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# Isolation and identification of fungi associated with grain discoloration of rice in Chittoor and Nellore districts of Andhra Pradesh

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**Abstract**
Grain discoloration of rice is a complex disease occurred, due to infection by certain microorganisms, especially fungi on glumes, kernels or both. The incidence of rice grain discoloration was ranging from 36.85% to least 20% in Nellore and Chittoor districts. So emphasis had been laid to isolate the mycoflora associated, with rice grain discoloration and their identification was carried out. A total of seven fungal genera using blotter method and twelve fungal genera using agar plate method were isolated *viz*; *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Bipolaris oryzae, Curvularia lunata, Curvularia clavata, Fusarium moniliforme, Fusarium oxysporum, Sarocladium oryzae, Rhizopus stolonifer, Chetomium* sp. and *Alternaria padwickii*. The pathogenic fungi species associated with rice grain discoloration in this study are of having Economical significance as it causes both qualitative and quantitative losses of grain yield and also results in seedling mortality, reduction in germination and Seedling vigour.

**Keywords:** Isolation, identification, fungi, rice, *Aspergillus niger*, *Bipolaris oryzae*

## Introduction
Rice (*Oryza sativa* L.) is the primary staple food in many countries. In India it is cultivated in an area of 437.8 L ha (Lakh hectares) with 118.4 M T (Million Tonnes) of production and 2705 kg $ha^{-1}$ of productivity. In Andhra Pradesh, the area under cultivation of rice is approximately 23.56 L ha with 13.71 M T of production and 2381 kg $ha^{-1}$ of productivity (Govt. of India, Ministry of Agriculture, Dept. of Agriculture & Cooperation, Directorate of Economics & Statistics, Press Information Bureau 2021).

Rice crop suffers from many biotic and abiotic stresses that incite diseases. It is affected by several fungal, bacterial and viral diseases. As many as thirty-five fungal, eight bacterial, twenty viral and mycoplasmal diseases were reported on rice (Ou, 1985) [15]. The major diseases of rice are blast, sheath rot, sheath blight, brown spot and bacterial leaf blight that account yield losses by 15-20 per cent. Besides the major diseases, grain discoloration of rice is gaining importance in almost all rice growing areas of the world (Biswas, 2003) [5], it is increasing year after year by decreasing the yield potential of rice crop upto six per cent (Savary *et al*., 2000) [17].

Some plant diseases, which were less significant earlier, are now gradually gaining importance and posing a serious threat to the crop production. Among these, the rice grain discoloration disease is one, which is also known as “Glume discoloration”, “Dirty panicle” etc. (Plate 1). Discoloration has been prevalent in almost all part of the world where rice is grown. It is presently posing a serious threat in rice growing areas of India, the early and medium rice cultivars grown particularly in wet seasons are generally exposed to high humidity and warm environmental conditions during flowering and post flowering stages, which significantly induced the disease incidence. These fungi individually or in combination were demonstrated to be infectious (Dash and Narain, 1988) [9].

Grain discoloration of rice is a complex disease occurred, due to infection by certain microorganisms on glumes, kernels or both. The disease is causing both qualitative and quantitative losses of grain yield and also results in seedling mortality, reduction in germination and seedling vigour. Seed (or) grain discoloration is an early indication of poor seed or grain quality which is generally associated with micro-organisms and sometimes insect pests. Such grains are of poor market value and low consumption quality due to degradation in nutritional value.

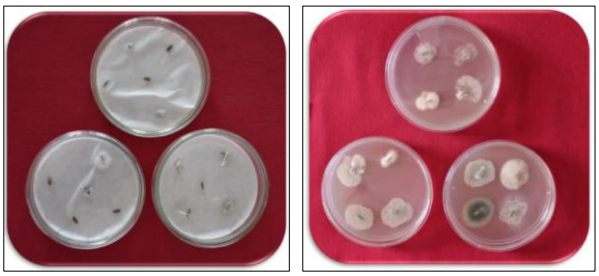
Except for other factors several microorganisms especially fungi play a major role in the development of this disease. Under humid conditions, the fungal growth may be prominently seen.

