# SYMPTOMATOLOGY ETIOLOGY AND CONTROL OF SHEATH ROT DISEASE OF RICE CAUSED BY *Acrocylindrium oryzae*


BY

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THESIS

SUBMITTED IN PARTIAL FULFILMENT OF

THE REQUIREMENT FOR THE DEGREE

**MASTER OF SCIENCE IN AGRICULTURE**

FACULTY OF AGRICULTURE

KERALA AGRICULTURAL UNIVERSITY

DEPARTMENT OF PLANT PATHOLOGY

COLLEGE OF AGRICULTURE, VELLAYANI

TRIVANDRUM

**1981**

DECLARATION

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

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CERTIFICATE

Certified that this thesis is a record of research work done independently by Sri B. BALAKRISHNAN, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, followship or associateship to him.

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ACKNOWLEDGEMENT

I wish to express my deep sense of gratitude to Dr. N. Chandrasekharan Nair, Professor of Plant Pathology for suggesting the problem and for his valuable guidance and helpful criticism during the course of the investigations and in the preparation of the thesis.

I am grateful to Dr. M. Ramanatha Menon, former Professor of Plant Pathology for his constant encouragement and help for research.

My sincere thanks are due to late Sri S.N. Shanmugham, Assistant Professor of Plant Pathology and late Sri E.J. Thomas, Professor of Agricultural Statistics, for their valuable suggestions rendered for the conduct of the experiments.

I wish to thank Sri Sivasankara Pillai, Associate Professor and other staff members of Model Agronomic Research Station, Karamana for providing land and other facilities and their overwilling help and co-operation for the conduct of the field experiments.

I am also thankful to Dr. S. Balakrishnan and Dr. James Mathew Associate Professors of Plant Pathology and Sri Abdul Hameed, Assistant Professor of Agricultural Chemistry for their keen interest and valuable suggestions and help rendered during the investigations and preparation of the thesis.

I use this opportunity to thank Sri Girija Sankaranarayanan, Junior Assistant Professor (Fisheries), Rice Research Station, Moncompu for the illustrations.

The help and co-operation rendered by Sri P. Karunakaran, Ph.D. Scholar and other Post-graduate students and staff members of Plant Pathology Division is gratefully acknowledged.

I am highly grateful to Kerala Agricultural University for sanctioning study leave to carry out the M.Sc.(Ag.) Programme.

B. BALAKRISHNAN

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# INTRODUCTION

# INTRODUCTION

Sheath rot of rice was first described by Sawada (1922) from Formosa and the causal fungus was named Acrocylindrium oryzae. This disease was considered to be a minor disease and has now gained much importance in recent years in many parts of the world including India. Chen (1957) reported 5 - 20 per cent damage, and it may sometimes be as much as 85 per cent in Taiwan.

The occurrence of this disease in India was first described by Agnihothrudu (1975) from Karnataka. In Kerala, sheath rot was first reported by Nair and Sathyarajan (1975) from Trivandrum area. It is known to cause much damage in many rice growing areas in the State.

Eventhough the disease was reported long back, not much work has been carried out on this disease in this country or abroad. The exact mode of survival of the pathogen, factors favouring the incidence and development of the disease, control measures etc. were not studied under Kerala conditions.

In the present investigations an attempt was made to acquire a better understanding of the disease on various important aspects such as symptomatology of the disease, morphological variations of different isolates of the

organism, its pathogenicity, role of associated organisms, host range of the pathogen, mode of survival and important physiological characters of the organism, evaluation of fungicides in laboratory and field and role of climatological factors on the incidence and development of the disease.

# REVIEW OF LITERATURE

# REVIEW OF LITERATURE

Sheath rot of rice caused by Acrocylindrium oryzae Saw., was first reported by Sawada from Formosa in 1922. Tasugi and Ikeda (1956) artificially inoculated the fungus on rice plant and established its pathogenicity and they provided more informations on cultural and physiological characteristics of A.oryzae from Japan. Chen (1957) observed 3 to 20 per cent damage of rice due to sheath rot in Taiwan. Subsequently this disease was reported from Vietnam (Anon., 1962) and from Thailand (Ou, 1963).

In India this disease was first reported by Agnihothrudu in 1973 from Karnataka. Prabakaran et al.(1974) reported A.oryzae from Tamil Nadu. They recorded a yield loss of about 85 per cent due to this disease. Ahamed et al.(1975) proved the pathogenicity of A.oryzae isolate in rice plants. Rao et al.(1974) from Andhra Pradesh gave a detailed account of the disease. They furnished information on occurrence, losses and the type of symptoms observed under natural conditions. Nair and Sathyarajan (1975) reported a heavy incidence of sheath rot of rice caused by A.oryzae from the experimental plots of College of Agriculture, Vellayani in Kerala.

Attabhanyo and Rush (1973) reported that this disease was a serious problem in breeding nurseries at the Rice Experimental Station, Louisiana in the United States of America.

During 1974, Shajahan et al. after conducting detailed survey of five major rice growing areas of South Western Louisiana, reported that 11.4 per cent of tillers showed symptom of sheath rot.

Gams and Hawksworth (1975) from Netherlands compared isolates of A.oryzae and Sawada's holotype collection and showed that two fungi have passed under this name in the literature. So they 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.

Datta and Purkayastha (1978) from Calcutta reported that although the new name proposed for A.oryzae was Sarocladium oryzae by Gams and Hawksworth, the controversy among Mycologists was not yet resolved. Chakravarty and Biswas (1970) 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.

Symptomatology

Tsugi and Ikeda (1956) reported that the fungus chiefly attacked the uppermost leaf sheath and caused rotting. The greyish brown lesions coalesce and form large irregular blotches.

Ou (1972) described the symptoms of the disease as follows:-

" The rot occurred on the uppermost leaf sheath enclosing the panicles. The lesions started as oblong or somewhat irregular spots, 0.5 to 1.5 cm long with brown margins and grey centres, or they were greyish brown throughout. They enlarged and often coalesced and covered most of the leaf sheath. The young panicles remained within the sheath or only partially emerged. Abundant whitish powdery growth could be found inside the affected sheaths and young panicles were rotted".

Amin et al.(1974) from their field observations found that the initial lesions were 0.5 to 1 cm long and 0.2 to 0.5 cm wide, oval, dark chocolate brown in colour surrounded by a diffused light brown halo, whereas the healthy sheath around

the lesion remained green. Lesions occurred on the sheaths of all leaves, but most conspicuous on the flag leaf sheath. At times, because of severe infection the entire sheath became dark chocolate brown in colour with irregular outlines of several overlaping lesions. Older lesions had dark brown border 0.2 to 0.3 cm wide and the green colour of the sheath progressively became light green and finally straw coloured. When severely affected, the panicles did not emerge and were compressed inside the flag leaf sheath, and dark brown lesions were evident on the outer side of the sheath. They referred to this stage choking. Some times small holes were also noticed on the affected sheaths which indicated insect injury Leaves with affected sheaths lost their green colour, dehydrated and became straw-yellow in colour.

Shajahan et al.(1974) found that the disease occurred primarily on the upper leaf sheaths most conspicuous on the flag leaf sheath. Lesion colour varied from grey-brown to purple-brown depending upon the varieties attacked. 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.

Nair and Sathyarajan (1975) described the symptoms as follows:-

" The symptoms appeared only on the sheath which cover the panicles. They appeared as long oblong lesions. The fully developed lesions varied in size from 0.5 to 2.0 cm in length and 0.5 to 1.0 cm in width. Young spots appeared uniformly greyish - brown but on maturity turned whitish grey with a dark brown margin. The individual lesions coalesced together and in advanced stages covered the sheath almost completely. As a result of infection the panicles were shy to emerge or even rotted while inside the leaf sheath. In the infected field the panicles could be observed at various stages of emergence. Whitish powdery mass of fungal growth could be detected at the central portion of the spots developed and inside the affected sheath".

Morphology of the fungus

A.oryzae was first described by Sawada in 1922, from Formosa.

Tasugi and Ikeda (1956) gave the conidial measurements. According to them the rod shaped hyaline conidia from the host measured 2.1 to 8.5 x 0.5 to 1.6 µm and 1.8 to 13 x 1 to 1.6 µm from culture.

Ou (1972) gave descriptions of the organism as follows:-

" White sparsely branched septate mycelium, 1.5 to 3.0 µm in diameter. Conidiophore arise from the mycelium, slightly thicker than the vegetative hyphae, branched once or twice each time with 3 to 4 branches in a whorl. The main axis measured 15.0 to 22.0 x 2.0 to 2.5 µm and terminal branches 23.0 to 45.0 µm long, 1.5 µm wide at the base. The conidia were formed consecutively on the tip. Conidia were hyaline, smooth, single celled, cylindrical, which measured 4.0 to 9.0 x 1.0 to 2.5 µm.

Shajahan et al.(1974) described in detail the morphological characters of the fungus. They reported that the mycelium was colourless, septate, 1.5 to 3.0 µm in diameter. Conidiophores were single or branched 15.0 to 25.0 µm long or with secondary branches in whorls of 2 to 5 phialides and 13.0 to 19.0 µm long. The conidia were found singly at the tip of the conidiophore and they were hyaline, smooth, cylindrical, single celled and measured 3.0 to 17.0 x 1.0 to 2.0 µm on PDA culture incubated at 32°C. Variations were noticed in the measurements with different media. In potato dextrose broth, the conidial measurements were

5.0 to 22.0 x 1.5 to 4.5 µm with an average of 9.6 x 2.8 µm. Conidia from the infected leaf sheath measured 2.5 to 8.0 x 1.0 to 2.0 µm with an average of 5.0 x 1.0 µm.

Nair and Sathyarajan (1975) described the fungus isolated from Kerala. The mycelium was septate, purplish white, profusely branched and 1.25 to 2.0 µm in diameter. The conidiophores were slightly thicker than the ordinary vegetative hyphae, short and were ending in a whorl of 3 to 6 branches. Often one or two side branches were also noticed from the main conidiophores. The main branch measured 10.0 to 15.0 µm in length and 2.0 to 2.5 µm in breadth. The terminal cylindrical branches were tapering towards the tip and measured 19.5 to 22.5 µm in length and the base measured 1.0 to 1.5 µm in breadth. Conidia were consecutively formed at the tip of the conidiophores. Conidia were single celled hyaline and measured 3.5 to 7.0 x 1.0 to 1.5 µm in size from the host and 4.0 to 8.0 x 1.0 to 1.5 µm from the culture.

Pathogenicity

Tsuda and Ikeda (1956) artificially inoculated the fungus on rice plants with conidial suspension and established its pathogenicity.

Chen (1957) reported that different isolates of the fungus differed in their pathogenicity. The fungus could not infect other graminaceous plants tested. Shajahan et al.(1974) obtained best results with rice plants grown in green house with hyphal and conidial suspension when injected behind the outer sheath with a hypodermic needle.

Stem-tape inoculation method or spraying of conidial suspension of the fungus was found effective to prove the pathogenicity of this organism in glasshouse grown rice plants (Amin et al. 1974).

Inoculation of rice plants at boot leaf stage with conidial suspension of the fungus could produce typical symptoms of sheath rot disease (Nair and Sathyarajan, 1975). A.oryzae isolates from sterile rice plants collected from various localities in Taiwan could produce typical sheath rot symptoms and sterility on artificially inoculated rice plants (Anon., 1978a).

Associated organisms

Fusarium roseum Link ex Fries, and an unidentified species of Hyalostachybotrys, were reported as associated

organisms in sheath rot disease (Shajahan et al., 1974). They reported that these organisms could be frequently isolated from parts of the rice plant with sheath rot symptoms. Both these organisms could infect sheath tissues and produce light brown lesions atypical of sheath rot symptom. But when these organisms were tested along with A.oryzae in rice plants no other peculiarities were observed than that of typical sheath rot symptoms, which the A.oryzae alone would produce.

Varietal reactions

Chen and Chien (1964) observed that Indica types were most susceptible than Japonica types. Shajahan et al.(1974) and Chung (1975) also reported similar varietal reaction.

Subramanian and Ramakrishnan (1975) reported that Annapurna was comparatively more susceptible while varieties like IR-1, TKM-6, Kanto and Sigadis showed least susceptibility to the disease.

Amin (1976a) from Andhra Pradesh and Naik (1976) from Orissa have also reported certain rice varieties as resistant to this disease (Ramtulsi, Manoharsali, Sigadis, Zenith, Tadukan and Ramnad).

Datta and Purkayastha (1978) suggested that sheath rot disease was most severe on high yielding dwarf cultivars. Mohan and Subramanian (1979) also observed variation in the intensity of the disease and the damage in different varieties were recorded upto 57 per cent.

Host range of the pathogen

The host range of the fungus is highly restrictive. However, the fungus has been isolated from the collar and roots of chestnut plants from Spain (Anon., 1943).

Factors favouring the incidence and development of the disease

Age of the plant, climatological factors and injuries brought about by some pests or other external agencies were found to be the most important factors favouring the incidence of sheath rot disease of rice.

Kasugi and Ikeda (1956) observed that wounding of the rice plants facilitated infection by the fungus. They also reported that the young ears were most susceptible, while mature grains and young rice seedlings were only rarely infected. Chen and Chien (1964) found more damage when the

disease was associated with stem borer infestation and yellow dwarf infection. This proved the fact that the external injuries of rice plants at the panicle emergence stage could be viewed as a most vulnerable pre-disposing factor for the infection by the fungus, which will retard the emergence of the young panicle.

Chin (1974) claimed that sheath rot disease was favoured by plant injuries especially those caused by stem borers.

Shajahan et al.(1974) correlated certain climatological factors with this disease. They found that a hot and humid weather favoured the incidence and development of the disease in Louisiana.

Amin et al.(1974) reported that the disease was severe on densely planted high yielding dwarf rice varieties. Varieties susceptible to stem borer were also severely affected by sheath rot. They also reported that the disease incidence was higher during Rabi crop season in Andhra Pradesh.

Nair and Sathyarajan (1975) observed that slight wounding of the sheath would favour the infection.

Singh and Raju (1979) recorded maximum disease incidence at the time of flowering for four different varieties of rice tested. Maximum disease development was favoured with a minimum temperature range of 17-20°C and minimum relative humidity range of 40 to 56 per cent at the time of flowering. They also reported that the maximum temperature, relative humidity, rainfall and sunshine had no direct influence on the severity of the disease.

Source of inoculum and mode of survival of the fungus

Kawamura (1940) found that mycelium of this fungus could survive in diseased tissues such as sheath, grain and rachis for more than six months.

Shajahan et al.(1974) reported that A.oryzae could survive in dry rice, straw and grain for more than a year. They also reported that sheath rot of rice was seed borne.

Biological characters

Kawamura (1940) reported that the fungus grew best at 30°C to 31°C, sparsely at 37°C, poorly at 13°C and was killed after five minutes at 50°C.

Tsuchi and Ikeda (1956) suggested that, the optimum conditions for growth of the fungus were 20°C to 28°C and a

pH of 6.4. For conidial germination, the optimum conditions were 25°C to 26°C and a pH of 5.5 to 6.4.

Chen (1957) reported that, isolates of the fungus differed in their response to temperature, pH and carbon and nitrogen sources as well as in their pathogenicity.

Chen and Chien (1964) observed that the viability of cultures of A.oryzae was greatly reduced after 10 months and the viability was completely lost after 12 months at 15 to 28°C.

Chajchan et al.(1974) reported that A.oryzae was very slow growing in cultures and reached a colony diameter of about 3.0 to 3.5 cm in 10 days on PDA incubated at 28°C. The colony appeared white and cottony with a light pinkish orange colour on the reverse. The fungus grew and formed conidia best on PDA at 32°C and on cornmeal agar at 28°C. The maximum growth and sporulation were obtained at a pH of 7.5 on potato dextrose agar medium and at 6.5 in potato dextrose broth.

Mohan and Subramanian (1978) found that A.oryzae Sav., grew well on potato dextrose agar and oats agar media. With regard to liquid media, maximum growth and sporulation

were obtained in Czapeks' medium. The optimum temperature and pH for growth and sporulation were 30 ± 1°C and 6.5 respectively. Sucrose and starch were found to be the best sources of carbon. Ammonium nitrate and ammonium sulphate were better growth promoting nitrogen sources.

Datta and Purkayastha (1978) observed that, the spore germination was inhibited on highly concentrated spore suspension. The optimum temperature and pH for the germination of spores were 30°C and 5.5 to 6.0 respectively. They also reported that the spores lost their viability with age. Spores from 60 to 120 days old culture showed considerable reduction in germination.

Chung (1975) reported that the culture filtrates of Aspergillus could inhibit seed germination of rice, barley, wheat, sago and rape. It was also reported that the culture filtrate could inhibit the conidial germination of Pyricularia oryzae.

Development of the intensity of sheath rot disease in field

Amin (1976 a) reported that a disease index of 1 to 9 would be convenient to evaluate a large number of cultivars under field conditions based on symptoms.

IRRI (Anon., 1976) published a standard evaluation system of 1 to 9 scale for measuring the intensity of sheath rot disease.

Singh and Raju (1979) scored the disease intensity in 1-9 scale on all plants at dough stage and converted into disease index.

Satyanarayana and Reddy (1979) suggested a modified system of scoring of 1 to 9 scale based on the coverage of lesions on top 4 leaf sheath and infection in panicle due to sheath rot disease.

Effect of fungicides on control of A.oryzae (sheath rot of rice)

The laboratory evaluation of fungicides by Ragunathan and Vijayaraghavan (1976) revealed that, Benlate and Hinosan at 0.005 and 0.05 per cent respectively could effectively inhibit the growth of A.oryzae. But Dithane M-22 and Dithane Z-78 were inhibitory only at higher concentrations. Kitazin Phos was also found effective at 0.4 per cent concentration for inhibiting the growth of this fungus under laboratory conditions.

Chinnaswamy et al.(1977) conducted a randomised replicated field experiment to study the comparative efficacy of six

fungicides in controlling the sheath rot disease of rice caused by A.oryzae. The treatments were Bavistin (0.1%), HMP-MBC (0.1%), Aureofungin Sol (100 ppm), Difolatan (0.15%), Hinosan (0.1%) and Dithane Z-78 (0.40%) and the control (no spray). They found that Bavistin was the best fungicide in checking the infection as well as in reducing the intensity of the disease. Bavistin was followed by HMP-MBC, Auriofungin sol, Hinosan and Difolatan.

