STUDIES ON THE SEED BORNE DISEASES OF RICE IN KUTTANADU

By

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Thesis

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D E C L A R A T I O N

I hereby declare that this thesis entitled "Studies on the seed borne diseases of rice in Kuttanadu" 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.

(JOSE JOSEPH.)

Vellayani,

1986.

C E R T I F I C A T E

Certified that this thesis entitled "Studies on the seed borne disease of rice in Kuttanadu" is a record of research work done independently by Sri. JOSE JOSEPH, B.Sc.(Ag.) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to him.

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INTRODUCTION

INTRODUCTION

Kuttanadu, the rice bowl of Kerala is a unique agroclimatic tract comprising of the deltaic formation of four major river systems. This area is generally situated 1 - 2 metres below MSL and is submerged under water for a considerable period of the year. Indiscriminate introduction and local multiplication of different varieties of rice is a common practice noticed in this region. Such practices often lead to the introduction of a multitude of diseases in rice also.

It has been estimated that up to 10 per cent loss is caused annually due to seed borne diseases alone in Rice. Vydhyasekaran and Ramadoss (1974) recorded severe seed infection in the cultivar IR-5 resulting in quantitative yield losses of 20 - 40 per cent in different parts of Tamil Nadu. The seed borne nature of the different diseases of rice in Kerala has not been investigated so far.

Seed borne fungi in general cause germination failure, seedling blights, rots and also diseases in adult plants (Christensen and Kauffman, 1965). Besides their spreading to newer localities. The metabolic products of seed borne microorganism also have serious consequences on the health of human and animal beings (Brook and White, 1966).

Determination of the associated microorganisms and the damages caused on seed viability, seed germination, establishment of seedlings, etc. is highly imperative to understand the extent of damage caused by seed infection. Appropriate corrective or protective measures can be adopted only in the light of these information.

Many plant pathogens are disseminated through seeds and the effective control measures include exclusion through seed certification and seed treatment. Seed treatment is probably the cheapest and often the safest method of direct plant disease control. The traditionally used organo-mercurials have been discontinued in view of their build up in soil and plant systems. They are largely replaced by various systemic fungicides like carboxin, benomyl, etc.

Choice of the type and concentration of the chemicals to be used for this purpose is also an important aspect to be investigated.

In the light of the above, the present investigation viz. "Studies on the seed borne diseases of rice in Kuttanadu" was undertaken with the following main objectives.

1. Isolation and identification of seed borne pathogens associated with the commonly cultivated varieties of rice in Kuttanadu.

2. Assessment of the extent of damage caused on seed germination and seedling vigour.

3. Effect of culture filtrate on seedling vigour.

4. Biochemical changes due to seed infection.

5. Effect of seed treatment chemicals in controlling infection.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Isolation of Seed-borne Microorganisms.

Several fungi have been reported to be associated with rice seeds all over the world. Sharangapani (1930) reported the scorching of grain coats and the association of a species of Helminthosporium. Suzuki (1930) suggested the possibility of primary infection of Pyricularia oryzae and Ophiobolus miyabeanus in rice seeds. Ganguly (1946) reported the seed-borne nature of Helminthosporium oryzae in rice.

Padmanabhan (1949) isolated Trichoconis padwickii from rice grains and found that the pathogen was internally seed-borne. He also isolated Curvularia lunata, Ophiobolus miyabeanus and Nigrospora oryzae from rice seeds from Cuttack. Machacek et al. (1951) reported different genera of fungi from rice seed and found Alternaria spp., Helminthosporium spp. and Fusarium spp. to be internally seed-borne. Del Prado and Christensen (1952) observed the association of Aspergillus glaucus, A. niger, Penicillium sp., Alternaria terreus, Fusarium sp., Hormodendrum sp. and Curvularia sp. in stored rice seeds. Bugnicourt (1952) isolated many fungi like Helminthosporium, Curvularia and many species of Fusarium such as F. decemecellulae, F. kuehnii, F. moniliforme and and F. scripi from rice seeds.

Rao and Salam (1954) in their studies on rice grain discoloration found that 60 per cent of the darkened grains were infected by Curvularia spp. viz., C. geniculata, C. affinis and C. specifera. Hirayama and Udagawa (1958) reported different species of Aspergillus viz., A. flavus, A. candidus, A. glaucus, A. versicolor, A. terreus and A. pheenicis from the rice seeds from Japan.

Baldacci and Corbetta (1964) reported the principal fungi infecting stored rice grains to be Epicoccum spp., Alternaria spp., Helminthosporium oryzae, Penicillium spp., Fusarium spp., Aspergillus sp. and Pyricularia oryzae. They also suggested that the extent of infection was determined by the storage conditions. Soraya (1964) suggested surface sterilization of rice seeds for detection of internal infection by P. oryzae and H. oryzae. Pavgi et al. (1966) reported that Trichoconis indica and Myrothecium indicum along with other common fungi were associated with rice seeds collected from Varanasi.

Hasany et al. (1968) isolated several species of Aspergillus from different varieties of rice. Aspergillus flavus was recorded as the most prevalent followed by A. niger, A. sydowi, A. tamarii, A. versicolor and A. candidus. Other fungi isolated included Penicillium spp., Rhizopus nigricans (R. stolonifer), Actinomucor sp., Circinella sp., Mucor mucedo and Phaeoramularia sp. Benoit and Mathus (1970) isolated

several species of Curvularia viz., C. siddiquii, C. oryzae, C. lunata, C. pallescens, C. trifolii, C. elavata, C. geneculata, C. inaequalis, C. uncinata and C. cymbopagonis from rice seeds by standard blotter method.

Nath et al. (1970) observed that in three rice seed lots in the blotter test, counts for Fusarium moniliforme increased considerably when the incubation period was increased. They also recorded Drechslera longirostrata for the first time on rice seeds by blotter method. Addison (1971) isolated 21 species of fungi from rice seeds in Ghana using blotter method at room temperature (26 - 28°C). Agarwal and Singh (1974) compared the percentage incidence of seed-borne fungi in different varieties of rice by blotter technique and several species of fungi were isolated. Trichoconis padwickii was the most common, followed by C. lunata, F. moniliforme, F. semitectum and Trichothecium spp. Lau and Sheridan (1975) recorded Alternaria alternata, Curvularia state of Cochliobolus lunata, Curvularia trifolii, Drechslera australiensis, D. hawaiiensis, Drechslera state of Cochliobolus bicolor, Epicoccum purpurascens, Nigrospora state of Khuskia oryzae, Pithomyces chartarum, Fusarium spp. and Alternaria padwickii from stored rice sees in Newzealand.

Furlani et al. (1975) reported the presence of Helminthosporium, Curvularia and Nigrospora spp. in the

descending order on the spotted panicles from Brazil. Esuruoso et al. (1975) in their studies on rice seeds by blotter method over three years, observed the seed-borne nature of fungi like D. oryzae, P. oryzae and T. padwickii in Nigeria. Presence of P. oryzae on seed was limited and infection was generally low. A survey conducted by Zainun and Nik (1977) on the seed-borne fungi of rice in Malaysia, revealed the presence of 33 fungal isolates. The most common pathogens were T. padwickii, D. oryzae, F. moniliforme, N. oryzae and P. oryzae. Mittal and Sharma (1978) reported that Stachybotrys lobulata was externally seed-borne while Mycogone nigra, Memnoniella echinata and Stemphylium botryosum were internally seed-borne in rice.

Sharm and Siddiqui (1979) recorded for the first time Drechslera holmi and F. camptoceras on rice seeds obtained from Assam and West Bengal. Azim and Khalil (1979) detected the predominance of Aspergillus spp. on the discoloured grains from Egypt. Supriaman and Palmer (1979) surveyed 133 samples of rice seeds in Indonasia and recorded several fungi associated with them; T. padwickii, D. oryzae, C. lunata and F. semitectum were the most common. Sanchez et al. (1979) detected Rynchosporium oryzae in paddy seeds collected from naturally infected fields in Philippines. The pathogen survived for seven months in storage at 20 - 30°C.

Richardson (1979) gave an annotated list of rice seed-borne fungi, which included Sarocladium oryzae, Alternaria longissima, Ascochyta oryzae, Brachysporium spp., Diplodia oryzae, Drechslera halodes, D. neergaardii, Giberella zea, Helicoceras spp., Hendersonia oryzae, Magnaporthe salvinii, Melanoma glumarum, Monascus sp., Mycosphaerella sp., Ophiobolus oryzinis and Sclerotium rolfsii in addition to the organisms reported earlier. Reddy and Khare (1979) from a study in Madhya Pradesh reported that, D. oryzae and A. padwickii were commonly associated with rice samples and both were externally as well as internally seed-borne. Ranganathiah et al. (1979) detected seed-borne infection of rice by P. oryzae in Karnataka State by blotter method. Kuthubutheen (1979) recorded frequent association of Aspergillus fumigatus from six rice cultivars.

Ribeiro (1980) found that the washings of the seeds ~~was~~ were better than the filter paper method for detecting of Pyricularia oryzae infection, while filter paper method was better for the detection of Helminthosporium oryzae from rice seeds. Arunganart et al. (1981) found that several fungi including Sarocladium oryzae and Cercospora oryzae associated with rice seed caused seed discoloration. Acrocylindrium (Sarocladium) oryzae was reported to be externally and internally seed-borne by Mohan and Subramonian (1981). Singh et al. (1982) detected 75 per cent infection of rice seeds by Alternaria padwickii

in modified blotter method. Caratelli and Saponaro (1982) isolated D. oryzae, P. oryzae, A. padwickii and Curvularia spp. from rice seeds in Brazil, while Zakeri and Zad (1983) reported internal seed infection by P. oryzae, C. lunata, C. pallescense, T. padwickii, Cochliobolus miyabeanus and Nigrospora oryzae from Iran. The first four were isolated from the endosperm and the latter two along with P. oryzae from the rice embryo.

Damages due to Seed-borne Fungi.

The results of the earlier experiments by Thomas (1941) on the influence of infected seed on development of Helmintho-sporium disease in rice seedlings proved the importance of seed-borne inoculum. He obtained a reasonable amount of infection on seedlings. When artificially infected seeds were sown in sand, the percentage of germination was affected. The post emergence mortality of the seedlings was also more. Mead (1942) reported different degrees of infection on cereal seedlings due to seed-borne fungi. Padwick and Ganguly (1945) detected severe germination failure in paddy seeds infected with H. oryzae, C. lunata and T. caudata while Padmanabhan et al. (1948), recorded loss in weight and germination failure of paddy seeds infected with H. oryzae.

Hingorani and Prasad (1951) demonstrated blight and leaf spot symptoms in rice seedlings raised from seeds inoculated with C. lunata and Helminthosporium spp. up to 80 and 70 per cent respectively, while there was no infection in seedlings raised from uninoculated controls. Govindaswamy (1955) reported the adverse effects of Helminthosporium and Fusarium species on the germination and seedling emergence of paddy seeds infected with them, while Curvularia and Chaetomium spp. isolated from paddy seeds did not cause much damage.

Abi Cheeran and Samraj (1965) reported germination failure and seedling loss due to seed infection by Trichoconis padwickii, while Ibrahim and Forag (1966) reported damping off and stunting of rice seedlings due to the association of Alternaria tenuis, Aspergillus ustus, F. lateritium, F. oxysporum and F. solani with rice seeds. According to Aguicro et al. (1966) germination failure, rotting of root and coleoptile as well as stem and seedling blight of rice were due to seed-borne fungi like F. moniliforme, C. lunata, Penicillium spp., T. padwickii, H. oryzae and N. oryzae. Fanse and Christensen (1966) observed a reduction in the germination capacity of grains due to infection by fungi after eight months of storage.

Christensen (1967) reported that Aspergillus spp. under some circumstances were responsible for germination failure of grain seeds. Vidhyasekaran et al. (1970) studied the effect of A. flavus, C. pallescens, C. lunata and F. moniliforme on the germination of rice seeds and found a reduction of 24 - 67 per cent. Teplyakova and Dudenko (1970) reported that 32 per cent of the fungi isolated from rice, inhibited seed germination. Chantarasnit (1971) also reported germination failure of rice seeds due to infection by Phoma indianensis, P. glomerata and P. julavana in Ghana, India, Nigeria, Philippines and Thailand. Mathur et al. (1972) observed poor germination in rice seeds infected with T. padwickii along with seed, root and coleoptile rot, and ultimate death of the germinated seedlings. Rath (1974) demonstrated significant decrease in the germination of rice seeds of five cultivars due to infection by D. oryzae.

Danquah et al. (1976) recorded significant germination failure in discolored paddy seeds. Hirrera and Seidel (1978) recorded that rice seed infection by Cochliobolus miyabeanus (H. oryzae) decreased germination by 66 per cent and caused 40 per cent seedling damping off in Cuba. Bilgrami et al. (1979) pointed out that in paddy, D. oryzae, F. moniliforme and T. padwickii mainly showed characteristic symptoms on the seedlings. Ashokan et al. (1979) reported that Helminthosporium spp., Curvularia spp. and Fusarium spp. were inhibitory on

rice seed germination and led to post emergence mortality. Positive correlation between the seed infection and consequent field damage were reported in several cereal crops by workers like Semenov (1980) and Mathur (1980). Rajan (1981) reported loss in weight of paddy seeds infected with Corticium sasakii and Acrocylindrium oryzae. Zakeri and Zad (1981) pointed out that paddy seeds infected with P. oryzae produced abnormal seedlings while Bernaux (1981) observed mycelium and spores of P. oryzae and D. oryzae on the glume and caryopsis of paddy seeds during germination. Necrosis was noticed on coleoptile and leaf sheath. Martin and Johnston (1982) reported that Fusarium infection on cereal seeds generally affected the germination and seedling vigour, while Inolehin (1983) could not obtain any correlation between seed infection and germination failure in paddy for many other fungi. The fungi tried were H. oryzae, F. moniliforme, Penicillium spp., C. lunata, Aspergillus spp. and Alternaria spp. Vidhyasekaran et al. (1984) recorded reduced grain weight and germination failure in seedlings due to infection by Sarocladium oryzae. Ramadoss (1985) gave indications for possible varietal preferences of different fungi to seed infection and further damage.

Yoshi (1950) studied the effect of metabolites of Fusarium moniliforme on cereal seedlings and reported

different levels of reaction by the seedlings. Brian et al. (1952) during the course of investigation on the phytotoxic properties of the fungal metabolites, reported the production of some toxins which were harmful to seeds even at very low concentrations. Root and shoot elongation of rice seedlings were recorded by Venkataram (1956) due to reaction with the metabolites of fungi like Fusarium spp. while, Ludwig (1957) demonstrated the growth inhibitory property of culture filtrates of Helminthosporium sp. in common cereals.

Vidhyasekaran et al. (1970) suggested that toxin produced by different seed-borne fungi inhibited root length of rice seedlings to a marked extent. Nair (1969) reported inhibition of germination of rice seeds by the culture filtrate of Alternaria padwickii. Bhale et al. (1982) gave a detailed account of the inhibitory effect of culture filtrate of T. padwickii on rice during the course of their studies on the effect of seed-borne fungal metabolites on seeds. They obtained indications for the killing of plumule by the fungal metabolites resulting in germination failure. Ramadoss (1985) reported that the toxic fraction of three fungi namely D. oryzae, A. padwickii and C. lunata induced grain discoloration and significantly inhibited seed germination.

Biochemical changes due to Fingal infection.

a. Changes in Starch content.

The starch content of cereal seeds has been reported to decrease due to fungal infection; Allen (1942) and Mirocha and Zaki (1966) reported reduction in starch content of cereal seeds due to microbial infection. Reduction in starch content followed by an increase in the content of reducing sugars due to infection by fungi was reported by Vidhyasekaran and Govindaswamy (1968). Vidhyasekaran and Kandaswamy (1972) have reported a reduction in starch content of many crop seeds infected with fungi, even at an increased level of prophylase activity. Vidhyasekaran and Ramadoss (1973) also reported a decrease in starch as well as soluble sugar content of Helminthosporium infected rice grains. Bilgrami et al. (1979) found a general reduction in starch content of rice seeds due to fungal association, accompanied by a rise in the total sugars, indicating the possible hydrolysis of starch.

b. Changes in Protein content.

Changes in the protein content of cereal seeds due to fungal infection have been investigated by several workers. Vidhyasekaran and Ramadoss (1973) and Vidhyasekaran et al.(1973)

recorded a slight increase in the protein content of rice seeds due to Helminthosporium infection. Oblisami et al. (1972) recorded increased amino nitrogen content of rice seeds due to infection by Aspergillus sp., Penicillium sp., Fusarium sp., Cladosporium sp., Alternaria sp. and Helminthosporium spp. Bilgrami et al. (1979) also reported variations in the protein level of fungus infected rice seeds.

c. Changes in Amino acid content.

The changes in the amino acid content of seeds infected with fungus have been reported by several workers. Vidhyasekaran et al. (1973) reported that amino acids like lysine, leucine and isoleucine decreased in rice seeds due to infection by Helminthosporiose. Eventhough the content of some amino acids showed an increase, there was a slight decrease in the total amino acid content. Bilgrami et al. (1979) reported that amino acids either increased or decreased or disappeared in fungal infected rice seeds, depending upon the organism, time of storage, etc. Ramadoss (1985) found that levels of amino nitrogen, phenolics and amino acids like lysine, glycine and tryptophan increased with increase in the levels of rice grain contamination by fungi, while, levels of amino acids like alanine, serine, aspartic acid and threonine tended to decrease.

Effect of Seed treatment Chemicals in controlling Infection.

Cereal seed treatment with systemic fungicides like vitavax, benlate, plant vax, etc. were tried successfully by Nene et al. (1971). Marsh (1972) reported that benomyl at 400 g per 100 kg of seed gave almost complete control of blast disease during the seedling stage of rice. He also reported that carboxin in a formulated mixture with thiram increased stand counts of rice infected with several other seed-borne fungi.

