

## Studies on Seed-Borne Fungi of Rice

by

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**Introduction:** The paddy crop is subjected to a number of diseases both in the seedbed and in the mainfield. Most of the trouble arises as a result of either using diseased seed material or the seed is attacked by the microflora present in the soil. The damage becomes very serious when soil and weather conditions favour the growth and development of the harmful microflora of the seed and soil. As a result of infection, there is seed decay resulting in the failure of germination of seed, destruction of young seedlings before emergence (pre-emergence blight) and even the seedlings that emerge out survive in a weakened state until they finally succumb to some trouble or other (post-emergence blight). The damage to seedlings is quite enormous resulting in the non-availability of sufficient seedlings and at times even necessitating the raising of seedlings again.

**Object:** The present study was undertaken at the Agricultural College, Bapatla, with the following objects in view: (1) to determine the commonly associated fungi of the paddy seed and (2) to study the effect of seed-borne fungi on the germination of seed and emergence of seedlings.

**Materials and Methods:** The seed material used in the investigation was obtained from ten rice research stations distributed all over the state (composite state of Madras and Andhra), each station representing an important rice growing belt. From each station seed samples of three popular varieties were obtained. The seedlings were raised in mud pots of the size 9" X 9", using pulverized clay soil collected from paddy fields. The soil was well powdered, sieved and sterilized at thirty pounds pressure for two hours whenever sterilized soil was used. The sowings in all the experiments were done with seeds selected at random at the rate of 25 seeds per pot.

The epiphytic microflora of the seed samples was determined by the centrifugal method. The seeds from each variety were picked at random and transferred to a clean test tube. In each tube 10 c. c. of sterilized water were added, vigorously shaken for five minutes and the water extract transferred to centrifuge tubes.

The sterilized water extracts of the seed samples were centrifuged in a Clay Adam's centrifuge for ten minutes. After centrifuging, the supernatant liquid was decanted and the sediment examined under the microscope for the presence of fungal spores or other characteristic fruiting bodies of the fungal flora.

The determination of endophytic fungi was done by the use of agar plate method. Fifty seeds selected at random from each variety were surface sterilized with mercuric chloride (1:1000) for three minutes and washed with five changes of sterilized water. The seeds were spaced equidistantly in oats agar medium in Petri dishes and the plates were incubated at laboratory temperature (mentioned in the proper context). Throughout the investigation Petri dishes of ten centimeters only were used. The mycelium arising from the colonies around each seed, on the third or fourth day, was transferred to fresh agar slants. The cultures were further purified, if necessary, and the purified cultures were maintained in oats agar medium.

All the cultures, excepting *Piricularia* and *Sclerotium* used in the investigation, were isolated and purified in this laboratory from the seed samples obtained from different localities. The two cultures viz., *Piricularia* and *Sclerotium* were kindly supplied by the Government Mycologist, Coimbatore. The cultures when required for inoculation purposes, were multiplied on 50 c. c. of oats agar medium in 250 c. c. Erlenmeyer flasks for two weeks and then used as inoculum. A sterilized water suspension of the spores or sclerotia of the respective fungi were prepared and surface sterilized seed of MTU. 3 were treated with fungi for half an hour. After treatment, the seeds were dried on a clean drying sheet and then sown in pots containing sterilized soil.

**Experimental:** Seed-borne diseases are responsible for serious losses in almost every crop grown from seed. It is, therefore, important to test the seed before sowing regarding its freedom from diseases. The object of testing the seed is not only to determine the seed-borne infections but also to control them, if found in time, by some kind of seed treatment. It is also desirable to determine whether the infection is only superficial (epiphytic) or whether it penetrates more deeply into the seed (endophytic). In general the epiphytic infections occur more frequently than the endophytic ones and can be effectively controlled by seed treatment.