It has been reported that another field study under Kerala conditions proved the lesser incidence of sheath rot in the treatments in which the insecticide Furadan was applied along with Bavistin or Hinosan (Anon., 1978 c).

Chien and Huang (1979) found that Bavistin (Carbendazim) Fusan ( TCMTB ) and Benlate (Benomyl) were very effective in controlling the in vitro growth of the fungus.

# MATERIALS AND METHODS

## MATERIALS AND METHODS

### Symptomatology

Symptoms of the disease were studied by observing the naturally infected rice plants in the field and also by noting the course of development of the disease on the plants artificially inoculated and incubated.

### Isolation of the pathogen

Isolate of *A.oryzae*, used for the present study was obtained from naturally infected rice plants, collected from rice fields at Model Agronomic Research Station, Karamana and College of Agriculture, Vellayani, Kerala. For isolation of the pathogen, portions of the sheath showing typical symptoms of the disease in its early stages of development, were cut into small bits, surface sterilized with 0.1 per cent mercuric chloride solution for two minutes and washed with three changes of sterile distilled water. These bits were then placed in sterilized petri dishes previously poured with potato dextrose agar medium (PDA).. The dishes were then incubated at room temperature ($28 \pm 2°C$). After 2 to 3 days, when the growth of the fungus was visible, mycelial bits were transferred aseptically to PDA slants. Culture was then purified by single conidium isolation and the stock culture was maintained on PDA by sub-culturing periodically.

Comparative studies on the morphology of six isolates of *A.oryzae*

A detailed comparative study of the morphological characters namely, nature of mycelium and colour, hyphal thickness nature and formation of conidiophores and conidia and their measurements were carried out for six isolates of *A.oryzae* as detailed below:-

Isolates from rice varieties

Triveni (A)

Jaya (B)

Jyothi (C)

Salem1 (D)

Isolates from field weeds

*Cyperus difformis* (E)

*Echinochloa crusgalli* (F)

The morphological characters of all the above six isolates were studied by growing them in 9 cm petri dishes on PDA incubated at laboratory conditions first for noting the growth and colour of mycelium and then to study other characters. After 10 days of growth, slide cultures were

prepared and other characters were studied following standard laboratory techniques. For preparation of slide cultures, method described by Riddel (1950) was followed.

Suitable sterile agar medium was poured in previously sterilized petri dish and after solidification, blocks of 6 mm square and 2 mm deep were cut out using a sterile scalpel. One square was placed in centre of each sterile microscope slide and each of the four sides of the agar block was inoculated with small culture bits of the required isolate of the fungus. A cover slip was placed on top of the square of agar and the slide was kept in a damp chamber (Petri dish 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 to 3 days. After this the coverslip was lifted off gently, a drop of 95 per cent alcohol was placed in the centre and before drying, the cover slip was mounted using lactophenol on another slide. One 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.

Pathogenicity tests

Pathogenicity of the fungus was tested by artificially inoculating rice plants at boot leaf stage. Plants were raised in 32 x 38 cm earthen pots and were artificially inoculated separately on three sheaths from top to bottom (sheaths No.1, 2 and 3 respectively). The inoculated plants were covered with polythene bags to maintain a high percentage of relative humidity. Inoculations were done with and without injury by applying the mycelial bit behind the sheaths or by injecting conidial suspension behind the sheath with a hypodermic needle. The concentration of conidial suspension was adjusted to give approximately $10^7$ spores per ml of the suspension prepared in sterile distilled water. Controls were maintained on identical conditions sprayed with sterile distilled water.

Inoculated plants were observed for the development of symptoms and observations were recorded 5 to 8 days after inoculation.

Role of associated organisms in the incidence and development of the disease

Specimens of rice infected by sheath rot were extensively collected from rice fields of Karamana area of Trivandrum district

and all the fungi found associated with the disease were isolated and brought into pure culture as per the method already described. The pure cultures of fungi thus obtained were artificially inoculated on rice plants raised in earthen pots, singly and in combination with A.oryzae, following the method described under pathogenicity tests. Observations on the incidence of the disease and the course of symptom development were recorded.

Varietal reaction to rice varieties

The following nine rice varieties were grown on earthen pots under identical conditions. Artificial inoculations were done on those plants with conidial suspension from ten day old culture of some isolate of A.oryzae as per the method described under pathogenicity test and kept the plants under controlled condition. Two replications were maintained for each variety. The inoculated plants were observed for the incidence of sheath rot disease and the variations in symptom development was recorded.

List of varieties tested

Jyothi

Ahriveni

Annapurna

Rohini

Co-25

Sabari

IR-8

CB-12

Jaya

Host range of the pathogen

The host range of the pathogen was studied by artificially inoculating ten different field weeds listed out below. The weeds were raised under controlled conditions in earthen pots and artificially inoculated as described in the case of pathogenicity tests. Control plants were also kept under identical conditions sprayed with sterile distilled water.

Weeds used for host range study

1. Fimbristylis miliacea Vahl.
2. Panicum repens Linn.
3. Paspalum notatum Flugge
4. Paspalum conjugatum Berg.
5. Echinochloa crusgalli Linn.

6. Monochoria vaginalis Presel.
7. Cyperus difformis Linn.
8. Cyperus iria Linn.
9. Cyperus teneriffae Poin.
10. Eleusine indica Gaertn.

Survival of the pathogen in infected paddy straw and grains

Infected paddy straw and grains were collected from the field and dried in the usual manner as for storage of the produce and kept in polythene bags for long periods under laboratory conditions. Samples were taken from these at 30 days interval and surface sterilized with 0.1 per cent mercuric chloride solution for two minutes and washed with three changes of sterile distilled water. The sterilized bits and grains were then planted on PDA and observed for growth of the organism. This study was conducted at 30 days interval upto six months.

A. Growth and sporulation of the fungus on different culture media

The following solid culture media were used to study the growth and sporulation of the fungus.

1. Potato dextrose agar

2. Czapeks' agar.

3. Richards' agar

4. Coon's agar

The composition of media used are given in appendix I.

The media were prepared and sterilized by autoclaving at 1.05 kg/cm$^2$ for 15 minutes. It was then melted and poured into sterilized petri dishes at the rate of 15 ml in each dish and allowed to solidify. Circular mycelial discs of 5 mm diameter were cut out by means of a sterile corkborer from the outer edge of 7 day old culture of the fungus and placed in the centre of each dish. The isolate from the rice variety Triveni was used for all physiological experiments. The plates were then incubated at room temperature (28 ± 2°C). Observations were taken when full growth of the fungus was obtained in any of the media tested. Five replications were maintained for each treatment.

B. Liquid media

The following liquid media were used to study the growth of the fungus.

1. Potato dextrose medium

2. Czapeks' medium

3. Richards' medium

4. Czapek's medium

5. Yeast leaf extract medium

Composition of the media are given under appendix I.

Each medium was prepared and poured into 250 ml conical flasks at the rate of 30 ml and sterilized by autoclaving at 1.05 kg/cm$^2$ for a period of 15 minutes. 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. For each treatment five replications were kept.

Effect of temperature and pH on growth and sporulation of A.oryzae

The fungus was grown on PDA and incubated at different temperatures, viz., 15 ± 1°C, 20 ± 1°C, 25 ± 1°C, 30 ± 1°C and 40 ± 1°C. The pH levels tried were 5.5, 6.5, 7.0 and 7.5. Before sterilization of the medium pH was adjusted using a systronics pH meter. The pH range was adjusted by adding 0.1 N sodiumhydroxide or 0.1 N hydrochloric acid solution. In both the cases four replications were maintained. The dishes were poured and inoculated as described earlier.

Assessment of sporulation

In all the above growth studies sporulation of the fungus was also assessed, culture discs of 5 mm diameter were taken from 5 places at random in the petri dishes, transferred each disc to 10 ml of sterile distilled water, macerated well and strained through a thin cloth. Each filtrate were then diluted ten times with sterile distilled water and 5 samples from each filtrate were taken for spore count. Average spore count of 5 microscopic fields 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 | (+) |
| 50 $\angle$ 100 | (++) |
| $\angle$ 100 | (+++) |

Production of toxin by the pathogen

A preliminary study on the production of toxin by the pathogen was conducted.

Toxin production

The fungus was grown in liquid Czapek's' medium for a period of 15 days at room temperature (28$\pm$ 2°C) and the mycelial growth was filtered through a previously weighed Whatman No.1

filter paper. The filtrate was then centrifuged at 2000 rpm for 15 minutes and the supernatant taken. It was then observed for the presence of any spores. This culture filtrate was assayed for the presence of any exotoxin secreted by the fungus. The test solution was assayed on growing rice plants at earhead stage. Plants were grown on earthen pots. Behind each of the three sheaths (Sheath No. 1,2 and 3 from top to bottom), 0.1 ml of test solution was injected with a hypodermic needle. The plants were then covered with polythene bags and kept under laboratory conditions. Controls injected with sterile distilled water were also kept under identical conditions. Observations for symptom development were taken after 24 hours 56 hours and 120 hours and recorded.

Changes in total sugars and total phenolics in leaf sheath of rice due to *A.oryzae* inoculation

Quantitative changes in total sugars and total phenolics in three different leaf sheaths of rice inoculated with *A.oryzae* were studied. Plants for this purpose were raised in uniform size pots under identical conditions using the variety Triveni. One set of plants were inoculated by injecting spore suspensions standardised as already described, below the different leaf sheaths. The sheath covering the panicle (No.1). the next immediately lower leaf (No.2), and

sheath of the third leaf from the boot leaf (No.3) were separately inoculated. Control plants were inoculated with sterile distilled water and all plants were incubated under identical conditions. On 15th day after inoculation samples of diseased leaf sheath and those of control were collected separately.

a. Total sugars

The total sugars in various samples were determined by following the method described by Yem and Willis (1954). One gram of the plant sample was ground with 70 per cent ethanol and dried in vacuum. It was then mixed with warm water and cleared with aluminium hydroxide.

Five ml of anthrone reagent (Anthrone 0.2 g was dissolved in 100 ml of dilute sulphuric acid, 5:2 acid and water) was pipetted out into a thick walled pyrex tube and chilled in ice water. One ml of the test solution was layered on the acid, cooled for a further five minutes and then thoroughly mixed while still immersed in cooled water. The tubes were loosely fitted with corks, heated for 2 minutes in a boiling water bath and then cooled in water. Distilled water was used as blanks. Readings were taken using a spectronic

20 Spectrophotometer at 590 nm. The quantity of the total sugars in the samples were expressed as /ug per g of sample as glucose equivalent.

4. Total phenolics

The method of Bray and Thrope (1954) was followed to determine the total phenolic content of both healthy and diseased sheath samples.

One gram of fresh leaf sample was ground with 80 per cent hot ethanol, boiled and filtered. The filtrate was evaporated to dryness and dissolved in 1 ml of 80 per cent ethanol. An aliquot of 0.1 ml of this solution was taken in a boiling tube and made upto 70 ml with distilled water.

One ml of Folin-Ciocalteau reagent (sodium tungstate 10 g, phosphomolybdic acid 2 g, 85 per cent phosphoric acid 5 ml and water 75 ml were mixed together and boiled for 2 hours, filtered the mixture and diluted to 100 ml) and 2 ml of saturated sodium bicarbonate were added and the tube heated for one minute in a boiling water bath, cooled and the colour read in Spectronic 20 Spectrophotometer with red filter (645 nm). The total phenolics expressed as /ug per g of the plant sample as pyrogallol equivalent.

Evaluation of fungicides against the pathogen

The following ten fungicides at different concentrations were used for laboratory assay against the pathogen.

1. Inhibition of growth of the fungus by poisoned food technique on solid medium

| Fungicides | Active ingredient | concentrations used (ppm) |
|---|---|---|
| 1. Vitavax | 5,6, dihydro 2 methyl 4,4, oxathin 3-carboxa nilide | 250<br>500<br>750 |
| 2. Dithane Z-78 | Zinc ethylene bisdithiocarbamate | 2000<br>4000<br>5000 |
| 3. Hinosan | O-ethyl S, S-diphenyl dithiophosphate | 500<br>1000<br>1500 |
| 4. Blue | Copper oxychloride | 2000<br>4000<br>5000 |
| 5. Difolaten | Cis-N (1-1, 2,2, tetra chloro ethyl) thio-4-cyclohexene 1,2, carboximide | 1000<br>1500<br>2000 |
| 6. Cuman.L. | Zinc dimethyl dithiocarbamate | 2000<br>4000<br>5000 |
| 7. Bavistin | 2 (methoxy-carbamyl) benzimidazole | 500<br>1000<br>1500 |
| 8. Kitazin | O,O-Di-isopropyl-S-benzyl thiophosphate | 500<br>1000<br>1500 |

| Fungicides | Active ingredient | Concentrations used (ppm) |
|---|---|---|
| 9. Aureofungin | N-Methyl-P-amino aceto phenonemycosamine heptane | 25<br>50<br>100 |
| 10. Dytolan | Copper oxychloride | 2000<br>3000<br>4000 |
| C. Control | PDA- without fungicide | |

The poisoned food technique described by Zentmeyer (1955) was adopted in order to study the effect of different fungicides on the growth of the fungus. Required quantity of each fungicides was weighed out and added to 50 ml of sterilized potato dextrose agar medium to give the required concentration, mixed well and poured into sterile petri dishes at the rate of 15 ml per dish. After solidification of the medium, the dishes were inoculated by mycelial discs of 5 mm diameter, cut out from an actively growing colony of the fungus. Controls consisted of unamended PDA inoculated in the same way. All the dishes were incubated at room temperature ($28 \pm 2°C$). The growth of the fungus was observed daily and final observations were taken on 10th day of incubation. Per cent inhibition of growth over control was calculated by using the formula.

$$\text{Per cent inhibition} = \frac{C-T}{C} \times 100$$

where C = radial growth in control

T = radial growth in treatment

2. Inhibition of spore germination of the fungus on glass slides

The method described by Rajagopalan and Wilson (1972) was followed. Spores obtained from 10 day old cultures of the fungus grown on PDA were used to assess the effect of fungicides on the spore germination of the fungus. Spore suspension was prepared in sterile distilled water. The concentration was adjusted to 50 to 60 spores in a drop of spore suspension examined under the low power of a microscope. The fungicidal solutions were prepared in sterile distilled water in double the concentration as that required for the experiment. Equal volumes of the fungicidal solution and spore suspension were mixed and two drops of the same were placed on sterile, clean grease free glass slides placed in petri dish moist chambers and incubated at room temperature. Observations were taken at 6 and 24 hours after incubation. The per cent inhibition of spore germination based on 20 microscopic fields was calculated from these observations.

3. Field assay of fungicides against sheath rot of rice

A field experiment was laid out during the second crop season (September-January - 1979-80) to study the effect of certain common fungicides on the incidence and intensity of

sheath rot of rice. The details of the experiment were as follows:-

| | |
|---|---|
| Layout design | RBD |
| Variety | Jyothi |
| Spacing | 15 x 15 cm |
| Gross plot size | 6.30 x 4.80 m |
| Net plot size | 6.00 x 4.50 m |
| Replications | 4 |
| Number of treatments | 7 (Including control) |

| Treatments | Active ingredients | Concentrations used |
|---|---|---|
| 1.Vitavax | 5,6,dihydro-2 methyl 4,4, oxathin 3-carboxanilide | 0.02% |
| 2.Dithane Z-78 | Zinc ethylene bis-dithio-carbamate | 0.40% |
| 3. Hinosan | O-ethyl S-S, diphenyl dithio-phosphate | 0.10% |
| 4. Fycop | Copper oxychloride | 0.40% |
| 5.Difolatan | Cis-N (1-1, 2,2, tetrachloro ethyl) thio-4-cyclohexene 1,2, carboximide | 0.15% |
| 6. Cuman-L | Zinc dimethyl dithio carbamate | 0.12% |
| 7. Control | (No spray) | |

Nursery

Ten kg of seeds were sown on 25-9-1979 in a wet nursery of 150 sq.m. The nursery was given a top dressing at the rate of 15 kg nitrogen per hectare. Prophylactic sprayings with carbaryl were given to prevent insect attack.

Main field

The crop was raised following the cultivation methods described in package of practices recommendations of Kerala Agricultural University (Anon., 1978 b). Each plot was given a basal dressing of 30:35:17.5 kg NPK per hectare in the form of urea, superphosphate and muriate of potash. Twenty two day old seedlings were transplanted and 15 days after transplanting (tillering stage) all plots were uniformly top dressed at the rate of 15 kg N per hectare in the form of urea and 17.5 kg per hectare of muriate of potash. The remaining 25 per cent of nitrogen was top dressed on 25th day of transplanting (Flower initiation state). The crop was sprayed with carbaryl as per recommended dose, on 25th and 40th day of planting against pest attack. At earhead stage methyl parathion spray was given to check earhead bug.

Fungicidal application

Two sprayings were given. The first spraying on 45th day after transplanting and the second at the earhead stage of the crop (80th day).

Observations

a. Per cent of hill infection

The observation was recorded 16 days before harvest. The per cent of hills infected was recorded by selecting five rows at random and examining all the hills in the rows leaving border two hills.

b. Disease intensity

The intensity of attack was recorded 15 days before harvest. For recording the intensity of disease, three rows were selected at random from each treatment and twelve random hills from each row were again selected. The intensity was scored as per the " Standard Assessment of Diseases of Rice " (Amin, 1976 b).

| Disease Index | Description |
|---|---|
| | No visible symptoms on sheath of any leaves. Panicles are fully emerged and grains are free from discolouration |

| Disease Index | Description |
|---|---|
| 3 | Two to three small lesions 0.5 to 1.0 cm long and 0.2 to 0.5 cm wide developed on flag leaf sheath, which are oval, dark chocolate brown and are surrounded by diffused light brown halo, while the colour of the healthy sheaths around the lesion remain green. Grains are not discoloured. |
| 5 | Large lesions 2 to 3 cm long and 1 cm wide are most conspicuous on flag leaf sheath, but occur on all the leaf sheaths. Lesions overlap and form irregular large chocolate brown blotches of flag sheaths. Emergence of panicles is affected and it is half way from flag leaf sheath. Grains inside are partially chaffy and are covered with white pink mycelium and spore masses. Affected panicles range upto an estimated 25 per cent. |
| 7 | Flag leaf sheaths are completely chocolate brown in colour due to many overlaping lesions. Flag leaves of affected sheaths gradually became yellow to straw coloured. Affected panicles are fully compressed by flag leaf sheaths 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. |

| Disease Index | Description |
|---|---|
| 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. |

Harvest

The crop was harvested at 110th day leaving two border rows near the bunds. The grain weight and straw weight were recorded after proper drying.