Bidari et al. (1978) found that benomyl had significant effect on cereal seedling vigour. Sherstyanykh and Lukyanchikov (1978) noted that pre-sowing seed treatment with many fungicides was very effective against the seed-borne disease of rice caused by F. oxysporum, H. oryzae and Alternaria oryzae.

Hampton (1979) observed an increase in germination percentage and viability of cereal seeds treated with carboxin and thiram in Newzealand. Paddy seeds treatment with systemic fungicides like benomyl were found to be effective against blast in nurseries by Bandong et al. (1979). Based on in vitro tests with eight fungicides against Trichoconis padwickii, Kumaraswamy (1979) reported that

bavistin completely inhibited growth and spore germination at 0.1 per cent while benlate and dithane M-45 were inhibitory at 0.2 per cent only. Cereal seed treatment with many other systemic fungicides was successfully attempted by workers like Moore et al. (1979) and Hampel and Saur (1979).

Kannaiyan and Prasad (1980) in tests for control of seed-borne Rhizoctonia solani in rice, reported that seed treatment with benomyl and carboxin increased germination while oxycarboxin, carboxin and benomyl increased shoot growth significantly. Root growth was increased by carbendazim, oxycarboxin and benomyl at 0.2 per cent. They also found a 90 per cent increase in the viability of the rice seeds treated with oxycarboxin and MEMC during storage for eight months. Dharam Vir (1980) reported that when systemic fungicides like bavistin, benlate and vitavax were used for rice seed treatment they remained biologically active and retained residual efficacy when tested after one year of storage. Storage of treated seeds did not adversely affect subsequent germination. Ashokan et al. (1980) reported that rice seeds treated with vitavax gave better germination even after periods of long storage. Esuruoso and Joaquiim (1980) recorded reduction in seed infection by Drechslera oryzae and Curvularia spp. in Nigeria, by treating the rice seeds

with benlate and dithane M-45. Pandy *et al.* (1981) found that most of the seed microflora were reduced by seed treatment with fungicides like dithane M-45 while, benomyl and captafol were less effective eventhough all treatments were reported to increase seed germination. According to Singh *et al.* (1982) cereal seed treatment with carbendazim and thiram at 0.25 per cent gave excellent protection against *Aspergillus* and *Penicillium* spp. while carbendazim alone was effective for only 30 days. Seed treatment of paddy with benomyl at 0.04 per cent was reported to have given almost complete control of blast (*Pyricularia oryzae*) by Vyas (1984) also.

MATERIALS AND METHODS

MATERIALS AND METHODS

Collection of Rice seeds.

The seeds used for the present investigations were collected during the main crop season (November-December to March-April) and additional crop season (April-May to September-October of 1984-85) from different parts of Kuttanadu namely, Edathua, Moncombu, Kidangara, Pulinkunnu, Kavalam and Champakulam in Alleppey District.

Seeds of the following varieties of paddy were used in the present study.

1. Athikrazhy (local cultivar)
2. Bharathy
3. Cheruvally (local cultivar)
4. Jaya
5. Jyothi
6. Karthika
7. MO-4
8. MO-7
9. M-329 (Pre-release culture)
10. M-310 (Pre-release culture)
11. Njavaravithu (local culture)
12. Pavizham.

Isolation of Seed-borne fungi.

Seed-borne fungi were isolated from all the 12 varieties using standard methods (Annon., 1966) with slight modifications as indicated below.

Seed samples were taken from the freshly harvested dried and winnowed seed heaps following the standard sampling procedure. From each sample 400 seeds were taken at random for isolating the microorganisms. Isolation was done within 12 days after collection.

Externally seed-borne microorganisms were isolated by plating the seeds without surface sterilization on a thin layer of Potato dextrose agar medium in sterilized petri dishes. The seeds were arranged at equal distances on the medium at the rate of ten seeds per dish. The petri dishes were incubated at room temperature ($28^{o}C \pm 1$) for seven days in alternating cycles of 12 hours darkness and light.

Isolation of internally seed-borne fungi was done following the modified blotter method as well as endosperm plating method of Ngala (1983) and Vidhyasekaran (1980) respectively. In the blotter technique the seeds were surface sterilized in 0.1 per cent mercuric chloride for three minutes and washed in three changes of sterile distilled water.

Twenty seeds were then placed at equal distances on a two layered blotter kept in sterile petri dishes and moistened with sterile water. The plates were then incubated for seven days at room temperature as in the previous case.

In the endosperm plating method, the seeds were surface sterilized with 0.1 per cent mercuric chloride for three minutes and washed in three changes of sterile distilled water and dehusked aseptically. The endosperm was cut into pieces and plated on a thin layer of Potato dextrose agar medium in sterile petri dishes at the rate of 20 cut pieces per dish and incubated for seven days at room temperature as in the previous case.

In all the above experiments the plates were examined regularly. The cultures were purified by single spore isolation and maintained on Potato dextrose agar slants. The cultures were identified through the courtsey of Commonwealth Mycological Institute, London.

Damages due to Seed-borne Fungi.

a. Effect of Inoculum on Germination and Post emergence vigour.

The seeds of four popular varieties of rice namely, Pavizham, Jyothi, Jaya and Karthika were used for the study. Five hundred seeds from each variety were divided into five

lots of 100 seeds each and surface sterilized by 0.1 per cent mercuric chloride. Each lot was inoculated separately with the following isolates grown on Czapeks dox agar (Appendix I) using the procedure of Ashokan et al. (1979).

Ten culture discs of eight mm diameter cut with a sterile cork borer from the growing edge of a five day old culture on PDA medium were mixed with the seeds in 100 ml sterile water and shaken continuously for 10 minutes on a mechanical shaker. The seeds were then taken out and dried thoroughly under shade to remove excess moisture and kept in sterilized glass containers following the procedure of Vidhyasekaran et al. (1970). Surface sterilized uninoculated lots treated with sterile water and shade dried as in the previous case served as control. Two replications were maintained for each treatment. After 45 days of storage the inoculated rice seeds were sown in sterile petri dishes with blotters at the rate of 25 seeds per dish. The seeds were then incubated for seven days at room temperature. Three replications were maintained for each treatment. Surface sterilized and uninoculated seeds treated in a similar manner served as control. The germination count was taken on the third day and the percentage of shoot and root rot were recorded on the seventh day. The seedling vigour as indicated by the length of shoot and root was also assessed on the same day.

b. Effect of culture filtrates on Seeds.

The study was carried out following the method of Vidhyasekaran et al. (1970) with slight modifications. Five selected isolates namely Aspergillus flavus, Alternaria padwickii, Bipolaris oryzae, Curvularia lunata and Sarocladium oryzae were used. Conical flasks of 250 ml capacity each containing 100 ml of Czapekdox liquid medium were then inoculated with three discs each (8 mm diameter) of seven days old cultures grown on potato dextrose agar medium. The cultures were incubated for 30 days at room temperature. These cultures were then filtered through sintered glass filter and the filtrate thus obtained was used for further studies.

Four hundred seeds each of four varieties namely Jaya, Jyothi, Karthika and Pavizham were used. Two replications of 200 seeds were maintained for each treatment. They were surface sterilized as in the previous experiments. Two hundred seeds were soaked in 80 ml of culture filtrate for 24 hours and then 25 seeds each were spread on a blotter in a sterile petri dish moistened with 5 ml of the filtrate. Healthy, surface sterilized seeds of each variety soaked in sterile Czapek Dox solution served as the control. After seven days of incubation at room temperature ($28^{\circ}C \pm 1$) the percentage of germination and inhibition of root initiation along with root and shoot length were recorded.

Estimation of Bio-chemical changes in Infected seeds.

Four isolates of fungi, namely, Aspergillus flavus, Bipolaris oryzae, Alternaria padwickii and Sarocladium oryzae were used for the study. The four varieties included Pavizhum, Jyothi, Jaya and Karthika. The seeds were inoculated by the method detailed earlier and stored for 45 days. Uninoculated, surface sterilized and apparently healthy seeds served as control. Changes in the starch, protein and total free amino acid content in each sample were estimated.

i. Estimation of Starch.

The seeds were powdered finely after drying in an air oven at 65 ± 5°C for two hours. The contents were sieved through a 0.5 mm sieve and the powder was kept in air tight glass bottles. Two grams of the seed samples were then digested with a dilute solution of hydrochloric acid in a waterbath. The hydrolysed solution was then filtered, made up to a volume and titrated against Fehling's solution and the content of starch was calculated (Piper, 1950). Samples from uninoculated seeds served as control. Two replications were maintained for each treatment.

ii. Estimation of Protein.

The inoculated as well as uninoculated seed samples were dried at 65 ± 5°C for two hours in an air oven and ground well.

The powdered samples were digested with concentrated sulphuric acid and the nitrogen content was estimated by the modified micro-kjeldahl method (Jackson, 1967). Two replications were maintained for each treatment. Samples from uninoculated seeds served as control. The protein content was calculated by multiplying the nitrogen percentage by the factor 6.25.

iii. Estimation of Total Free Amino acids.

The total free amino acids in the seed samples were estimated by the method of Moore and Stein (1948).

A five per cent TCA (Trichloro acetic acid) extract of the seed samples was prepared, centrifuged at 3000 rpm for 30 minutes and the supernatant was collected. Residue was extracted with another aliquot of five per cent TCA and centrifuged. The supernatants were pooled together and made up to 50 ml. One millilitre aliquots were taken from this and neutralized with 0.05 N sodium hydroxide. To this one millilitre of Ninhydrin reagent was added and kept in a boiling waterbath for 20 minutes. After colour development five millilitre of diluent solvent (1 : 1 n-propanol : water) was added and the optical density was measured at 570 m/u in a spectrophotometer against a blank of diluent solvent. The total free amino acids were estimated with reference to a

standard curve prepared with leucine. Two replications were maintained. Samples taken from uninoculated seeds served as control.

Studies on the Effect of Seed treatment chemicals.

Rice seeds of three varieties namely Pavizham, Jyothi and Jaya were inoculated with the following isolates namely Aspergillus flavus, Alternaria padwickii, Bipolaris oryzae and Sarocladium oryzae. The following fungicides were tested for seed treatment at a concentration of 0.3 per cent both in vitro and in pot culture.

1. Benomyl (Benomyl)	(methyl-N (butylcarbamoyl)-2 benzimidazole carbamate)
2. Carbendazim (Agrizim)	(methyl- 2-benzimidazole carbamate)
3. Carboxin (Vitavux)	(5-6-dihydro-2-methyl-1,-4-oxathiin-3-carboxanilide)
4. Mancozeb (Dithane M-45)	(Manganese ethylene bisdithio carbamate + Zinc ions).

Effect of Fungicides on Seedling Emergence.

For evaluation of the effect of seed treatment on the emergence of seedlings, the method followed by Grewal and Kapoor (1966) and Hiremath and Hegde (1981) was used.

The seeds were inoculated separately with different isolates as detailed under assessment of damage and stored

for 30 days. Two replications of 200 seeds were maintained for each treatment. Each treatment was then divided into five sub-samples. In four sub-samples, seed dressing was done as dry seed treatment by shaking the seeds with fungicides in 250 ml conical flasks in a mechanical shaker for 20 minutes. The treated seeds were stored for 48 hours and later plated on blotters moistened with sterile water in sterile petri dishes at the rate of 25 seeds per dish. The untreated seeds plated on blotters moistened with sterile water in sterile petri dishes served as control. The plates were incubated for eight days at room temperature ($28^{o}C \pm 1$) and examined for the following.

i) Germination count
ii) Inoculum recovery
iii) Root and shoot length
iv) Root and shoot rot.

On the eighth day, the seedlings on the blotter along with a bit of filter paper were transplanted in earthen pots containing sterilized soil of uniform nutrient status, (Appendix II). Twenty five seedlings were planted in a pot and maintained for 30 days. Two replications were used for

each treatment. Untreated seeds grown in sterile blotters and transplanted to sterile soil in pots served as the control. Observations were made on the 30th day for post-emergence mortality, height of seedling and seedling weight.

RESULTS

RESULTS

Isolation of Seed borne Fungi.

Several fungi were isolated from the seeds of the 12 varieties / cultivars of rice used in the present investigation (Table 1).

Externally Seed borne fungi.

Aspergillus flavus was the most common externally seed borne fungus isolated from nine cultivars. Bipolaris oryzae and Curvularia lunata were isolated from seven cultivars each while Rhizopus stolonifer, Chaetomium gracile and Emericella nidulans were found to be associated with three cultivars each. Syncephalastrum racemosum, Fusarium moniliforme, Trichoderma viride, Sarocladium oryzae, Aspergillus niger and Alternaria padwickii were associated with two cultivars only. Fusarium graminearum, Pyricularia oryzae, Aspergillus fumigatus, A. quadrilineatus, Nigrospora oryzae and Penicillium spp. were less frequently observed.

Internally Seed borne fungi.

Only six fungi were found to be internally associated with the tested seeds. Bipolaris oryzae, Curvularia lunata and Aspergillus flavus were associated internally with

Table 1. Fungi associated with different varieties of rice seeds from Kuttanadu.

Sl. No.	Name of variety	Externally seed-borne	Internally seed-borne
1.	Athikarazhi	Aspergillus flavus Link:Fr Bipolaris oryzae (Breda de Haar) Shoem Chaetomium gracile Udagawa Curvularia lunata (Wakker) Boedijn Fusarium moniliforme	Aspergillus flavus Link:Fr Bipolaris oryzae (Breda de Haan) Shoem Curvularia lunata (Wakker) Boedijn
2.	Bharathy	Aspergillus flavus Link: Fr Nigrospora oryzae Berk & Br. Rhizopus stolonifer (Fr)Lind Syncephalastrum racemosum Cohn ex Schroter	Aspergillus flavus Link: Fr
3.	Cheruvally	Aspergillus quadrilineatus Thom and Raper Bipolaris oryzae (Bredade Haan) Shoem Chaetomium gracile Udagawa Curvularia lunata (Wakker) Boedijn Sarocladium oryzae (Sawada) W.Gams & D.Hawksw	

Sl. No.	Name of variety	Externally seed-borne	Internally seed-borne
4.	Jaya	Aspergillus flavus Link: Fr Aspergillus niger Van Tiegh Bipolaris oryzae (Breda de Haah) Shoem Curvaluria lunata (Wakker)Boedijn Pyricularia oryzae Cav. Rhizopus stolonifer (Fr)Lind	Aspergillus flavus Link:Fr Bipolaris oryzae (Breda de Haah) Shoem Curvularia lunata (Wakker)Boedijn
5.	Jyothi	Aspergillus flavus Link Fr Aspergillus niger Van Tiegh Alternaria padwickii (Ganguly) M.B. Ellis Bipolaris oryzae (Breda de Haah) Shoem Myrothecium roridum Tode ex Fr.	Aspergillus fumigatus Fres Alternaria padwickii (Ganguly) M.B.Ellis Bipolaris oryzae (Breda de Haah) Shoem
6.	Karthika	Aspergillus flavus Link: Fr Curvularia lunata (Wakker)Boedijn Sarocladium oryzae (Sawada) W.Gams & D.Hawkaw Syncephalastrum racemosum Cohn ex schroter	Aspergillus flavus Link: Fr Curvularia lunata (Wakker)Boedijn Sarocladium oryzae (Sawada) W.Gams & D.Hawkaw

Sl. No.	Name of variety	Externally seed-borne	Internally seed borne
7.	MO-4	Alternaria padwickii (Ganguly) M.B.Ellis Bipolaris oryzae (Breda de Haah) Emericella nidulans (Eidam)Vr Trichoderma viride Pers.ex S.F.Gray	Alternaria padwickii (Ganguly) M.B.Ellis Bipolaris oryzae (Breda de Haah
8.	MO-7	Aspergillus flavus Link:Fr Curvularia lunata (Wakker) Boedijn Fusarium graminearum Schwabe Rhizopus stolonifer (Fr) Lind	Aspergillus flavus Link: Fr Curvularia lunata (Wakker) Boedijn
9.	M--310	Emericella nidulans (Eidam) Penicillium sp. Trichoderma viride	
10.	M--329	Aspergillus flavus Link:Fr Curvularia lunata (Wakker) Boedijn Emericella nidulans (Eidam)	Curvularia lunata (Wakker) Boedijn

Sl. No.	Name of variety	Externally seed-borne	Internally seed-borne
11.	Njavara vithu	Aspergillus flavus Link: Fr Bipolaris oryzae (Breda de Haah) Shoem Chaetomium gracile Udagawa Fusarium moniliforme	Aspergillus flavus Link:Fr Bipolaris oryzae (Breda de Haah) Shoem
12.	Pavizham	Aspergillus flavus Link:Fr Aspergillus fumigatus Fres Bipolaris oryzae (Breda de Haah) Shoem Curvularia lunata (Wakker)Boedijn Trichoderma viridie Pers. ex S.F.Gray	Aspergillus flavus Link: Fr Aspergillus fumigatus Fres Bipolaris oryzae (Breda de Haah) Shoem

seven cultivars while Aspergillus fumigatus was associated with only two cultivars. Alternaria padwickii and Sarocladium oryzae were internally seed borne in two cultivars only. All the above fungi were found to be externally seed borne also.

Among the varieties, Jaya seeds recorded the maximum external count of fungi with six different species, followed by Jyothi, Cheruvally, Athirakazhy and Pavizham with five numbers each, while minimum infection was recorded by the two pre-release cultures viz., M-329 and M-310. Similarly maximum internal infection was recorded by five varieties viz., Pavizham, Jaya, Jyothi, Karthika and Athirakazhi, while M-310, the pre-release culture recorded no infection.

M-310, the pre-release culture was completely free of external and internal infection.

Damages due to Seed-borne Fungi.

a. Effect of inoculum on germination and post emergence vigour.

i. Influence of Inoculum on Germination.

The germination percentage of the seeds of four varieties of rice inoculated with five different fungi were not found to be significantly affected (Table 2, and Appendix III).

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ii. Influence of Inoculum on Root and Shoot Rot.