**Plate 1:** Discoloured panicle

## Materials and Methods

**Isolation of pathogens by agar plate method:** The discoloured grain samples were collected in a polythene bag and labeled the samples properly and then the samples were brought to the laboratory for further studies. Fungi associated with discoloured grains had been isolated by following the agar plate and blotter method (ISTA, 1976) [12]. The discoloured grains were surface sterilized with 1.0 per cent sodium hypochlorite solution for 1 min and rinsed aseptically in three changes of sterilized distilled water. The surface sterilized discoloured rice grains were placed on sterile Petri plates containing 20 ml of potato dextrose agar medium and were incubated at 27±1 °C for 5 - 8 days under alternate cycles of light and darkness (ISTA, 1976) [12]. The fungi grown on PDA medium were purified by single hyphal tip method then sub cultured and transferred aseptically to PDA slants for temporary preservation and stored at 4 °C (Plate 2).

**Isolation of the pathogen by blotter plate method:** Fungi were isolated by blotter method from the discoloured grains of rice which were collected from the rice fields. Firstly, Petri plates were surface sterilized by hot air oven and blotter papers are cut into 9 cm discs to fit into the Petri plate, then under aseptic conditions in the laminar airflow, blotter paper was placed in the sterilized Petri plate. Later, surface sterilized discoloured grains with 1% sodium hypochlorite were placed on the blotter paper. Finally, 200 µl of sterilized distilled water was sprinkled on it and incubated at room temperature (27±1 °C) for allowing the growth of the fungi associated with grain discoloration (Plate 2).



a) Blotter paper method B) Agar plate method

**Plate 2:** Isoaltion of mycoflora associated with grain discolouration

After observing the fungal growth on grains, the fungi were isolated and purified by single spore isolation method. Later, sub cultured and transferred aseptically to PDA slants for temporary preservation and stored at 4 °C.

**Identification of Pathogens:** From pure cultures of the fungi, which were grown in PDA separately, mycelium was transferred onto the microscopic slide and observed under the microscope. The isolated test pathogens were identified based on their mycelia growth, fungal colony and spore characters (Barnett and Hunter, 1972) [4].

## Results and Discussion

**Blotter method:** A total number of seven fungal genera were found to be associated with the seed samples collected from different Mandals in Chittoor and Nellore districts. The fungal genera *viz*; *F. moniliforme*, *C. lunata*, *A. padwickii*, *A. niger*, *A. flavus*, *Chaetomium* sp. and *R. stolonifer* were isolated using blotter method.

**Agar plate method:** A total number of twelve fungal genera were found to be associated with the seed samples collected from different mandals in Chittoor and Nellore districts. The fungal genera *viz*; *F. moniliforme*, *F. oxysporum*, *B. oryzae*, *C. clavata*, *S. oryzae C. lunata*, *A. padwickii*, *P. grisea*, *A. niger*, *A. flavus*, *Chaetomium* sp., and *R. stolonifer* were isolated using agar plate method.

**Identification and characterization:** The different fungi isolated from the rice seed samples were purified by fungal hyphal tip method. The identification of the isolated fungi was done based on cultural and morphological characters and fructifications produced using research compound microscope (Olympus CX 41). The pure cultures of different fungi and photo micrographic pictures are produced in Plate 3.

### *Alternaria alternata*

Colonies appeared black on PDA. Septation of hyphae was quite conspicuous under microscope. Conidiophores were pale brown, simple or branched, bearing catenulate conidia at the apex and apical part. Multicellular conidia with both transverse and longitudinal septa were broader at the base than tip portion. Conidial formation in acropetal manner was in chain. Sometimes, well developed beaks were present at the tip portion of the conidia. The cultural and morphological characters of the fungal isolate indicated its close identity with *A. alternata*.

### *Aspergillus flavus*

Fungal isolate was fast growing and developed olive green colonies. The mycelium of the fungus was septate. Long and hyaline stipes with little rough wall surface terminated into subglobose vesicles. The vesicles were uniseriate as well as biseriate. Conidiophores were erect and simple. Conidia were globose to subglobose, hyaline, single celled and produced in chains. The morphological characters of the fungal isolate indicated its close identity with *A. flavus*.

