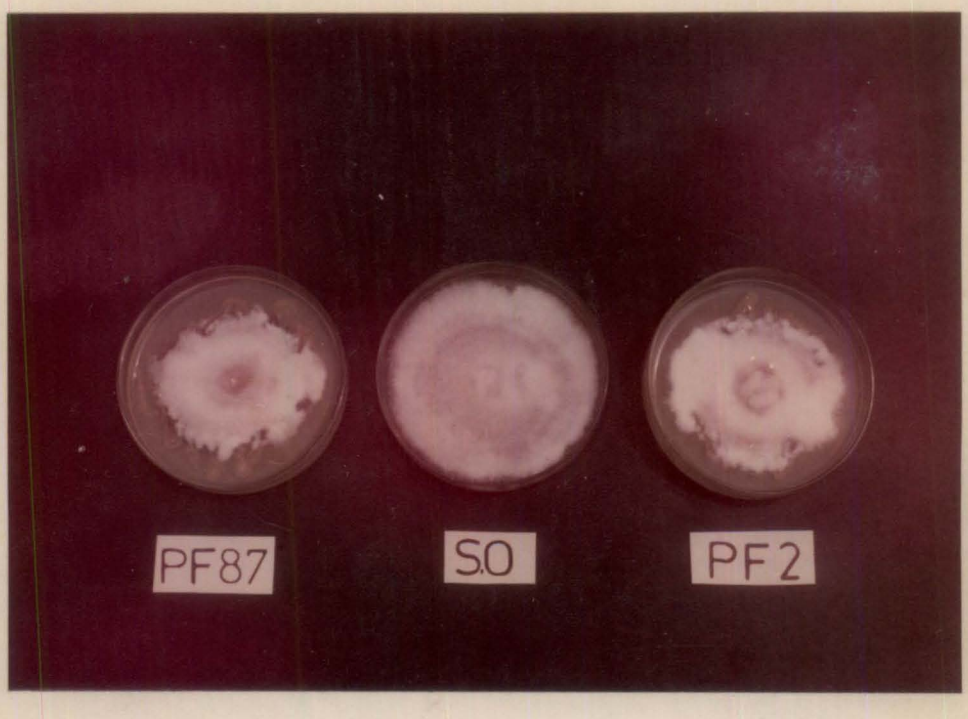


Plate /- 7.7 Antagonism between Pseudomonas fluorescens and Sarocladium oryzae

PF - 87 - Pseudomonas fluorescens- 87

PF - 2 - Pseudomonas fluorescens - 2

S.O. - Sarocladium oryzae

4.6.2 Evaluation of the efficiency of the biological agents in the control of sheath rot under field conditions

Inoculations with S. oryzae alone and S. oryzae + Biological control agents were carried out under field conditions in the rice plants raised in cement troughs.Complete choking and nonemergence of the panicle was observed in the case of inoculation done with S. oryzae alone. Partial emergence of the panicle was observed when biological control agents like Chaetomium sp., P. fluorescens - 2 and P. fluorescens - 87 were used along with S. orzyae. Among the organism tested Chaetomium sp. was found to be the most efficient in reducing the severity of sheath rot disease under field conditions. (Table 19;Plate-8.1 to 8.3).

Table 19

Efficiency of biological agents on control of sheath rot disease under field condition

Treatment	Type of Reaction
Sacrocladium oryzae	Typical disease symptom with brown discolouration and complete choking of the panicle
Sarocladium oryzae + Chaetomium sp.	Partial emergence of the panicle with very light brown discolouration. Grains also discoloured.
Sarocladium oryzae + Pestalotia sp.	Partial emergence of the panicle with brown discolouration. Grains also discoloured.
Sarocladium oryzae + Pseudomonas fluorescens 87	Partial emergence of the panicle with dark brown discolouration on the boot leaf. Grains also discoloured.
Sarocladium oryzae + Pseudomonas fluorescens 2	Partial emergence of the panicle with dark brown discolouration on the boot leaf. Grains also discoloured.