1. **Determination of epiphytic fungi by the centrifugal method:** The seed samples were examined for the presence of epiphytic fungi. For this purpose, ten seeds from each variety were picked at random and examined for the presence of prevalent fungi as per the method described in the foregoing page. The examination of thirty varieties of paddy constantly revealed the presence of the spores of the fungi mentioned in table 1. (Vide Appendix)

It will be seen from the data presented in table 1 that *Helminthosporium* was predominating in most of the varieties and it was followed by *Fusarium*. The prevalence of other fungi viz., *Curvularia*, *Alternaria*, *Nigrospora* and different groups of moulds viz., *Aspergillus*, *Penicillium* and *Rhizopus* were of equal intensity and noticed only on few varieties. Stray spores of *Piricularia* were observed only on two strains of paddy viz., ADT. 3 from Aduthurai and BCP. 2 from Buchireddipalem.

2. **Determination of endophytic fungal flora by agar plate method.** The different seed samples were examined for the prevalence of endophytic fungi by agar plate method. The seed material from each variety was divided into three separate lots, each consisting of fifty seeds selected at random. The first lot was surface sterilized with mercuric chloride (1:1000) and then incubated in oats agar medium. The second lot was treated with an organomercury fungicide viz., Agrosan GN and after removing the excess fungicide on the seed coat, the seeds were incubated in the agar medium. The third lot was directly incubated in the agar medium without any treatment to serve as control. The plating was done with seeds spaced at equidistant intervals under aseptic conditions. The plates were incubated at the laboratory temperature (25°C-30°C). On the third or fourth day the mycelium arising from the colonies around the seeds was transferred to fresh agar slants. The growth of the fungus in the slants was observed and examined under a microscope to make sure of its freedom from contamination. In all cases, the purity of the cultures was ascertained before proceeding to their identification. The cultures were identified mainly by observing their spores and other characteristic fructifications. No efforts were made to identify the species of the organisms. More than hundred isolations were made and the most prevalent genera of fungi frequently isolated from thirty different varieties are presented in table 2. (Vide Appendix)



The data presented in table 2 reveal that in the case of unsterilized seed (control) there was a preponderance of *Rhizopus* sp. in all the varieties. The prevalence of different groups of moulds viz., *Aspergillus*, *Penicillium* and bacteria was observed in low intensities only on few varieties. In the case of seed sterilized with mercuric chloride, the preponderance of *Curvularia* sp. over other fungi was very striking in all the varieties examined. The prevalence of *Helminthosporium* sp. was less than *Curvularia* sp. although its occurrence was more frequent than the rest of the fungi. Among the other fungi isolated \**Chaetomium* and moulds particularly species of *Aspergillus*, were significant while *Fusarium* and \**Sphaeropsis* were encountered less frequently. In the case of seed treated with Agrosan GN there was complete absence of fungi but bacteria were observed on few varieties.

3. Effect of associated seed-borne fungi on germination of seed and emergence of seedlings: The presence of micro-organisms either on the seed coat or inside affects the seed in several ways. The discolouration of rice grains on account of the presence of fungi on the seed coat is a very common phenomenon known to everyone familiar with growing of rice. The seedling blights and seed rots have been reported by many investigators as a result of using diseased seed material. With a view to studying the effect of different seed-borne fungi isolated from various samples, on the germination of seed and emergence of seedlings an experiment was conducted. The isolates of *Curvularia*, *Helminthosporium*, *Fusarium*, *Chaetomium*, *Sphaeropsis*, *Piricularia* and *Sclerotium* were selected for the experiment. The last mentioned fungus was included for the trial because of its frequent association with seed in the soil. The seed of a medium duration variety viz., MTU. 3 was surface sterilized with mercuric chloride (1:1000) and then divided into eight different lots. The seeds of seven lots were treated with spores or sclerotial suspension of the seven fungi mentioned above, for half an hour. The treated seeds were air dried and sown in pots containing sterilized soil. The eighth lot was also sown, without any treatment of fungus, for the purpose of control. The sowings were done in duplicate at the rate of 25 seeds per pot. The experiment was conducted during the regular paddy season and conditions were ideal for the normal germination of seed and development of seedlings.

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\*Kindly identified by the Government Mycologist, Coimbatore.