### *Aspergillus niger*

Mycelial growth was quite black, which revealed somewhat pale yellow colour in reverse side of the Petri plates. Stripes were long, smooth walled and terminated into spherical vesicles. It was biseriate. Conidiophores were pale brown,

erect, simple, thick walled and with foot cell basally. Conidia were globose in shape showing rough surface texture under high power of magnification. The cultural and morphological characters of the fungal isolate revealed it to be *A. niger*.

***Bipolaris oryzae***

Colonies of the isolate grew rapidly and texture was velvety to wooly. The colonies were initially white to grayish brown and become olive green to black. Hyphae were septate. Conidiophores were simple or branched, pale brown in color, geniculate and sympodial. Conidia were cylindrical in shape. Germination of conidium was bipolar. The cultural and morphological characters of the fungal isolate indicated its close identity with *B. oryzae*.

***Curvularia lunata***

Septate mycelial colonies of the isolate were black with irregular margin on PDA. Conidiophores were erect, brown, branched, curved, bearing conidia apically and laterally. Conidia were curved, transversally septate and dark brown in two central cells. Isolate was identified as *C. lunata*.

***Curvularia clavata***

Colonies were black and mycelium was septate. Conidia were straight and transversally septate. The cultural and morphological characters of the fungal isolate indicated its close identity with *C. clavata*.

***Fusarium moniliforme***

The isolate produced initially white colonies later, turned into pinkish and floccose colony. Hyphae are branched and septate. Microconidia formed in a chain or false head on laterally borne conidiophores. Conidia are 1-2 celled, fusiform to ovate and hyaline. Macroconidia are borne on phialides, 3 to 7 septate, straight or slightly curved, basal cell slightly or distinctly pedicellate, formed in sporodochia. It was identified as *F. moniliforme*.

***Fusarium oxysporum***

Colonies were initially white, become tinged with salmon and lavender at maturity. Reverse was lavender to purple. Conidiophores were short, hyaline and bearing spore masses at the apex. Macroconidia were usually abundant and slightly sickle-shaped, thin-walled, with attenuated apical cell and a foot-shaped basal cell. Micro conidia were abundant, mostly non-septate and slightly curved or straight. Clamydospores were brown, globose and solitary. The cultural and morphological characters revealed its close identity with *F. oxysporum*.