Effect of microclimatological factors on the incidence and intensity of sheath rot disease of rice

The microclimate (temperature and relative humidity) prevailed in the plots of the experimental area under fungicidal trial was observed using hand whirling psychrometer. Observations were taken from 5 places at random from each plot by operating the instrument at the level of the leaf sheath of the crop from ground level. These observations were taken from the stage of flower initiation of the crop and continued upto the earhead stage. Observations were taken daily at three times, early morning, midnoon and afternoon. Finally the average microclimate prevailed in the cropped

field during the infection stage and the period of disease development were recorded by averaging the daily observations. Percentage of hills infected were recorded at seven days interval during the period. Seven random squares ( 5 x 5 = 25 hills) were observed from each block for watching the incidence of the disease. Therefore altogether 700 hills (25x7x4) were observed each time.

Effects of the relative humidity and temperature on the symptom development were also studied by keeping inoculated rice plants under high percentage of relative humidity artificially provided.

Rice plants (variety - Triveni) grown on earthen pots were removed to a wirenet cage at the boot leaf stage. The cage was completely covered with thin cloth and this covering was always kept wet by periodical sprayings with tap water all over the cloth. Plants were inoculated with 10 day old culture of A.oryzae. Inside the cage a moderately hot and humid condition was maintained throughout the experimental period. These conditions were maintained upto the 15th day of inoculation. Controls of rice plants of the same age inoculated under identical conditions were also maintained in

the ordinary atmospheric conditions. Forty pots with rice plants were kept for study under each set of conditions. Maximum humidity and temperature prevailed in both the cases were observed daily, for 15 days and recorded. Percentage of plants infected were recorded at three days interval after inoculation and the results were compared.

# RESULTS

## RESULTS

### Symptomatology

The sheath rot disease of rice initiated on the middle portion of flag leaf sheaths as light purplish - brown oblong lesions. The young lesions were surrounded by a light yellow-brown halo, which on maturity turned dark brown with papery white or grey white centre. Lesions were 0.5 to 2.5 cm long and 0.5 to 1.5 cm broad. In severely affected plants symptoms were seen on second and third sheaths also. But most conspicuous sympton was seen only on the flag leaf sheath. The number of lesions on flag leaf sheath varied from 3 to 10. The individual lesions coalesced together and in advanced stages covered almost the entire sheath.

Plants infected early in its growing period showed severe symptoms at the heading stage of the crop. Panicles from such plants did not emerge fully or only partially emerged with greyish brown or dark-brown grains. Due to this, panicles could be observed at various stages of emergence in the affected field. The leaves along with the diseased sheath gradually dehydrated and became straw-yellow or grey-white in colour. In certain cases the panicles as a whole were

soon rotten inside the leaf sheath. The fungal growth could be observed as whitish powdery mass in the centre of the lesions and inside the affected sheaths (Plate Nos. 1 and 2).

Morphology of the causal organism

The morphological characters, viz., nature of mycelium, hyphal thickness, nature of conidiophore formation and its measurements, attachment of conidia and their measurements etc. were studied for six isolates of the organism and the results are presented in tables 1 and 2.

Mycelium was septate, profusely branched and purple-white in colour. Conidiophores were branched in single or double whorl with 2 to 5 branches in each whorl. The conidia were single celled, hyaline, cylindrical and were borne single or consecutively at the tip of each conidiophore branch. The isolates from different varieties of rice compared well in the morphological characters. But the hyphae of isolates A, B, D and F were slightly thicker than those of isolates C and E. The size of conidia from culture and from infected plant parts did not show much variation. However, the conidia from weed hosts were smaller than those from rice ( Table 2, Fig.1).

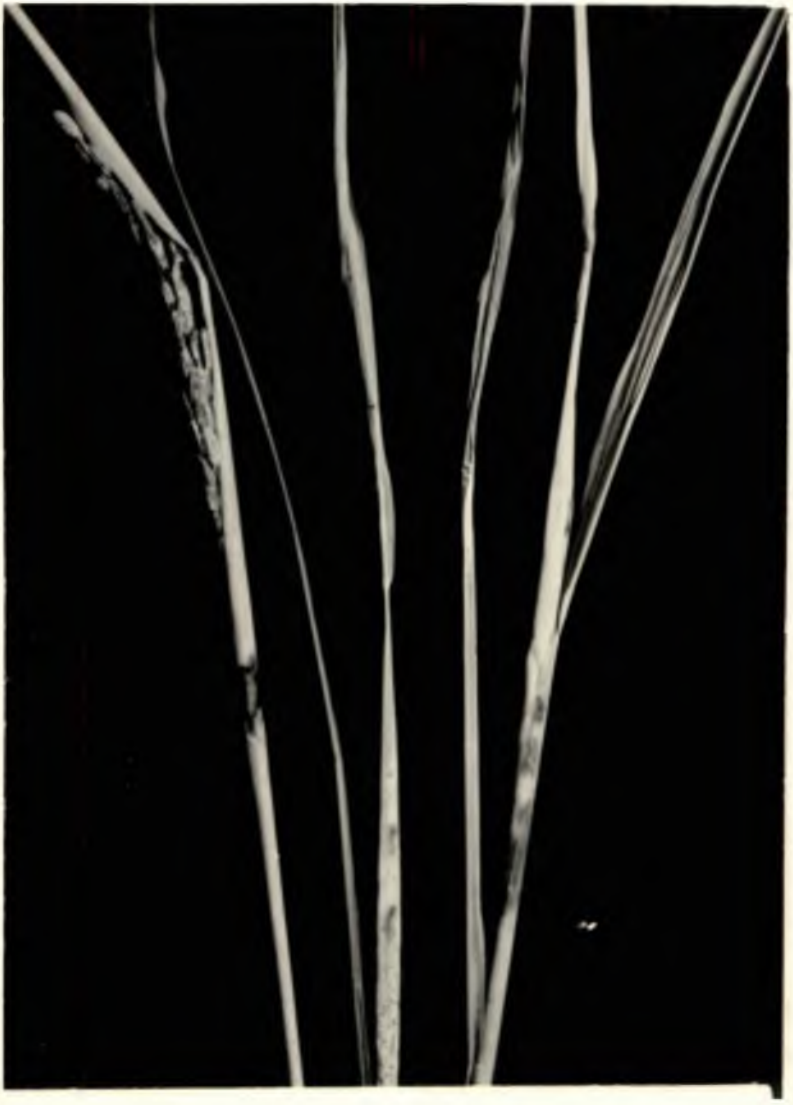


Plate 1. Rice plants showing sheath rot symptoms (Natural infection).

Plate.2. Sheath rot affected rice plants showing choking of panicles.

Fig.1

Comparative morphological characters of six isolates of Acrocylindrium oryzae

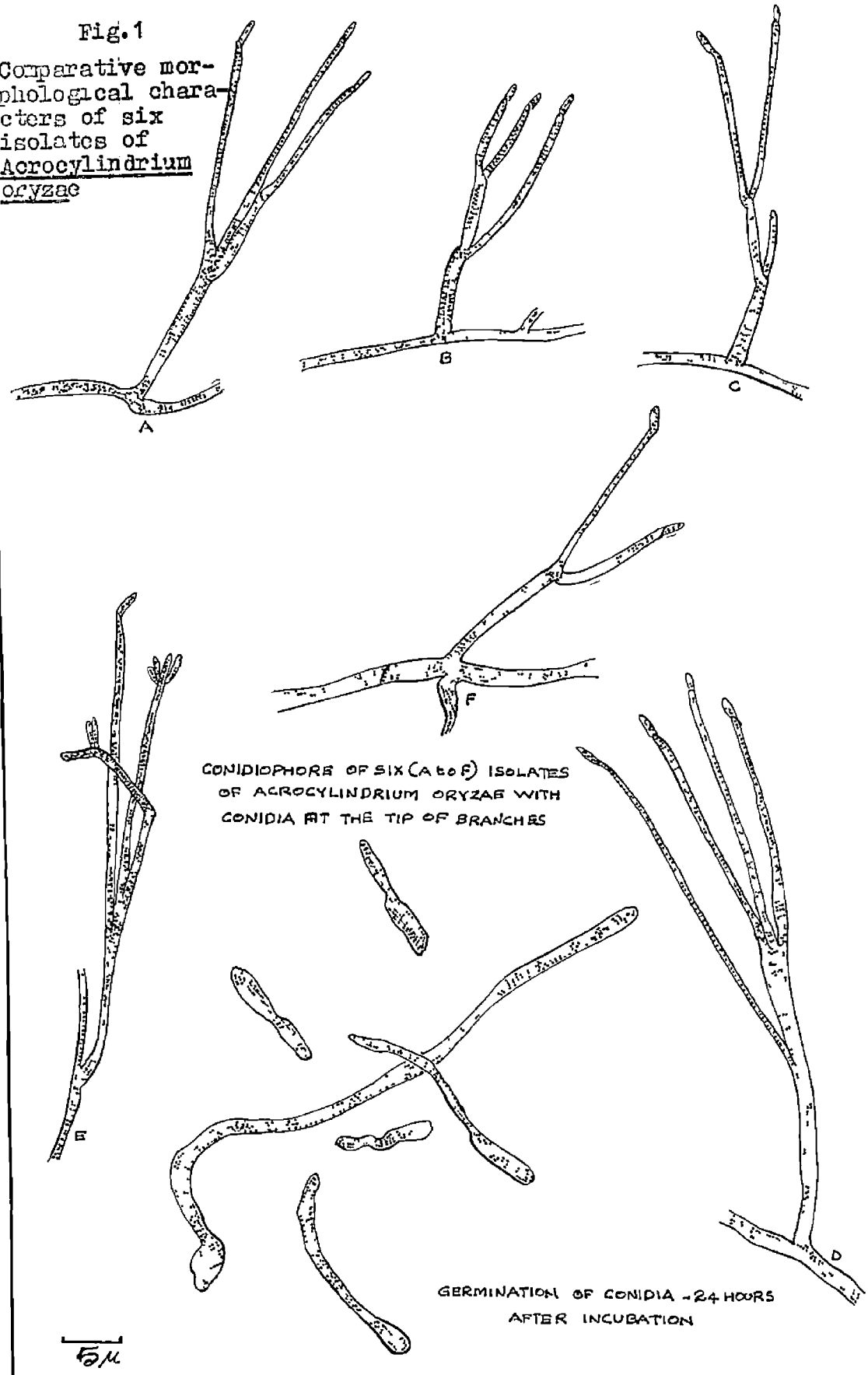


Table 1

Comparative morphological characters of six isolates of *Acrocylindrium oryzae*
(General characters)

| Isolates | Different morphological characters | | |
|---|---|---|---|
| | Mycelium | Conidiophore | Conidia |
| 1. Isolate from rice variety-Triveni(A) | Septate highly branched and purple white in culture medium. Good growth on PDA. A pink colour could be noticed on other side of the culture dish. | Conidiophores are branched in single whorl of 2 to 5 branched. | Single celled, cylindrical hyaline conidia are attached single or in consecutively at the tip of the conidiophore branches. |
| 2. Isolate from rice var. Jaya (B) | do | Branching was noticed both in single and double whorls of 2 to 5 branches in each whorl. | do |
| 3. Isolate from rice var.Jyothi (C) | do | Branched only in single whorl | do |
| 4. Isolate from rice var. Sabari (D) | do | Branched in single and double whorls | do |
| 5. Isolate from field weed *Cyperus difformis* (E) | Poor growth on PDA. Very slow growing. Pink colour was very prominent. | Branched only in single whorl. | 2 to 5 conidia are borne consecutively at the tip of each branch. |
| 6. Isolate from field weed *Echinochloa crusgalli* (F) | More or less white cottony mycelium. Grow well on PDA | do | Single conidium was noticed on tip of each branch |

Plate 3. Rice plants artificially inoculated with *Acrocylindrium oryzae* showing symptoms on different sheaths.

Plate 4. Sheath rot symptoms produced on artificial inoculation.

symptoms were studied critically in both the treatments. When F.roseum alone was inoculated in rice plants small light brown lesions with an yellow halo appeared here and there on the flag leaf sheath on 8th day of inoculation. These lesions slowly increased in area and after thirteen days of inoculation the plants showed a general chlorosis. When the infected plants were given sufficient moisture the fungal growth could be observed over the affected portions. These portions later turned grey in colour with light yellow margins. The grains were free of attack in all the inoculated plants (Plate No.5).

In the case of combined inoculation of F.roseum along with A.oryzae the initial symptoms could be observed two days earlier i.e., on the 6th day of inoculation. The development of symptoms was similar to that which A.oryzae alone. Here the second leaf sheath was also found to be infected. The development of lesions was faster than in the case of A.oryzae alone. On 12th day of inoculation the partially emerged panicle showed the fungal out growth and by 15th day the unfilled grains showed brown discolourations (Plate No.6).


Plate 5. Rice plants artificially inoculated with *Fusarium roseum* showing mild symptoms atypical to sheath rot disease.

Plate 6. Rice plants artificially inoculated with Acrocylindrium oryzae and Fusarium roseum - combined infection showing typical sheath rot symptoms.

Rice-varietal variations in development of sheath rot symptoms

Nine rice varieties were tested to study the variation in symptom development. Slight variations were noticed in the symptom development on different varieties. The majority of them showed symptom development seven days after inoculation. The colour of lesions varied according to variety also (Table 3).

Host range of the pathogen

A total number of ten weeds (listed out under materials and methods) from rice fields were raised in earthen pots and inoculated with the spore suspension of the fungus. Out of this, six weeds were found infected and symptoms produced within 5 to 8 days after inoculation. It was noticed that the isolates from these infected weeds were able to infect rice plants again and produce typical sheath rot symptoms.

Following are the weeds which were detected as effective field hosts of the fungus by pathogenicity tests.

1. Echinochloa crusgalli
2. Eleusine indica
3. Leptochloa vaginalis

Table 3

Rice-varietal variations in development of sheath rot symptoms

| Varieties | Appearance of initial symptoms days after inoculation | Nature of symptom development after 10th day of inoculation |
|---|---|---|
| 1.Jyothi | 6th day | Purple-brown lesions began to spread in the flag leaf sheath. Lesions were of 0.5 to 2.5 cm long and 0.5 to 1.5 cm broad in size. Second and third leaf sheaths were found free of symptoms. |
| 2.Triveni | 6th day | Purple-brown lesions. Lesions on flag leaf began to spread by 10th day of inoculation. The disease free areas in sheath appeared dark green in colour. Emergence of panicle was retarded. The diseased sheath became brittle in nature. |
| 3. Annapurna | 6th day | Grey-brown lesions appeared almost in the middle region of flag leaf sheath. Lesion length were same as in Jyothi. |
| 4.Rohini | 7th day | The second leaf sheath also showed mild symptoms. |
| 5.Co-25 | 7th day | Small light-brown lesions were noticed. Symptoms were noticed only on flag leaf sheath. The flag leaf sheaths turned rough and brittle. |
| 6.Sabari | 7th day | Symptoms on flag leaf sheath only. Lesions were light-brown in flag leaf sheath. Lesion on other sheaths were not conspicuous. |
| 7.IR-8 | 6th day | do |
| 8.PTB-12 | 8th day | Only few number of lesions were seen on the flag leaf sheath, comparing to the other varieties. Lesions were also comparatively smaller and light brown in colour. |
| 9.Jaya | 7th day | Oblong dark-brown lesions were seen. Lesions were comparatively larger in size. One or two small lesions were seen on the second sheath also. |

4. Cyperus difformis
5. Cyperus iria
6. Cyperus tenuriflee

The details of symptoms observed on these weeds were as follows:-

Echinochloa crusgalli: Symptoms appeared on 6th day of inoculation. Purple-brown elongated lesions were noticed on first and second leaf sheaths. The lesions coalesced rapidly and covered three fourth of sheath area within fifteen days of inoculation. Sheaths with their leaves were dehydrated and became straw coloured and dried. The mycelial growth of the fungus could be observed on the panicle and the grains turned dark brown (Plate No.7).

Eleusine indica: Infection initiated on the fifth day of inoculation, as elongated light brown lesions on the first leaf sheath. These lesions gradually coalesced and the whole leaf sheath was covered. White mycelial growth could be observed in the panicles. The panicles turned chaffy and dried (Plate No.8).

Leptochloa vaginalis: Symptoms started on the 8th day of inoculation. At first dark-brown to black lesions were

Plate 7. Symptoms produced on weed hosts *Echinochloa crusgalli* and *Cyperus tenuiflorus* on artificial inoculation with *Acrocylindrium oryzae*.


Plate 8. Symptoms produced on weed host *Eleusine indica* - on artificial inoculation with *Aspergylindrium oryzae*.

noticed on the neck region of leaf petioles. Soon these portions started to rot and the rotting extended downwards in certain petioles. Meanwhile the petioles toppled down at the affected region. The leaves and petioles gradually dried with white mycelial growth on affected portions (Plate No.9).

Cymerus differnia: Initial symptoms were noticed on 6th day of inoculation. Small oblong, purple red spots appeared first on stems towards the top region which turned purple-brown later. These spots gradually coalesced and formed into irregular necrotic patches and covered almost one third of the top portion of the stem. The flower heads were seen distorted and rotten. Mycelial mat could be observed on the flower heads. In certain plants the leaves were also seen rotten in advanced stages of the disease (Plate No.10).

Cymerus iria: Brown lesions were noticed first on 6th day of inoculation on basal portions of the leaf sheath. All the sheaths were found infected. Gradually the lesions began to spread upwards and downwards. The infected portions of the stem rotted and toppled down. Fungal growth could be detected on the flower heads. In advanced stages the whole leaf sheath along with the stem rotted and the flower heads turned black in colour (Plate No.11).