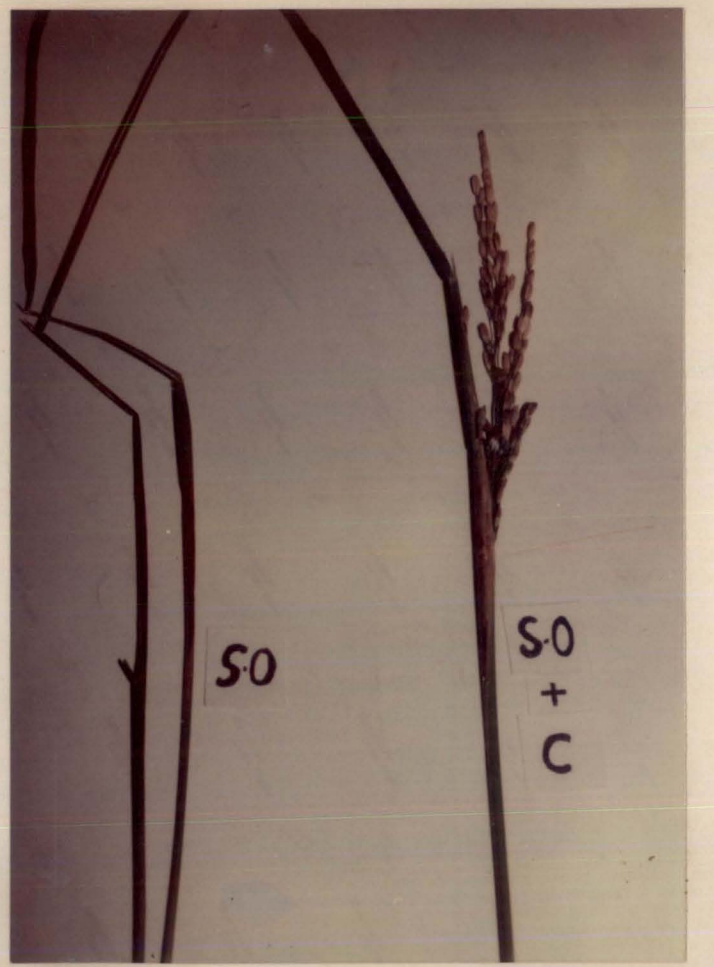


Plate - 8.1 Field evaluation of Chaetomium sp

S.O - Sarocladium oryzae

C - Chaetomium sp

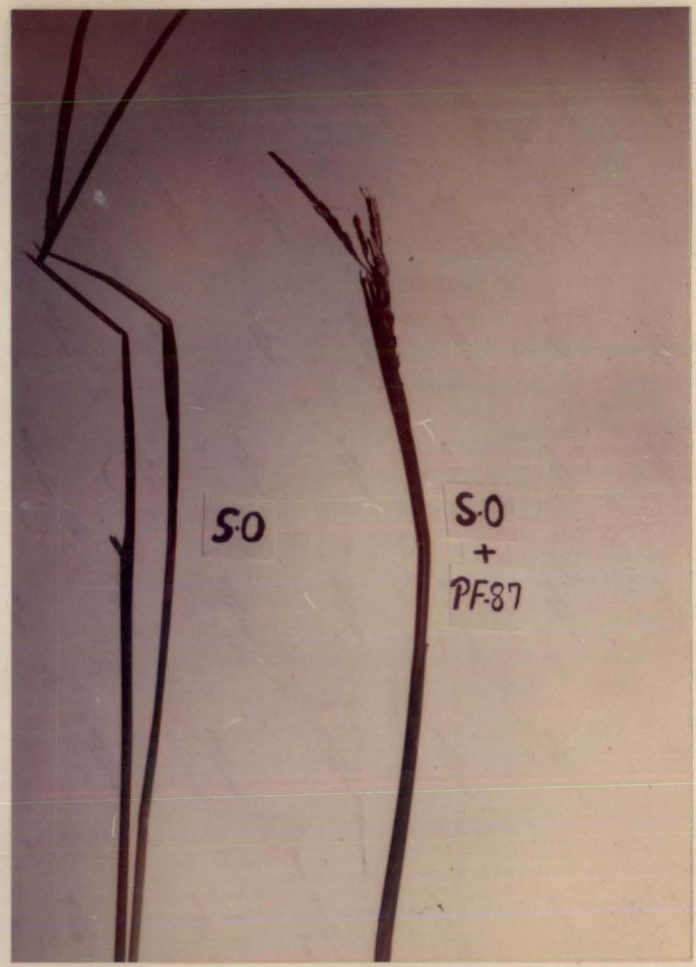


Plate - 8.2 Field evaluation of Pseudononas fluorescen

S.O. - Sarocladium oryzae

PF - 87 - Pseudomonas fluorescens- 87

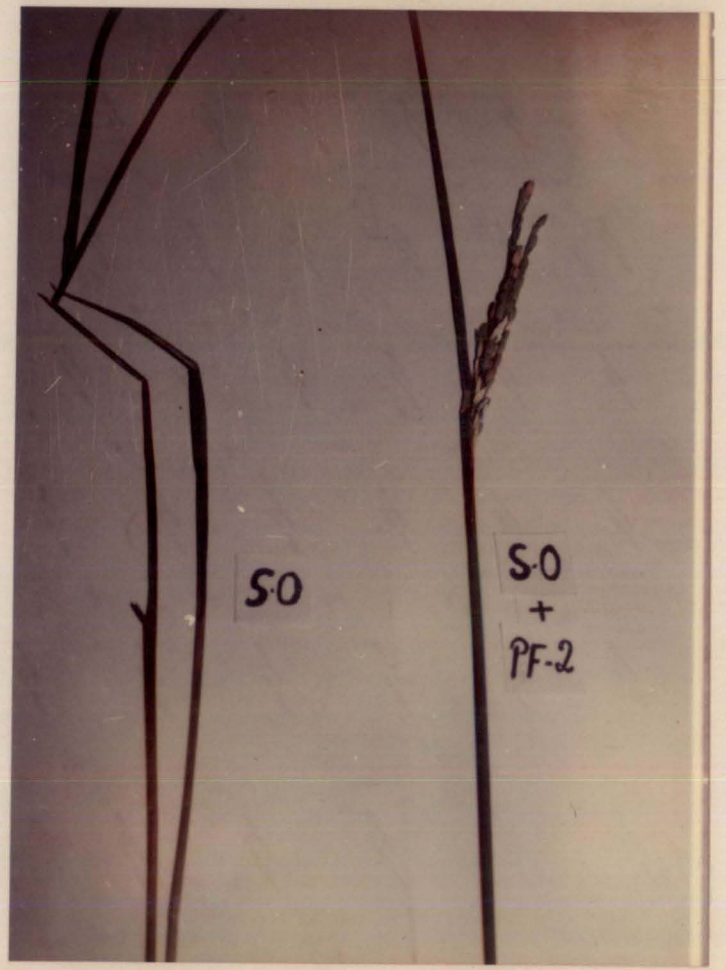


Plate - 8.3 Field evaluation of Pseudomonas fluorescens-2.

PF - 2 - Pseudomonas fluorescens - 2

S.O. - Sarocladium oryzae

DISCUSSION

5. DISCUSSION

Sheath rot disease of rice caused by Sarocladium oryzae (Sawada) Gams & Hawks. has gained much importance in India especially Kerala during the last decade. Damages due to this disease in severe forms have been observed in various rice growing tracts of the state.

In the present investigation attempts were made to study the symptomatology and etiology of the disease in detail. The symptoms of the disease have been observed in the naturally infected plants and described. The present observations made on the typical forms of sheath rot symptoms are in full agreement with the symptoms described by the earlier workers.(Tasugi and Ikeda, 1956; Ou, 1972; Amin et al., 1974; Nair and Sathyarajan,1975 ; Shajahan et al., 1977.)