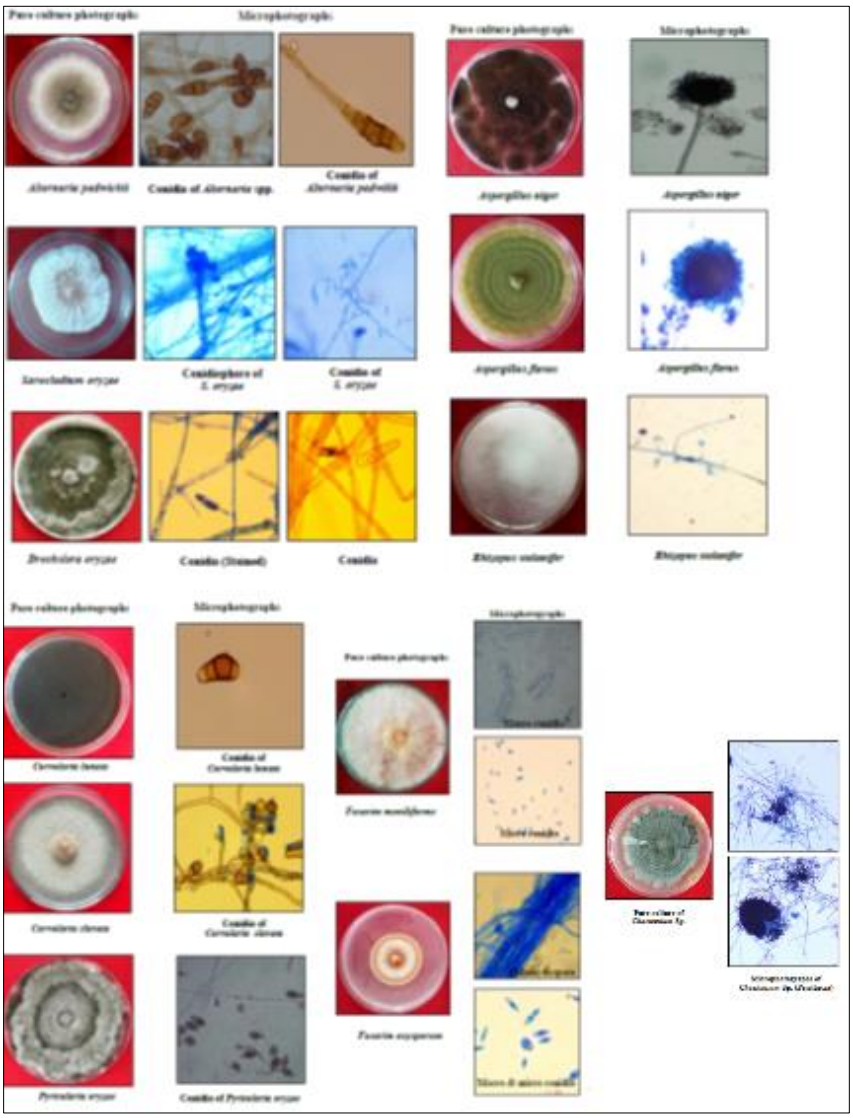


**Plate 3:** Pure cultures and photo microphotographs of isolated mycoflora associated with grain discoloration

***Sarocladium oryzae***

The mycelium was white or pinkish, which grow slowly and sporulate abundant in the central parts of colonies. The conidiophores were irregularly branched and produced hyaline conidia. Conidiophores arising from the mycelium were hyaline, smooth, branched, monophialidic discrete, elongate, and cylindrical with apical conidiogenous cells in groups of 2-5. Single, individual intercalary phialides were also observed on the conidiophores. The phialides being flask shaped, elongated and narrow towards the apex. The conidia formed in slimy masses were cylindrical with rounded ends, sometimes becoming slightly curved, hyaline, thin walled, smooth and one-celled. Isolates revealed its close identity with *S. oryzae*.

***Rhizopus stolonifer***

The isolate on PDA produced fast growing colonies. Texture was typically cotton candy like. Hyphae were coenocytic. Sporangiophores, rhizoids and sporangia are rapidly visualized. Sporangiophores were erect, yellowish to dark brown and rhizoidal. Sporangia were globose, dark brown to black, minutely spiny and apparently subglobose after maturity. Sporangiophores were subglobose to sub elliptical and pale brown with bluish stripes. The cultural and morphological characters indicated its close identity with *R. stolonifer*.

***Chaetomium* sp.**

Pale yellow to grayish green colony. Hyaline and septate hyphae. Ascoma (Perithecia) are spherical to Ovoidal to obovoidal with numerous hairs, usually unbranched, flexsulose, undulating or coiled, septate, brownish in colour. The cultural and morphological characters indicated its close identity with *Chaetomium* sp.

***Alternaria padwickii***

Light salmon to dark grayish brown colony, the profusely growing mycelia are grayish brown, thin, well-developed, copiously branched, hyaline, while young and become salmon to dark brown at maturity, are common characteristic of this fungus. Sometimes pinkish to brownish areas are seen over the blotter around the seed. Aerial hyphae with straw-colored to dark brown conidia with long terminal appendages. Conidiophores are swollen apically and conidia are fusiform, nondeciduous, have three to five transverse septa. It was identified as *A. padwickii*.

The results were in agreement with the work done by Chandramani (2007) [7], who conducted studies on grain discoloration of rice and isolated twelve fungal genera associated with discoloured grain samples of ten different rice varieties using blotter and agar plate method. Among there, most commonly associated fungal genera were *D. oryzae*, *F. moniliforme*, *C. lunata*, *S. oryzae* and *A. padwickii*. The cultural characterization of the fungi was done by using colour, texture and mycelia growth of the fungal colony on PDA plates and morphological characterization was done by studying the conidial characters colour, shape, size and cell number.