The observations were recorded on the germination of seed, emergence and vigour of seedlings. The germination of seed on the fourth day of sowing was taken as the basis for determining the percentage of emergence was obtained by recording the number of seedlings that have emerged completely and growing vigorously on the tenth day of sowing and the total number of seeds germinated in each pot. The results are presented in table 3.

TABLE 3.

*Effect of seed-borne fungi on the germination of seed and emergence of seedlings.*

No.	Name of fungus	Percentage of germination	Percentage of emergence	*Remarks
1.	<i>Chaetomium</i>	.. 52	100	
2.	<i>Curvularia</i>	.. 54	100	
3.	<i>Helminthosporium</i>	.. 42	80	
4.	<i>Fusarium</i>	.. 42	68	
5.	<i>Piricularia</i>	.. 50	88	
6.	<i>Sclerotium</i>	60	84	
7.	<i>Sphaeropsis</i>	58	80	
8.	Control (Untreated)	88	100	

\*The results obtained from both the pots were identical and the percentages given above represent the average of two pots.

It will be seen from the above data that the percentage of germination was affected in varying degrees by different fungi. The two parasitic fungi viz., *Fusarium* and *Helminthosporium* have caused failure of germination of seed to a marked extent while there was only slight reduction in germination percentage with *Piricularia*, *Chaetomium*, and *Curvularia*. The effect of *Sclerotium* and *Sphaeropsis* on germination was negligible in comparison with other fungi. The results also indicated that the emergence of seedlings was appreciably affected only by *Fusarium* while other fungi viz., *Helminthosporium*, *Sphaeropsis*, *Piricularia* and *Sclerotium* have affected the emergence only to a small degree. The normal emergence was not affected by *Curvularia* and *Chaetomium*. It was further observed that only those seed-borne fungi that are known to cause serious diseases on paddy like *Fusarium* and *Helminthosporium*, are capable of causing damage to seed and young seedlings while the saprophytic seed-borne fungi are not likely to cause any damage, under optimum conditions for the normal germination of seed and the growth of seedlings.

**Discussion:** Several investigators have reported that the fungi associated with seeds are responsible for bringing about reduced emergence and seed decay. Many fungi have been reported to cause pre-emergence seedling blights. These troubles are further aggravated when the weather and soil conditions favour the growth and development of seed and soil-borne fungi. A number of fungi are able to attack the grains, and Padwick (1950) includes *Piricularia oryza*, *Cochliobolus miyabeanus*, *Trichoconis padwickii*, *Gibberella zeae*, *Neovossia horrida*, *Ustilaginoidea virens* and *Nigrospora* spp. in that list. He has also listed some of the fungi that have been more frequently observed or isolated from the grains. Mundkur (1946) reported, after examining the diseased specimens of leaves and seeds collected from all over India, said to be affected by *Helminthosporium oryzae* (*C. miyabeanus*), that there was a preponderance of *Curvularia* spp. He also concluded that in all probability, the *Curvularia* spp. are responsible for losses similar to those caused by *H. oryzae*. In the present investigation the determination of epiphytic fungal flora by the centrifugal method has revealed the presence of many fungi viz., *Helminthosporium*, *Curvularia*, *Fusarium*, *Alternaria*, *Nigrospora*, *Piricularia* and several groups of moulds. But among these, there was a preponderance of *Helminthosporium* only over others and *Curvularia* was met with less frequently. The prevalence of other fungi was only of stray occurrence and observed only on some varieties.

The isolations of endophytic fungi by agar plate method yielded species of *Curvularia*, *Helminthosporium*, *Fusarium*, *Chaetomium*, *Sphaeropsis*, *Piricularia* and various moulds particularly species of *Aspergillus*. In this case there was a preponderance of *Curvularia* spp. over other fungi. *Helminthosporium* was also isolated readily from most of the varieties but less frequently than *Curvularia*. The other fungi were encountered in small degrees with few varieties only. It is evident from the results obtained that almost all varieties are infected with *Helminthosporium* and *Curvularia* to an appreciable extent and to a lesser degree with *Fusarium*. The other fungi isolated in the course of the investigation, excepting *Piricularia* which is mainly an air-borne parasite, are only saprophytes and are not known to cause any serious damage to paddy crop. Therefore a knowledge of the most commonly prevalent types of fungi on the seed material will be of immense help in devising the most economic and effective method of seed treatment.