Plate 9. Symptoms produced on weed host Monochoria vaginalis - on artificial inoculation with Acrocylindrium oryzae.

Plate 10. Symptoms produced on weed host *Cyperus difformis* - on artificial inoculation with *Acrocylindrium oryzae*.


Plate 11. Symptoms produced on weed host *Cyperus iria* - on artificial inoculation with *Aerocylindrium oryzae*.

Cyperus teneriffae: Small purple red spots appeared on 8th day of inoculation, on the middle as well as on the basal portions of the stem. Gradually a general chlorosis was noticed on the plants. Fungal growth was observed on flower heads also. But it was noticed that the heads were not considerably affected due to the infection (Plate No.7).

Survival of A.oryzae

Viability of the pathogen in severely infected paddy straw and grains kept dried under laboratory conditions were studied. Paddy straw and grains collected from field infected by sheath rot were kept under laboratory conditions, and the viability of the pathogen was observed at thirty days intervals. The results revealed that the pathogen was able to remain viable upto 60 days in infected paddy straw and 120 days in paddy grains (Table 4).

A. Growth and sporulation of A.oryzae on different culture media

The effect of different solid media on the growth of the pathogen was studied. Potato dextrose agar, Czapeks' Dox agar, Richards' agar, Coon's agar and host leaf extract agar were used for the study. The mean radial growth and the growth characters of the organism in different culture media are presented in Table 5. The results of the study

Table 4

Viability of Acrocylindrium oryzae in severely infected paddy straw and grain kept under laboratory conditions

| Days on storage | Survival of the pathogen | |
|---|---|---|
| | On paddy straw | On paddy grains |
| 30th day | Survived | Survived |
| 60th day | Survived | Survived |
| 90th day | Not survived | Survived |
| 120th day | Not survived | Survived |
| 150th day | Not survived | Not survived |
| 180th day | Not survived | Not survived |

revealed that Richards' agar was the best medium for its growth followed by Czapeks' Dox agar and potato dextrose agar, respectively. Best sporulation was obtained on PDA followed by the other two media which showed equal grades of sporulation (Fig.2).

Statistical analysis of the data revealed that Richards' agar was significantly superior to all other media used for growth.

Fig.2 Growth of *Acrocylindrium oryzae* on different solid media.

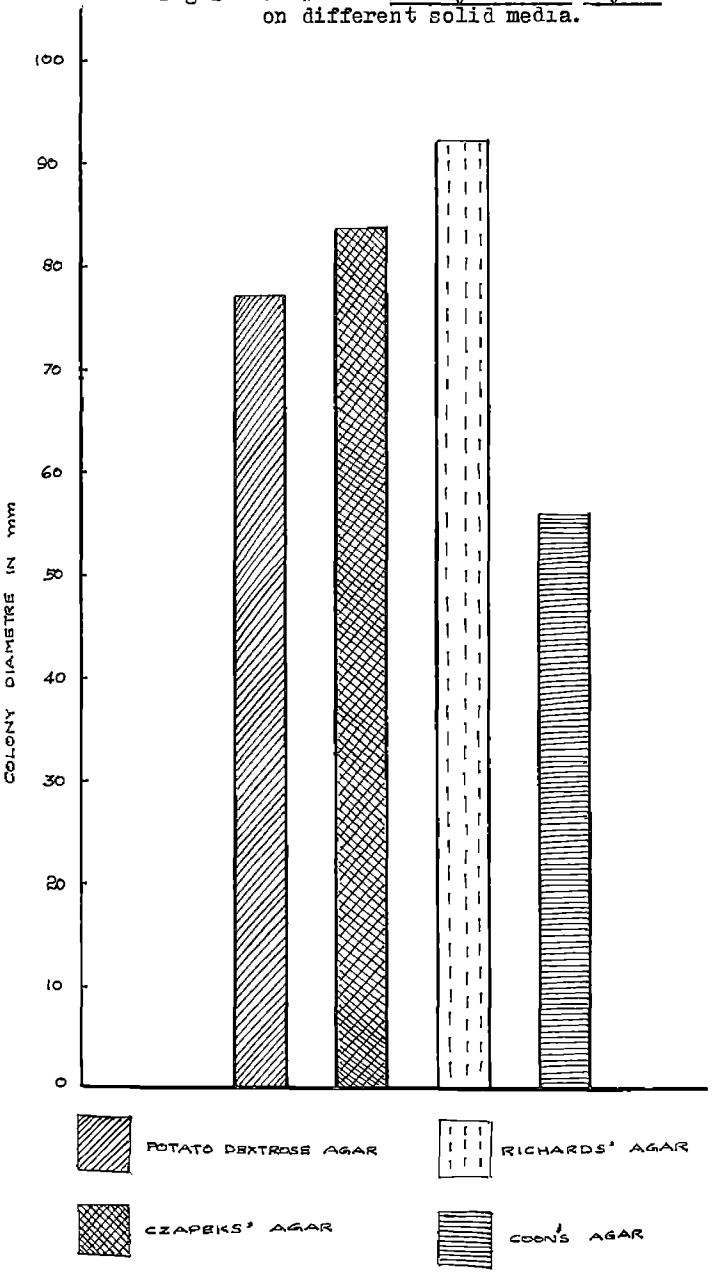


Table 5

Growth and sporulation of *A. oryzae* on different solid media

| Sl.No. | Medium | Mean colony diameter in mm* | Sporulation |
|---|---|---|---|
| 1 | Potato dextrose agar | 77.2 | ( +++ ) |
| 2 | Czapeks' agar | 83.6 | ( ++ ) |
| 3 | Richards' agar | 92.4 | ( ++ ) |
| 4 | Coon's agar | 55.7 | ( + ) |

C.D. = 5.3

* Average of five replications

(+) Less than 50 average spore count per microscopic field

(++) 50 < 100 ,,

(+++) > 100 ,,

D. Liquid media

Of the five different liquid media tested maximum dry weight of mycelium was obtained on Richards' medium followed by Czapeks' broth and PD broth. Coon's medium and host leaf

extract medium were found to be poor substrates for the growth of the fungus. On all media, growth started on the third day of incubation. Regarding sporulation, Richards' medium was found to be the best, followed by Czapeks' and potato dextrose broth which were ranked equal (Table 6, Appendix I and Fig.3).

Table 6

Growth and sporulation of A.oryzae on different liquid media

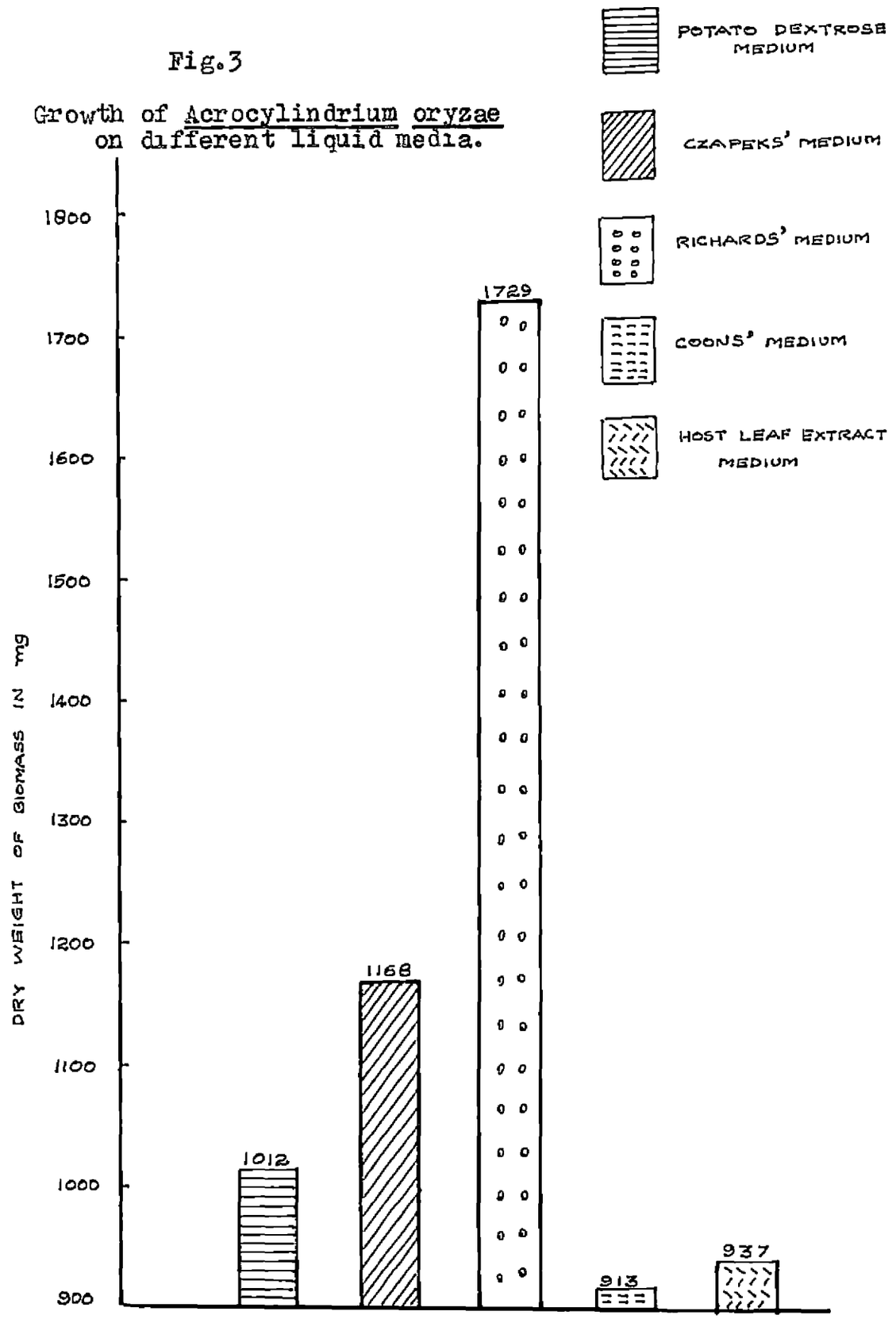
| Sl. No. | Medium | Mean dry weight of mycelium in mgms * | Sporulation |
|---|---|---|---|
| 1 | Potato dextrose medium | 1012.00 | (++) |
| 2 | Czapeks' medium | 1168.00 | (++) |
| 3 | Richards' medium | 1729.00 | (+++) |
| 4 | Coon's medium | 913.00 | (+) |
| 5 | Host leaf extract medium | 937.00 | (+) |

C.D. = 83.36

* Average of five replications.

Fig.3

Growth of *Acrocylindrium oryzae* on different liquid media.



Effect of temperature and pH on radial growth of A.oryzae

a. Effect of temperature

Five different temperature levels ranging from 15 ± 1°C to 40 ± 1°C were tested. It was found that below 20°C and above 30°C the radial growth of mycelium was decreasing. The optimum temperature range for best growth and sporulation of the fungus was found to be between 20 and 30°C (Table 7; Fig.4).

Table 7

Effect of temperature on radial growth and sporulation of A.oryzae

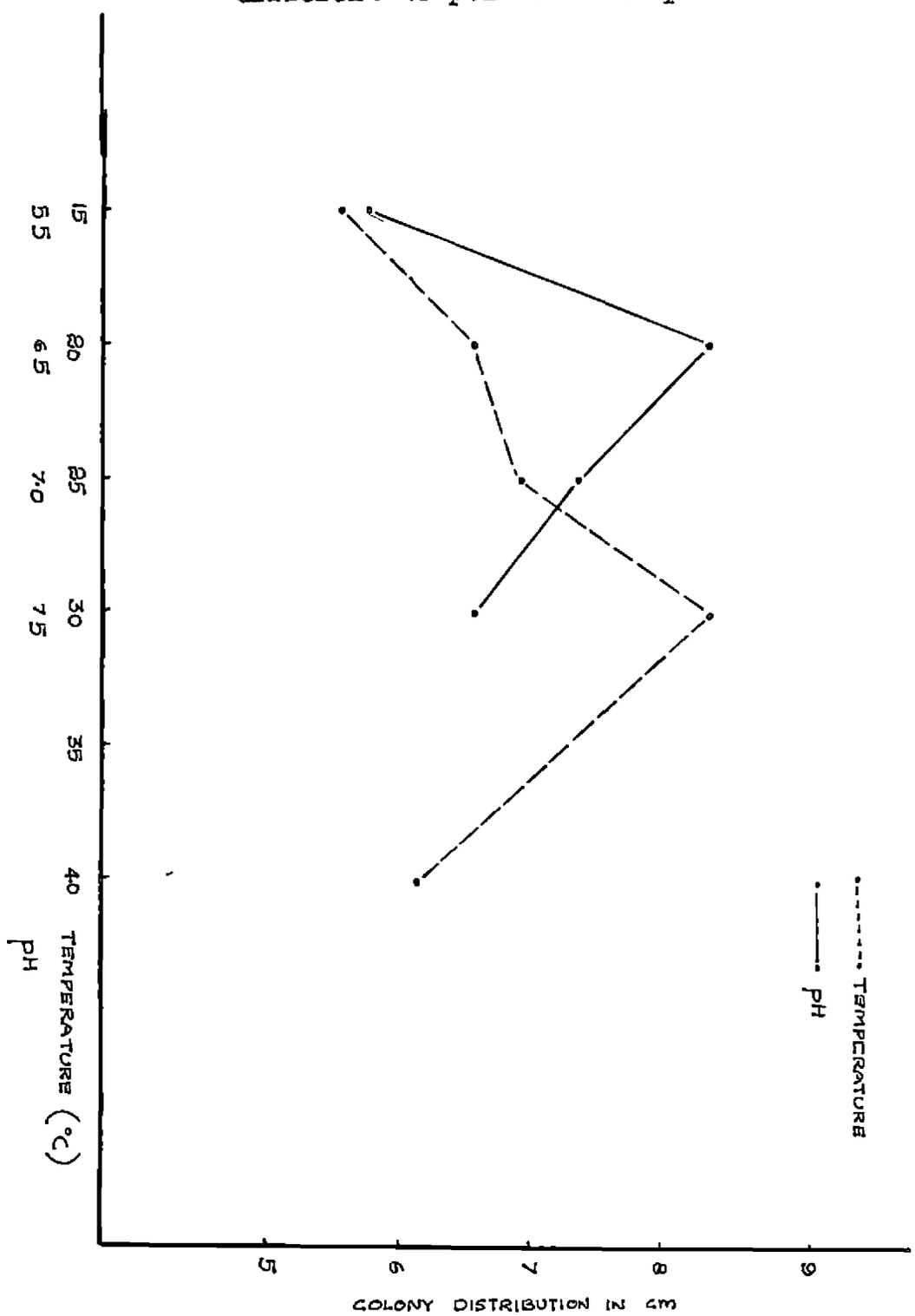
| Sl.No. | Temperature | * Mean colony diameter in cm | Sporulation |
|---|---|---|---|
| 1 | 15± 1°C | 5.54 | (++) |
| 2 | 20 ± 1°C | 6.56 | (+++) |
| 3 | 25 ± 1°C | 6.90 | (+++) |
| 4 | 30 ± 1°C | 8.32 | (+++) |
| 5 | 40 ± 1°C | 6.13 | (++) |

C.D. = 0.97

* Average of four replications

Fig.4

Growth of Acrocylindrium oryzae at different temperature and pH levels.



b). Effect of pH

Among four different pH levels tested ranging from 5 to 7.5, maximum mycelial growth was obtained at pH 6.5, followed by 7 and 7.5. The optimum pH range for the growth of the fungus was found to be 6.5 to 7.5 (Table 8; Fig.4).

Table 8

Effect of pH on growth and sporulation of A.oryzae

| Sl.No. | pH levels | * Mean colony diameter in cm. | Sporulation |
|---|---|---|---|
| 1 | 5.5 | 5.75 | (+++) |
| 2 | 6.5 | 8.37 | (+++) |
| 3 | 7.0 | 7.35 | (+++) |
| 4 | 7.5 | 6.53 | ( ++) |

C.D. = 0.65

(* Averages of 4 replications)

Production of toxin by A.oryzae

The effect of the exo-toxin extracted from the fungus by growing on Czapeks' broth was made use of for this test. The

tested rice plants showed a slight yellow discolouration on the first leaf sheath, 24 hours after injection. The culture filtrate of the organism by growing in Czapeks' broth showed that it was able to produce typical disease symptoms similar to that of sheath rot caused by A.oryzae on leaf sheath after inoculation. After 120 hours it was noticed that the first and second leaf sheaths were completely turned to purple brown with grey or papery dried portions at certain parts (Plate No.12).

Changes in total sugars and phenolics of rice plants due to A.oryzae inoculation

Artificially infected rice plants were used for this study. The infected leaf sheaths were chemically analysed 15 days after inoculation. Samples of sheaths No.1, 2 and 3 were separately analysed for both total sugars and phenolics. Corresponding leaf sheaths from healthy rice plants of same age raised under identical conditions were also analysed for comparison.

a. Changes in total sugars

The results obtained showed that in healthy plants the first leaf sheath contained the highest quantity of sugars followed by the second and third leaf sheaths respectively.


Plate 12. Effect of Exotoxin of *Acrocylindrium oryzae* on rice plant sheaths.

Leaf infection by the pathogen caused a reduction in total sugars in each of the three sheaths. The maximum per cent of decrease in total sugars due to the infection over the corresponding healthy one was noticed in the case of third sheath (93.0 per cent) and the least decrease was noticed in first sheath (55.37 per cent). The details are presented in table 9.

b. Changes in total phenolics

In healthy plants the highest phenolic content was recorded in the first leaf sheath followed by the second and third sheaths. Inoculation caused a reduction in total phenolic contents in each of the three leaf sheaths. The highest per cent reduction over healthy was noticed in the case of first

Table 9

Changes in total sugars and phenolics on leaf sheaths of rice plants due to infection by A.oryzae

| Sheath No. | * Total sugars | | * Total phenolics | |
|---|---|---|---|---|
| | Healthy | Diseased | Healthy | Diseased |
| 1 | 168 | 75(-55.37) | 725 | 105(-85.5) |
| 2 | 160 | 16(-90.00) | 630 | 160(-74.0) |
| 3 | 90 | 6(-93.00) | 615 | 210(-65.85) |

* µg / g of fresh sheath sample

The values in the parenthesis represent Per cent decrease in quantity over healthy

leaf sheath (85.5 per cent) and the least reduction (65.85 per cent) in the third sheath due to the infection by the fungus (Table 9 and Fig.5).