All the fungi caused significant rotting of the root (Table 3, Appendix IV) and shoot (Table 4, Appendix V) as compared to control.

Maximum root rot was observed in the case of seeds inoculated with B. oryzae and was on par with A. padwickii and A. flavus. Treatment with S. oryzae recorded minimum rotting.

Significant difference was also noticed with respect to the different varieties (Table 3). Varieties and treatment interactions were also found to be significant. Seeds of Pavizham inoculated with B. oryzae recorded the maximum rotting (Plate I) and the minimum was recorded by seeds of Jyothi inoculated with S. oryzae.

Maximum shoot rot was caused by A. padwickii and was on par with the other fungi (Plate II). The variety Karthika showed a significantly higher percentage of rotted shoots compared to other varieties. There was no significant difference between the varieties Pavizham, Jaya and Jyothi.

Interaction effects due to varieties and treatment were also significant. Seeds of Karthika inoculated with A. flavus

Table 2. Mean value showing the effect of different inoculum on rice seed germination. (Three days after incubation)

Inoculum tested	Per cent germination				
	Pavizham	Jyothi	Jaya	Karthika	Mean
A. flavus	80.78 (95.00)	90.00 (100.00)	90.00 (100.00)	67.50 (85.00)	82.07
A. padwickii	90.00 (100.00)	90.00 (100.00)	90.00 (100.00)	80.78 (95.00)	87.69
B. oryzae	90.00 (100.00)	80.78 (95.00)	90.00 (100.00)	80.78 (95.00)	85.39
C. lunata	80.78 (95.00)	90.00 (100.00)	90.00 (100.00)	80.78 (95.00)	85.30
S. oryzae	90.00 (100.00)	90.00 (100.00)	90.00 (100.00)	67.50 (85.00)	87.69
Control	90.00 (100.00)	90.00 (100.00)	90.00 (100.00)	90.00 (100.00)	90.00
Mean	86.92	88.46	90.00	77.89	

Figures in parentheses are values in original units.

C.D. 5% inoculum = N.S.

C.D. 5% Variety = 5.591

C.D. 5% Interation = N.S.

recorded maximum rotting (Plate III) and Pavizham inoculated with A. flavus recorded the minimum.

iii. Influence of Inoculum on Root and Shoot Elongation.

Significant difference on primary root elongation was noticed in some treatments. Seeds treated with S. oryzae and A. padwickii were not found to differ from the control, while B. oryzae recorded the minimum root length and was on par with C. lunata and A. flavus. Neither varieties nor the interactions due to varieties and treatments were significant (Table 5 and Appendix VI).

All the treatments had significant influence on reduction of shoot length in comparison to control (Table 6 and Appendix VII). Bipolaris oryzae recorded the minimum shoot length even after seven days of incubation and was observed to be on par with all other fungi (viz., A. flavus, A. padwickii, C. lunata and S. oryzae).

Varieties differed significantly in shoot elongation. Karthika recorded the least shoot length and was observed to be on par with Jaya. Pavizham recorded the highest shoot length. Interaction effects due to varieties and treatments were observed to be not significant.

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Table 3. Mean value showing the effect of different inoculum on the root rot of emerging rice seedlings. (Seven days after incubation)

Inoculum tested	Per cent root rot				
	Pavizham	Jyothi	Jaya	Karthika	Mean
A. flavus	33.21 (30.00)	53.78 (65.10)	53.78 (65.10)	47.88 (55.00)	47.16
A. padwickii	42.11 (45.00)	45.00 (50.00)	53.78 (65.10)	47.88 (55.00)	49.39
B. oryzae	67.50 (85.35)	42.11 (45.00)	47.78 (54.85)	53.78 (65.10)	52.79
C. lunata	42.11 (45.00)	48.01 (55.25)	44.90 (49.80)	47.88 (55.00)	45.72
S. oryzae	47.88 (55.00)	32.89 (29.50)	36.22 34.90	42.11 45.00	39.77
Control	0.00	0.00	0.00	0.00	0.00
Mean	38.80	36.96	39.41	39.92	

Figures in parentheses are values in original units.

C.D. 5% Inoculum = 5.246

C.D. 5% Varieties = 4.284

C.D. 5% Interaction = 10.719

Table 4. Mean value showing the effect of different inoculum on the shoot rot of emerging rice seedlings. (Seven days after incubation)

Inoculum tested	Per cent shoot rot				
	Pavizham	Jyothi	Jaya	Karthika	Mean
A. flavus	22.50 (14.65)	29.88 (24.80)	33.21 (30.00)	56.78 (70.00)	35.59
A. padwickii	33.21 (30.00)	29.88 (24.80)	33.21 (30.00)	47.88 (55.00)	36.04
B. oryzae	29.88 (24.80)	29.88 (24.80)	29.88 (24.80)	42.11 (44.95)	32.94
C. lunata	22.50 (14.65)	36.22 (34.90)	36.31 (35.05)	36.31 (35.05)	32.83
S. Oryzae	36.22 (34.90)	29.88 (24.80)	29.98 (24.95)	45.09 (50.15)	35.29
Control	0.00	0.00	0.00	0.00	0.00
Mean	24.05	25.96	27.10	38.03	

Figures in parentheses are values in original units

C.D. 5% Inoculum = 5.086

C.D. 5% Varieties = 4.152

C.D. 5% Interactions = 10.390

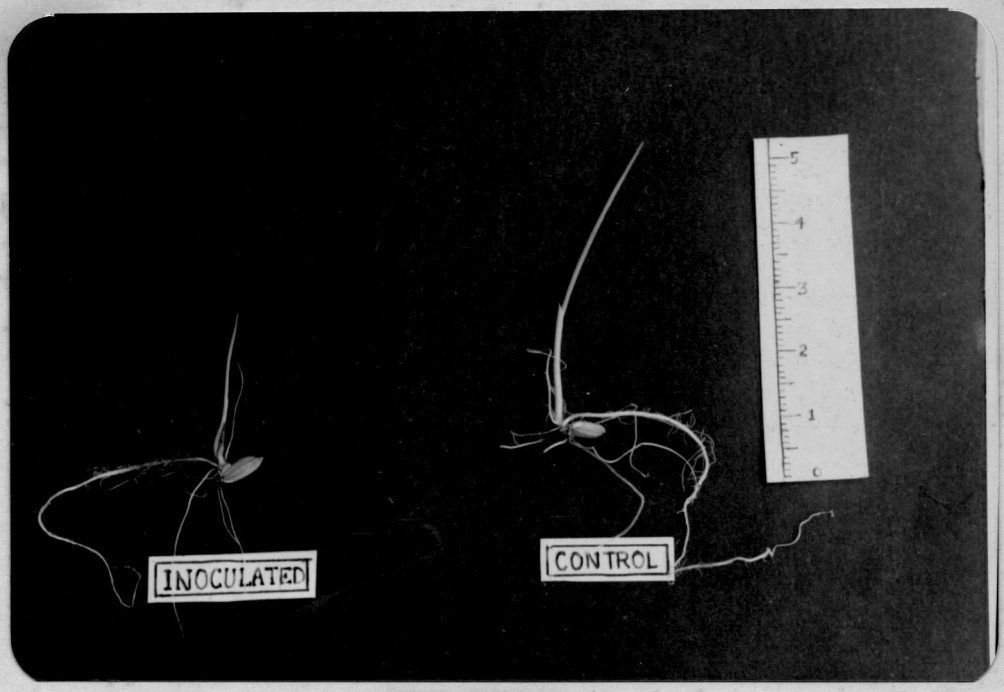

PLATE I

Effect of seed inoculation with B. oryzae on Pavizham seeds in causing root rot as compared to uninoculated control.

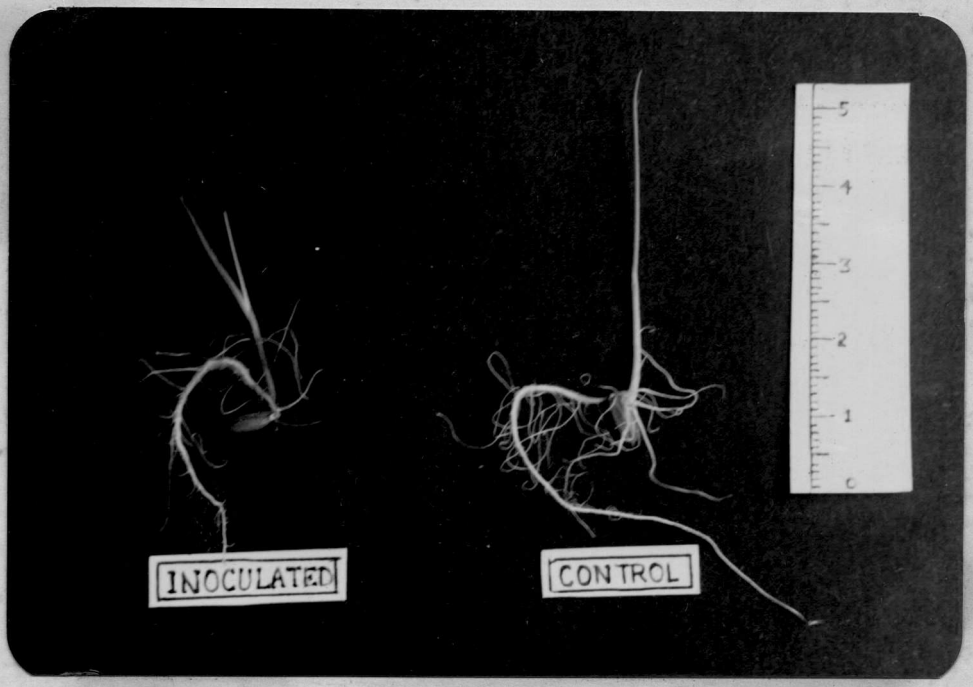

PLATE II

Effect of seed inoculation with A.padwickii on Karthika seeds in causing shoot rots as compared to control.

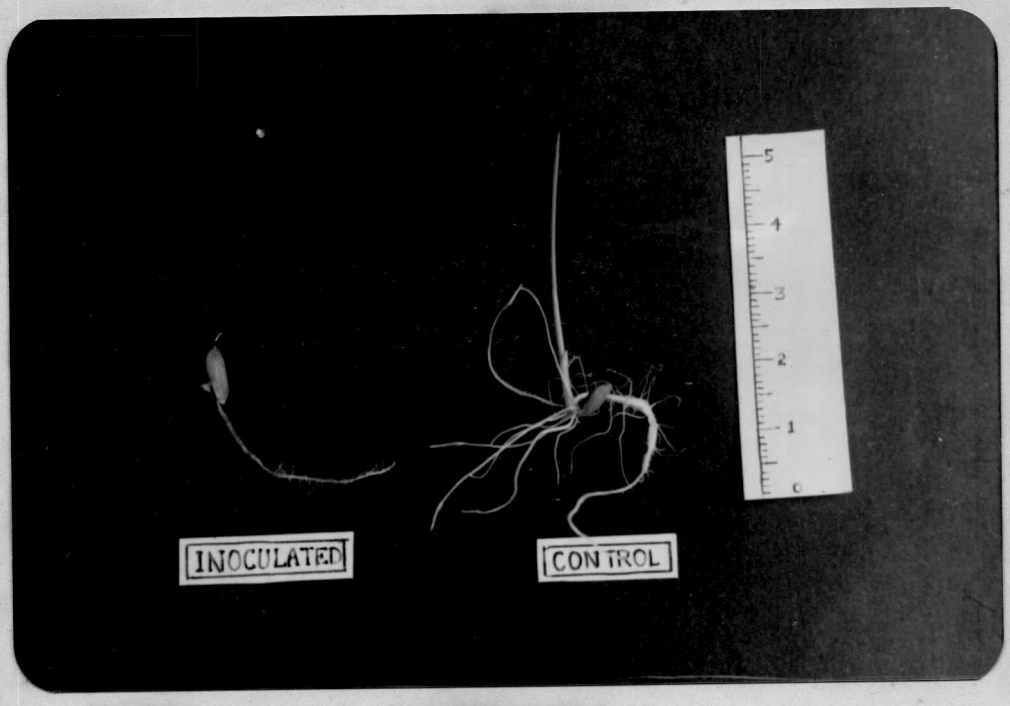

PLATE III

Effect of seed inoculation with A. flavus in Karthika seeds in causing shoot rot as compared to control.

Table 5. Mean value showing the effect of different inoculum on the primary root length of emerging rice seedlings. (Seven days after incubation)

Inoculum tested	Root length (cm)				
	Pavizham	Jyothi	Jaya	Karthika	Mean
A. flavus	8.67	5.57	6.16	3.64	6.01
A. padwickii	8.66	6.98	5.64	8.68	7.49
B. oryzae	4.58	6.78	4.44	3.97	4.94
C. lunata	6.36	6.17	4.83	5.64	5.75
S. oryzae	7.85	7.64	9.77	7.00	8.06
Control	8.69	9.54	10.21	8.95	9.35
Mean	7.47	7.45	6.84	6.31	

C.D. 5% Inoculum = 2.120

C.D. 5% Varieties N.S.

C.D. 5% Interaction N.S.

Table 6. Mean value showing the effect of different inoculum on shoot length of emerging rice seedlings. (Seven days after incubation)

Inoculum tested	Shoot length (cm)				
	Pavizham	Jyothi	Jaya	Karthika	Mean
A. flavus	4.74	4.30	3.19	3.09	3.83
A. padwickii	5.52	3.96	2.90	3.65	4.01
B. oryzae	3.76	4.11	3.70	3.03	3.65
C. lunata	4.88	3.85	3.41	4.33	4.11
S. oryzae	4.11	4.31	3.41	3.14	3.74
Control	5.74	5.90	5.70	4.74	5.52
Mean	4.79	4.11	3.72	3.66	

C.D. 5% Inoculum 0.540

C.D. 5% Varieties 0.441

C.D. 5% Interaction N.S.

b. Effect of Culture filtrates on Seeds.

i. Influence of Culture filtrates on Germination.

The germination percentage of seeds was found to be significantly reduced due to the effect of all culture filtrates (Table 7 and Appendix VIII).

The maximum reduction in germination was noted with the filtrates of C. lunata and observed to be on par with A. flavus, S. oryzae and B. oryzae. Minimum reduction was recorded with A. Padwickii.

Among the varieties, Jyothi recorded the maximum germination failure and was observed to be on par with Karthika, followed by Jaya which was on par with Pavizham. Interaction due to varieties and treatments was not found to be significant.

ii. Influence of Culture filtrates on Inhibition of Root initiation.

Culture filtrates of B. oryzae and S. oryzae had no inhibitory action on root initiation while A. flavus recorded maximum inhibition followed by C. lunata and A. padwickii (Table 8 and Appendix IX).

Varieties were found to differ significantly in inhibition of root initiation. The maximum inhibition was noticed for Pavizham and the least inhibition was for Jaya which was on par with Karthika.

Interaction effects due to varieties and treatments were noticed to be significant. All combinations of varieties with B. oryzae and S. oryzae were found to have no inhibitory effect and behaved like control. The maximum inhibition was noticed for Pavizham with culture filtrate of A. flavus (Plate IV). Jaya and Jyothi also recorded similar results with A. flavus (Plate V), while A. padwickii caused maximum inhibition in Karthika.

iii. Influence of Culture filtrates on Primary Root Elongation.

The culture filtrates of all fungi tested showed significant influence in reduction of root length on the seedlings emerged (Table 9 and Appendix X).

The maximum reduction was observed in treatments with A. flavus and was found to be on par with A. padwickii and C. lunata. Metabolites of S. oryzae recorded the minimum reduction in root length.

Table 7. Mean value showing the effect of different culture filtrates on rice seed germination.

Culture filtrates	Per cent germination				
	Pavizham	Jyothi	Jaya	Karthika	Mean
A. flavus	76.28 (94.35)	71.64 (90.10)	76.66 (94.70)	72.14 (90.60)	74.18
A. padwickii	80.78 (97.45)	72.56 (91.00)	82.41 (98.25)	81.93 (98.00)	79.42
B. oryzae	76.28 (94.35)	76.28 (94.35)	78.10 (95.75)	79.68 (96.80)	77.58
C. lunata	76.28 (94.35)	70.74 (89.10)	73.58 (92.00)	75.75 (93.95)	74.09
S. oryzae	90.00 (100.00)	71.57 (90.00)	81.38 (97.75)	66.69 (84.35)	77.41
Control	90.00 (100.00)	84.22 (99.00)	80.54 (97.30)	85.93 (99.50)	85.17
Mean	81.60	74.50	78.78	77.02	

Figures in parentheses are values in original units

C.D. 5% Filtrates 4.486

C.D. 5% Varieties 3.663

C.D. 5% Interaction N.S.

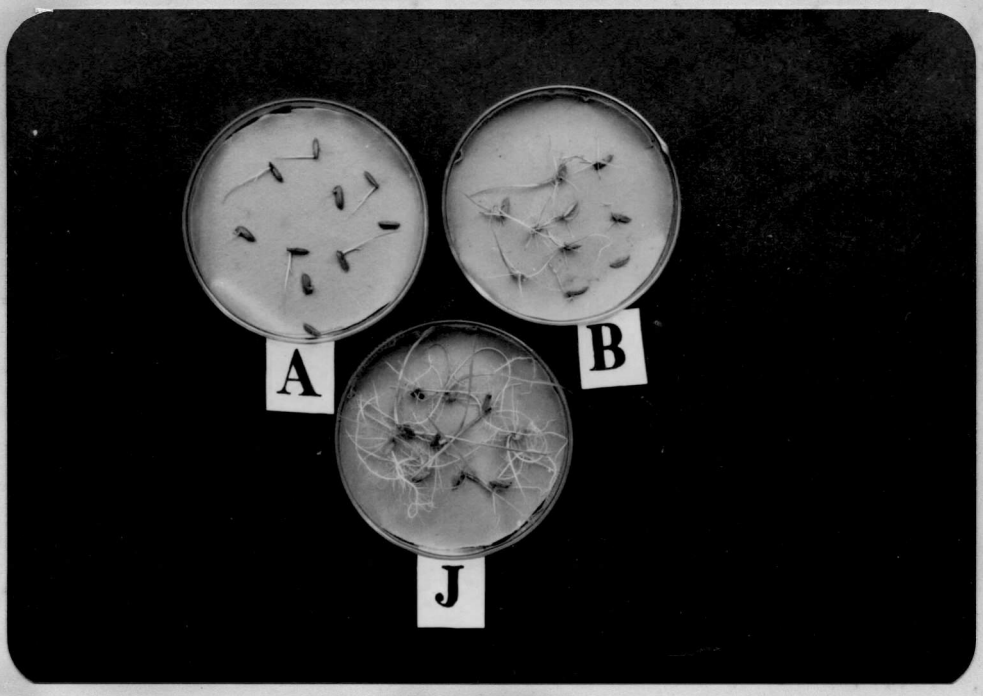

PLATE IV

Influence of culture filtrates of A.flavus (A) and B.oryzae (B) in causing inhibition of root initiation in Pavizham seeds as compared to control (J).