**This study summarized and concluded as follow**

Two groups of fungi are associated in grain discoloration of rice. One group is field fungi, more or less parasitic and infects grain before harvest *viz*; *Drechslera oryzae*, *Pyricularia oryzae*, *Alternaria padwikii*, *Fusarium moniliforme*, *Curvularia geniculata*, *Sarocladium oryzae* etc. Other groups are storage molds, saprophytes *viz*; *Aspergillus* sp., *Penicillium* sp., *Mucor* sp., *Rhizopus* sp. etc.

The seed-borne inoculum of *A. alternata* is responsible for ashy grey discoloration and *D. oryzae* (*Cochliobolus miyabeanus*) is responsible for black discoloration, dark brown spots and light to dark brown dot like spots are found in the seed coat and endosperm of discoloured seed. Whereas, Curvularia *geniculata* found responsible for eye shaped spots. Besides, *Fusarium equiseti*, *Fusarium oxysporum* (*Gibberella zeae*), *F. moniliforme* (*Gibberella fujikuroi*) found responsible for pink discoloration and *S. oryzae* is responsible for light brown discoloration on the seed coat, endosperm and embryo of discoloured seed (Sachan and Agarwal, 1994) [16].

**References**


1. Ahmed M, Hossain M, Hassan K, Dash CK. Efficacy of different plant extracts on reducing seed borne infection and increasing germination of collected rice seed samples. Universal Journal of Plant Sciences. 2013;1(3):66-73.
2. Akila R, Ebenezar EG. Ecofriendly approaches for the management of grain discoloration in rice. Journal of Biological Control. 2009;23(2):175-180.
3. Anonymous. 24th PPSC report, NARP, GAU, Navsari path, 1988, 27-30.
4. Barnett HL, Hunter BB. Illustrated genera of imperfect fungi. Burgess Publishing Company, 1972, 273-275.
5. Biswas A. Grain discoloration disease of rice. Journal Mycopathological Research. 2003;4(11):7-13.
6. Chandramani B, Awadhiya GK. Assessment of percent grain discoloration in important rice varieties. International Journal of Current Research in Biosciences and Plant Biology. 2014;1(5):61-64.
7. Chandramani R. Studies on grain discoloration of rice. M.Sc. (Ag.) Thesis submitted to Indira Gandhi Krishi Viswavidyalaya, Raipur, 2007, 51.
8. Dash AN, Narain A. Detection of grain discoloration fungal organisms of rice and production of disease free seeds. Indian Journal of Mycology and Plant Pathology. 1988;18(1):24-30.
9. Dash AN. Further studies on paddy seed discoloration and its nature with relation to associated fungi. M.Sc. (Ag) Thesis. Orissa University of Agriculture and Technology, Bhubaneswar, 1986, 78.
10. Government of India, Ministry of Agriculture, Department of agriculture and cooperation, Directorate of Economics and Statistics, 2016. Area and production of Rice in India, 2016-17.
11. Gurjar MS, Ali S, Akhtar M, Singh KS. Efficacy of plant extracts in plant disease management. Agricultural Sciences. 2012;3:425-433.
12. ISTA. International rules for seed testing. Seed Science and Technology. 1976;4:3-49.
13. ISTA. International rules for seed testing. Seed science and Technology. 1995;21:141-146.
14. Nene YL, Thapliyal PN. Fungicides in plant disease control, 3rd ed. Oxford and IBH Publishing Co. Pvt. Ltd. Calcutta, 1993, 531-550.
15. Ou SH. Commonwealth Mycological Institute, England. Rice Diseases, 1985, 61-96.
16. Sachan IP, Agarwal VK. Efficacy of seed treatment of

discolored seeds of rice on seed borne inoculum, germination and seedling vigour. Seed Research. 1994;22(1):45-49.
17. Savary S, Willocquet L, Elazegui FA, Castilla NP, Teng PS. Rice pest constraints in tropical Asia: quantification of yield losses due to rice pests in a range of production situations. Plant Disease. 2000;84:357-369.