Evaluation of fungicides against Acrocylindrium oryzae

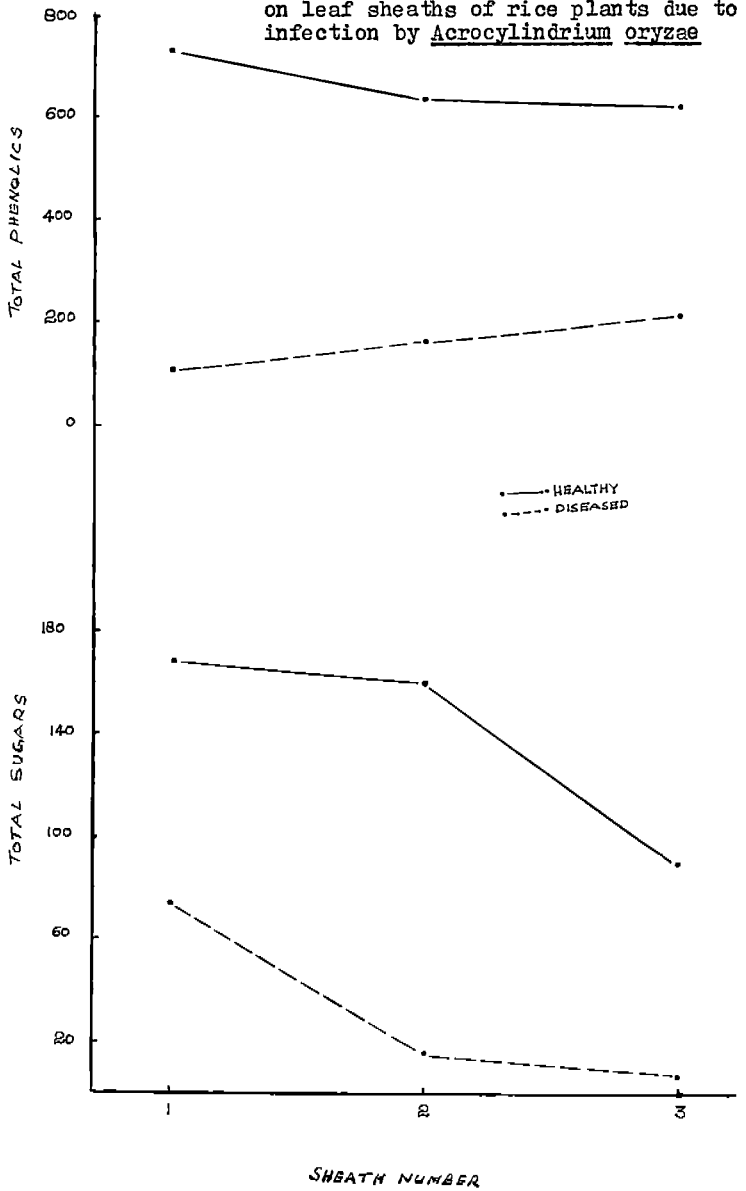
A. Laboratory assay

1. Inhibition of growth of the fungus:(Poisoned food technique on solid medium)

Of the ten fungicides tested, each at three concentrations, there was complete inhibition of growth of the fungus on potato dextrose agar medium incorporated with Dycop 2000 ppm, 4000 ppm and 5000 ppm: Bavistin 500 ppm, 1000 ppm and 1500 ppm and Bytolan 2000 ppm, 3000 ppm, and 4000 ppm (Table 10). They were found to be significantly superior to all other fungicides tested. Of the remaining seven fungicides Kitazin 1000 ppm was found most superior one. Hinosan 1500 ppm Cuman L 3000 ppm, Kitazin 1500 ppm and Difolatan 2000 ppm were also equally effective in inhibiting the growth of the fungus.

Hinosan and Cuman L at all the three levels were equally effective as Difolatan 1500 ppm and 1000 ppm. Vitavax 500 ppm and 750 ppm were found superior to Aureofungin and Dithane Z-78. But Aureofungin 25 ppm was superior to Dithane Z-78.

Fig.5. Changes in total sugars and phenolics on leaf sheaths of rice plants due to infection by Acrocylindrium oryzae



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Evaluation of fungicides against Acrocylindrium oryzae

A. Laboratory assay

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Fig.5. Changes in total sugars and phenolics on leaf sheaths of rice plants due to infection by Acrocylindrium oryzae

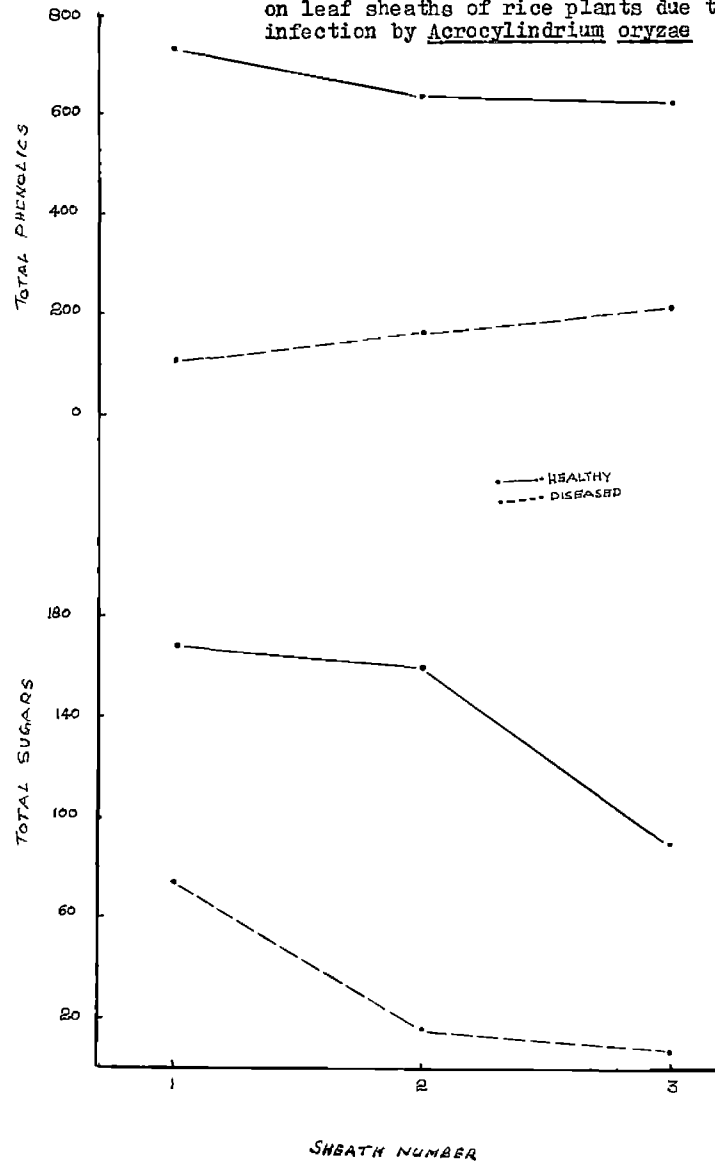


Table 10

Laboratory evaluation of Fungicides against A.oryzae effect on radial growth (poisoned food technique)

| Sl. No. | Fungicides | Concentration in(ppm) | *Mean colony diameter in (mm) | Per cent inhibition over control |
|---|---|---|---|---|
| 1 | Vitavax | 250 | 57.33 | 23.72 |
| | | 500 | 33.16 | 55.88 |
| | | 750 | 31.66 | 57.87 |
| 2 | Dithane Z-78 | 2000 | 73.66 | 1.99 |
| | | 4000 | 61.83 | 17.55 |
| | | 5000 | 59.16 | 21.28 |
| 3 | Hinosan | 500 | 35.00 | 53.43 |
| | | 1000 | 33.16 | 55.88 |
| | | 1500 | 28.16 | 62.55 |
| 4 | Fycop | 2000 | 0.00 | 100.00 |
| | | 4000 | 0.00 | 100.00 |
| | | 5000 | 0.00 | 100.00 |
| 5. | Difoletan | 1000 | 31.33 | 58.31 |
| | | 1500 | 28.66 | 61.86 |
| | | 2000 | 24.83 | 66.96 |
| 6 | Cuman L | 2000 | 36.50 | 51.44 |
| | | 4000 | 36.33 | 51.66 |
| | | 5000 | 26.33 | 64.96 |
| 7 | Bavistin | 500 | 0.00 | 100.00 |
| | | 1000 | 0.00 | 100.00 |
| | | 1500 | 0.00 | 100.00 |
| 8 | Kitazin | 500 | 42.00 | 44.12 |
| | | 1000 | 25.83 | 65.63 |
| | | 1500 | 27.16 | 63.86 |
| 9 | Aureofungin | 25 | 51.66 | 31.26 |
| | | 50 | 41.83 | 44.35 |
| | | 100 | 41.83 | 44.34 |
| 10 | Fytolan | 2000 | 0.00 | 100.00 |
| | | 3000 | 0.00 | 100.00 |
| | | 4000 | 0.00 | 100.00 |
| 0 | Control | .. | 75.16 | .. |

* Average value of three replications

Dithane Z-78 2000 ppm was least effective in inhibiting the growth of the fungus (Plate Nos.13, 14 and 15 and Fig.6).

2. Inhibition of spore germination of the fungus on glass slides

Complete inhibition of spore germination was observed for all the three concentrations of the fungicides in the case of Dithane Z-78, Fycop, Bavistin and Kitazin even after 24 hours. Hinosan and Difolatan were able to inhibit the germination of spores completely only at 200 ppm after 12 hours. After 24 hours Difolatan could inhibit complete spore germination at all the three concentrations and Fytolan at 100 and 200 ppm concentrations only. Hinosan and Fytolan were also able to inhibit more than 90 per cent spore germination even at 50 ppm concentration both after 12 hours and 24 hours. Cuman L showed more than 80 per cent inhibition only at 200 ppm after 12 and 24 hours. Vitavax at lower concentration (50 ppm and 100 ppm) showed very low per cent inhibition. Aureofungin and Vitavax were the least effective fungicides in inhibiting the spore germination of the pathogen (Table 11).

B. Field assay of fungicides against sheath rot disease of rice

A randomised replicated field experiment was laid out to access the efficacy of six different fungicides in controlling the disease.

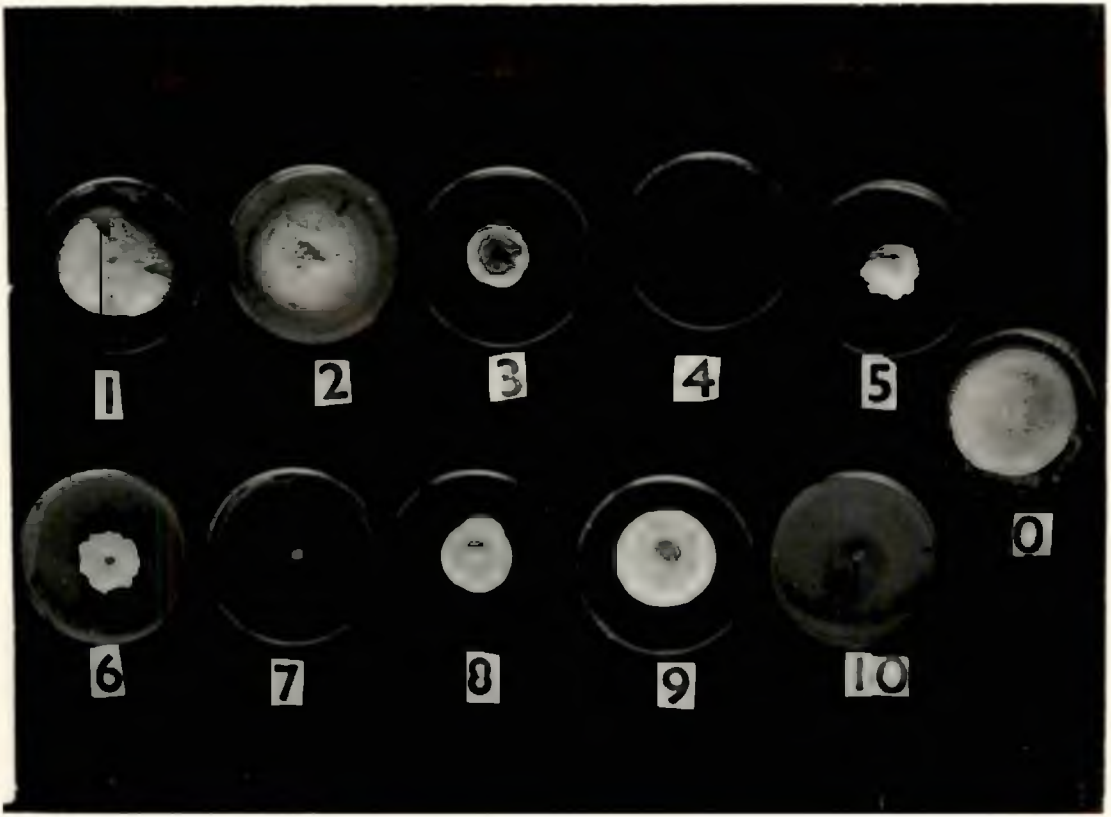



Plate 13. Growth of Acrocylindrium oryzae in fungicide incorporated potato dextrose agar medium - lower concentrations.

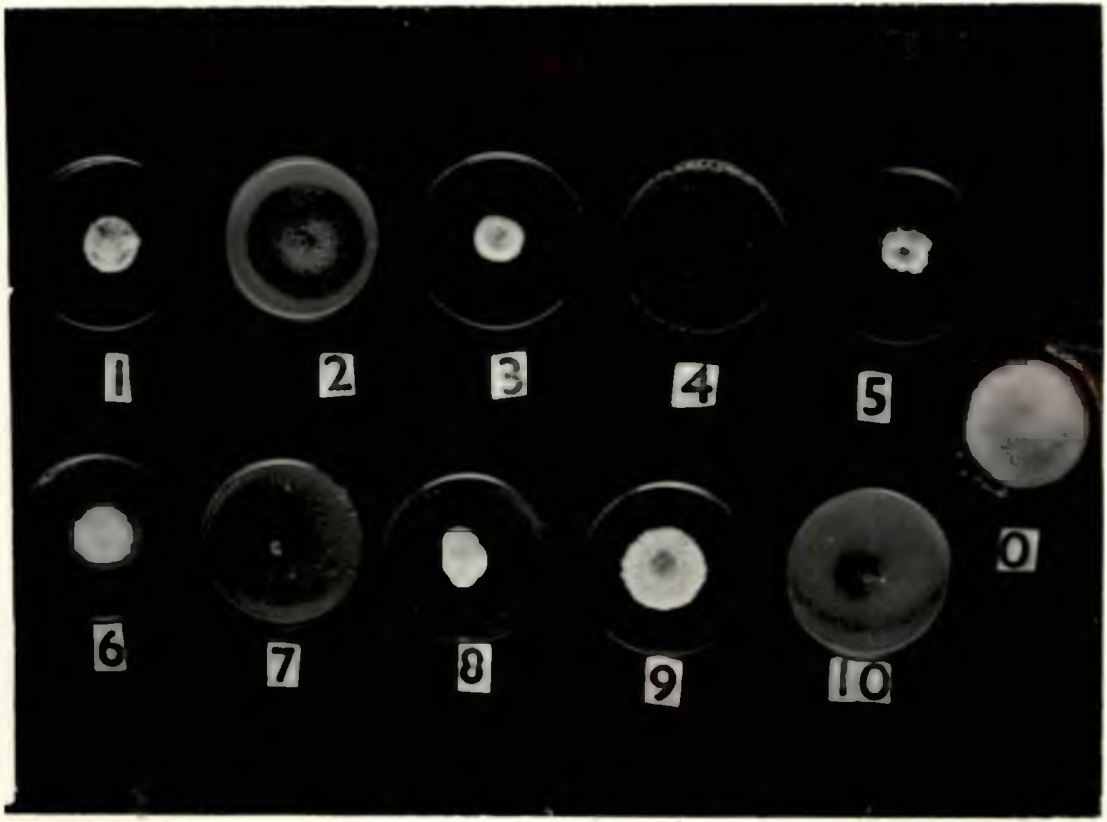



Plate 14. Growth of Acrocylindrium oryzae in fungicide incorporated potato dextrose agar medium - middle concentrations.


Plate 15. Growth of Agrocylindrium oryzae in fungicide incorporated potato dextrose agar medium - highest concentrations.

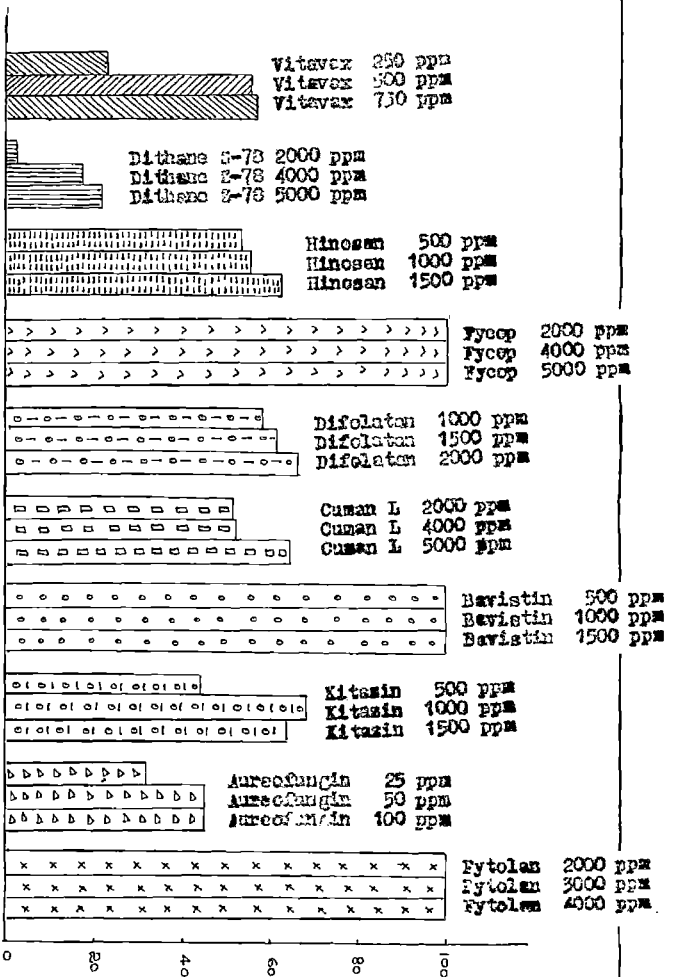



Fig.6. Effect of radial growth of Acrocylindrium oryzae on different fungicides incorporated at varying levels in potato dextrose agar medium.