PLATE V

Influence of culture filtrates of B.oryzae (G) and A.flavus (A) on Jyothi seeds in causing inhibition of root initiation as compared to control (J).

There was no significant difference among the varieties in their response to the culture filtrates. Interaction effect due to the varieties and treatment was noticed to be significant. The minimum root length was recorded for the combination of Pavizham with A. flavus and the maximum root length for Pavizham with S. oryzae, baring control. For Jyothi and Karthika also metabolites of A. flavus showed similar results, while C. lunata caused the maximum reduction in Jaya.

iv. Influence of Culture filtrates on Shoot Elongation.

The shoot length of seedlings was also significantly reduced due to the influence of all culture filtrate in comparison to control (Table 10 and Appendix XI).

The effects of the culture filtrates of B. oryzae was observed to be maximum in reducing the shoot length and was found to be on par with those of A. flavus and C. lunata. The culture filtrate of S. oryzae recorded the minimum reduction.

Among the varieties, Karthika recorded the maximum reduction. Pavizham ranked next and was on par with Jaya. The variety Jyothi recorded minimum reduction in shoot length. Interaction due to varieties and treatments was

Table 8. Mean value showing the effect of different culture filtrates on root initiation in germinating rice seeds. (Seven days after incubation)

Culture filtrates	Per cent inhibition				
	Pavizham	Jyothi	Jaya	Karthika	Mean
A. flavus	60.58 (75.90)	58.39 (72.40)	41.68 (44.20)	23.99 (16.50)	46.16
A. padwickii	51.97 (62.05)	36.36 (35.15)	0.00 (0.00)	41.40 (43.75)	32.43
B. oryzae	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00
C. lunata	53.92 (65.30)	18.66 (10.20)	38.03 (37.95)	22.65 (14.85)	33.31
S. oryzae	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00
Mean	27.74	18.90	13.28	14.67	

Figures in parentheses are values in original units

C.D. 5% Filtrates 3.132

C.D. 5% Varieties 2.557

C.D. 5% Interaction 6.119

Table 9. Mean value showing the effect of different culture filtrates on the primary root length of emerging rice seedlings. (Seven days after incubation)

Culture filtrates	Reoot length (cm)				
	Pavizham	Jyothi	Jaya	Karthika	Mean
A. flavus	0.56	0.60	2.08	1.00	1.33
A. padwickii	0.78	0.87	2.26	2.08	1.49
B. oryzae	0.92	4.80	3.13	1.77	2.67
C. lunata	2.99	2.89	1.53	1.88	2.32
S. oryzae	6.41	5.19	2.98	5.15	4.93
Control	7.02	6.94	5.10	6.72	6.44
Mean	3.11	3.56	2.84	3.10	

C.D. 5%	Filtrates	1.001
C.D. 5%	Varieties	N.S.
C.D. 5%	Interactions	2.00

significant. The combination of Karthika and culture filtrate of B. oryzae recorded the maximum reduction in the shoot length (Plate VI). The minimum reduction in shoot length was noticed in combination of Jyothi with S. oryzae barring control. For Pavizham also the combination of B. oryzae caused the maximum reduction in shoot length. For Jyothi and Jaya also filtrates of C. lunata and A. flavus respectively recorded maximum reduction. Culture filtrates of S. oryzae had the least inhibitory action on shoot length in all varieties.

Bio-chemical Changes due to Fungal infection.

i. Changes in Starch.

All the fungi significantly reduced the starch content of infected seeds (Table 11 and Appendix XII). Significant difference among different fungi also noticed. Treatment with B. oryzae recorded the maximum reduction of starch by about 32 per cent. Treatment with A. flavus ranked next in reducing the starch followed by A. padwickii. Among the fungi tested, the minimum reduction of 10.8 per cent was noted for the infection by S. oryzae.

Varieties also recorded significant differences. Jaya recorded the maximum reduction in starch followed by Pavizham

Table 10. Mean value showing the effect of different culture filtrates on the shoot length of emerging rice seedlings.
(Seven days after incubation)

Culture filtrates	Shoot length (cm)				
	Pavisham	Jyothi	Jaya	Karthika	Mean
A. flavus	2.05	2.34	1.42	1.62	1.85
A. padwickii	2.35	2.96	2.62	2.65	2.65
B. oryzae	0.83	2.74	2.75	0.67	1.75
C. lunata	2.16	2.05	1.73	1.46	2.08
S. oryzae	3.88	5.16	2.95	2.76	3.69
Control	3.89	5.43	4.20	3.33	4.21
Mean	2.52	3.45	2.61	2.08	

C.D. 5% Filtrates 0.457
C.D. 5% Varieties 0.373
C.D. 5% Interaction 0.914

PLATE VI

Effect of culture filtrates of A.flavus (E) and B.oryzae (F) on Karthika seeds in causing reduction in shoot length as compared to control (J).

and was observed to be on par with Karthika. Minimum reduction in starch content was noticed in Jyothi. Interaction due to varieties and treatment was also found to be significant. The combination of B. oryzae with Jaya recorded the maximum reduction of starch by about 39.7 per cent, while Jyothi with S. oryzae recorded the least. Treatment with B. oryzae reduced the starch content of both Pavizham and Karthika to the maximum. Similarly A. flavus caused maximum reduction in Jyothi. Minimum starch reducing effect was recorded by S. oryzae in all combinations.

ii. Changes in Protein.

The protein content of seeds was not found to be significantly affected due to infection and subsequent storage, (Table 12 and Appendix XIII). However, among the treatments, seeds treated with A. padwickii recorded the maximum protein content. Similarly among the varieties, the maximum protein content was observed for Pavizham. The combination of Pavizham seeds inoculated with A. padwickii recorded the maximum protein content in the interaction effect.

iii. Changes in Total Free Amino Acid.

Significant reduction in the total free amino acid content was noticed due to fungal inoculation as compared

Table 11. Changes in starch content of rice seeds due to fungal infection. (Mean of two replications)

(Forty five days after inoculation)

Particulars	Percentage of starch content				
	Pavizham	Jyothi	Jaya	Karthika	Mean
A. flavus	52.04	63.85	55.23	73.17	61.07
A. padwickii	68.23	78.26	54.22	60.85	65.39
B. oryzae	45.45	69.24	45.23	51.78	52.92
S. oryzae	75.02	78.96	60.82	65.34	70.03
Control	81.08	81.84	75.02	76.29	78.55
Mean	64.36	74.43	58.10	65.48	

C.D. 5% Inoculum 2.000

C.D. 5% Varieties 1.789

C.D. 5% Interactions 4.104

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Table 12. Changes in protein content (N x 6.25) of rice seeds due to fungal infection.

(Forty five days after inoculation)

Particulars	Percentage of protein content				
	Pavizham	Jyothi	Jaya	Karthika	Mean
A. flavus	11.27	10.75	10.92	11.62	11.14
A. padwickii	12.15	11.77	12.05	10.75	11.68
B. oryzae	10.40	11.62	11.25	10.92	11.05
S. oryzae	11.95	10.75	10.57	11.27	11.13
Control	10.92	11.10	11.10	11.10	11.05
Mean	11.34	11.20	11.18	11.13	

C.D. 5% Inoculum N.S.

C.D. 5% Varieties N.S.

C.D. 5% interaction N.S.

Table 13. Changes in total free amino acid content of rice seeds due to fungal infection. (Mean of two replications)

(Forty five days after inoculation)

Particulars	Total free amino acid (mg/g)				
	Pavizham	Jyothi	Jaya	Karthika	Mean
A. flavus	96.00	90.00	40.00	58.50	71.12
A. padwickii	97.00	91.00	48.00	46.00	70.50
B. oryzae	41.25	47.50	39.00	45.00	43.18
S. oryzae	104.00	92.00	40.50	45.50	70.50
Control	116.00	106.00	62.50	80.50	91.25
Mean	90.85	85.30	46.00	55.10	

C.D. 5%	Inoculum	7.212
C.D. 5%	Varieties	6.450
C.D. 5%	Interaction	14.424

to control (Table 13 and Appendix XIV). The maximum reduction of 52.6 per cent was noticed for the treatment with B. oryzae. All other treatments of A. flavus, A. padwickii and S. oryzae were similar in their effects.

Varieties were also observed to be significantly different. Pavizham recorded the maximum amino acid content and was on par with Jyothi, while Jaya recorded the minimum. Interaction due to variety and treatment was also noticed to be significant. The combination of Jaya with B. oryzae recorded the maximum reduction in the total free amino acid. Combination of B. oryzae with Pavizham, Jyothi and Karthika also recorded similar results.

Effect of Seed treatment Chemicals in controlling Infection.

A. In vitro Studies.

a) Effect on Germination.

i) Influence of Fungicides.

Seeds treated with fungicides viz., Benomyl, Carbendazim, Carboxin and Mancozeb recorded significantly higher germination than control (Table 14 and Appendix XV). Benomyl treated seeds recorded the maximum germination and was on par with all other fungicides.

ii) Influence of Inoculum.

No significant difference in seed germination was observed for different fungal inoculum (Table 15).

iii) Influence of Varieties.

No significant difference on seed germination was noticed for different varieties (Table 16).

iv) Interaction Effects.

The interaction due to varieties and inoculum was found to be significant (Table 17 and Appendix XV). The minimum germination was observed for Jaya seeds inoculated with A. flavus other interaction viz., Inoculum x Fungicide, Variety x Fungicide and Variety x Inoculum x Fungicide were not found to be significant (Appendix XV).

b) Effect on Inoculum recovery.

i) Influence of fungicides.

All the fungicides significantly reduced the fungal inoculum applied on the seeds compared to control (Table 14). Among the fungicides, mancozeb was found to reduce the fungal inoculum to the maximum and was on par with benomyl and carboxin.

ii) Influence of Inoculum.

The type of inoculum applied on seeds had significant effect on their recovery (Table 15). The effect of S. oryzae was on par with A. flavus. The minimum recovery was observed for A. padwickii which was on par with B. oryzae.

iii) Influence of Varieties.

The varieties also differed significantly with respect to inoculum recovery (Table 16). Jaya seeds recorded minimum recovery and was on par with Jyothi. The maximum recovery was recorded on Pavizham.

iv) Interaction effects.

The interaction effect due to fungicides and fungal inoculum was noticed to be significant (Table 18 and Appendix XVI). In the case of seeds inoculated with B. oryzae and treated with different fungicides, benomyl treated seeds recorded no recovery of fungal mycelium. This was found to be on par with mancozeb. Carbendazim and carboxin treatments were also on par with each other. In seeds, inoculated with A. flavus and treated with different fungicides carboxin treatment recorded no recovery of fungal mycelium, followed by benomyl and mancozeb, all the three being on par. With

A. padwikii inoculated, no inoculum recovery was recorded from the seeds treated with benomyl and mancozeb, and was on par with carboxin. Similarly with S. oryzae inoculated seeds also carboxin and mancozeb recorded no recovery and was on par with benomyl.

Carbendazim recorded comparatively higher inoculum recovery from treated seeds inoculated with A. flavus, A. padwickii and S. oryzae eventhough the effect was significant.

Interaction effects due to fungicides and varieties were also noticed to be significant (Table 19 and Appendix XVI). Seeds of Pavizham treated with mancozeb recorded no fungal recovery and was on par with benomyl and carboxin. For Jyothi also, the same trend was noticed. But for Jaya, seed treatment with carboxin was the most effective and was on par with benomyl and mancozeb. In all the three varieties carbendazim recorded the higher fungal recovery.

Interaction due to fungal inoculum and varieties were also significant (Table 20 and Appendix XVI). The minimum inoculum recovery was noticed for Jaya seeds infected with A. padwickii while, Pavizham seeds inoculated with A. padwickii recorded the maximum.

c) Effect on Root length of Seedlings.

i) Influence of Fungicide.

Seed treatment with fungicides had significant influence on elongation of primary roots in seedlings (Table 14). Significant difference was noticed between the fungicides also. Seedlings from mancozeb treated seeds recorded the maximum root length. Carbendazim ranked next, followed by benomyl which was on par with carboxin.

ii) Influence of the Inoculum.

Effect of fungal inoculation also differed significantly with respect to the seedling root length (Table 15). Seedlings raised from seeds infected with S. oryzae recorded the maximum root length after seed treatment. This was followed by B. oryzae which was on par with A. padwickii.

iii) Influence of the Varieties.

Varieties also differed significantly with respect to the mean root length (Table 16). Jyothi was found to have the maximum root length and was on par with Pavizham. Jaya recorded the minimum root length.

iv) Interaction effects.

The interaction effect due to fungicide and fungal inoculum on root length was found to be significant (Table 21 and Appendix XVII). Seeds infected with B. oryzae on treatment with mancozeb recorded maximum root length. Carbendazim ranked next followed by benomyl and carboxin which were on par. Seeds infected with A. flavus showed maximum root length on treatment with mancozeb, followed by carbendazim and were on par. The effect of carboxin was lowest. In the case of A. padwickii also mancozeb recorded maximum root length and was on par with other fungicides except benomyl. Against S. oryzae also mancozeb proved superior with respect to root elongation followed by carbendazim and carboxin.

Among the different combinations, B. oryzae infected seeds treated with mancozeb recorded the maximum root length.

Interaction effect due to fungicides and varieties was also found to be significant (Table 22 and Appendix XVII). The Pavizham seeds, treatment with carbendazim proved to be the best in producing maximum root elongation followed by mancozeb. For Jyothi, mancozeb was found to be the best with respect to root length, followed by carbendazim which was

on par with benomyl. For Jaya also mancozeb treatment resulted in the maximum root length followed by carbendazim.

Among all the combinations, Jyothi with mancozeb produced the maximum root length.

Interaction due to fungal inoculum and varieties was also found to be significant (Table 23 and Appendix XVII). Jyothi seeds infected with S. oryzae after seed treatment recorded maximum root length while minimum root length was observed for seeds of Jyothi infected with A. flavus.

d) Effect on Shoot length of Seedlings.

i) Influence of the Fungicides.

All the fungicides used for seed treatment were found to have significant influence on the shoot length of seedlings emerged (Table 14). Fungicides also differed significantly among themselves. Benomyl was observed to be significantly superior and was on par with carboxin. Mancozeb ranked next, followed by carbendazim.

ii) Influence of the Inoculum.

The fungal infection also had significant influence on shoot length (Table 15). Seeds infected with B. oryzae

after seed treatment gave seedlings with maximum shoot length. Minimum shoot length was recorded for seeds infected with A. flavus.

iii) Influence of Varieties.

Varieties also differed significantly with respect to seedling shoot length (Table 16). Pavizham and Jaya recorded the maximum and minimum shoot length respectively after seed treatment.

iv) Interaction effects.

The interaction effect due to the fungal inoculum and fungicides was observed to be significant (Table 24 and Appendix XVIII). With B. oryzae infected seeds, benomyl treatment resulted in the maximum shoot length and was on par with mancozeb. Carboxin ranked next followed by carbendazim. Seed treatment with carboxin, was found to be the best with respect to seedling shoot length against A. flavus infection. Mancozeb ranked next and was on par with benomyl. The minimum shoot length was recorded with carbendazim treatment. With A. padwickii, treatment with carboxin gave the maximum shoot length and was on par with benomyl. Mancozeb ranked next and was on par with carbendazim. In the case of S. oryzae, treatment with mancozeb recorded maximum shoot length and was

on par with benomyl. Carbendazim ranked next and was on par with carboxin.

Among all combinations tried, the maximum shoot length was observed for the seeds infected with B. oryzae and treated with benomyl followed by mancozeb.

The interaction effect due to fungicides and varieties was also significant (Table 25). Seed treatment with benomyl gave the maximum shoot length for the variety Pavizham and was on par with carboxin. Carbendazim treatment produced the minimum shoot length for the variety Pavizham. For Jyothi, manocozeb was the best with respect to seedling shoot length and all other fungicides were on par with each other. Jaya seeds treated with benomyl gave the maximum shoot length and was observed to be on par with carboxin. Treatment with carbendazim gave the minimum shoot length.

The interaction effect due to fungal inoculum and varieties was also significant (Table 26). Pavizham seeds infected with S. oryzae gave seedlings with the maximum shoot length due to seed treatment, while Jyothi seeds infected with A. flavus gave seedlings with the minimum shoot length.

e) Effect on Control of Root rot.

i) Influence of Fungicides.

All fungicides tested were found to have significant influence in reducing the root rot as compared to control (Table 14). Fungicides themselves differed significantly. Benomyl treated seeds recorded minimum root rot and was observed to be on par with carboxin. Mancozeb ranked next. Carbendazim was least effective among the fungicides tested.

ii) Influence of the Inoculum.

Root rot was also found to be significantly influenced by the type of inoculum (Table 15). The minimum rotting was observed for seedlings raised from seeds inoculated with B. oryzae while maximum rotting was observed in seeds inoculated with A. padwickii.

iii) Influence of Varieties.

No significant difference was observed among the varieties due to seed treatment with respect to root rot (Table 16).

iv) Interaction Effects.