Typical symptoms were seen in the variety Pavizhachempavu collected from Kalliyoor area of Trivandrum district. Variations from the typical symptom were also seen in various varieties collected from different locations. In variety Matta collected from Pattambi, boot leaf was found

to be straw coloured with pinkish growth of the fungus. The same variety collected from Pattambi itself showed variation in symptoms as yellowish and brownish discolouration of the boot leaf with whitish growth of the fungus. Presence of black discolouration on the partially emerged panicles was seen in the varieties Annapoorna and White Thriveni. Most of the varieties collected from majority of the locations showed brownish discolouration on the stalk region of the panicle without any symptom on the boot leaf and the panicles were left unaffected. In variety Bhagya, Jyothy, Matta and Red Mashoori collected from Kayamkulam and Pattambi showed brownish and yellowish discolouration on the boot leaf and the panicle was affected. These variations in symptoms may be due to the difference in virluence of the pathogen. Chen (1957) proved that the nature of symptom varies with the virulence of the pathogen.

Isolations from the sheath rot infected rice plants yielded Sarocladium oryzae (Sawada) Gams and Hawks. The identity of the pathogen was earlier established by Sawada (1922), Tasugi and Ikeda (1956), Chen (1957), Ou (1963) and Jiminez and Panizo (1977). While tissue isolations were made a few other fungi were also found to be associated with the

disease frequently which included Fusarium sp. and Alternaria padwickii. Shajahan et al. (1977) has reported the association of Fusarium roseum Link ex Fries from sheath rot infected rice plants. The presence of Fusarium along with sheath rot disease was reported by Balakrishnan (1981), Sung et al. (1982), Kang and Rattan (1983), Singh and Devi (1991). Nair (1986) reported the presence of Alternaria padwickii from sheath rot infested panicles. Lakshmanan (1991) reported the presence of different pathogens causing the disease.

Morphological characters of two different isolates of the pathogen, S.oryzae compared with those reported by earlier workers (Tasugi and Ikeda, 1956; Ou,1972; Nair and Sathyarajan, 1975; Shajahan et al., 1977 ; Balakrishnan, 1981; and Nair, 1986. The results of the present study on the morphology of the pathogen were in agreement with the descriptions made by the above workers. However, slight variations were seen in the morphology of certain isolates with respect to the colour of the colony, conidiophore length, size of conidia etc. The colour of the colony was different for different isolates. Isolate I$_5$ had the longest condiophore and measured twice the length of other isolates.

Isolate I_6 showed two conidia attached at the tip of the conidiophore while all the other isolates showed single conidial attachment. Balakrishnan (1981) reported a peculiar bunch type of conidial attachment for the isolate of the fungus obtained from the weed host, Cyperus difformis Linn.

Morphological characters of the associated fungi were also studied. When the Fusarium sp. was compared with the earlier reported isolates of Nair (1986), it was found that the colour of the colony and length of the conidiophore differed in the present isolates. Alternaria padwickii obtained in the present study differed in the colour of the colony from the isolate described by Nair (1986).

Pathogenicity tests done with various isolates of **S. oryzae** and the associated fungi showed that they individually and in combination produced disease symptoms on different rice varieties tested. The nature of the symptoms varied with the virulence of the pathogen and rice varieties. Chen (1957) found that the nature of symptom varies with the virulence of the pathogen.

Inoculation of rice plants with **S. oryzae** alone at boot leaf stage produced typical sheath rot symptom

in severe forms while inoculation done at active tillering stage more often produced mild symptoms. This was same in the case of inoculation done with the other associated fungi. Nair and Sathyarajan (1975) has proved that inoculation of rice plants at boot leaf stage with the conidial suspension of the fungus could produce typical sheath rot symptom. Balakrishnan (1981), Mukerjee et al. (1981) and Nair (1986) also proved that inoculation of the pathogen at the boot leaf stage of the crop could produce typical sheath rot symptom.