Table 11

Effect of different fungicides on the germination of spores of A.oryzae

| Sl.No. | Fungicides | Per cent inhibition of spore germination | | | | | |
|---|---|---|---|---|---|---|---|
| | | After 12 hours | | | After 24 hours | | |
| | | 50 ppm | 100 ppm | 200 ppm | 50 ppm | 100 ppm | 200 ppm |
| 1 | Vitavax | 30.00 | 30.00 | 90.00 | 13.33 | 20.00 | 83.00 |
| 2 | Dithane Z-78 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 3 | Hinosan | 96.66 | 96.66 | 100.00 | 90.00 | 96.66 | 100.00 |
| 4 | Fycop | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 5 | Difolatan | 80.00 | 80.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 6 | Cuman L | 31.66 | 60.00 | 90.00 | 13.33 | 50.33 | 83.33 |
| 7 | Bavistin | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 8 | Kitazin | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 9 | Aureofungin | 13.33 | 13.33 | 16.66 | 11.66 | 13.33 | 16.66 |
| 10 | Fytolan | 93.33 | 100.00 | 100.00 | 93.33 | 100.00 | 100.00 |
| 0 | Control | 6.66 | 6.66 | 6.66 | 5.00 | 5.00 | 5.00 |

a. Per cent of hill infection

The per cent of hill infection with respect to the different fungicides are presented in Table 12. Hinosan was found to be superior to all other treatments in reducing the per cent of hill infection which was followed by Vitavax, Dithane Z-78 and Cuman-L. The latter three fungicides were on par with each other and they were superior to Difolatan and Fycop. Difolatan and Fycop were on par with control and so they were least effective in reducing the hill infection.

b. Disease intensity

With regard to intensity of attack, the results revealed that Hinosan was superior to all other fungicides used in reducing the intensity (Table 13). Hinosan was followed by Vitavax and Dithane Z-78 which were on par with Hinosan. All the other treatments were not effective in reducing the intensity of the disease.

c. Grain yields and straw yield

With regard to higher grain yield the plots treated with Dithane Z-78 ranked first followed by Hinosan (Average yield recorded were 2503 and 2441 kg respectively of

Table 12

Comparative efficacy of different fungicides on sheath rot of rice

| | | Per cent of hill infection (Mean values after angular transformation) |
|---|---|---|
| Sl. No. | Fungicides | Observation |
| 1 | Vitavax | 17.38 |
| 2 | Dithane Z-78 | 20.15 |
| 3 | Hinosan | 12.93 |
| 4 | Fycop | 26.19 |
| 5 | Difolatan | 25.91 |
| 6 | Cuman L | 21.00 |
| 7 | Control | 29.17 |
| | C.D. = 3.84 | |

Table 13

Comparative efficacy of different fungicides on disease intensity of sheath rot of rice

| Sl.No. | Fungicides | Disease intensity |
|---|---|---|
| 1 | Vitavax | 2.305 |
| 2 | Dithane Z-78 | 2.365 |
| 3 | Hinosan | 2.158 |
| 4 | Fycop | 3.315 |
| 5 | Difolatan | 3.129 |
| 6 | Cumen L | 3.066 |
| 7 | Control | 3.480 |

C.D. = 0.690

processed paddy per hectare as against 2147 kg per hectare for the control).

In the case of straw yield Difolatan and Fycop ranked first and second places (i.e., an average yield of 2038 kg and 1977 kg, respectively, of processed straw per hectare as against an average yield of 1737 kg per hectare from the control).

Statistical analysis of the yield data revealed that Hinosan and Cuman L were also on par with Difolatan and Fycop in the case of increased straw yield. All the other treatments were insignificant with regard to straw yield. However statistical analysis of grain yield data showed that fungicidal applications did not enhance the grain yield significantly (Table 14)

Table 14

Comparative efficacy of different fungicides on grain and straw yield

| Sl.No. | Fungicides | Grain yield (kg/ha) | Straw yield (kg/ha) |
|---|---|---|---|
| 1 | Vitavax | 2210.185 | 1791.66 |
| 2 | Dithane Z-78 | 2503.24 | 1701.85 |
| 3 | Hinosan | 2441.66 | 1881.47 |
| 4 | Fycop | 2030.55 | 1976.84 |
| 5 | Difolatan | 2379.63 | 2038.60 |
| 6 | Cuman L | 2398.14 | 1862.96 |
| 7 | Control | 2147.22 | 1737.95 |

C.D. for comparison of straw yield = 256.54

Effect of microclimatological factors on the incidence and intensity of sheath rot disease of rice

a. Under field conditions

The microclimatological factors such as maximum temperature and relative humidity prevailed in the rice field during the incidence and developmental periods of the disease were observed (Table 15 and Fig.7). The period of these observations were selected from 45th day after sowing of the crop (variety:Jyothi) till 65th day, i.e., from flower initiation to complete heading of the crop. It was noticed that during the first ten days of the above mentioned period, which was considered as most critical period of initiation of infection, the maximum temperature prevailed in the field ranged between 30.5°C to 34°C. Minimum temperature and relative humidity were ranging from 27.3 to 29°C and 65 to 89 respectively. Evidences of initiation of disease symptoms were first noticed on 58th day after sowing.

From 55th day to 65th day, there was a little fall in the maximum temperature range (29 to 31°C). Similar decrease was observed in the case of minimum temperature and Relative humidity ranges also (25.5 to 28.3°C and 71 to 82). On 58th day of the crop, a hill infection of 12.5 per cent was observed followed by 21.7 per cent on 65th day.

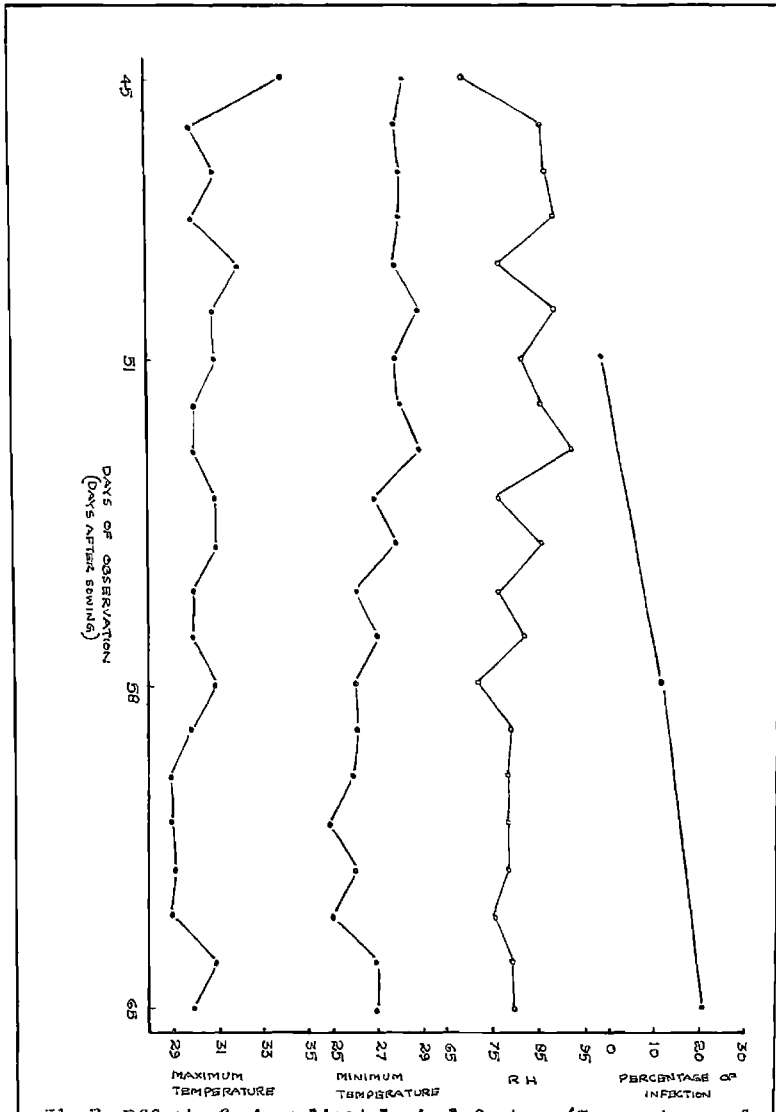



Fig.7. Effect of microclimatological factors (Temperature and relative humidity) on the incidence and intensity of sheath rot disease of rice - under field conditions.

Table 15

Microclimatological factors prevailed in the rice field during the incidence and development of the disease

| Date of observation (Days after sowing of the crop) | * Microclimate prevailed inside the crop | | | |
|---|---|---|---|---|
| | Maximum temperature (°C) | Minimum temperature (°C) | Relative humidity | Percentage of infection |
| 45 | 30.0 | 28.5 | 65 | .. |
| 46 | 30.75 | 28.0 | 82 | .. |
| 47 | 31.5 | 28.3 | 83 | .. |
| 48 | 30.6 | 28.3 | 85 | .. |
| 49 | 32.0 | 28.0 | 73 | .. |
| 50 | 31.0 | 29.0 | 85 | .. |
| 51 | 31.0 | 28.0 | 78 | Nil |
| 52 | 30.8 | 28.3 | 82 | .. |
| 53 | 30.5 | 29.0 | 89 | .. |
| 54 | 31.16 | 27.3 | 72 | .. |
| 55 | 31.0 | 28.3 | 82 | .. |
| 56 | 30.6 | 26.6 | 72 | .. |
| 57 | 30.0 | 27.1 | 78 | .. |
| 58 | 31.0 | 26.6 | 68 | 12.5 |
| 59 | 30.0 | 26.6 | 75 | .. |
| 60 | 29.6 | 26.6 | 74 | .. |
| 61 | 29.0 | 25.5 | 74 | .. |
| 62 | 29.5 | 26.5 | 74 | .. |
| 63 | 29.0 | 25.0 | 71 | .. |
| 64 | 31.0 | 27.5 | 75 | .. |
| 65 | 30.6 | 27.3 | 75 | 21.4 |

* Averages of three observations of 5 places from each block.

Any way the data showed that the microclimatical factors were not steady during the infection and disease development stages.

b) Under artificial conditions

The effects of maximum temperature and maximum relative humidity which were provided under artificial conditions were also studied. The period of artificial conditions maintained was fifteen days from the date of inoculation of rice plants (during boot leaf stage of the plants).

The infection by the pathogen could be detected on 6th day of inoculation on rice plants kept at both artificial and ordinary conditions. The maximum temperature range maintained during the first five days after inoculation was 29.5 to 33.4°C under artificial conditions and 29.5 to 31°C in the case of ordinary atmospheric conditions. Almost this range of temperature itself was prevailed upto 15th day of inoculation in both the conditions. But the R.H. maintained in artificial conditions was 95 to 97.7 whereas under atmospheric conditions R.H. of 62.5 to 76 was prevailing during the period (Table 16).

It was noticed that under artificial conditions, where a high range of R.H. and temperature were maintained 17.0

Table 16

Effect of temperature and relative humidity on the incidence of sheath rot disease under artificial conditions

| Days after inoculation | Artificial conditions | | | Ordinary atmospheric conditions | | |
|---|---|---|---|---|---|---|
| | Maximum temperature | R.H. | % of plants infected | Maximum temperature | R.H. | % of plants infected |
| 1 | 31.6 | 96.5 | .. | 28.5 | 67.0 | .. |
| 2 | 32.0 | 97.0 | .. | 27.6 | 65.0 | .. |
| 3 | 32.4 | 96.5 | Nil | 28.0 | 63.4 | Nil |
| 4 | 31.0 | 94.0 | .. | 31.0 | 67.0 | .. |
| 5 | 29.5 | 97.7 | .. | 29.5 | 67.0 | .. |
| 6 | 29.5 | 97.0 | 17.0 | 29.5 | 65.8 | 12.0 |
| 7 | 31.0 | 96.8 | .. | 29.0 | 72.5 | .. |
| 8 | 30.5 | 96.5 | .. | 30.0 | 76.0 | .. |
| 9 | 32.0 | 95.0 | 21.0 | 31.0 | 65.5 | 16.0 |
| 10 | 31.0 | 96.3 | .. | 31.0 | 65.5 | .. |
| 11 | 31.4 | 96.7 | .. | 31.5 | 67.0 | .. |
| 12 | 27.5 | 96.0 | 27.0 | 27.5 | 67.0 | 19.0 |
| 13 | 27.8 | 96.4 | .. | 27.0 | 72.4 | .. |
| 14 | 31.5 | 95.8 | .. | 27.0 | 72.0 | .. |
| 15 | 30.0 | 95.0 | 35.0 | 28.4 | 65.5 | 24.0 |

per cent of plants were found infected (Fig.8), as against only 12 per cent in ordinary atmospheric conditions on 6th day after inoculation(Fig.9). This trend was noticed till 15th day of inoculation. On 15th day 11 per cent higher infection was noticed in the artificial conditions than in the plants kept under ordinary atmospheric conditions.

The other peculiarities noticed were more darker and larger rotted areas were visible on the boot leaf sheaths in the case of plants kept under hot humid conditions. Similarly fungal out growths over the rotted areas and panicle were more prominent only on plants kept under artificial conditions.

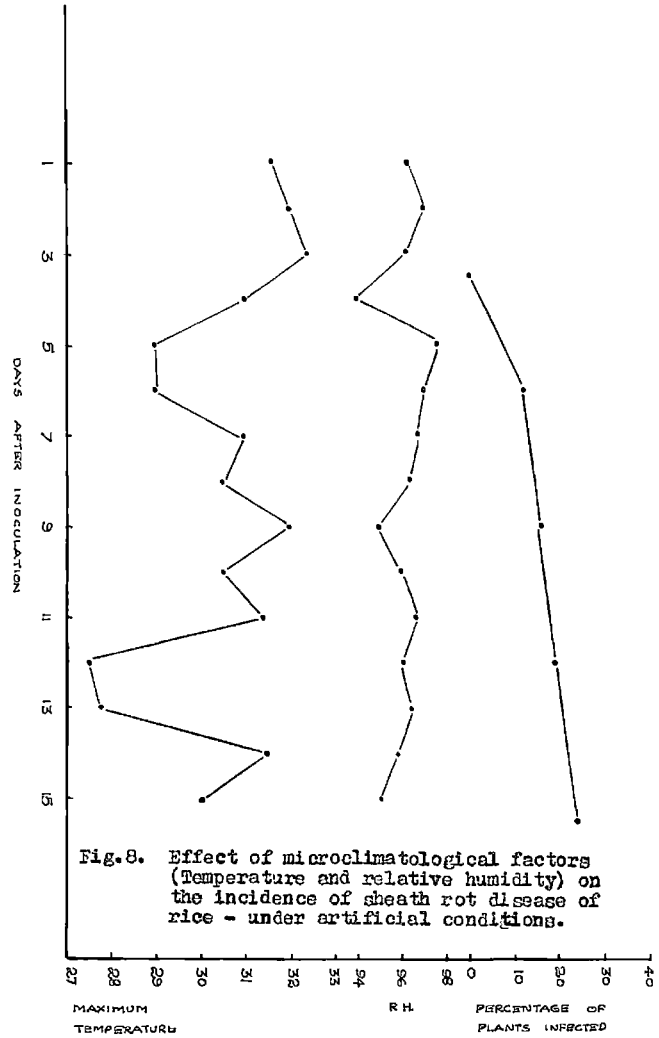
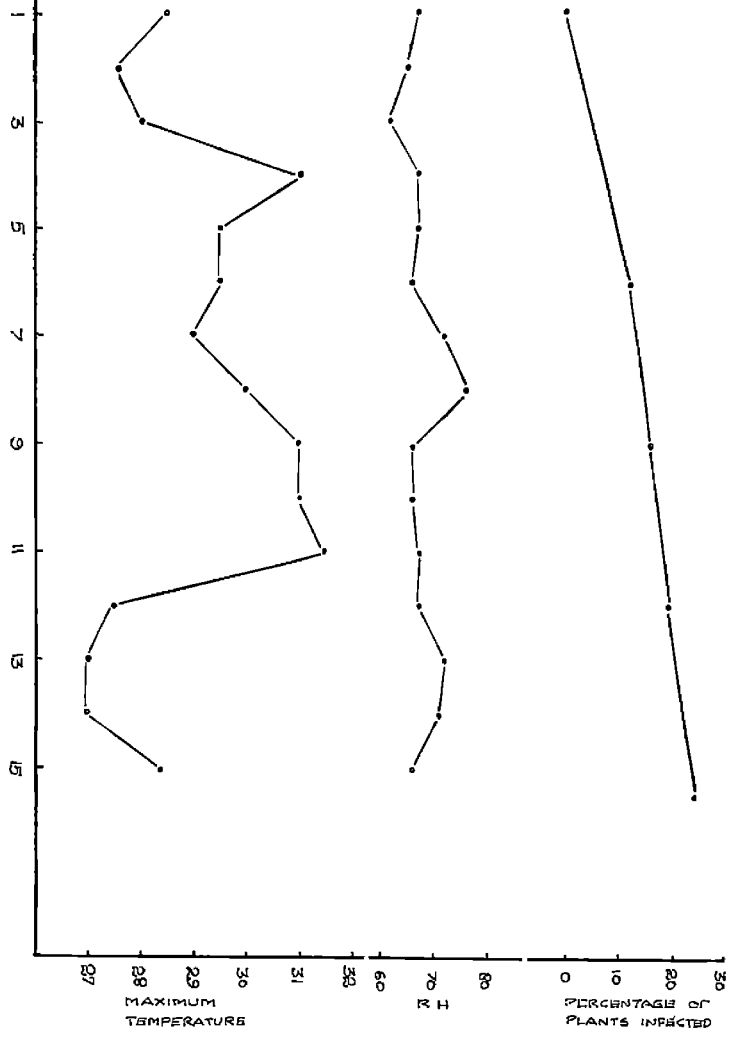



Fig.8. Effect of microclimatological factors (Temperature and relative humidity) on the incidence of sheath rot disease of rice - under artificial conditions.