The three factor interaction was noticed to be significant (Appendix XIX). Interaction effect due to

fungicides and fungal inoculum was observed to be significant (Table 27). For B. oryzae infected seeds, treatment with benomyl recorded no root rot and was on par with carboxin and mancozeb. For seeds infected with A. flavus, carboxin treatment produced no root rot and was on par with benomyl. Seeds infected with A. padwickii followed the same trend of B. oryzae infection. Here also, benomyl recorded no rotting and was on par with carboxin and mancozeb. In all the above cases carbendazim was found to have the minimum effect among the fungicides tested.

Interaction due to fungicides and varieties was also significant (Table 28). For the variety Pavizham, benomyl was found to be the best and recorded no root rot. Carboxin and mancozeb which were on par, while carbendazim was the least effective. For Jyothi, mancozeb and carboxin recorded no root rot and was on par with benomyl. Here also carbendazim was least effective. For Jaya, benomyl recorded no root rot and was on par with carboxin. Maximum root rot was observed for carbendazim treatment which was on par with mancozeb.

Interaction due to fungal inoculum and varieties was also significant (Table 29). Among all combinations Jyothi seeds infected with B. oryzae on seed treatment recorded the

minimum root rot while Jyothi seeds infected with A. flavus recorded the maximum root rot after seed treatment.

f) Effect on Control of Shoot Rot.

i) Influence of Fungicides.

All fungicides were observed to have significant influence in reducing shoot rot compared to control (Table 14 and Appendix XX). Fungicides differed significantly among themselves. Shoot rot was not recorded for seeds treated with benomyl and was on par with carboxin and mancozeb.

ii) Influence of Inoculum.

The inoculum was found to have no significant effect on shoot rot (Table 15).

iii) Influence of Varieties.

Varieties also had no significant effect on shoot rot (Table 16).

iv) Interaction Effects.

Among the studies on the interaction effects (Appendix XX), significant results were obtained only with inoculum and varieties (Table 30). Jyothi seeds infected with A. padwickii

recorded the minimum rotting while Jaya seeds infected with S. oryzae recorded maximum rotting after seed treatment.

B. Pot Culture Experiment.

i. Effect on Post emergence Mortality.

Post emergence mortality of seedlings was observed to be reduced significantly by all fungicides as compared to control (Table 31 and Appendix XXI). Carbendazim recorded the least seedling mortality and was observed to be on par with benomyl, mancozeb and carboxin.

Neither inoculum nor varieties (Table 32 and 33) were noticed to be significantly influencing the post emergence mortality of treated seeds.

All interactions were not significant with respect to post emergence mortality after seed treatment (Appendix XXI).

ii. Effect on Seedling height.

All the fungicides influenced the seedling vigour (Table 31). Maximum seedling height was observed for treatment with mancozeb and was on par with all other fungicides (Plate VII).

Fungal inoculum has no significant effect on seedling height (Table 32). But varieties differed significantly

Table 14. Effect of seed treatment chemicals on seed quality and seedling vigour seven days after inoculation. (As influenced by fungicides)

Fungicides	Germination (per cent)	Inoculum recovery (per cent)	Root length (cm)	Shoot length (cm)	Root rot (per cent)	Shoot rot (per cent)
Benomyl	86.05 (99.50)	1.89 (0.11)	5.17	5.45	1.09 (0.05)	0.00 (0.00)
Carbendazim	85.64 (99.40)	15.43 (7.00)	6.33	4.86	17.24 (8.70)	9.12 (2.50)
Carboxin	84.06 (98.90)	3.06 (0.28)	5.97	5.38	3.35 (0.35)	1.84 (0.10)
Mancozeb	83.22 (98.60)	1.84 (0.10)	6.86	5.31	7.99 (1.90)	2.77 (0.25)
Control	77.12 (95.00)	34.29 (31.75)	4.36	3.75	33.90 (31.10)	27.15 (20.80)
C.D. (5%)	5.352	3.258	0.183	0.124	3.491	3.647

Figures in parentheses are values in original units

Table 15. Effect of seed treatment chemicals on seed quality and seedling vigour seven days after inoculation (As influenced by fungal inoculum)

Fungal inoculum	Per cent germination	Per cent inoculum recovery	Root length (cm)	Shoot length (cm)	Root rot (per cent)	Shoot rot (per cent)
A. flavus	81.67 (97.90)	14.66 (6.40)	4.72	4.53	15.25 (6.90)	8.96 (2.40)
A. padwickii	82.42 (98.25)	6.82 (1.40)	5.66	4.97	30.30 (25.45)	7.03 (1.50)
B. oryzae	85.68 (99.43)	9.79 (2.90)	5.76	5.23	10.20 (3.15)	6.62 (1.30)
S. oryzae	83.10 (98.55)	11.77 (4.15)	6.12	5.07	15.31 (6.95)	10.08 (3.05)
C.D. (5%)	N.S	2.914	0.163	0.111	3.122	N.S

Figures in parentheses are values in original units

Table 16. Effect of seed treatment chemicals on seed quality and seedling vigour seven days after inoculation.

(As influenced by varieties)

Varieties	Per cent germination	Per cent inoculum recovery	Root length (cm)	Shoot length (cm)	Root rot (per cent)	Shoot rot (per cent)
Pavizham	82.37 (98.20)	14.02 (5.85)	5.76	5.35	13.08 (5.10)	8.70 (2.30)
Jyothi	84.18 (98.95)	10.12 (3.10)	5.84	4.88	11.92 (4.25)	6.81 (1.40)
Jaya	83.10 (98.55)	9.76 (2.85)	5.09	4.61	13.14 (5.15)	9.00 (2.45)
C.D. (5%)	N.S	2.524	0.141	0.096	N.S	N.S

Figures in parentheses are values in original units

Table 17. Interaction effect of fungal inoculum and varieties due to seed treatment on seed germination.

Fungal inoculum	Per cent germination		
	Pavizham	Jyothi	Jaya
A. flavus	85.08 (99.25)	85.14 (99.30)	74.79 (93.10)
A. padwickii	79.74 (96.80)	80.55 (97.30)	86.98 (99.70)
B. oryzae	85.58 (99.40)	85.95 (99.50)	85.51 (99.35)
S. oryzae	79.06 (96.40)	85.08 (99.20)	85.14 (99.30)

C.D. (5%) = 8.291

Figures in parentheses are values in original units

Table 18. Interaction effect of fungicide and inoculum due to seed treatment on inoculum recovery

Fungicides	Per cent inoculum recovery			
	B. oryzae	*A. flavus*	*A. padwickii*	*S. oryzae*
Benomyl	0.00 (0.00)	4.92 (0.75)	0.00 (0.00)	2.64 (0.20)
Carbendazim	8.44 (2.15)	22.89 (15.15)	11.95 (4.30)	18.43 (10.00)
Carboxin	9.80 (2.90)	0.00 (0.00)	2.46 (0.20)	0.00 (0.00)
Mancozeb	2.46 (0.20)	4.92 (0.75)	0.00 (0.00)	0.00 (0.00)
Control	28.24 (22.40)	40.27 (41.80)	30.85 (26.30)	37.81 (37.50)

C.D. (5%) = 6.517

Figures in parentheses are values in original units.

Table 19. Interaction effect of fungicides and varieties due to seed treatment on inoculum recovery

Fungicides	Per cent inoculum recovery		
	Pavizham	Jyothi	Jaya
Benomyl	1.98 (0.12)	1.84 (0.10)	1.84 (0.10)
Carbendazim	25.84 (19.00)	9.63 (2.80)	10.82 (3.50)
Carboxin	4.59 (0.65)	4.61 (0.65)	0.00 (0.00)
Mancozeb	0.00 (0.00)	0.00 (0.00)	5.53 (0.90)
Control	37.72 (37.40)	34.55 (32.15)	30.62 (25.95)

C.D. (5%) = 5.644

Figures in parentheses are values in original units.

Table 20. Interaction effect of fungal inoculum and varieties due to seed treatment on inoculum recovery.

Fungal inoculum	Per cent inoculum recovery		
	Pavizham	Jyothi	Jaya
A. flavus	14.24 (6.00)	14.64 (6.40)	14.92 (6.60)
A. padwickii	15.59 (7.20)	6.68 (1.30)	4.88 (0.70)
B. oryzae	13.82 (5.70)	8.57 (2.20)	6.97 (1.40)
S. oryzae	12.44 (4.60)	10.61 (3.40)	12.27 (4.50)

C.D. (5%) = 5.048

Figures in parentheses are values in original units.

Table 21. Interaction effect of fungicides and fungal inoculum due to seed treatment on primary root length of rice seedlings

Fungicides	Primary root length (cm)			
	B. oryzae	*A. flavus*	*A.padwickii*	*S.oryzae*
Benomyl	5.25	4.58	5.25	5.61
Carbendazim	6.47	5.93	6.12	6.81
Carboxin	5.10	3.66	5.99	5.63
Mancozeb	7.68	6.04	6.18	7.56
Control	4.32	3.39	4.77	4.97

C.D. 5% = 0.366

Table 22. Interaction effect of fungicides and varieties due to seed treatment on primary root length of rice seedlings

Fungicides	Primary root length (cm)		
	Pavizham	Jyothi	Jaya
Benomyl	5.12	5.64	4.76
Carbenxazim	7.57	5.78	5.64
Carboxin	5.08	5.00	5.20
Mancozeb	6.49	8.19	5.92
Control	4.56	4.61	3.91

C.D. 5% = 0.317

Table 23. Interaction effect of fungal inoculum and varieties due to seed treatment on primary root length of rice seedlings.

Fungal inoculum	Root length (cm)		
	Pavizham	Jyothi	Jaya
A. flavus	4.79	4.64	4.72
A. padwickii	5.81	6.26	4.98
B. oryzae	5.97	5.68	5.64
S. oryzae	6.48	6.78	5.08

C.D. 5% = 0.283

Table 24. Interaction effect of fungal inoculum and fungicides due to seed treatment on shoot length of rice seedlings.

Fungicides	Shoot length (cm)			
	B.oryzae	A.flavus	A.padwickii	S.oryzae
Benomyl	5.92	4.90	5.48	5.49
Carbendazim	4.82	4.53	4.83	5.27
Carboxin	5.45	5.32	5.57	5.18
Mancozeb	5.82	4.93	4.97	5.51
Control	4.15	2.97	3.99	3.90

C.D. (5%) = 0.249

Table 25. Interaction effect of fungicides and varieties due to seed treatment on shoot length of rice seedlings

Fungicides	Shoot length (cm)		
	Pavizham	Jyothi	Jaya
Benomyl	6.12	4.98	5.24
Carbendazim	5.04	5.06	4.48
Carboxin	5.91	5.01	5.22
Mancozeb	5.41	5.71	4.80
Control	4.29	3.64	3.33

C.D. (5%) = 0.215

Table 26. Interaction effect of fungal inoculum and varieties due to seed treatment on shoot length of rice seedlings

Fungal inoculum	Shoot length (cm)		
	Pavizham	Jyothi	Jaya
A. flavus	5.47	4.01	4.12
A. padwickii	5.07	4.91	4.92
B. oryzae	5.36	5.50	4.83
S. oryzae	5.52	5.10	4.60

C.D. (5%) = 0.192

Table 27. Interaction effect of fungicides and fungal inoculum due to seed treatment on root rot of rice seedlings

Fungicides	Root rot (per cent)			
	B.oryzae	*A.flavus*	*A.padwickii*	*S.oryzae*
Benomyl	0.00 (0.00)	4.38 (0.60)	0.00 (0.00)	0.00 (0.00)
Carbendazim	8.75 (2.30)	23.42 (15.80)	3.40 (5.40)	23.39 (15.70)
Carboxin	2.55 (0.20)	0.00 (0.00)	4.82 (0.70)	6.05 (1.10)
Mancozeb	6.05 (1.10)	7.94 (1.90)	5.29 (0.80)	12.69 (4.80)
Control	33.67 (30.80)	40.54 (42.20)	26.98 (20.60)	34.43 (32.00)

C.D. (5%) = 6.982

Figures in parentheses are values in original units.

Table 28. Interaction effect of fungicides and varieties due to seed treatment on root rot of rice seedlings.

Fungicides	Per cent root rot		
	Pavizham	Jyothi	Jaya
Benomyl	0.00 (0.00)	3.28 (0.30)	0.00 (0.00)
Carbendazim	16.51 (8.00)	20.53 (12.30)	14.68 (6.40)
Carboxin	8.23 (2.00)	0.00 (0.00)	1.84 (0.10)
Mancozeb	10.58 (3.40)	0.00 (0.00)	13.41 (5.40)
Control	30.11 (25.00)	35.81 (34.30)	35.79 (34.20)

C.D. (5%) = 6.047

Figures in parentheses are values in original units.

Table 29. Interaction effect of fungal inoculum and varieties due to seed treatment on root rot of rice seedlings.

Fungal inoculum	Per cent root rot		
	Pavizham	Jyothi	Jaya
A. flavus	12.97 (5.00)	23.16 (15.50)	9.63 (2.80)
A. padwickii	15.16 (6.80)	7.47 (1.70)	7.67 (1.80)
P. oryzae	9.78 (2.90)	4.22 (0.50)	16.61 (8.10)
S. oryzae	14.22 (6.20)	12.85 (4.90)	18.66 (10.20)

C.D. (5%) = 5.408

Figures in parentheses are values in original units.

Table 30. Interaction effect of inoculum and varieties due to seed treatment on shoot rot of rice seedlings.

Fungal inoculum	Per cent shoot rot		
	Pavizham	Jyothi	Jaya
A. flavus	8.03 (1.95)	11.02 (3.65)	7.83 (1.85)
A. padwickii	11.97 (4.30)	3.67 (0.40)	5.46 (0.90)
B. oryzae	8.16 (2.00)	4.31 (0.55)	7.38 (1.65)
S. oryzae	6.65 (1.35)	8.25 (2.05)	15.33 (7.00)

C.D. (5%) = 5.651

Figures in parentheses are values in original units.

(Table 33). Jyothi recorded the maximum seedling height and Pavizham showed the minimum.

The interaction effect due to fungicides and fungal inoculum observed to be significant (Table 34). The seedling height was the maximum for the combination of carboxin with A. padwickii. Against B. oryzae, mancozeb was observed to be superior and was on par with carboxin. Carbendazim ranked next and was on par with benomyl. Against A. flavus, benomyl was superior and was on par with carboxin. This was followed by carbendazim which was on par with mancozeb. But with A. padwickii carboxin proved to be superior. In the case of S. oryzae, carbendazim was the most effective and was on par with benomyl and mancozeb, while carboxin recorded the minimum seedling height.

Fungicide and varietal interaction was also noticed to be significant (Table 35). For Pavizham carbendazim was observed to be most effective and was on par with mancozeb (Plate VIII). Benomyl ranked next and was on par with carboxin. For Jyothi, carboxin was the most effective and was on par with mancozeb and benomyl. For Jaya also the same trend was observed.

The interaction effect due to variety and inoculum was not significant with respect to the seedling height (Appendix XXII). But the three factor interaction of fungicide, variety and inoculum was significant.

iii. Effect on Seedling weight.

All the fungicides had significant influence on increasing the seedling weight (Table 31 and Appendix XXIII). Mancozeb treated seeds recorded the maximum seedling weight and was on par with other fungicides tested.

Fungal inoculum had no significant effect on the wet weight of the seedlings on seed treatment (Table 32).

Varieties differed significantly with respect to seedling weight (Table 33). Jyothi recorded the maximum seedling weight followed by Jaya. Pavizham which recorded the least seedling weight.

The interaction due to fungicides and varieties was noticed to be significant (Table 36). For Pavizham, treatment with mancozeb recorded the maximum seedling wet weight and was on par with carbendazim. Benomyl ranked next and was on par with carboxin. For Jyothi and Jaya, carboxin was the best in increasing seedling weight.

The interaction effects due to fungicides and inoculum (Table 37) and inoculum and variety (Table 38) were also significant.

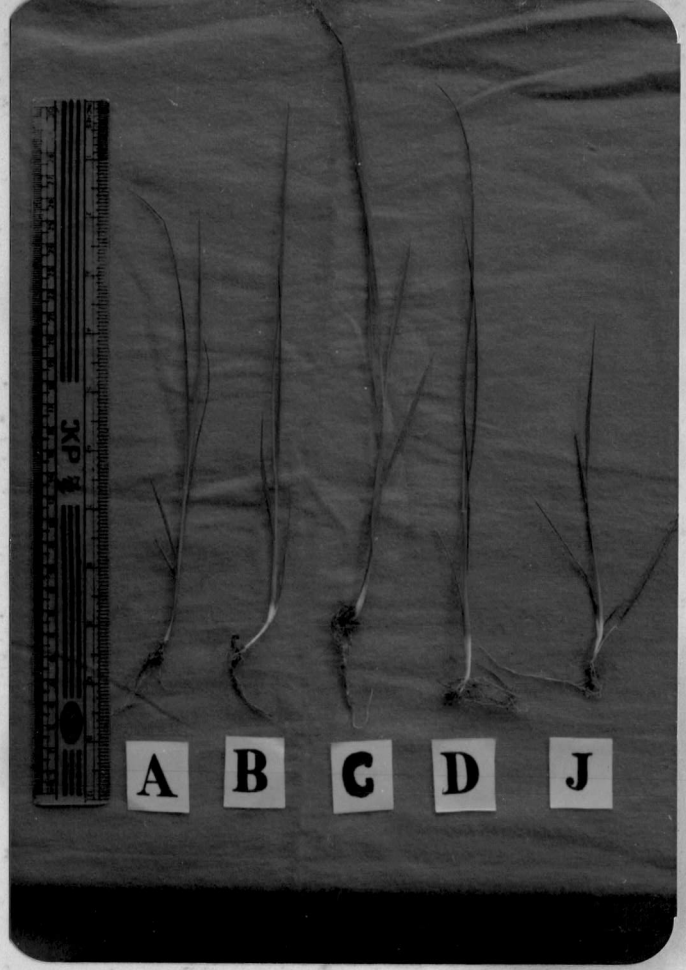


PLATE VII

Effect of seed treatment on Pavizham seeds infected by A.padwickii with carbendazim (E), benomyl (F), mancozeb (G) and carboxin (H) in improving seedling height as compared to control (J).