Inoculation of **S. oryzae** and the associated fungi in combination gave the typical choking symptom more often than their individual action. Shajahan et al. (1977), Balakrishnan (1981), Sung et al. (1982), Kang and Rattan. (1983) and Singh and Devi (1991) reported the presence of Fusarium sp. along with the sheath rot pathogen. Nair (1986) also found that the inoculation with **S. oryzae** and the associated fungi could hasten the initiation and development of sheath rot symptoms.

Eight of the common varieties of rice used in the study showed variations in their symptom expression for the

different isolates of the pathogen tested. Jaya showed the highest susceptibility to the disease. In most of the varieties disease symptoms started as brown discolouration on boot leaf which later developed in to mild, moderate or severe symptoms. Varietal variations on symptom development was earlier established by Srinivasan (1980), Balakrishnan and Rajan (1981), Singh and Raju (1981), Chien and Thseng (1982).

Studies on the survival of *S. oryzae* showed that the fungus remained viable for 6 months in infected grains eventhough the viability of the pathogen was found to be reduced in progress of time. Kawamura (1940) found that the fungus could remain viable in diseased tissues, sheaths, grains and rachis for more than six months. Shajahan *et al*. (1977) reported the seed borne nature of the pathogen. Mukerjee and Yadov (1989) reported the presence of the fungus on the seed husks, floral parts, rachis, rachilla, outer leaf sheath, panicle stem and the flag leaf sheath. Maite *et al*. (1991) found that sheath rot pathogen could effectively survive in infected seeds and weed inflorescence for about seven months under dry storage conditions.

Present study showed that S. oryzae could survive for about three months in soil. Hsien et al. (1980) found that the fungus could survive for 75 days in stubbles but it failed to survive beyond 55 days when buried in soil and puddled. Maite et al. (1991) reported that the fungus could survive in wet rhizosphere soils for more than six months after sowing of the infected seeds.

Among the different media tested for the growth of different isolates Coon's media was found to be the least effective media since it gave less radial growth of mycelium. For most of the isolates Czapel's agar, Oatmeal agar and Carrot agar supported the maximum growth for majority of the isolates. Mohan and Subramanian (1978) described Oat meal agar media as the best solid media. PDA gave the least radial growth for isolate I_2. This may be due to difference in the response of the fungus to the carbon and nitrogen sources of the media as suggested by Chen (1957).

Of the different liquid media tested, Czapek's media was found to be the best to give maximum dry weight of biomass. Mohan and Subramanian (1978) reported that Czapek's media was the best liquid media for the growth of the fungus.

Among the different nitrogen sources substituted in Czapek's media, Ammonium nitrate and Sodium nitrate gave the best results. Mohan and Subramanian (1978) reported that Ammonium sulphate and Ammonium nitrate were the best nitrogen sources for supporting maximum growth of S. oryzae

Studies on the effect of different carbon sources proved that mannitol was the best carbon source followed by starch and sucrose. Mohan and Subramanian (1978) suggested sucrose and starch as the best carbon sources.

Studies showed that different spectra of light could influence the growth of S. oryzae . Red, Blue and Green light were found to reduce the growth of the fungus when compared to the ordinary room light. Sporulation was found to be enhanced when the fungus was grown under blue light and least sporulation noted was under red light. Kumagai and Hsiao (1983) found that Verticillium agaricum sporulated abundantly in blue light. Wulf and Schauz (1983) found out that blue light was effective in the spore germination of Ustilago maydis.

Effect of different plant extracts on the spore germination of S. oryzae was also tested. Both 1% and 10% concentration of extracts of Ocimum sanctum, Phyllanthus niruri, Allium sativum and Azadirachta indica were found to be inhibitory to the spore germination of S. oryzae. Naidu and John (1981) found that the radial growth and mycelial dry weight of S. oryzae were reduced by garlic bulb extract Kanagarajan (1988) reported that Neem leaf extract reduced the growth of S. oryzae. Komala et al. (1988) also found the effect of Ocimum sanctum and Neem on the inhibition of spore germination of this fungi.