Fig.9

Effect of microclimatological factors (Temperature and relative humidity) on the incidence of sheath rot disease of rice - under ordinary atmospheric conditions.



# DISCUSSION

# DISCUSSION

Sheath rot of rice caused by Acrocylindrium oryzae, considered to be a minor disease has gained much attention in recent years as a severe disease of rice crop in many parts of the world including India. In Kerala also it is known to cause much damage in many parts and in the present study also it was found to occur in a severe form in many parts of Trivandrum district.

The nomenclature of the organism causing the disease is still under debate. Eventhough Gams and Hawksworth (1975) suggested the new combination Sarocladium oryzae for the pathogen, this name has not been accepted widely by Mycologists and many still retain Sawada's old nomenclature Acrocylindrium oryzae. Prabakaran et al. (1974), Agnihothrudu (1973), Chung (1975), Datta and Purkayastha (1970), Kannaiyan (1979), Sathyanarayana and Reddy (1979) and Raina and Singh (1980) have followed Sawada's old nomenclature and designated the organism as A.oryzae. Since the old name is widely used in the literature the same is retained in the present study also.

The boot leaf stage of rice plants was found to be the most susceptible stage of infection by the fungus. In addition to inciting rotting of the sheaths, the pathogen

was also found to infect the panicle of the affected plants. The disease is at present known to occur widely. In India also its occurrence has been reported from different parts by Agnihothrudu (1973), Amin et al.(1974), Nair and Sathyarajan (1975). The symptomatology observed in the present study is in agreement with those already described in literature reviewed under the chapter, Review of Literature.

The pathogen was isolated and brought into pure culture on potato dextrose agar medium. Morphological characters of the different isolates of the fungus compared well with those reported by other investigators, Tsugi and Ikeda (1956), Ou (1972), Shajahan et al.(1974), Nair and Sathyarajan (1975). The results of the present studies were in agreement with those of the above investigators with negligible variations. No appreciable differences were noted in the morphological characters of the isolates of the causal organism made from different sources, especially those from different rice varieties. However, the hyphae of the isolates from varieties Ambika (A), Jaya (B), Sabari (D), and isolate from field weed namely Echinochloa crysgalli (F) were slightly thicker than those of isolates from Jyothi (C) and Cyperus difformis (E). Similarly, the conidium from weed hosts were

comparatively smaller than those from rice varieties.

Artificial inoculation studies conducted with the conidial suspension or culture bits have shown that the fungus could easily infect rice plants at boot leaf stage. If the plants were infected early in the boot leaf stage, rotting of the whole leaf sheath occurs resulting in the complete choking of the panicles. Similar observations were recorded by Inagi and Ikeda (1956), Singh and Raju (1979).

During the investigation a mixed infection of A.oryzae Jaw. and Fusarium roseum Link ex Fries could also be detected which was able to produce typical sheath rot symptoms as A.oryzae alone could produce. Artificial inoculation studies of rice plants with F.roseum alone showed that it could produce mild symptoms on the sheaths atypical of sheath rot disease. But combined inoculation along with A.oryzae resulted in typical sheath rot symptoms, the initiation of which could be detected two days earlier than when it was inoculated alone. Shajahan et al.(1974) have observed that F.roseum and an unidentified species of Hyalostachybotrys could be frequently isolated from plant parts with sheath rot symptoms. They also reported that these organisms alone

could infect rice sheath tissues and produce light brown lesions atypical of sheath rot symptoms. Similar associations have been reported in the case of Helminthosporium brown leaf spot disease of rice. In addition to H.oryzae (Breda de Haan), Helminthosporium rostratum Drechs., and H.halodes Drechs., were also associated with brown spot disease of rice (Chattopadhyay and Das Gupta, 1959; Atkins, 1972; Ramakrishnan and Subramanian, 1977). They found that these fungi could produce varying symptoms on different varieties when they were inoculated alone. Similarly in the case of Darhead Complex of rice it was reported that the combined infection of H.bipolaris, H.oryzae and Trichoconis (Alternaria) padwickii which were together responsible for the glume discolouration and blackening of grains (Anon.,1978 c).

Inter-varietal variations in development of sheath rot symptoms

From the nine rice varieties tested for varietal variation in symptom development, slight variations in the colour and size of initial lesions could be detected. Both short duration and medium duration commonly used high yielding rice varieties were found to be infected by the organism. However, development of infection on short duration varieties

such as Jyothi, Annapurna and Triveni were one day earlier than the medium duration varieties tested. Shajahan et al.(1974), Chung (1975), Datta and Purkayastha (1978) have reported that sheath rot disease was most severe on high yielding dwarf rice varieties. Shajahan et al.(1974) have also found that lesion colour varied from grey-brown to purple-brown depending upon the varieties attacked.

Host range studies carried out indicated that the fungus could infect a number of graminaceous and cyperaceous weeds which are commonly found in and around rice fields. This showed the fact that these weeds can remain as potential sources of inoculum of the sheath rot pathogen, especially, during the off seasons in and around the paddy fields.

Out of the ten weed plants tested by artificial inoculation positive results were obtained with six weeds viz., Echinochloa crusgalli, Eleucine indica, Monochoria vaginalis, Cyperus difformis, Cyperus iria and Cyperus tenuriffae. These weeds are recorded for the first time as hosts of the pathogen.

Studies on artificial inoculation revealed the fact that 5 to 8 days of incubation period was necessary for the fungus to produce initial symptoms which did not vary

according to varieties. Purple red or light brown to dark brown or black patches of rotted portions were the characteristic symptoms noticed on almost all weed hosts. In these weeds symptoms were observed on all the above ground plant parts, viz., stems, leaf sheaths, flower heads or panicles and grains etc. were found affected in the case of Echinochloa crusgalli, Eleusine indica, Cyperus iria, Cyperus difformis and Cyperus teneriffae, whereas leaf petioles and leaf blades, flower heads etc. were found affected in Monochoria vaginalis.

A period of fifteen days after inoculation was sufficient for the fungus to cover the whole, vegetative as well as reproductive parts of the weed hosts which resulted in complete damage of the affected plants except in the case of Cyperus teneriffae, which was able to remain without much damage even after fifteen days of inoculation with a slight chlorotic appearance only in the plants.

Factors favouring the incidence of sheath rot disease

Injury on the leaf sheaths of the host plant was reported to be a pre-requisite for successful infection by the fungus by many workers. Chen and Chien (1964) and Chin (1974) have observed that rice stem borer was associated with severe sheath rot disease. They claimed that the injuries brought

about by the stem borers on leaf sheaths were a pre-disposing factor for the easy entry of the sheath rot pathogen in rice plants. They considered this as a main reason for the non-emergence of panicles. Nair and Sathyarajan (1975) also observed that slight wounding of the sheaths could favour the infection. In the present study it was observed that the fungus could infect rice plants equally with and without any injury.

Studies on the viability of the pathogen in severely affected paddy straw and grains showed that the fungus was able to remain viable in infected dried paddy straw and grains for about 60 days and 120 days, respectively, under laboratory conditions. Kawamura (1940) found that the fungus could remain viable in diseased tissues - sheaths, grains and rachis for more than six months. Shajahan et al.(1974) also have mentioned about the survival of the organism. They found that the fungus could remain viable in dried rice straw and grains for more than one year.

Growth and sporulation of the fungus on different media

The fungus was able to grow well and sporulate on a number of solid and liquid media. Richards' agar was found

most superior for the radial growth of the fungus followed by Czapeks' agar and potato dextrose agar among the solid media tested. Best sporulation was noticed on PDA. In the case of liquid media best sporulation was obtained in Richards' medium. Maximum dry weight of biomass was also obtained from Richards' medium followed by Czapeks' and potato dextrose medium. Mohan and Subramanian (1978) have reported that A.oryzae grew well in potato dextrose agar and regarding, liquid medium, they found Czapeks' medium as the best.

Effect of temperature and pH on growth and sporulation

From the present studies on the effect of temperature and pH on growth and sporulation, the results showed that the optimum temperature range for the best growth and sporulation was between 20°C and 30°C. Regarding pH, the optimum pH range noticed was between 6.5 and 7.5. Tsuzi and Ahoda (1956) suggested that the optimum conditions for the best growth of the fungus were 20°C to 28°C and a pH of 6.4 Mohan and Subramanian (1978) found that the optimum temperature and pH for best growth and sporulation were 30°C and 6.5 respectively. An initial pH of 5 to 7 is satisfactory for majority of fungi (Cochrane, 1958).

Toxin production by the fungus

The culture filtrate obtained from Czapeks' broth could produce typical sheath rot symptoms when injected behind the sheath of the flag leaf as well as the sheaths of lower leaves, 120 hours after injection without providing any external injury on the sheaths. This showed the ability of the pathogen to produce toxic metabolites and its role in the pathogenesis of sheath rot disease of rice. The toxic effect of culture filtrate of A.oryzae on the inhibition of seed germination of rice, barley, wheat, rye and rape have already reported by Chung (1975). He also reported that the culture filtrate of the pathogen could inhibit the conidial germination of Cylicularia oryzae.

From the preliminary studies conducted it can be well presumed that the fungus produces a toxin or toxin like material which may induce the pathogenesis.

Changes in total sugars and phenolics of rice plants due to A.oryzae inoculation

Total sugars

In the present investigation it was observed that there was a gradual quantitative fall in the content of total sugars

in leaf sheaths from top to bottom both in the case of healthy as well as inoculated rice plants. The flag leaf sheath (No.1) contained the highest quantity of total sugars followed by the second and third sheaths. But the inoculation of each of the leaf sheaths with A.oryzae caused a considerable reduction of total sugars with reference to the corresponding leaf sheaths of the healthy plants. The maximum per cent of decrease due to infection was noticed in the case of third leaf sheath (93 per cent) and the least decrease in the first sheath (55.37 per cent).

It was reported earlier that the soluble sugar level influences the susceptibility of a host plant (Allen 1942; Inman, 1962). Horsfall and Dimond (1957) classified rusts, powdery mildews and chocolate leaf spot of beans which attack tissues with high sugar level, as " high sugar diseases", while Helminthosporial and Alternarial diseases occurring in tissue with . low sugar content were grouped under " low sugar disease". Sridhar (1972a) found that sugar reserve of susceptible tissues was higher than that of resistant ones in the case of Blast disease of rice. This might be the reason attributed to the easy infection of flag leaf sheath

of rice plants by A.oryzae, which contained the highest quantity of total sugars compared to the other lower leaf sheaths which contained only lesser quantities of sugars. Hence the sheath rot of rice caused by A.oryzae can also be considered as a high sugar disease.

The levels of tissue sugars in the host decreased followed by infection. Asada (1957), Dayal and Joshi (1968) have reported this phenomenon in several host parasite interactions. Reddy and Sridhar (1976) claimed that the reduction of sugars in the infected sheaths were either due to the utilization of these compounds by the pathogen itself or the decreased synthetic ability of the tissues of the severely infected leaves. They showed the same principle in rice plants infected with Xanthomonas oryzae. The presence of more sugars in the tissues, tended to increase the susceptibility of the host to invading pathogens and they served as sources of energy to the pathogen for its growth and multiplication.

Changes in total phenolics

The results of the present study showed that in healthy plants the highest phenolic content was recorded in the case of

flag leaf sheath followed by the second and third leaf sheaths. Inoculation caused a reduction in total phenolic content of each of the three leaf sheaths.

Phenolic compounds and their related oxidases have been found to be associated with the defense mechanisms of plants, because of their general accumulation near the wounded and infected tissues. Phenols and their oxidation products are highly toxic to pathogens (Walker and Stahmann 1955; Farkas and Kiraly, 1962; Tomiyama, 1963; Suzuki, 1965).

The present observations showed a general decrease of total phenolics due to infection by the fungus. In the case of rice blast disease, Jayachandran Nair (1975) reported that inoculation decreased the total phenol level in less susceptible cultivar Ratna, while highly susceptible cultivar Co.15 showed a general increase especially in the later stages of disease development. Toyoda and Suzuki (1960) and Sridhar (1972b) have correlated the resistance of rice cultivars to high peroxidase activity which oxidised the phenolic compounds in the absence of polyphenol oxidase. This was found to be true in rice affected by blast disease particularly during lesion formation stage (Sridhar and Ou, 1974). They suggested that the less susceptible variety

might have also possessed an augmented level of this oxidase enzyme during the disease development which might have oxidised the phenolics more effectively. This might be the reason for the decreased level of total phenolics in the infected sheaths of rice plants observed in the present study also.

Evaluation of fungicides

Results of the laboratory evaluation of fungicides indicated that the growth of the fungus was completely inhibited by Tycop at concentration of 2000 ppm, 4000 ppm and 5000 ppm; Bavistin at 500 ppm, 1000 ppm and 1500 ppm, Fytolan 2000 ppm, 3000 ppm and 4000 ppm when tested by the poisoned food technique using potato dextrose agar as basal medium. The effect of Bavistin in checking the growth of many fungi in nutrient media have been reported (Zachos et al., 1963; Sen and Kapoor, 1975; Kataria and Grover, 1977). Chinnaswamy et al. (1977) in a field study it was observed that Bavistin was the best fungicide in checking the infection as well as reducing the intensity of sheath rot disease. Chien and Huang (1979) found that Bavistin was very effective in controlling the in vitro growth of the fungus. Of the remaining seven fungicides tested Hinosan at concentration of 1500 ppm, 2000 ppm, Difolaten

1500 ppm and 2000 ppm, Cuman L 5000 ppm, Kitazin 1000 ppm and 1500 ppm were able to inhibit more than 60 per cent of the growth of the fungus. Vitavax 500 ppm, 750 ppm, Hinosan 500 ppm and 1000 ppm, Difolatan 1000 ppm, Cuman L 2000 ppm and 4000 ppm were able to inhibit the growth of the fungus more than 50 per cent and at these concentrations, the above said fungicides were almost equally effective in inhibiting the growth of the fungus.

Ragunathan and Vijayaraghavan (1976) from their laboratory studies observed that Hinosan at 0.005 per cent could effectively inhibit the growth of A.oryzae. They reported that Dithane Z-78 could inhibit only at higher concentrations (0.4 per cent).

Present study revealed that Dithane Z-78 and Aureofungin solveven at higher concentrations were not able to inhibit the growth of the fungus effectively. But it was found that Aureofungin at 250 ppm concentration was superior to Dithane Z-78 even at a concentration of 5000 ppm. Chinnaswamy et al.(1977) found that Aureofungin sol was effective in checking the infection as well as reducing the intensity of sheath rot disease of rice under field conditions.

In the experiments conducted to study the inhibition of spore germination of the fungus on glass slides by different fungicides it was found that complete inhibition of spore germination was observed for all the three concentrations tested in the case of Dithane Z-78, Tycop, Bavistin and Kitazin even after 24 hours. Hinosan and Difolatan were able to inhibit cent per cent spore germination only at 200 ppm after 12 hours. This effect was maintained after 24 hours also. Fytolan could inhibit complete spore germination after 12 hours and 24 hours at 100 and 200 ppm concentrations only. Hinosan, Difolatan and Fytolan could inhibit 80 per cent and above of spore germination even at 50 ppm concentration for both 12 hours and 24 hours of incubation. Cuman L showed more than 80 per cent inhibition only at 200 ppm after 12 and 24 hours. Vitavax at lower concentration (50 ppm and 100 ppm) showed poor results. Aureofungin and Vitavax were the least effective fungicides noticed in this particular study.

In the field assay of fungicides the average means for per cent of hill infection and disease intensity are presented in tables 12 and 13. The data clearly demonstrated that fungicidal application reduced the intensity of sheath rot disease of rice. Hinosan was found superior in both reducing

the per cent of hill infection and intensity of the disease at a concentration of 0.1 per cent. Vitavax, Dithane Z-78 and Cuman L 0.02%, 0.40%; and 0.12% respectively were found equally effective in reducing the per cent of hill infection. In reducing the disease intensity, Vitavax and Dithane Z-78 were found equally effective as Hinosan, whereas Difolatan was found to be poor in reducing the intensity of the disease. Grain yield was found slightly increased with the application of Dithane Z-78 followed by Hinosan. Regarding straw yield, Difolatan and Fycop have significantly increased the straw yield compared to the other treatments. Statistically Hinosan and Cuman L were also on par with Difolatan and Fycop with regard to increased straw yield. All the other treatments could not enhance the straw yield. However, the increase in grain yield was statistically insignificant.

From a field experiment Chinnaswamy et al.(1977) have observed that Dithane Z-78 was best in reducing the percentage of infection followed by Hinosan, Difolatan, and Bavistin. Another field study under Kerala conditions proved that sheath rot incidence was reduced when Eupadan (an insecticide) was applied along with the fungicide, Bavistin or Hinosan (Anon,1978 c).

Laboratory evaluation of fungicides by Nagunathan and Vijayaraghavan (1976) have also revealed that Hinosan even at very low concentrations (0.05 per cent) could effectively control A.oryzae. It was also reported that Vitavax was found superior in reducing the per cent of hill infection in the case of sheath blight disease of rice (Lakshmanan, 1979). He has also claimed that the treatments with Dithane Z-78 and Hinosan could give increased grain yield in sheath blight affected rice.

The results of the present study and earlier findings indicated that Hinosan, Vitavax and Dithane Z-78 can be recommended for the effective control of sheath rot disease of rice in field. However, large scale recommendations can be made only after detailed critical studies on the correct stage of crop growth, number and frequency of application of those fungicides required for obtaining least incidence of the disease in field.