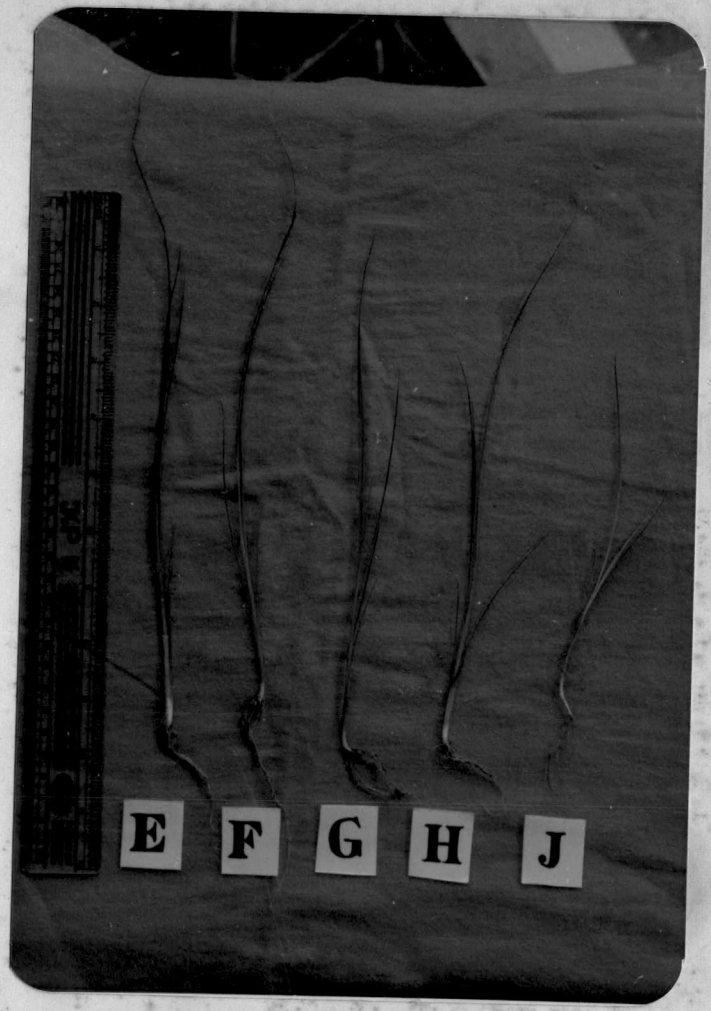


PLATE VIII

Effect of seed treatment on Jyothi seeds infected by B.oryzae with carbendazim (A), benomyl (B) mancozeb (C) and carboxin (D) in improving the seedling height as compared to control.

Table 31. Mean value showing the effect of seed treatment chemicals on seedling vigour in pot culture experiments.

(As influenced by fungicides)

Fungicides	Post emergence mortality (per cent)	Seedling height (cm)	Seedling weight (wet) (mg)
Benomyl	10.80 (3.50)	29.91	255.08
Carbendazim	8.08 (1.95)	29.94	259.75
Carboxin	11.54 (4.00)	30.65	271.15
Mancozeb	9.40 (2.65)	31.17	279.26
Control	32.46 (29.00)	20.74	135.35
C.D. (5%)	5.584	2.005	25.079

(Figures in parentheses are values in original units)

Table 32. Mean value showing the effect of seed treatment chemicals on rice seedling vigour in pot culture experiments.

(As influenced by inoculum)

Fungal inoculum	Post emergence mortality (per cent)	Seedling height (cm)	Seedling wet weight (mg)
A. flavus	13.72 (5.60)	28.17	229.50
A. nadwickii	14.10 (5.95)	29.29	255.63
B. oryzae	15.07 (6.75)	28.91	235.78
S. oryzae	15.35 (7.00)	27.57	239.55
C.D. (5%)	N.S	N.S	N.S

Figures in parentheses are values in original units.

Table 33. Mean value showing the effect of seed treatment chemicals on rice seedling vigour in pot culture experiments.

(As influenced by varieties)

Varieties	Post emergence mortality (per cent)	Seedling height (cm)	Seedling wet weight (mg)
Pavizham	14.62 (6.35)	24.89	188.21
Jyothi	13.47 (5.40)	32.13	281.39
Jaya	15.64 (7.25)	28.43	250.75
C.D. (5%)	N.S.	1.553	19.426

Figures in parentheses are values in original units.

Table 34. Interaction effect of fungicides and inoculum due to seed treatment on rice seedling height in pot culture experiments. (Thirty days after transplantation)

Fungicides	Seedling height (cm)			
	B.oryzae	*A.flavus*	*A.padwickii*	*S.oryzae*
Benomyl	26.58	33.88	28.18	30.83
Carbendazim	29.08	28.53	29.41	32.75
Carboxin	32.26	31.52	37.87	26.96
Mancozeb	33.58	25.55	32.93	29.63
Control	22.83	18.36	24.09	17.70

C.D. (5%) = 4.010

Table 35. Interaction effect of fungicides and varieties due to seed treatment on rice seedling height in pot culture experiments.
(Thirty days after transplantation)

Fungicides	Seedling height (cm)		
	Pavizham	Jyothi	Jaya
Benomyl	25.36	34.43	29.95
Carbendazim	29.60	32.64	27.58
Carboxin	23.39	36.41	32.16
Mancozeb	28.53	35.35	29.64
Control	17.57	21.84	22.82

C.D. (5%) = 3.473

Table 36. Interaction effect of fungicides and inoculum due to seed treatment on rice seedling weight in pot culture (Thirty days after transplantation)

Fungicides	Seedling wet weight (mg)			
	Pavizham	Jyothi	Jaya	Mean
Benomyl	186.69	302.06	276.49	255.08
Carbendazim	229.87	306.24	243.13	259.75
Carboxin	170.38	334.93	308.15	271.15
Mancozeb	231.11	328.33	278.34	279.26
Control	123.01	135.41	147.63	135.35

C.D. (5%) = 43.438

Table 37. Interaction effect of fungicides and inoculum due to seed treatment on rice seedling weight in pot culture experiments. (Thirty days after transplantation)

Fungicides	Seedling wet weight (mg)			
	B.oryzae	A.flavus	A.padwickii	S.oryzae
Benomyl	206.77	280.73	224.99	307.83
Carbendazim	221.82	248.93	273.47	294.77
Carboxin	300.95	274.19	301.42	208.04
Mancozeb	311.93	240.58	307.12	257.41
Control	137.45	103.07	171.17	129.70

C.D. (5%) = 50.158

Table 38. Interaction effect of inoculum and varieties due to seed treatment on rice seedling weight in pot culture experiments.
(Thirty days after transplantation)

Fungal inoculum	Seedling wet weight (mg)		
	Pavizham	Jyothi	Jaya
A. flavus	145.02	288.82	254.61
A. padwickii	249.10	259.02	258.78
B. oryzae	174.83	309.82	222.71

C.D. (5%) = 38.852

DISCUSSION

DISCUSSION

Isolation of Seed-borne Fungi.

Several fungi have been found to be associated with the commonly cultivated varieties of rice seeds collected from Kuttanadu, a major rice growing tract of Kerala. The casual agents of some important diseases like Bipolaris oryzae, Alternaria padwickii, Sarocladium oryzae and Curvularia lunata and storage fungi like Aspergillus flavus and A. fumigatus were encountered externally as well as internally. Pathogens like Piricularia oryzae and Fusarium moniliforme were present only on the external surface of seeds (Table 1).

Among the externally seed-borne fungi, A. flavus was the predominant one followed by B. oryzae and C. lunata with its association on nine and seven varieties respectively. The local cultivar Cheruvally recorded the association of a different species of Aspergillus viz. A. quadrilineatus.

Among the internally seed-borne fungi, A. flavus, B. oryzae and C. lunata were common on almost all the varieties.

Seeds of Jaya, Jyothi, Karthika and Pavizham showed maximum internal infection while M-310, the pre-release

culture and Cheruvally the local cultivar recorded no internal infection.

The seed-borne nature of P. oryzae, B. oryzae, C. lunata, S. oryzae and A. padwickii was already reported (Suzuki, 1930; Sharangapani, 1930; Ganguly, 1946; Padmanabhan, 1949; Bugnicourt, 1952 and Abi Cheeran, 1964). Similar observations were also made by Baldacci and Corbetta (1964) from Africa. In addition to P. oryzae, B. oryzae and A. padwickii they have also recorded C. oryzae, Fusarium sp. and Aspergillus sp. from infected rice seeds.

The seed infection by fungi clearly indicated that the pathogens were widely distributed in the paddy growing tracts of Kuttanadu under varied type of climatic conditions. P. oryzae infection was observed in few seed sample only. The environmental factors and the cropping pattern may be limiting its seed-borne nature.

Eventhough the fungi isolated in the present study were already reported from different parts of the world, such a location specific survey of this type in the main rice growing tract of Kerala viz., Kuttanadu for two successive seasons incorporating the commonly cultivated varieties is done for the first time. In Kuttanadu the fungal inoculum is available all throughout the year due to the indiscriminate use of

different varieties by farmers. The detailed survey on the percentage incidence of all the fungi associated with different seeds was not attempted.

Damages due to Seed-borne Fungi.

a. Effect of Inoculum on Germination and Post emergence Vigour.

i. Influence of Inoculum on Germination.

Germination of seeds was not found to be affected due to fungal inoculation (Table 2). Similar results were obtained by Inolehin (1983) with paddy seeds. He also could not obtain any correlation between seed infection and germination failure with fungi like B. oryzae, C. lunata, Aspergillus sp. and Alternaria sp. But reports of workers like Thomas (1941), Padwick and Ganguly (1945), Padmanabhan et al. (1948) and Alice and Philip (1985) showed that fungal infections reduced the germination potential of paddy seeds. For successful infection the fungus should gain entry into the embryo by penetrating the seed coat with the help of enzymes (Christensen and Kaufmann, 1965 and Vidhyasekaran et al., 1966). Death of embryo will eventually result in germination failure. So it can be assumed that in the present study

penetration of seed coat and invasion of embryo might not have occurred within the short period of three days.

ii. Influence of Inoculum on Root and Shoot Rot.

Post emergence rotting of primary root and shoot was observed due to infection by all fungi (Table 3 and 4). Maximum rotting of the roots was produced by B. oryzae and minimum was recorded by S. oryzae. Similarly A. padwickii recorded maximum rotting of the shoots.

Similar observations on root and shoot rot were made by Hingorani and Prasad (1951) with B. oryzae and C. lunata inoculation on paddy. Ibrahim and Førag (1966) observed damping off in rice seedlings due to fungal infection and Aguicro et al. (1966) recorded root, coleoptile and stem rot along with seedling blight in rice due to infection of seeds with A. padwickii, B. oryzae and C. lunata.

The pathogenic effect of A. flavus on germinating seeds of crop plants has already been reported (Rati and Ramalingam, 1974). The fungus produces serious pre-emergence rotting and post-emergence killing depending upon the extent of colonisation. It also produces the characteristic aflaroot symptom (Rao et al., 1984) attributing to the production of aflatoxin. In the present study such typical symptom was not observed.

Varieties also showed differences in their response to different fungi. Thus Pavizham inoculated with B. oryzae recorded maximum root rot while minimum rotting was noticed in Jyothi seeds inoculated with S. oryzae. Similarly Karthika showed higher shoot rot compared to other varieties and Karthika inoculated with A. flavus recorded maximum rotting. Ramadoss (1985) has also observed similar varietal preferences by seed-borne fungi.

iii. Influence of Inoculum on Root and Shoot Elongation.

Variations in primary root length were observed in seeds due to infection (Table 5). Minimum root length was recorded by B. oryzae treated seeds. C. lunata and A. flavus also recorded similar results. While A. padwickii and S. oryzae behaved like control.

In the case of shoot elongation also (Table 6) minimum length was recorded by B. oryzae treated seeds. A. flavus, A. padwickii, C. lunata and S. oryzae also showed similar behaviour.

Among the varieties tried Karthika showed the minimum shoot length.

b. Effect of Culture Filtrates on Seeds.

When paddy seeds were treated with the culture filtrate of the same fungus, remarkable reduction in germination was

recorded (Table 7) as compared to inoculum applied on seeds (Fig. 1).

Fungi are known to produce substances toxic to plants which affect adversely the seed germination and seedling vigour. Inhibitory effects on seed germination by the metabolites of fungi have been reported in the case of many crops (Nair, 1969; Ramadoss, 1985 and Naseema, 1987). In the present study also the metabolites contained in the culture filtrates have exerted similar inhibitory effects on germination.

The fungal metabolites were found to exert toxic effects on the embryo resulting in its death (Brian et al., 1952; Ludwig, 1957 and Bhale et al., 1982). The toxic fractions of the 30 days old culture filtrates of the fungi might have penetrated the seed coat and infected the embryo unlike the fungal inoculum applied on the seed surface.

Complete inhibition of root initiation was also noticed by the action of culture filtrates like A. flavus, A. nadwickii and C. lunata (Table 8). The roots are considered to be the most susceptible tissues to toxic principles at the time of germination than shoots (Luke and Wheeler, 1955). Vidhyasekaran et al. (1970) suggested the presence of cell

toxic substances in the fungal metabolites. Hence it can be assumed that the culture filtrates which happened to react with the root primordia might have inhibited the root initiation in seedlings.

Among the varieties tried, Pavizham recorded the maximum inhibition and Pavizham seeds treated with A. flavus filtrate produced the maximum inhibitory effect. Varietal susceptibility of plants to toxins were already reported (Ramadoss, 1985).

The culture filtrate also exerted significant effect on the vigour of the seedlings. The root and shoot length were reduced (Table 9 and 10). Maximum reduction in root and shoot length was observed with the culture filtrates of A. flavus and B. oryzae respectively. In both cases S. oryzae recorded minimum reduction.

Seed inoculations with A. padwickii and S. oryzae did not alter the primary root length (Table 5, Fig. 2) but their culture filtrates exerted significant effect on germination as well as on root and shoot elongation.

The toxic effects of the fungal metabolites on the embryo resulting in the death of the plumule was already reported (Brian et al., 1952; Ludwig, 1957 and Bhale et al., 1982). The toxic principles contained in the 30 days old

FIG.1. INFLUENCE OF FUNGAL INOCULAM AND CULTURE FILTRATES ON RICE SEED GERMINATION

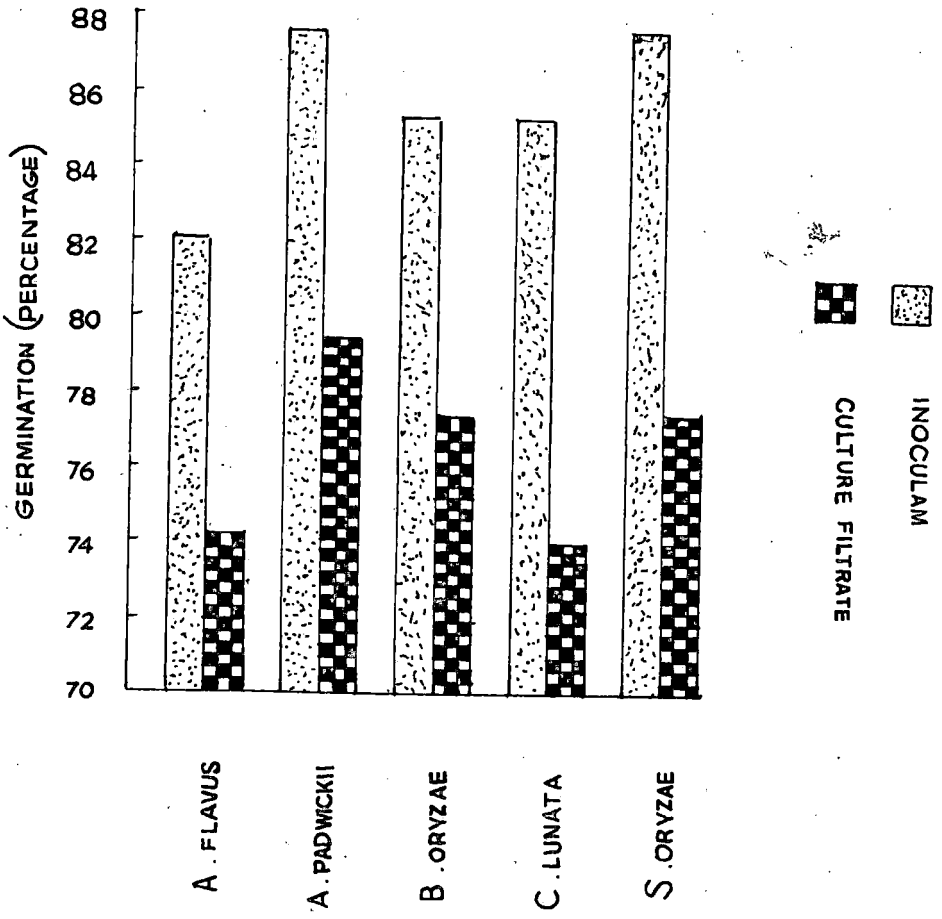
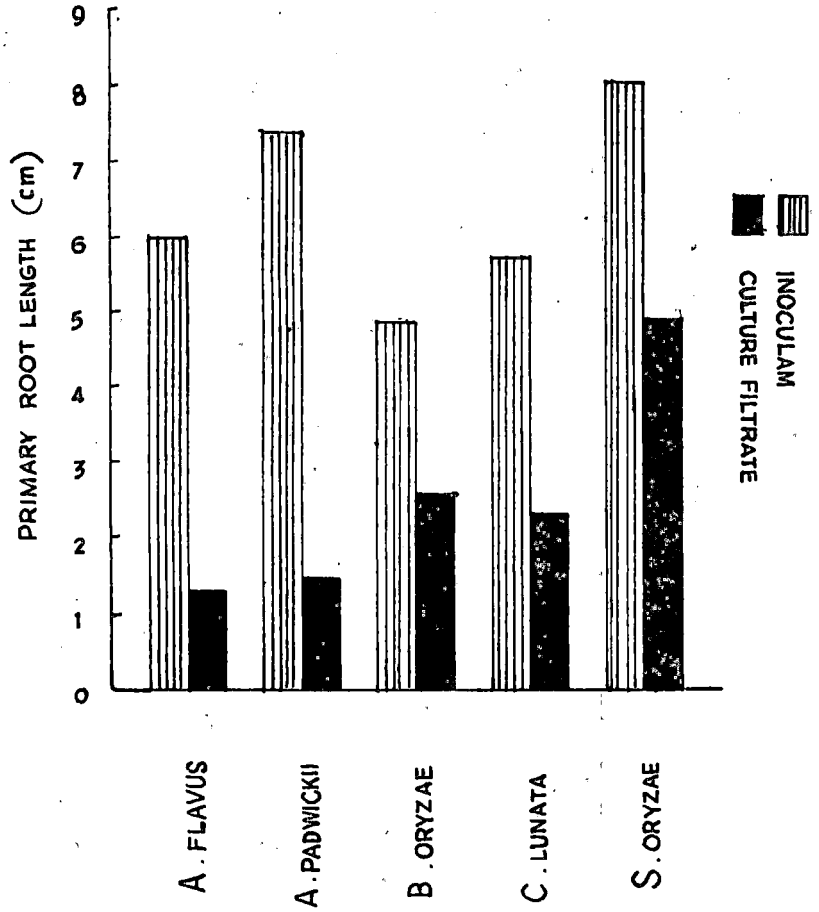


FIG.2. INFLUENCE OF FUNGAL INOCULAM AND CULTURE FILTRATE ON PRIMARY ROOT LENGTH OF RICE SEEDLINGS



culture filtrates might have acted on the embryo and resulted in reduced root and shoot growth. Vidhyasekaran et al. (1970) has also recorded similar results with the culture filtrates of B. oryzae. In the early stages of infection the seeds have escaped deliterious effect because the fungus might not have produced metabolites within that short period. When seeds were treated with 30 days old culture filtrate the response was immediate. The germination percentage was reduced along with a reduction in the length of the emerging root and shoot.