Phylloplane microflora of rice plants were isolated, brought in to pure culture and their antagonistic effect on S. oryzae both under in vitro and in vivo conditions were studied. Among the seven fungal isolates tested, Chaetomium sp. exhibited the maximum antagonistic behaviour. Joseph and Philip (1987) reported that Trichoderma viride, Myrothecium roridum and Chaetomium gracile exerted special antagonistic effect on the common pathogens associated with rice seeds.

P. fluorescens-2 and P. fluorescens-87 procurred from T.N.A.U, Coimbatore also exhibited antagonistic effect against sheath rot pathogen. Saktivel and Gnanamanickam (1986b) reported that P. fluorescens isolated from rice roots and citrus leaves were inhibitory to S. oryzae. Kumar (1992) observed that pre inoculation of P. fluorescens and Bacillus sp. were effective than their post inoculation in reducing sheath rot disease.

SUMMARY

6. SUMMARY

Detailed investigations were carried out on the symptomatology, etiology and biological control of sheath rot disease of rice caused by Sarocladium oryzae (Sawada) Gams and Hawksworth.

The symptoms of the disease initiated as brown oblong lesions on the boot leaf sheath which were initially surrounded by light yellow halo. On maturity, these lesions turned dark brown with grey centres. Even though symptoms were more pronounced in the boot leaf sheath, slight brown discolouration could be noticed on the second and third leaf sheaths. Panicles were found fully or partially emerged or choked inside the sheath. Infected grains were discoloured, brownish or pinkish in colour.

Naturally infected plants were collected from seven different locations viz., Kayamkulam, Pattambi, Kalliyoor, Vellayani, Kumarakom, Moncompu and Karamana and from ten different varieties viz., Bhagya, White Mashoori, Matta, Jyothy, Annapoorna, Red Mashoori, Pavizhachempavu, White Thriveni, Jaya and Basumathi. Pathogens were isolated and

out of the different isolates obtained six isolates of *S. oryzae* viz., I_1, I_2, I_3, I_4, I_5 and I_6 were selected for detailed study based on their difference in symptom expression under natural condition.

A few other fungi which were found frequently associated with the disease were also brought in to pure culture. Four such isolates of the associated fungi viz., A_1, A_2, A_3 and A_4 were selected based on the symptomatological vaiations noticed in naturally infected plants.

Detailed morphological studies of the six isolates of *S. oryzae* and four isolates of the associated fungi were done following the slide culture technique. Morphological studies on the nature of mycelium, conidiophore formation, conidial ontogeny and their measurement were observed.

Mycelium was septate and profusely branched and the colour varied from light pink to violet. Hyphal thickness ranged 3.33-4.9µm. Conidiophores were branched in one, two or three whorls and their main axis measured 29.7-81.5x1.66-4.1µm. Conidia were single celled, hyaline, cylindrical in

shape and borne singly or in two at the tip of each branch of the conidiophore which measured 4.9-9.9µmx1.66-4.1µm.

All the six isolates of S. oryzae and four isolates of associated fungi were subjected to pathogenicity test, both individually and in combination on the rice varieties namely, Jaya, Bharathi, Kanchana, Aiswarya, White Mashoori, Jayathi, Aathira and Sabari. All the isolates of S.oryzae and the associated fungi produced symptoms on all rice varieties tested. I_5 showed maximum virulence on symptom development.

Combined effect of S. oryzae and the associated fungi were tested on the variety Jaya. S. oryzae and the associated fungi in combination gave typical symptoms more often than their individual action. The combination $I_5 + A_1$ and $I_5 + A_2$ were found to be the most effective combination for typical disease development.

All the varieties tested viz., Jaya, Bharathi, Kanchana, Aiswarya, White Mashoori, Jayathi, Aathira and Sabari were found to be susceptible to the disease. The variety Jaya showed the maximum disease intensity.

The viability of the sheath rot pathogen both in infected grains and in soil was studied. It was found that the pathogen could remain viable for six months, both in the surface sterilized and non surface sterlized grains, but the frequency of occurrence was found to be reduced from the 3rd month onwards on grains. In soil the fungus was found to survive steadily for three months.