Effect of microclimate on the incidence and intensity of the disease

The preliminary field observations noted in the present study revealed that sheath rot infection of rice could be

initiated at a temperature round about 30°C with a relative humidity range of 65 to 89 per cent. Under these microclimatical conditions the infection rate was found to increase slowly and attained 21 per cent on 65th day of crop growth.

Studies under artificial conditions proved that, where a higher range of relative humidity and temperature were maintained during the infection stage, comparatively higher percentage of disease incidence also could be detected. Under artificial conditions, within a period of fifteen days of incubation after inoculation, a maximum increase of eleven per cent infection could be observed than the ordinary atmospheric conditions. It was also noticed that larger initial lesions and thereby faster spreading of lesions and prominent infection on panicles could be noticed only under the saturated microclimatical conditions.

Shajahan et al.(1974) found that a hot and humid weather condition could favour the incidence and development of sheath rot disease. But Singh and Raju (1979) had a difference of opinion on this. They reported that maximum disease development was favoured with a minimum temperature range of 17°C to 20°C., and minimum relative humidity range of 40 to 56 at the time of flowering. Sarkar and Gupta (1977) have claimed that

The relative humidity is most important than temperature for disease development in the case of Helminthosporium on rice. According to them the optimum temperature range for disease development was 25 to 30°C.

From the results of the present study and earlier findings it could be inferred that the incidence and development of sheath rot disease of rice was favoured by a hot and humid microclimate.

# SUMMARY

SUMMARY

The symptomatology, etiology and control aspects of sheath rot disease of rice caused by Acrocylindrium oryzae Saw. were studied in detail.

The sheath rot disease of rice initiated on the middle portion of flag leaf sheath as light purplish-brown oblong lesions with light yellow-brown halo, which on maturity turned dark brown with papery white or grey white centres. In severe cases the other sheaths also showed symptoms. In advanced stages the lesions coalesced and covered the entire sheath area. Plants infected early in the flower initiation stage showed severe symptoms at the heading stage. In such cases the choking of whole or part of panicles could be noticed.

The pathogen was isolated from diseased rice varieties and weeds in the field and brought into pure culture on potato dextrose agar. The morphological characters viz., nature of mycelium, conidiophore formation, attachment of conidia and their measurement were studied well for six isolates of the pathogen isolated from four different rice and two field weeds.

Mycelium was septate, profusely branched and purple white in colour. Conidiophore were branched in single or double whorls with 2 to 5 branches in each whorl. The conidia were

single celled, hyaline, cylindrical in shape and borne singly or consecutively at the tip of each branch. No appreciable differences were noticed in the morphological characters of the different isolates of rice varieties. However, the hyphae of the isolate from rice varieties Triveni (A), Jaya (B), Sabari (D) and isolate from field weed namely Echinochloa crusgalli (F) were slightly thicker than those of isolates from Jyothi (C) and Cyperus difformis (E). Similarly the conidium from weed hosts were smaller than those of rice.

The pathogenicity tests showed that the pathogen could easily infect rice plants at boot leaf stage. Symptoms were noticed on the sheath of all the leaves and prominent symptoms were seen on boot leaf only. Successful and uniform infections were noticed when inoculated with either spore suspension or culture bit of the organism. Initial symptoms were noticeable within 4 to 8 days of inoculation but in most cases it took 8 to 12 days to produce characteristic of natural symptoms.

During the investigation a mixed infection of A.oryzae Sav. and Fusarium roseum Link ex Fries was also noticed which was able to produce typical sheath rot symptoms as A.oryzae alone could produce. Artificial inoculation of rice plants with F.roseum alone showed mild symptoms on the sheaths

atypical of sheath rot disease. But combined inoculation with A.oryzae proved that it could influence the incidence and development of sheath rot disease typical to that of A.oryzae alone could produce.

Nine rice varieties were examined for varietal reactions to sheath rot disease. Slight variations were noticed in the colour and size of the initial lesions developed among different varieties. High yielding short duration varieties were found infected little earlier than those of medium duration varieties tested.

Host range studies showed that the fungus could infect a number of common field weeds. Six out of the ten field weeds tested showed positive results namely, Echinochloa crusgalli, Ploucine indica, Monochoria vaginalis, Cyperus difformis, Cyperus iria and Cyperus teneriffae. These weeds were recorded for the first time as hosts of the pathogen.

Regarding the factors favouring the incidence of sheath rot disease, it was noticed that injuries on sheath could favour the easy entry of the fungus. However, the present study revealed that the fungus could infect rice plants with and without any injury.

Studies on the viability of the pathogen showed that the fungus could remain viable in infected paddy straw and grains for about 60 and 120 days respectively after the usual processing and storage of the produce.

Regarding the physiological characters of the sheath rot pathogen, growth and sporulation on different solid and liquid culture media, effect of different levels of temperature and pH, ability of the fungus to produce toxic metabolites etc. were studied.

Richards' agar was found most suitable for radial growth of the fungus followed by Czapeks' agar and potato dextrose agar among the solid media tested. Best sporulation was noticed on PDA. Among the liquid media tested Richards' medium was found best both for growth as well as sporulation followed by Czapeks' and Potato dextrose medium. Optimum temperature range for best growth and sporulation was noticed between 20°C and 30°C. The optimum pH range was between 6.5 and 7.5.

The preliminary studies conducted on the ability of the pathogen to produce toxic metabolites and its role in pathogenesis gave positive results. The culture filtrate obtained

from Czapeks' broth could produce typical sheath rot symptoms when injected behind the sheaths, 120 hours after injection without providing any external injury on the sheath surface.

The studies on certain biochemical changes brought about by the pathogen on artificially inoculated rice plants, variety Co 1veni showed that there was a considerable reduction of both total sugars and total phenolics with reference to the corresponding leaf sheaths of healthy plants of same age.

In the study to control sheath rot fungus, the effect of various prominent fungicides were tested both in laboratory as well as in the fields. Results of the laboratory evaluation showed that, Fycop, Bavistin and Fytolan at various concentrations tested were able to inhibit the complete growth of the fungus. Hinosan, Difolatan, Cuman L and Kitazin were also found moderately effective in the inhibition of growth of the fungus.

Dithane Z-78, Fycop, Bavistin, and Kitazin showed complete inhibition of spore germination of the fungus on glass slides even after 24 hours of incubation. In addition to this, Hinosan, Difolatan and Fytolan also gave moderate results in the inhibition of spore germination.

In the control of sheath rot disease under field conditions the comparative efficacy of six fungicides namely Vitavax, Dithane Z-78, Hinosan, Fycop, Difolatan and Cumen L were tested. The results showed that Hinosan was superior in reducing both per cent of hill infection and intensity of the disease at a concentration of 0.10 per cent. Vitavax, Dithane Z-78 and Cumen L ,0.02%, 0.40% and 0.12% respectively showed equal effect in reducing the hill infection. Vitavax and Dithane Z-78 were found equally effective as Hinosan in reducing the intensity of the disease.

Increased grain yield was recorded for the treatments with Dithane Z-78 followed by Hinosan. Difolatan and Fycop have significantly increased the straw yield. Hinosan and Cumen L was also on par with Difolatan and Fycop in the case of increased straw yield.

The preliminary field observations noted in the present study with respect to the effect of microclimatical factors such as temperature and relative humidity showed that the sheath rot disease of rice could be initiated at temperature round about 30°C with a relative humidity range of 65 to 89. Studies under artificial conditions indicated that a hot and humid microclimate could favour the incidence as well as intensity of sheath rot disease of rice.

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# APPENDICES

## APPENDICES I

### Composition of media used

#### Potato dextrose agar medium

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

#### Czapeks' agar medium

| | | |
|---|---|---|
| $MgSO_4 \cdot 7H_2O$ | - | 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 |

#### Richards' agar medium

| | | |
|---|---|---|
| $KNO_3$ | - | 10.00g |
| $NH_2PO_4$ | - | 5.00 g |
| $MgSO_4$ | - | 0.50 g |
| NaCl | - | 100.00 mg |
| $CaCl_2$ | - | 130.00 mg |
| Sucrose | - | 30.00 g |

Coon's agar medium

| | |
|---|---|
| $MgSO_4 \cdot 7H_2O$ | - 1.23 g |
| Sucrose | - 7.20 g |
| Dextrose | - 3.60 g |
| $KNO_3$ | - 2.20 g |
| Agar agar | - 20.00 g |
| Pot.acid phosphate | - 2.72 g |
| Distilled water | - 1000 ml |

Host leaf extract medium

| | |
|---|---|
| Host leaves | - 200.00 g |
| Distilled water | -1000 ml |

Source: Source book of laboratory exercises in Plant Pathology, Source book committee of the American Phytopathological Society, pp. 366-368.

## APPENDIX II

### Analysis of variance table

Growth of *Acrocylindrium oryzae* on different solid media

| Source | Sum of squares | df | M.S. | F | Whether significant or not |
|---|---|---|---|---|---|
| Total | 3924.02 | 19 | .. | .. | .. |
| Treatment | 3671.24 | 3 | 1223.75 | 77.46 | Significant |
| Error | 252.78 | 16 | 15.798 | .. | |

C.D. = 5.33

| $T_3$ | $T_2$ | $T_1$ | $T_4$ |
|---|---|---|---|
| 92.4 | 83.6 | 77.2 | 55.7 |

APPENDIX III

Analysis of variance table

Growth of *Acrocylindrium oryzae* on liquid media

| Source | Sum of squares | df | M.S. | F | Whether significant or not |
|---|---|---|---|---|---|
| Total | 2370394.00 | 24 | .. | .. | .. |
| Treatment | 2280654.00 | 4 | 570163.50 | 127.07 | Significant |
| Error | 89740.00 | 20 | 4487.00 | .. | .. |

C.D. = 89.36

| $T_3$ | $T_2$ | $T_1$ | $T_5$ | $T_4$ |
|---|---|---|---|---|
| 1729 | 1168 | 1012 | 937 | 913 |

APPENDIX IV

Analysis of variance table

Effect of temperature on growth of the fungus

| Source | Sum of squares | df | M.S. | F. | Whether significant or not |
|---|---|---|---|---|---|
| Total | 23.64 | 19 | .. | .. | .. |
| Treatment | 17.46 | 4 | 4.365 | 10.59 | Significant |
| Error | 6.18 | 15 | 0.412 | .. | .. |

C.D. 0.97

| $T_4$ | $T_3$ | $T_2$ | $T_5$ | $T_1$ |
|---|---|---|---|---|
| 8.32 | 6.90 | 6.56 | 6.13 | 5.54 |

APPENDIX V

Analysis of variance table

Effect of pH on growth of the fungus

| Source | Sum of squares | df | M.S. | F | Whether significant or not |
|---|---|---|---|---|---|
| Total | 17.46 | 15 | .. | .. | .. |
| Treatment | 15.15 | 3 | 5.05 | 26.57 | Significant |
| Error | 2.31 | 12 | 0.19 | .. | .. |

C.D. = 0.65

| $T_2$ | $T_3$ | $T_4$ | $T_1$ |
|---|---|---|---|
| 8.37 | 7.35 | 6.53 | 5.75 |

APPENDIX VI

Analysis of variance table

Laboratory evaluation of fungicides against *Acrocylindrium oryzae*

| Source | Sum of squares | df | M.S. | F | Whether significant or not |
|---|---|---|---|---|---|
| Total | 635.06 | 92 | .. | .. | .. |
| Replication | 0.30 | 2 | .. | .. | .. |
| Treatment | 632.28 | 30 | 21.07 | 526.75 | Significant |
| Error | 2.48 | 60 | 0.04 | .. | .. |

C.D. = 0.37

## APPENDIX VII

### Analysis of variance table

Comparative efficacy of different fungicides on per cent of hill infection after angular transformation

| Source | Sum of squares | df | M.S. | F | Whether significant or not |
|---|---|---|---|---|---|
| Total | 911.270 | 27 | .. | .. | .. |
| Block | 22.520 | 3 | 7.506 | 1.12 | .. |
| Treatment | 768.509 | 6 | 128.084 | 19.17 | Significant |
| Error | 120.241 | 18 | 6.68 | .. | .. |

C.D. = 3.84

| $T_3$ | $T_1$ | $T_2$ | $T_6$ | $T_5$ | $T_4$ | $T_7$ |
|---|---|---|---|---|---|---|
| 12.93 | 17.38 | 20.15 | 21.00 | 25.91 | 26.19 | 29.17 |

## APPENDIX VIII
### Analysis of variance table

Comparative efficacy of different fungicides on disease intensity of sheath rot of rice

| Source | Sum of squares | df | M.S. | F | Whether significant or not |
|---|---|---|---|---|---|
| Total | 13.425 | 27 | .. | .. | .. |
| Block | 2.532 | 3 | 0.844 | 3.907 | .. |
| Treatment | 7.004 | 6 | 1.167 | 5.402 | Significant |
| Error | 3.889 | 18 | 0.216 | .. | .. |

C.D = 0.69

| $T_0$ | $T_1$ | $T_2$ | $T_6$ | $T_5$ | $T_4$ | $T_7$ |
|---|---|---|---|---|---|---|
| 2.450 | 2.305 | 2.365 | 3.066 | 3.129 | 3.315 | 3.48 |

APPENDIX IX

Analysis of variance table

Comparative efficacy of different fungicides on grain yield

| Source | Sum of squares | df | M.S. | F | Whether significant or not |
|---|---|---|---|---|---|
| Total | 3011974.703 | 27 | .. | .. | .. |
| Block | 873124.076 | 3 | 291041.358 | 3.706 | .. |
| Treatment | 725368.91 | 6 | 120894.82 | 1.54 | Not significant |
| Error | 1413481.71 | 18 | 78526.76 | .. | .. |

APPENDIX X

Analysis of variance table

Comparative efficacy of different fungicides on straw yield

| Source | Sum of squares | df | M.S. | F | Whether significant or not |
|---|---|---|---|---|---|
| Total | 882302.165 | 27 | .. | .. | .. |
| Block | 64074.88 | 3 | 21358.29 | 0.843 | .. |
| Treatment | 361934.42 | 6 | 60322.40 | 2.379 | Significant |
| Error | 456292.87 | 18 | 25349.60 | .. | .. |

C.D. = 236.54

| $T_5$ | $T_4$ | $T_3$ | $T_6$ | $T_1$ | $T_7$ | $T_2$ |
|---|---|---|---|---|---|---|
| 2039.60 | 1976.84 | 1881.47 | 1862.96 | 1791.66 | 1737.95 | 1701.85 |

# SYMPTOMATOLOGY ETIOLOGY AND CONTROL OF SHEATH ROT DISEASE OF RICE CAUSED BY *Acrocylindrium oryzae*

BY

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ABSTRACT OF A THESIS
SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENT FOR THE DEGREE
**MASTER OF SCIENCE IN AGRICULTURE**
FACULTY OF AGRICULTURE
KERALA AGRICULTURAL UNIVERSITY

DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE, VELLAYANI
TRIVANDRUM

**1981**

## ABSTRACT

Sheath rot disease of rice caused by Acrocylindrium oryzae Saw. was investigated. The fungus was found to infect rice plants at boot leaf stage. The leaf sheath covering the panicle was found to be comparatively more susceptible to the fungus than the sheath of other leaves. Severe infection caused choking of the whole panicle inside the sheath itself before emergence.

The pathogen was isolated from infected tissues of host plants and brought into pure culture. Comparative studies of six isolates of Acrocylindrium oryzae from four rice varieties and two weed hosts did not show much appreciable difference in their morphological characters except slight variations in the hyphal thickness and smaller conidial size from those on weed hosts.

Pathogenicity tests conducted with either spore suspension or culture bits showed that the pathogen could easily infect rice plants at boot leaf stage. Eventhough it could invade all the leaf sheaths, prominent symptom was noticed on boot leaf sheath only.

A mixed infection of Acrocylindrium oryzae Saw., and Fusarium roseum Link. ex Fries was also observed in rice during the investigation. The symptom observed was typical to that

of Acrocylindrium oryzae alone could produce. Artificial inoculation studies revealed that Fusarium roseum alone was not able to produce typical sheath rot symptoms on rice. But along with Acrocylindrium oryzae it could influence the infection and symptom development processes.

Studies on varietal reactions to sheath rot disease with nine varieties showed that in general high yielding short duration varieties were infected by the organism earlier to that of medium duration varieties tested. No other appreciable variations could be noticed between varieties on symptom expression except slight differences in colour and size of initial lesions developed.

Host range studies of the causal organism showed that six out of ten field weeds tested were effective weed hosts of the fungus. They were Echinochloa crusgalli, Eleusine indica, Monochoria vaginalis, Cyperus iria, C.difformis and C.teneriffae. These plants were the first record of Acrocylindrium oryzae as host plants.

Present study showed that the fungus can survive in paddy straw and grains upto 60 and 120 days respectively under ordinary conditions.

Richards' medium was found best for the growth of the fungus, followed by Czapeks' and potato dextrose medium in the case of both solid and liquid media tested. Best sporulation was obtained in potato dextrose agar and Richards' medium among solid and liquid media respectively. A temperature range of 20-30°C and a pH range of 6.5 to 7.5 were found optimum for best growth and sporulation of the fungus.

The preliminary studies conducted showed that the fungus was able to produce toxic metabolites which play a role in the pathogenesis of the sheath rot disease.

A comparative analysis of infected and healthy leaf sheaths of the rice variety Triveni showed a considerable reduction in both total sugars and phenolics.

Laboratory evaluation of fungicides showed that Fycop, Bavistin and Fytolan at various concentrations tested could inhibit complete growth of the pathogen. Hinosan, Difolatan, Cumen L, and Kitazin were found moderately effective in inhibiting the <u>in vitro</u> growth of the fungus.

Dithane Z-78, Fycop, Bavistin and Kitazin could inhibit complete spore germination on glass slides even after 24 hours of incubation.

Hinosan, Difolatan and Fytolan gave moderate results in inhibition of spore germination.

Under field conditions Hinosan at 0.1 per cent concentration followed by Vitavax 0.02 per cent and Dithane Z-78 0.4 per cent were found effective in controlling sheath rot disease of rice.

Preliminary studies on the microclimatological relations with the disease incidence, showed that a hot humid microclimate during the boot leaf stage of paddy crop could favour the disease development.