The effect of varieties was also very prominent. Karthika was found to be more susceptible to fungal toxins and showed the maximum reduction in shoot length when treated with the culture filtrate of B. oryzae (Table 10). Conversely Jyothi seeds treated with the culture filtrate of S. oryzae produced minimum reduction in shoot length.

Bio-chemical changes due to Fungal Infection.

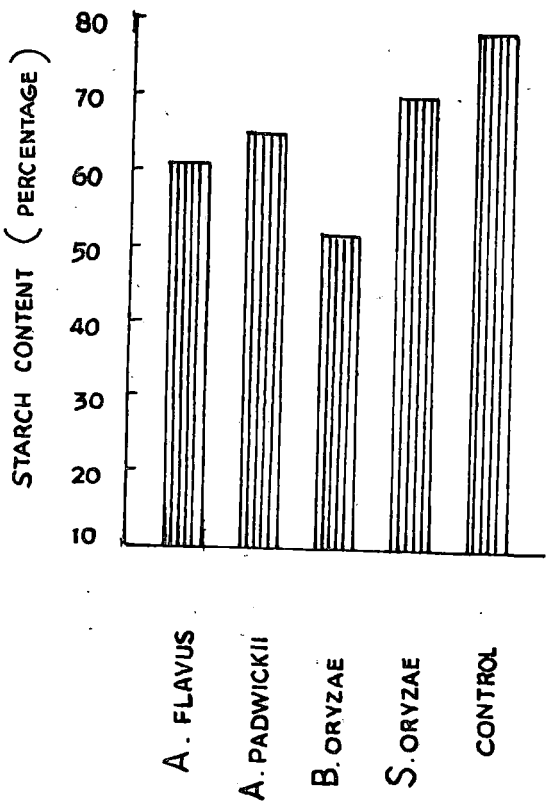
i. Changes in Starch.

From the results given in Table 11 it can be seen that the starch content of all the varieties were significantly reduced due to fungal infection during storage. Maximum reduction of starch was recorded due to the infection of B. oryzae while S. oryzae recorded the minimum effect (Fig. 3).

The results of the present investigation are in agreement with the previous reports on the subject. Allen (1942) and Mirocha and Zaki (1966) have reported a reduction of starch content due to fungal infection in wheat and barley respectively. Vidhyasekaran and Govindaswamy (1968) and Vidhyasekaran and Ramadoss (1973) also reported the starch reducing capacity of many fungi like B. oryzae on rice seeds.

Bilgrami et al. (1979) suggested the possibility of starch hydrolysis during the fungal infection of rice seeds. They considered that the availability of free sugars during starch hydrolysis might help in the perpetuation of the fungus in the infected tissues of the seed leading to decreased level of starch content in the seed. The decreese in starch content was also correlated by the activation of starch bydrolysing enzyme, -amylase (Vidhyasekaran and Kandaswamy, 1972). Enzyme amylase is considered to be widely distributed among fungi and they help in hydrolysis of starch (Cochrane, 1958). It is also thought that reduction of starch in fungus infected plant parts is due to rapid degradation of starch by enzymes produced by the pathogen (Mirocha and Zaki, 1966). Vidhyasekaran and Ramadoss (1973) also put forth the same explanation and

FIG. 3. CHANGES IN THE STARCH CONTENT OF FUNGUS INFECTED RICE SEEDS



recorded the starch content of Helminthosporium infected grains to be significantly less than the healthy grains.

The reduction in the starch content of infected seeds might also be due to some of the starch being utilised as a nutrient, supplying both energy and carbon for the growth of fungi. Starch is the major carbohydrate reserve of stored grains which can be easily hydrolysed to soluble glucose by a variety of microorganisms including fungi. Starch hydrolysing enzymes, the α-amylases are extracellular and inducible in the presence of starch as a substrate for microbial proliferation.

The detection of amylase activity in deteriorating grains is thus an evidence for the hydrolytic action on the starch leading to a reduction of its total quantity. The decrease in starch content of the infected grains obtained in the present study can be due to its utilisation as a nutrient by the parasitic fungi. The variations in the content of starch in grains infected by different species of fungi mught be attributed to the differences in their rate of proliferation in the grain resulting in a greater or lesser rate of assimilation.

ii. Changes in Protein content.

The protein content recorded no statistically significant changes due to fungal infection after 45 days of storage.

However, slight increases in the protein values were observed for infected seeds (Table 12). Similar observations were recorded by Vidhyasekaran and Ramadoss (1973) and Vidhyasekaran et al. (1973) and Bilgrami et al. (1979) in rice seeds due to Helminthosporium infection. McCombe and Winstead (1964) reported fluctuations in protein content with increased levels at the later stages after infection. This was represented as the sum total of protein from the seed and fungal mycelium. Vidhyasekaran and Durairaj (1971) reported a slight increase in the protein content due to the infection of Xanthomonas citri in citrus fruits. Cherry et al. (1974) pointed out that in peanuts infected with Aspergillus parasiticus, with fungus tissue included, the protein content decreased for two days and increased thereafter. However, reduction in protein content of Urd seeds and Arhar seeds due to fungal infection was also reported by Jamaluddin et al. (1977) and Sinha et al. (1978) respectively.

A comparatively greater utilisation of non-protein carbon sources with respect to proteins by fungi has been reported (Alexander, 1977). The trend to increase the percentage of protein, eventhough not significant, may be due to the utilisation of non-protein carbon source by the fungi, leading to an increase in the percentage of protein

(Philip, 1978). The free amino acids in the seeds are reported to be both in protein and non-protein form (Bewly and Black, 1978). The studies on the changes of protein during different periods of storage were not attempted in the present investigation.

As the starch and other polysaccharide constituents of the grain are assimilated, the proteins are also slowly degraded under the influence of proteolytic enzymes and provide nitrogen for the synthesis of fungal protoplasm. A part of the seed protein may then be converted to fungal protein without affecting the total protein content of the seed which is infected by the parasitic fungus. As the fungal assimilation is taking place, the C : N ratio of the seed (host) is getting narrower due to loss of part of carbon as CO_2, resulting in a slight increase in the nitrogen content of seed (Alexander, 1977).

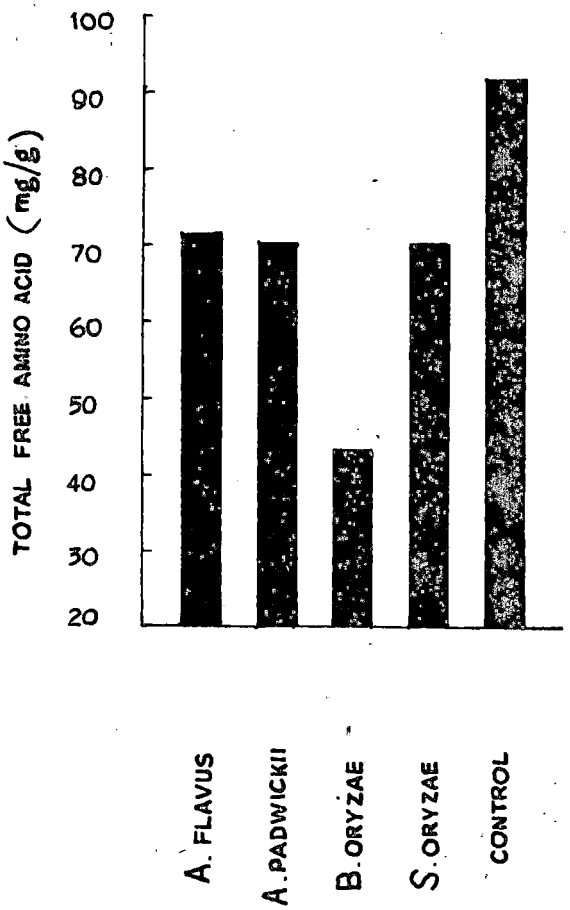
iii. Changes in Amino acids.

The total free amino acid content was found to be reduced significantly due to microbial infection after 45 days (Fig. 4). The maximum reduction was observed to be due to infection by B. oryzae (Table 13). Vidhyasekaran et al. (1973) also reported decrease in total amino acid

content due to B. oryzae infection in rice seeds. Influence of the varieties were also significantly evident as the variety Pavizham recorded the highest and Jaya the lowest total free amino acid content. Obviously, the infection by B. oryzae on Jaya seeds caused the maximum reduction in amino acid content. Prasad et al. (1970), Sekhawat and Kothari (1971), Bilgrami et al. (1979) and Ramadoss (1985) have also recorded similar results. The reason for the decline in amino acid content was thought to be due to the ready utilisation of the amino acid fraction present in the host seed by the pathogen for synthesising its own protein (Reisener et al., 1970 and Bilgrami et al., 1979). It was also thought that atleast a part of the amino acids served as carbon source after deamination by fungi (Sharma and Wahale, 1975 and Bilgrami et al., 1979).

The results of protein analysis showed no significant change in the infected seeds, though there was a slight increase after 45 days. The delay in protein synthesis of fungal infected seeds of many crops was suggested to be due to the sudden reduction in the amino acid pool (Harman et al., 1970). Rapid ageing of pea seeds infected with fungus, on storage resulting in germination failure or delayed germination, is attributed by them to many bio-chemical and physiological changes like a slower increase in oxygen

FIG. 4. CHANGES IN THE TOTAL FREE AMINO ACID CONTENT OF FUNGUS INFECTED RICE SEEDS



uptake, a delay in protein synthesis, smaller ATP and amino acid pool in embryonic axis etc. The aminoacid content of the infected seeds may also vary considerably depending on the period of storage, type of fungus and type of amino acid. Other than an observation on change in the total amino acid content, no attempts were made to study their periodic or qualitative changes in separated as well as bound form.

Effect of Seed treatment Chemicals in Controlling Infection.

A. In vitro Studies.

a. Effect on Germination.

Increase in seed germination was recorded in all cases due to seed treatment by all the chemicals viz. benomyl, carbendazim, carboxin and mancozeb (Table 14). Dharam Vir et al. (1970) observed increased germination for Drechslera oryzae infected rice seeds on treatment with benlate and mancozeb. Seed treatment with carboxin was also reported to increase the germination of wheat seeds (Nene and Saxena, 1971 and Tyagi, 1972). Ellis and Paschal (1979) reported that systemic fungicides on application to seeds penetrated the seed coat and embryo of pea seeds and acted directly on the infecting pathogen present on the plumule and radical resulting in enhanced germination. In the present study

also the effect of all the fungicides in increasing the germination of infected seeds was evident, but there was no relative merit for the three systemic fungicides over mancozeb, the dithiocarbamate fungicide.

b. Effect on Inoculum Recovery.

Studies on inoculum recovery revealed significant reduction in the inoculum due to seed treatment. Mancozeb recorded maximum reduction (Table 14). Among the effect of different fungal isolates, A. padwickii and B. oryzae treated seeds recorded minimum recovery suggesting a better response to seed treatment (Table 15). Similarly among the varieties tried, Jyothi and Jaya seeds showed better response with less inoculum (Table 16).

Studies on the interaction effect of fungicides on inoculum recovery revealed the effectiveness of all the fungicide tried. Benomyl, carboxin and mancozeb recorded slight fungal recovery while carbendazim recorded higher inoculum recovery of A. flavus, A. padwickii and S. oryzae (Table 18). On a varietal comparison also carbendazim recorded higher inoculum recovery on all the three varieties viz. Pavizham, Jyothi and Jaya (Table 19), while minimum inoculum recovery was noticed for Jaya seeds infected with A. padwickii (Table 20).

c. Effect on Root and Shoot Length.

Seed treatment with fungicides had significant influence on the elongation of primary root and shoot. Among the fungicides mancozeb and benomyl produced maximum elongation of root and shoot respectively (Table 14).

Fungal infection also had influence on root and shoot length. Seeds infected with S. oryzae and B. oryzae after seed treatment recorded maximum root and shoot length respectively (Table 15).

Varieties also differed. Among the varieties Jyothi produced maximum root length while Pavizham recorded maximum shoot length (Table 16).

Interaction effects were also significant. Mancozeb was the best in enhancing the root length of seeds infected with all the test fungi viz., B. oryzae, A. flavus, A.padwickii and S. oryzae (Table 21). Similarly mancozeb produced better results on varietal comparisons also (Table 22). For Jyothi and Jaya it proved best and Jyothi seeds infected with S. oryzae after seed treatment recorded maximum root length (Table 23).

In the case of shoot length also the interaction effects were significant. But no specificity was noted

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(Table 24) on varietal comparisons. Benomyl gave maximum shoot length for Pavizham and Jaya, while for Jyothi, mancozeb was the best (Table 25). Similarly Pavizham infected with S. oryzae recorded the maximum shoot length in seedlings (Table 26).

Increased shoot length was noticed in seeds on treatment with benomyl and carboxin. Vyas (1984) suggested that seed treatment with benomyl provided an initial source of active component for the systemic movement into the first few leaves of the developing seedlings. Carboxin also showed similar results. Carboximides in some conditions appeared to be directly beneficial to plants. Schmling and Clark (1970) have claimed that oxathiins showed unique growth stimulation properties when applied either to seed, soil or foliage. The results obtained in the present study with seed treatment agrees with their results.

d. Effect on Root and Shoot Rot.

All the fungicides were found to be effective in reducing root and shoot rot to the minimum. But benomyl treatment was found to be superior (Table 14). Benomyl was reported to be effective in controlling root rot and damping off of crops like tomato, garden peas, etc. Seed treatment

with mancozeb against Drechslera spp. on barley, rice and oats is also reported (Dharm Vir et al., 1970 and Rath, 1974).

B. Pot Culture Experiments.

The post emergence mortality of germinated seedlings after transfer to the soil was found to be considerably reduced due to the seed treatment with all the fungicides. Here, the effects of all fungicides tested were alike eventhough carbendazim was slightly superior over others (Table 31).

The seedling vigour after 30 days as indicated by the seedling height and fresh weight were found to be significantly influenced by fungicidal treatment. The effects of all the fungicides were similar. Seedling height and weight were increased (Table 34, 36). As far as the varietal preference for fungicidal treatments are concerned, Jyothi recorded maximum seedling height and weight against carboxin treatment (Table 35, 37). Detailed studies after 30 days were not attempted.

SUMMARY

SUMMARY

Several fungi were isolated from the seeds of twelve varieties of cultivars of rice collected from different localities of Kuttanadu, the major rice growing tract of Kerala. Fungi were encountered externally as well as internally. Among the externally seed borne fungi, Aspergillus flavus was the most common with its occurrence on nine varieties followed by Bipolaris oryzae and Curvularia lunata. Other fungi included Rhizopus stolonifer, Chaetomium gracile, Syncephalastrum racemosum, Fusarium moniliforme, Trichoderma virde, Sarocladium oryzae, Aspergillus niger, Alternaria padwickii, Fusarium graminearum, Piricularia oryzae, A. fumigatus, A. quadrilineatus, Nigrospora oryzae and Penicillium spp.

The germination capacity was not found to be altered due to seed inoculation with predominant fungi viz. A. flavus, A. padwickii, B. oryzae, C. lunata and S. oryzae. Rotting of the root and shoot were noticed due to the application of inoculum on the seeds. Varietal difference was also prominent. Seeds of Pavizham inoculated with B. oryzae and Jhothi with S. oryzae recorded maximum and minimum root rot respectively. In the case of shoot rot maximum damage was caused by A. padwickii. Among the varieties Karthika showed maximum shoot rot.

Root and shoot length were also found to be reduced due to infection by all the fungi tested. Among the varieties Karthika recorded minimum shoot length.