Cultural characters of the fungus were studied by growing them both in solid and liquid media. Solid media viz., PDA, Coon's agar, Czapek's agar, Carrot agar and Oat meal agar were used for the study. Czapek's agar, Carrot agar and Oat meal agar were found to support the maximum growth of most of the isolates. Coon's agar was the least effective medium.

Liquid media viz., Paddy leaf extract, Czapek's medium, PDA, Coon's medium and Richard's medium were used for the study. Czapek's medium was found to be the best liquid media which gave maximum dry weight of the biomass of the fungus followed by Richard's medium and Coon's medium.

Czapek's media was used to find out the best nitrogen source and carbon source. Sodium nitrate, the nitrogen

source in Czapek's medium was substituted by Tyrosine, Potassium nitrate, Ammonium nitrate and Leucine. Sodium nitrate and Ammonium nitrate were found to be the best nitrogen sources. Sucrose, the carbon source in the Czapek's medium was substituted by Mannitol, Lactose, Glucose and Starch. Mannitol was ranked as the best carbon source.

Effect of different spectra of light was studied on growth and sporulation of S. oryzae under laboratory conditions. All the spectra of light tested namely, red, blue and green showed a reduction in the mycelial growth. Sporulation was found to be enhanced by blue light and minimised by red light.

Studies on the effect of plant extracts showed that Neem, Garlic, Phyllanthus and Ocimum extracts could inhibit the spore germination of S.oryzae

Phylloplane microflora were isolated from rice plants and attempts were made to test their antagonistic effect on S.oryzae both under in vitro and in vivo condition. Phylloplane microflora were isolated from Virippu and Mundakan season crops both at active tillering and boot leaf stages of crop growth.

Fungal isolates namely, Chaetomium sp., Aspergillus sp., Botrytis sp., Fusarium sp., Pestalotia sp., Mucor sp., Yeast and Bacterial isolates namely, Bacillus sp., Xanthomonas sp. etc were evaluated. In addition to this P. fluorescens strains, 2 and 87 which were collected from T.N.A.U were also included in the study.

Results showed that fungi namely, Chaetomium sp., Pestalotia sp. etc. and bacterial species namely Pseudomonas fluorescens strain 2 and 87 were antagonistic against Sarocladium oryzae under in vitro conditions. The promising isolates were then evaluated for their efficiency under in vivo conditions against the pathogen. Chaetomium sp. was found to minimise the disease maximum among the fungal isolates followed by the bacterial strains P. fluorescens strain 2 and 87.

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APPENDICES

APPENDIX - I

Composition of solid media used

1. Potato dextrose agar medium

Peeled and sliced potato	-	200g
Dextrose	-	20g
Agar agar	-	20g
Distilled water	-	1000ml

2. Czapek's agar medium

$Mg\ SO_4 . 7H_2O$	-	0.50 g
KH_2PO_4	-	1.00 g
KCl	-	0.50 g
$FeSo_4$	-	0.01 g
$NaNO_3$	-	2.00 g
Sucrose	-	30.00 g
Agar agar	-	20.00 g
Distilled water	-	1000 ml

3. Coon's agar medium

$Mg\ SO_4 . 7H_2O$	-	1.23 g
Sucrose	-	7.20 g
Dextrose	-	3.60 g
KNO_3	-	2.20 g
Agar agar	-	20.00 g
Pot.acid phosphate		

4. Carrot agar medium

Peeled and sliced carrot	-	200 g
Agar agar	-	20 g
Distilled Water	-	1000ml

5. Oat meal agar medium

Oats	-	200 g
Agar agar	-	20 g
Distilled water	-	1000 ml

APPENDIX - II

Composition of liquid media used

1. Potato dextrose medium

Peeled and sliced potato	-	200 g
Dextrose	-	20 g
Distilled water	-	1000 ml

2. Czapek's medium

$Mg\ SO_4 . 7H_2O$	-	0.50 g
KH_2PO_4	-	1.00 g
KCl	-	0.50 g
$FeSo_4$	-	0.01 g
$NaNO_3$	-	2.00 g
Sucrose	-	30.00 g
Distilled water	-	1000 ml