The data regarding the effect of fungi isolated from seed material, on germination of seed and emergence of seedlings, revealed that *Fusarium* and *Helminthosporium* have markedly affected the germination of seed. The other fungi viz., *Chaetomium*, *Curvularia*, and *Piricularia* have affected the germination only to a small extent. The seedling emergence was affected to an appreciable degree only by *Fusarium* and to a little extent by *Helminthosporium*, *Piricularia*, *Sphaeropsis* and *Sclerotium*. The indications obtained with *Helminthosporium* and *Fusarium* in reducing the germination percentage and emergence of seedlings are in conformity with the results reported by other workers. Padwick (1950) stated that seeds infected with *H. oryzae* germinate poorly resulting in the wastage of some proportion of the seed. As a result of infection by *H. oryzae*, Ocfemia (1922) recorded a seedling mortality of 10 – 58 per cent in Philippines while Tucker (1927) recorded 15 per cent mortality and seedlings in Puerto Rico. Ito and Kimura (1931) reported from Japan that although accurate estimates of losses due to *Gibberella fujikuroi* (*Fusarium molini-forme*) are not known, they are frequently as high as 20 per cent or more, mainly in the seedbed but partly in the field. Thus the indications arrived at in this investigation emphasize the need for a critical examination of the seed material to ascertain the nature of the fungi present so that the most effective method of control can be devised.

Cralley and Tullis (1937) stated that rice seedling blight is a disease complex encountered in all rice growing tracts and the severity of seedling blight depends upon weather conditions and the microflora of the seed and the soil. They have further stated, that in Arkansas there was a tendency for the seedling blight to occur when the soil temperature was unfavourable for the rapid germination of the seed and maximum growth of seedlings. Tisdale (1922) stated that wet condition of the soil checked the normal growth of seedlings and favoured the development of the fungus resulting in the destruction of the seed. The results obtained in this investigation have indicated that only parasitic seed-borne organisms can cause damage to seed and young seedlings while the saprophytic seed-borne fungi are likely to be harmful only under conditions of temperature and soil moisture unfavourable for the normal germination of seed and development of seedlings. In the light of these findings, the evidence of sowing, at a time unfavourable for the germination of seed and development of seedlings.



eventhough the seed material is free from parasitic fungi, is a practical point of considerable importance to cultivators.

**Summary:** 1. The determination of epiphytic fungal flora by the centrifugal method, from thirty different varieties of paddy collected from all over the state, revealed constant association of the following fungi viz., *Helminthosporium*, *Curvularia*, *Fusarium*, *Alternaria*, *Nigrospora*, *Piricularia* and various groups of moulds.

2. The isolation of endophytic fungal flora by agar plate method repeatedly yield the following fungi viz., *Curvularia*, *Helminthosporium*, *Fusarium*, *Piricularia*, *Chaetomium*, *Sphaeropsis* and different groups of moulds particularly species of *Aspergillus*.

3. Among the several fungi isolated from different varieties, only two parasitic seed-borne fungi, *Helminthosporium* and *Fusarium* are found to cause adverse effects on germination of seed and emergence of seedlings.

4. The saprophytic seed-borne fungi viz., *Curvularia*, *Chaetomium* and *Sphaeropsis* are not likely to cause serious damage under optimum conditions for the normal germination of seed and the growth of seedlings.

**Acknowledgement:** The investigation was carried out at the Agricultural College, Bapatla, during 1953-54 as part of the research items of Mycology Section. I wish to express my sincere thanks to Sri T. S. Ramakrishnan, M. A., F. A. Sc., the then Government Mycologist, Coimbatore, for his helpful suggestions and critically going through the manuscript,

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