The culture filtrate of the above fungi (30 days old) also exerted considerable influence on the germination of seeds. Germination capacity of the seeds was reduced. Among the varieties Jyothi recorded maximum reduction in germination. The culture filtrates also inhibited root initiation in emerging seedlings. Maximum inhibition was observed in the variety Pavizham. The culture filtrates also reduced root and shoot elongation. The combination of Pavizham with A. flavus recorded minimum root length. Similarly Karthika with culture filtrate of B. oryzae recorded minimum shoot length.

Bio-chemical changes were also observed in infected seeds after 45 days of storage. Starch and total free amino acid content of the infected seeds were reduced while protein remained unchanged. Among the varieties Jaya recorded maximum reduction in starch. Similarly Jaya inoculated with B. oryzae recorded the maximum reduction in total free amino acids.

Fungicidal treatment with systemic fungicides like benomyl, carbendazim, carboxin and the dithio carbamate fungicide, mancozeb at 0.3 per cent concentration was found to be effective in improving the germination of artificially infected

seeds. Considerable reduction in the quantity of inoculum in the germinated seedlings was observed after seven days. The root and shoot length of rice seedlings were also found to increase with seed treatment. Among the fungicides, mancozeb and benomyl produced maximum elongation of root and shoot respectively. Varietal response was also noticed. Jyothi recorded maximum root length while Pavizham recorded the maximum shoot length. The root and shoot rot normally associated with infected seeds were found to be reduced due to fungicidal treatments. Benomyl was found to be superior in both cases. Root rot was significantly influenced by the type of inoculation.

Studies in pot culture experiment revealed a reduction in the post emergence mortality of seedlings, raised from seeds treated with the above fungicides. Among the fungicides carbendazim recorded the least seedling mortality eventhough all the fungicides were effective. The seedling vigour was also found to be increased. The height and weight of the seedling were improved. All the fungicides were effective in increasing the seedling vigour but mancozeb proved to be slightly superior. Among the varieties Jyothi recorded maximum height and weight on treatment with carboxin.

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* Original not seen.

APPENDICES

APPENDIX I

COMPOSITION OF CZAPEK (DOX) AGAR MEDIUM

Sucrose	20.00 g
Sodium nitrate ($Na\ NO_3$)	2.00 g
Potassium dihydrogen phosphate ($K\ H_2\ PO_4$)	1.00 g
Magnesium sulphate ($Mg\ SO_4.7H_2O$)	0.50 g
Potassium chloride ($K\ Cl$)	0.50 g
Ferrous sulphate ($Fe\ SO_4.7H_2O$)	0.01 g
Agar	20.00 g
Distilled water	1000 ml

The medium was sterilised by autoclaving at 15 lb pressure for 30 minutes.

Source: Plant Pathologist's Pocket Book, 2nd Edition. Commonwealth Mycological Institute, London, pp.394.

APPENDIX II

NUTRITIONAL STATUS OF VELLAYANI UPLAND SOIL

Total N	0.013%
Total P	0.014%
Total K	0.065%

Source: Department of Agronomy, College of Agriculture, Vellayani.

APPENDIX III

Analysis of variance Table

(Effect of fungi on germination of rice seeds)

Source	S.S.	Df.	M.S.	F.
A	1062.75	3	354.25	7.82*
B	300.03	5	60.00	1.32
A x B	843.56	15	56.23	1.24
Error	1086.09	24	45.25	
Total		47		

A = Variety
B = Inoculum

* Significant at 5% level.

APPENDIX IV

Analysis of variance Table

(Effect of fungi on per cent root rot of rice seedlings)

Source	S.S.	Df.	M.S.	F
A	59.96	3	19.98	74.23
B	15125.38	5	3025.07	113.87*
A x B	1676.90	15	111.79	4.20*
Error	637.57	24	26.56	
Total		47		

A = Variety
B = Inoculum

* Significant at 5% level

APPENDIX V

Analysis of variance Table

(Effect of fungi on per cent shoot rot of rice seedlings)

Source	S.S.	Df.	M.S.	F.
A	1424.61	3	474.87	19.02*
B	8030.13	5	1606.02	64.34*
A x B	1099.12	15	73.27	2.93*
Error	599.06	24	24.96	
Total		47		

A = Variety

B = Inoculum

* Significant at 5% level.

APPENDIX VI

Analysis of variance Table

(Effect of inoculum on the primary root length of emerging rice seedlings)

Source	S.S.	Df.	S.S.	F.
A	8.48	3	2.82	0.65
B	109.23	5	21.85	5.03*
A x B	53.66	15	3.57	0.82
Error	104.16	24	4.34	
Total		47		

A = Variety

B = Inoculum

* Significant at 5% level.

APPENDIX VII

Analysis of variance Table

(Effect of inoculum on shoot length of emerging rice seedlings)

Source	S.S.	Df.	M.S.	F.
A	10.74	3	3.58	12.69*
B	19.31	5	3.86	13.68*
A x B	7.68	15	0.51	1.81
Error	6.77	24	0.28	
Total		47		

A = Variety

B = Inoculum

* Significant at 5% level.

APPENDIX VIII

Analysis of variance Table

(Effect of culture filtrate on rice seed germination)

Source	S.S.	Df.	S.S.	F.
A	321.37	3	107.12	5.65*
B	671.06	5	134.21	7.08*
A x B	642.53	15	42.83	2.26
Error	454.81	24	18.95	
Total		47		

A.= Variety

B = Inoculum

* Significant at 5% level

APPENDIX IX

Analysis of variance Table

(Effect of culture filtrate on root initiation)

Source	S.S.	Df.	M.S.	F.
A	1528.84	3	509.61	13.94*
B	17645.20	5	3529.04	401.43*
A x B	4820.02	15	321.33	36.55*
Error	210.98	24	8.79	
Total		47		

A = Variety
B = Inoculum

* Significant at 5% level.

APPENDIX X

Analysis of Variance Table

(Effect of culture filtrate on primary root elongation of emerging rice seedlings)

Source	S.S.	Df.	M.S.	F.
A	3.15	3	1.05	1.11
B	176.44	5	35.28	37.38*
A x B	41.57	15	2.77	2.94*
Error	22.65	24	0.94	
Total		47		

A = Variety
B = Inoculum

* Significant at 5% level.

APPENDIX XI

Analysis of variance Table

(Effect of culture filtrates on mean shoot length of emerging rice seedlings)

Source	S.S.	Df.	M.S.	F.
A	11.68	3	3.89	20.76*
B	44.70	5	8.94	47.66*
A x B	10.27	15	0.68	3.65*
Error	4.50	24	0.18	
Total		47		

A = Variety

B = Inoculum

* Significant at 5% level.

APPENDIX XII

Analysis of variance Table

(Effect of fungal inoculation on starch content of rice seeds)

Source	S.S.	Df.	M.S.	F.
A	1357.46	3	452.48	123.86*
B	2949.25	4	737.31	201.83*
A x B	1078.50	12	89.87	24.60*
Error	73.06	20	3.65	
Total		39		

A = Variety

B = Inoculum

* Significant at 5% level.

APPENDIX XIII

Analysis of variance table

(Effect of fungal inoculation on the protein content of rice seeds)

Source	S.S.	Df.	M.S.	F.
A	0.23	3	0.07	0.06
B	2.24	4	0.56	0.48
A x B	7.08	12	0.59	0.51
Error	22.99	20	1.15	
Total		39		

A = Variety

B = Inoculum

* Significant at 5% level.

APPENDIX XIV

Analysis of variance Table

(Effect of fungal infection on the total free amino acid content of rice seeds)

Source	S.S.	Df.	M.S.	F.
A	14649.31	3	4883.10	110.02*
B	935.50	4	2339.75	52.72*
A x B	3864.90	12	322.07	7.25*
Error	956.30	20	47.81	
Total		39		

A = Variety

B = Inoculum

* Significant at 5% level.

APPENDIX XV

Analysis of variance Table

(Effect of seed treatment fungicides on germination of infected seeds

Source	S.S.	Df.	M.S.	F.
A	66.62	2	33.31	0.39
B	273.06	3	91.02	1.07
C	1241.62	4	310.40	3.64*
A x B	1204.68	6	200.78	2.36*
B x C	630.93	12	52.57	0.61
A x C	424.12	8	53.01	0.62
A x B x C	647.37	24	26.77	0.31
Error	5102.93	60	85.04	
Total		119		

A = Variety B = Inoculum C = Fungicide

*Significant at 5% level.

APPENDIX XVI

Analysis of variance Table

(Effect of seed treatment chemicals on inoculum recovery from infected seeds)

Source	S.S.	Df.	M.S.	F.
A	446.69	2	223.34	7.08*
B	553.73	3	184.57	5.85*
C	18998.76	4	4749.68	150.66*
A x B	491.30	6	81.88	2.59*
B x C	1368.38	12	114.03	3.61*
A x C	1337.93	8	167.24	5.30*
AxBxC	1463.78	24	60.99	1.93*
Error	1891.50	60	31.52	
Total		119		

A = Variety B = Inoculum C = Fungicide

* Significant at 5% level.

APPENDIX XVII

Analysis of variance Table

(Effect of seed treatment chemicals on root length of seedlings)

Source	S.S.	Df.	M.S.	F.
A	13.81	2	6.90	71.08*
B	32.12	3	10.70	110.17*
C	98.36	4	24.59	252.99*
A xB	12.85	6	2.14	22.03*
B x C	15.62	12	1.30	13.39*
A x C	32.72	8	4.09	42.08*
AxBxC	70.39	24	2.93	30.17*
Error	5.83	60	9.72	
Total		119		

A = Variety B = Inoculum C = Fungicide

* Significant at 5% level.

APPENDIX XVIII

Analysis of variance Table

(Effect of seed treatment chemicals on shoot length of seedlings)

Source	S.S.	Df.	M.S.	F.
A	11.23	2	5.61	125.18*
B	8.10	3	2.70	60.19*
C	48.06	4	12.01	267.75*
A x B	8.92	6	1.48	33.15*
B x C	5.70	12	0.47	10.60*
A x C	7.00	8	0.87	19.50*
A x B x C	20.49	24	0.85*	19.02*
Error	2.69	60	4.48	
Total		119		

A = Variety B = Inoculum C = Fungicide

* Significant at 5% level.

APPENDIX XIX

Analysis of variance Table

(Effect of seed treatment chemicals on root of seedlings)

Source	S.S.	Df.	M.S.	F.
A	37.71	2	18,25	0.52
B	789.98	3	263.32	7.27*
C	17147.52	4	4286.88	118.45*
A x B	2292.95	6	382.15	10.55*
B x C	1152.84	12	96.07	2.65*
A x C	1434.10	8	179.26	4.95*
A x B x C	3525.86	24	146.91	4.05*
Error	2171.36	60	36.18	
Total		119		

A = Variety B = Inoculum C = Fungicide

* Significant at 5% level.

APPENDIX XX

Analysis of variance Table

(Effect of seed treatment chemicals on shoot rot of seedlings)

Source	S.S.	Df.	M.S.	F.
A	112.58	2	56.29	1.42
B	238.75	3	79.58	2.01
C	19913.57	4	2978.39	75.39*
A x B	843.01	6	140.50	3.55*
B x C	351.84	12	29.32	0.74
A x C	539.57	8	67.44	1.70
A x B x C	1732.93	24	72.20	1.82
Error	2370.15	60	39.50	
Total		119		

A = Variety B = Inoculum C = Fungicide

*Significant at 5% level.

APPENDIX XXI

Analysis of variance Table

(Effect of seed treatment chemicals on post emergence mortality of seedlings)

Source	S.S.	Df.	M.S.	F.
A	94.62	2	47.31	0.52
B	52.73	3	17.57	0.19
C	9718.69	4	2429.67	26.91*
A x B	631.38	6	105.23	1.16
B x C	388.95	12	32.41	0.35
A x C	539.87	8	67.48	0.74
A x B x C	1386.97	24	57.79	0.64
Error	5416.31	60	90.27	
Total		119		

A = Variety B = Inoculum C = Fungicide

* Significant at 5% level

APPENDIX XXII

Analysis of variance Table

(Effect of seed treatment chemicals on seedling height)

Source	S.S.	Df.	M.S.	F.
A	1049.45	2	524.72	45.07*
B	52.84	3	17.61	1.51
C	1823.97	4	455.99	37.16*
A x B	177.79	6	29.63	2.54
B x C	592.15	12	49.34	4.23*
A x C	427.72	8	53.46	4.59*
A x B x C	1183.09	24	49.29	4.23*
Error	698.53	60	11.64	
Total		119		

A = Variety B = Inoculum C = Fungicide

* Significant at 5% level.

APPENDIX XXIII

Analysis of variance Table

(Effect of seed treatment chemicals on seedling weight)

Source	S.S.	Df.	M.S.	F.
A	180442.50	2	90221.25	49.55*
B	11179.00	3	3726.33	2.04
C	337935.50	4	84483.88	46.40*
A x B	73233.50	6	12205.58	6.70*
B x C	118568.00	12	9880.66	5.42*
A x C	69925.00	8	8740.62	4.80*
A x B x C	145338.50	24	6055.77	3.32*
Error	109245.50	60	1820.75	
Total		119		

A = Variety
B = Inoculum
C = Fungicide

*Significant at 5% level.

ABSTRACT

Several fungi were isolated from the seeds of twelve varieties/cultivars of rice collected from different localities of Kuttanadu, the major rice growing tract of Kerala. Fungi were encountered externally as well as internally. Among the externally seed borne fungi, Aspergillus flavus was the most common with its occurrence on nine varieties followed by Bipolaris oryzae and Curvularia lunata. Other fungi included Rhizopus stolonifer, Chaetomium gracile, Syncephalastrum racemosum, Fusarium moniliforme, Trichoderma virde, Sarocladium oryzae, Aspergillus niger, Alternaria padwickii, Fusarium graminearum, Piricularia oryzae, A. fumigatus, A. quadrilineatus, Nigrospora oryzae and Penicillium spp.

Among the varieties Jaya was found to harbour the maximum fungal population.

The germination per centage was not found to be affected due to seed inoculation with the predominant fungi viz. A. flavus, A. padwickii, B. oryzae, C. lunata and S. oryzae.

The above inoculum when applied on the seed caused significant rotting of the root and shoot as compared to the control after seven days. Root rot was maximum with B. oryzae inoculated treatment followed by A. padwickii and A. flavus.

Treatment with S. oryzae recorded minimum rotting. Varietal difference was also prominent. Seeds of Pavizham inoculated with B. oryzae and Jyothi with that of S. oryzae recorded maximum and minimum rotting respectively. In the case of shoot rot, maximum damage was caused by A. padwickii and among the varieties, Karthika ranked first in inducing shoot rot.

Seeds inoculated with fungi on germination also showed decreased root and shoot length. B. oryzae treated seeds recorded minimum root and shoot length eventhough it was on par with other fungi tried. Varieties differed significantly in shoot elongation and Karthika recorded minimum shoot length.

The culture filtrates of the above fungi also exerted considerable influence on the germination of seeds. Germination capacity was reduced on treatment with 30 days old culture filtrates in all cases. Varietal difference was also noted. Jyothi recorded maximum germination failure.

The root initiation of the emerging seedling was also found to be inhibited by the culture filtrates of A. flavus, C. lunata and A. padwickii. The variety Pavizham recorded maximum inhibition.

Root and shoot elongation were also reduced by all the culture filtrates. The combination of Pavizham with A. flavus

recorded minimum root length. Similarly the combination of Karthika with the culture filtrate of B. oryzae recorded minimum shoot length.

Biochemical changes were also conspicuous in infected seeds after 45 days. The starch content was found to be reduced. But minimum reduction was noticed with S. oryzae infected seeds. Among the varieties Jaya recorded maximum reduction.

The protein content of the seed was not found to be affected eventhough the total amino acids were reduced significantly. Among the treatments Jaya inoculated with B. oryzae recorded maximum reduction in total free amino acid.

Seeds inoculated with the common pathogens, after 30 days of storage, on treatment with fungicides viz., benomyl, carbendazim, carboxin and mancozeb at 0.3 per cent recorded improved germination in all cases. But it was observed that neither the inoculum nor the varieties had any effect in improving seed germination.

Studies on inoculum recovery from the above treated seeds revealed significant reduction in the quantity of inoculum after seven days. Mancozeb recorded maximum

reduction in inoculum. Among the fungal isolates tried A. padwickii and B. oryzae treated seeds recorded minimum inoculum recovery. Similarly among the varieties, Jaya and Jyothi showed better response with lesser quantity of recovered inoculum. Minimum inoculum was recorded for Jaya seeds infected with A. padwickii.

The root and shoot length of rice seedlings were found to be considerably increased due to seed treatment. Among the fungicides mancozeb and benomyl produced maximum elongation of root and shoot respectively. Influence of inoculum was also significant. Seeds infected with S. oryzae and B. oryzae recorded maximum root and shoot elongation after seed treatment. Varietal response was also conspicuous. Jyothi and Pavizham recorded maximum root and shoot length respectively.

The root and shoot rot normally associated with infected seeds were also found to be reduced by all fungicides. But benomyl treatment was found to be superior in both cases. Root rot was significantly influenced by the type of inoculum. Seeds infected with A. padwickii responded poorly to seed treatment and produced maximum rotting as compared to other inoculum in controlling root rot while the type of inoculum had no effect on shoot rot. Varietal response to fungicides in controlling root and shoot rot was also not prominent.

Post emergence mortality of seedlings raised from seeds treated with the above fungicides were found to be reduced after 30 days of transplantation in culture pots. Among the fungicides carbendazim recorded the least seedling mortality eventhough all the fungicides were effective. The seedling vigour as represented by height and weight was also increased. Maximum seedling height was observed for treatment with mancozeb eventhough all the other fungicides were also effective. Varietal difference was also significant. Jyothi recorded maximum seedling height and Pavizham recorded the minimum. The seedling height was maximum for the combination of carboxin with A. padwickii.

The seedling weight was also increased due to the action of all fungicides. Mancozeb treated seeds recorded the maximum seedling weight and among the varieties Jyothi recorded the maximum seedling weight.