3. Richard's medium

KNO_3	-	10.00 g
KH_2PO_4	-	5.00 g
$Mg\ SO_4$	-	0.50 g
NaCl	-	100.00 mg
$CaCl_2$	-	130.00 mg
Sucrose	-	30.00 g

4. Coon's medium

$MgSO_4 \cdot 7H_2O$	-	1.23 g
Sucrose	-	7.20 g
Dextrose	-	3.60 g
KNO_3	-	2.20g
Pot. acid phosphate	-	2.72 g
Distilled water	-	1000ml

5. Paddy leaf extract medium

Paddy leaves	-	200.00 g
Distilled water	-	1000 ml

ETIOLOGY AND BIOLOGICAL CONTROL OF SHEATH ROT DISEASE OF RICE

BY

MINI GEORGE

ABSTRACT OF THE THESIS
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9. ABSTRACT

Sympotmatology and etiology of sheath rot disease of rice was investigated and attempts were made in the biological control of the disease. The sheath rot disease of rice initiated as brown oblong lesions on the flag leaf sheath were surrounded by light yellow halo initially. On maturity, these lesions turned dark brown with grey centres. Slight brown discolouration was also seen on the second and third leaf sheaths. Panicles were found choked inside the sheath or partially emerged with discoloured grains.

The pathogen was isolated from diseased rice varieties collected from seven different locations and from ten different varieties. Out of these six isolates of Sarocladium oryzae and four isolates of associated fungi were selected based on the symptomatolgical variations for further study. Slide cultures of the above isolates were prepared and the morphological studies on the nature of mycelium, conidiophore formation, conidial ontogeny and their measurements were recorded. All the isolates were similar in many characters except slight variation in conidiophore length and the length of conidia.

Pathogenicity tests done on eight varieties showed that S. oryzae and the associated fungi inoculated could produce disease symptoms. The nature of symptoms varied with virulence of the pathogen and rice varieties. Combined inoculation of S. oryzae and the associated fungi in variety Jaya gave typical symptoms, than their individual action on symptom development. I_5 showed the maximum virulence in all the varieties.

Inoculation of the pathogen showed that all the varieties were susceptible to the different isolates of the pathogen tested. Variety Jaya showed the maximum disease intensity.

Studies on the viability of the pathogen in infected grains showed that the pathogen could remain viable for six months in paddy grains. But the frequency of occurrence was found to be reduced from the 3rd month onwards of the storage. Pathogen was viable in the soil up to three months steadily and after that their frequency of occurrence was reduced both at 2cm and 6cm depths.

Cultural characters of the fungus was studied by growing the fungus both in liquid and solid media. Studies

on the growth of the pathogen in different solid media showed that different isolates respond varyingly under different media conditions. Czapek's agar, Oat meal agar and Carrot agar were found to be the best media which supported maximum radial growth of the fungus.

Among the different liquid media tested Czapek's medium was found to be the best followed by Richard's medium and Coon's medium. Mannitol was found to be the best carbon source followed by starch and sucrose. Ammonium nitrate and Sodium nitrate ranked first in the nitrogen sources.

Effect of different spectra of light was studied on growth and sporulation of *S. oryzae* All the light spectra viz., red, blue and green showed a reduction in the growth. Blue light induced maximum sporulation while red light showed least effect.

Studies on the inhibition of spore germination using plant extracts showed that all the plant extracts tested viz., Neem, Garlic, Phyllanthus and Ocimum had inhibitory effect on the spore germination of the pathogen.

Studies on Biological control showed that fungi namely, Chaetomium sp., Pestalotia sp. etc. and bacterial species namely Pseudomonas fluorescens strain 2 and 87 were antagonistic against Sarocladium oryzae under in vitro conditions. Under in vivo conditions Chaetomium sp. was found to minimise the disease maximum among the fungal isolates followed by the bacterial strains P.fluorescens strain 2 and 87.